(EO2) Effects of track structure in radiation chemistry. Jay A. LaVerne. Universtiy of Notre Dame, Notre Dame, IN, USA.

This presentation will define and describe some of the factors responsible for the local geometry of decomposition products, track structure, induced by the passage of ionizing radiation and how that structure is responsible for much of the subsequent chemistry and biology. There have been many attempts to define track structure and provide quantitative predictability of product yields with various degrees of success. A number of recent experimental and model studies have provided new insights into the physico-chemical processes occurring in water on the picosecond time scale that have many long time consequences. Water will be the medium of choice in this presentation because of the obvious biological implications, but the fundamental physics and chemistry would equally apply to almost any condensed phase medium. A qualitative approach will be used to explain the primary processes occurring in passage of ionizing radiation through condensed matter including the deposition of energy, how the medium responds, and the fate of products. A brief description of the transient species and final products that make up the basic radiation chemistry of water will be given and possible biological implications described.

(EO3) Reassessment of effective dose of A-bomb radiation and its possible impact on risk evaluation. Masao S. Sasaki. National Institute of Biomedical Innovation, Osaka, Japan.

Follow-up study of A-bomb survivors in Hiroshima and Nagasaki continues to provide an important source of information on radiation effects on humans. The A-bomb radiation is a mixture of γ-rays and neutrons, and hence for the risk projection the neutron dose should be converted to the equivalent effective dose by weighting with relative biological effectiveness (RBE). However, the direct derivation of any meaningful estimate of neutron RBE has been reckoned impossible from the mortality data of survivors. To overcome this difficulty, we attempted an experimental approach first to simulate the chromosomal RBE of A-bomb spectrum energy neutrons relative to A-bomb γ-rays, then its validity being tested for chromosomal data in survivors, and next extended to the implication of cancer incidence. In the *in vitro* experiments using human blood lymphocytes, chromosomal RBE of fission neutrons was dependent on dose but not on the energy spectrum. The maximum RBE at low dose limit, RBE_M, was 75. When the dose-dependent RBE was applied to the chromosome aberration data in survivors, the current disparity in the effects between Hiroshima and Nagasaki was eliminated for DS86 system and with higher probability for DS02 system. Analysis of chromosome aberration frequencies in Nagasaki factory workers was consistent with partial-body exposure, where on average 25% of the body was additionally shielded. The RBE of fission neutrons in inducing tumors in experimental animals (mice and rats) was re-calculated for published data in literature which provided three or more dose points in low dose range before bending-over of the incidence. With these limitations, the RBE of fission neutrons was also dose dependent with its dose-response mode comparable to that of chromosomal effects, but with different RBE_M by tumor type; 86.7 ± 37.7 for solid tumors and 7.8 ± 2.4 for leukemia and lymphoma. Such dose-dependent and tumor-type specific RBE was successfully applied to the equality of Hiroshima and Nagasaki survivors in the risk of solid cancer and leukemia. The present neutron weighting system results in an increase of effective dose and hence reduction of cancer risk. The reduction factor is about 0.66 for solid cancer and 0.97 for leukemia as compared to the current risk estimates using constant RBE of 10.

(EO4) DNA repair foci: what are they, and what are they good for? Peggy L. Olive. BC Cancer Research Centre, Vancouver, BC, Canada.

Ionizing radiation produces complex DNA lesions including double-strand breaks that can lead to chromosome damage and cell death. Accompanying changes in chromatin organization may activate signaling molecules like the ATM kinase that phosphorylate proteins involved in cell cycle arrest and DNA repair. Proof that DNA repair molecules like Mre11 can aggegate at sites of DNA damage was first shown by Petrini, and Bonner's group subsequently identified a minor nucleosomal histone protein, H2AX, that became phosphorylated in megabase regions surrounding each double-strand break. These "γH2AX foci" made it possible to determine the number and placement of individual double-strand breaks within a nucleus. Several groups have employed γH2AX foci in radiation biodosimetry and track structure analysis as well as examining chromatin behavior after irradiation. We now know that more than two dozen molecules involved in DNA repair or chromatin organization can aggregate as large clusters within irradiated nuclei. Many but not all co-localize with γH2AX. Repair foci that form after irradiation may be immediate or delayed, cell cycle dependent or independent, and may be lost before mitosis or persist through cell division. RAD51, γH2AX and RPA can also appear as foci within micronuclei. Double-strand break-induced foci are sufficiently large (> 1000 molecules per focus), that antibody labeling provides a suitable method for their detection. Why these molecules aggregate in such large clusters is still under debate. One possibility is that they signify damage that cannot be easily repaired. This would explain why the fraction of cells that retain γH2AX or RAD51 foci 24 hours after irradiation is similar to the fraction of cells that die. This refresher course will discuss the nature of DNA repair foci and their applications in low dose radiobiology and DNA repair studies.

(EO5) Target for radiation cell kill: rafts or DNA? Richard N. Kolesnick. Sloan-Kettering Institute, New York, NY, USA.

Emerging evidence suggests clustering of plasma membrane rafts into ceramide-enriched platforms serves as a transmembrane signaling mechanism for a subset of cell surface receptors and environmental stresses. These cell membrane domains transmit signals for stimuli as diverse as CD95, CD40, TCR, chemotherapy, infection with pathogenic bacteria and viruses, UV-C as well as exposure to ionizing radiation. Our data show that upon stimulation, a secreted form of acid sphingomyelinase (ASM) translocates into and generates ceramide within rafts by sphingomyelin hydrolysis. Ceramide, which possesses the unique capability to self-associate, provides the driving force for coalescence of these sub-microscopic rafts into the large signaling platforms. These platforms, although existing only transiently, serve as sites for select protein concentration and oligomerization, a requirement for transmembrane signaling. Genetic and pharmacologic inhibition of these structures indicate that these structures are required for most of the clonogenic death of irradiated Jurkat T cells and apoptotic death of endothelium. This system thus provides a new set of targets for pharmacologic intervention in radiation responses that utilize ASM-based signaling for induction of cell death.

(EO6) Heavy ion radiobiology in therapy and space. Marco Durante. University Federico II, Napoli, Italy.

Research in the field of biological effects of heavy charged particles is needed for both heavy-ion therapy (hadrontherapy) and protection from the exposure to galactic cosmic radiation in long-term manned space missions. Although the exposure conditions (e.g. high- vs. low-dose rate) are different in therapy and space, it is clear that a substantial overlap exists in several research topics, such as individual radiosensitivity, mixed radiation fields, and tissue degenerative effects. Late effects of heavy ions are arguably the main health risk for human space exploration, and with the increasing number of cancer patients treated by heavy-ion therapy, including young adults and children, this issue is now becoming the main source of uncertainty for the success of hadrontherapy as well. Reducing uncertainty in both cancer and noncancer late risk estimates is therefore the first priority in heavy-ion radiobiology. In addition, researchers involved either in experimental studies on space radiation protection or heavy-ion therapy often use the same accelerator facilities. Several heavy-ion therapy facilities are now under construction or planned in Europe, USA, and Japan. Beamtime will be available at these facilities for clinical radiobiology and basic heavy-ion effects experimental research, as

already happens since several years at the HIMAC in Japan. The NASA Space Radiation Laboratory (NSRL) in Brookhaven (Long Island, NY) provides beams of very heavy ions at energies around 1 GeV/n which are of specific interest for space radiobiology. In Europe, these very high energy beams are available at GSI in Germany, where the new Facility for Antiprotons and Ion Research (FAIR) is currently under construction. It is foreseeable that the availability of beamtime and the presence of many dedicated research programs will lead to great improvements in our knowledge of biological effects of heavy ions in the coming few years.

(EO7) Low dose radiation exposure, polymorphisms, and thyroid cancer risks. Alice Sigurdson. National Cancer Institute, Bethesda, MD, USA.

Thyroid cancer is relatively rare, but among US women has recently increased in frequency so that in 2007 it will be the sixth most common female malignancy. Incidence patterns in adults by age and gender are different from those for many other solid cancers in that thyroid cancer is most common in females (by about three to one compared to males) and the average diagnosis age is in the fourth rather than the sixth decade of life. Risk factors for thyroid cancer are not well understood except for exposure to ionizing radiation in the head and neck region at a young age, which increases risk substantially compared to adult exposure. A clear dose-response relationship has been shown in many different populations exposed to ionizing radiation from medical or environmental sources and this appears to be linear at relatively low doses, with iodine deficiency potentially increasing risk. At much higher doses (>20 Gy), risk of thyroid cancer decreases likely due to the effect of cell killing. Papillary thyroid cancer is the most common type and is the histology associated with ionizing radiation exposure. Genetic variation may contribute to the risk of papillary thyroid cancer and several studies have examined the effect of polymorphisms. However, most studies have been small and few, if any, associations have been confirmed. Large consortia will be required to uncover and replicate individual study findings. Similarly, the detection of gene-radiation interaction is hampered by small sample sizes and robust dose-response relationships unless doses were high or age at exposure was low. Some groups have considered thyroid nodules as a surrogate endpoint, such as the study of residents near the Semipalatinsk nuclear test site in Kazakhstan in which several polymorphic variants in *RET*-related and other genes were evaluated. In this eye-opener session, several studies will be reviewed and suggestions for future directions will be discussed.

(EO8) Targeted radiotherapy: light at the end of the tunnel? Robert J. Mairs, Marie Boyd. Cancer Research UK Beatson Laboratories, Glasgow, United Kingdom.

Indirect effects may contribute to the efficacy of radiotherapy by sterilizing malignant cells that are not directly irradiated. However, little is known of the influence of indirect effects in targeted radionuclide treatment. We compared γ-radiation-induced bystander effects with those resulting from exposure to three radiohaloanalogues of *meta*-iodobenzylguanidine (MIBG): [^{131}I]MIBG (low linear energy transfer (LET) β-emitter), [^{123}I]MIBG (high LET Auger electron emitter), and *meta*-[^{211}At]astatobenzylguanidine ([^{211}At]MABG) (high LET α-emitter). Cells derived from a human glioma (UVW) and a bladder carcinoma (EJ138) were exposed to media from γ-irradiated cells. They exhibited a dose-dependent reduction in survival fraction at low dosage and a plateau in cell kill at > 2 Gy. Cells treated with media from [^{131}I]MIBG demonstrated a dose-response relationship with respect to clonogenic cell death and no annihilation of this effect at high radiopharmaceutical dosage. In contrast, cells receiving media from cultures treated with [^{211}At]MABG or [^{123}I]MIBG exhibited dose-dependent toxicity at low dose but elimination of cytotoxicity with increasing radiation dose (i.e. U-shaped survival curves). Therefore radionuclides emitting high LET radiation may elicit toxic or protective effects on neighboring untargeted cells at low and high dose respectively. We conclude that radiopharmaceutical-induced bystander effects are potent, may depend on LET and be distinct from those elicited by conventional radiotherapy.

(EO10) New mechanistic approaches to modelling radiation carcinogenesis. Herwig G. Paretzke, Wolfgang Heidenreich, Werner Friedland. GSF-Institute of Radiation Protection, Neuherberg, Germany.

Ionizing radiation in the medium (0.1 - 1 Sv) to high (> 1 Gy) dose ranges can induce and can cure cancers. The risks of significant radiation induced human health effects in the low dose (< 0.1 Sv) and low dose rate range, however, are largely speculative and -if existent at all- most likely will ever be buried in the signal noise of epidemiological data sets (until the advent of reliable molecular radiation markers in tumour cells). Therefore mechanistic, mathematical models for testing hypotheses describing the complex processes set into motion by radiation interactions in humans are needed to permit extrapolations and interpolations in the multi-parameter space regarding the radiation field and the individual human data of solid, relevant experimental observations. Such models have been developed by many authors over many years. In this Eye-Opener-Lecture a survey will be given on the present status of this field and on possible developments, primarily along the concepts of multi-level systems radiation biology describing the reversible and irreversible biological and medical processes following radiation interactions with a living organism. The importance and necessity of close and successful cooperation between experimental and theoretical researchers as well as across disciplines will be emphasized.

(EO11) The silent treatment: delivering RNA interference. Judy Lieberman. CBR Institute for Biomedical Research, Boston, MA, USA.

Harnessing RNAi presents an opportunity for treating a variety of diseases. The main obstacle is delivering siRNAs into target cells *in vivo*. Topical delivery at mucosal surfaces may be especially effective. Intravaginal application of siRNAs could protect mice from lethal herpes simple virus type 2 (HSV-2) sexually transmitted infection. siRNAs mixed with lipid silence gene expression deep in the mouse vagina and ectocervix for at least 9 days. Intravaginal application of siRNAs targeting HSV-2 genes and a host receptor was well tolerated, did not induce interferon responsive genes or cause inflammation, and protected mice when administered before and/or following lethal HSV-2 challenge. Therefore siRNAs are attractive candidates for the active component of a topical microbicide to prevent or treat viral infection. We devised a method for cell-specific systemic delivery of siRNAs by mixing siRNAs with an antibody fragment fused to protamine. These siRNA complexes silence gene expression *in vitro* or *in vivo* with exquisite specificity in cells bearing the receptor recognized by the antibody and can target cells, such as primary lymphocytes, that are refractory to lipid-mediated transfection. Intravenously injected siRNA-fusion protein complexes inhibit subcutaneous or pulmonary tumors in mice. Fusion proteins have been engineered to deliver siRNAs into all human (or only activated) leukocytes.

(EO12) Novel radioprotectors. Roger F. Martin. Peter MacCallum Cancer Centre, Melbourne, Australia.

Amifostine, the only radioprotector with FDA-approval for use in radiotherapy, emerged from a research program at the Walter Reed Army Research Institute in the 1950s-60s. Thousands of compounds were synthesised and evaluated for radioprotective activity, amid the concerns of the nuclear era. The most promising compounds were radical-scavenging aminothiols, and Amifostine (initially known as WR2721), a phosphorothioester prodrug of an active aminothiol, emerged as the lead. Although Amifostine was

considered too toxic to be adopted for military use, the potential use of radioprotectors to ameliorate damage to normal tissues during cancer radiotherapy motivated continuing attention. The fact that the road to FDA-approval was such a long one for Amifostine, may reflect a combination of factors, including some limiting toxicities (principally hypotension), the pressing clinical need for an effective radioprotector, and the lack of competition. Interestingly, half a century later, a new impetus for radioprotector research again came from outside the experimental radiation oncology arena. The US government funding of several new Centres for Medical Countermeasures Against Radiation rejuvenated interest in developing new radioprotectors. While the most important feature of the response to 9/11 in the radiological context, was the realisation of the dearth of effective radioprotectors, the language that emerged clarified some ambiguities. The general term "countermeasures" embraces three distinct types of pharmacological intervention. *Protectors/radioprotectors* now refer to prophylactic agents, which reduce initial radiochemical damage when present before and during irradiation. *Mitigators* can be effective if administered after irradiation, but before the appearance of overt biological consequences, and *treatments*, such as haematopoietic growth factors, are employed after clinical symptoms develop. This review of radioprotectors, old and new, will focus on more recent developments, such as SOD mimetics and those agents with IND status for Acute Radiation Syndrome (ARS), namely the isoflavone Bio 300, and 5-androstenediol. The development of new antioxidants, particularly the DNA-binding bibenzimidazoles characterised by methylproamine, will be reviewed in some detail.

(EO13) Host-tumor interactions in metastasis: a pase double. Ruth J. Muschel. Oxford University, Oxford, United Kingdom.

Much of the literature about cancer is centered upon attributing the properties of cancer to intrinsic properties of the cancer cell itself. However, many of these properties are seen now as additionally depending upon attributes of the host response to the cancer. This includes metastasis. The host response includes cellular, inflammatory, coagulation and extracellular matrix components. Cancer cell motility, homing to and survival at distant sites are now seen as having required host elements. The most studied of these components has been the vascular response, including those of circulating endothelial stem cells. But there is also conclusive evidence for the involvement of other host components in metastasis. Coagulation is required for metastasis to occur in animal models. Attachment to extracellular matrix components helps confer site specificity as does response to host cytokine production. The induction of anti apoptotic and the extracellular remodeling enzyme lysyl oxidase by hypoxia enhances metastatic potential indicating a contribution by the tumor metabolic environment. Macrophage involvement is required for metastasis at several steps, and there is less well documented evidence supporting the involvement of other members of the innate immune system. Some of this information has been revealed by new microscopic methods for examining tumors. This dance between the host and tumor will be reviewed in this session.

(EO14) Nanoparticles and nanomaterials in cancer and radiation biology. Gayle Woloschak. Northwestern University, Chicago, IL, USA.

Nanoparticles and nanomaterials are becoming increasingly more important for radiation therapy, hyperthermia delivery, imaging, and cancer therapy. Nanomaterials are being used to provide nanobeams for delivery of radiation to cells and animals; nanoparticles are being used as docking platforms for delivery of chemotherapeutic agents and other treatment modalities to cells and even to intracellular locations. Nanoparticles are providing approaches to concentrate contrast agents so that large concentrations can be built up in cells and tissues and thus permit visualization of tumors too small to identify by conventional means. Iron nanoparticles are permitting the development of hyperthermia agents that can achieve locally high concentrations in subcellular locations. There are numerous other applications that are being developed in the broad field. The goal of this talk is to provide a basic understanding of nanotechnology and novel approaches that are being developed as a consequence of nanotechnology that are impacting radiation and cancer biology, therapy, and diagnosis.

(EO15) Understanding the chemistry of stored defense nuclear waste: studies of waste simulants. Donald M. Camaioni. Pacific Northwest National Laboratory, Richland, WA, USA.

This presentation will highlight research over the past decade that has advanced the understanding of high-level waste stored at Department of Energy (DOE) nuclear materials production sites. The waste invariably contains high concentrations, often above saturation levels, of nitrate and nitrite salts. Because of the high efficiency of scavenging of the primary radicals from water radiolysis by NO_3^-/NO_2^-, a majority of the radiolytically generated radicals are of the NO_x family. Therefore, the early events that follow the absorption of radiation, and the subsequent redox chemistry of NO_x radicals and ions are highly relevant to understanding radiolytic aging of wastes. Pulse radiolysis in conjunction with time-resolved electron spin resonance detection was used to identify the initial products of radiolysis of NO_x^- solutions. Continuous gamma-radiolysis and NO_2 gas contacted with waste simulants, with subsequent product analyses by ion chromatography and nuclear magnetic resonance spectroscopy, was used to identify later events in the evolving chemistry of these systems. Intermediates were characterized using ab initio theory and dielectric continuum models of solvation. Insights from the science and some of the quantitative rate determinations have been incorporated into safety analyses at the sites. In that context they contributed to the resolution of several of the safety issues, including the organic tanks and gas generation issues at the Hanford site. The research benefited from a coordinated effort by groups at the Pacific Northwest National Laboratory and the Notre Dame Radiation Laboratory with funding provide by the DOE Environmental Management Science Program and the Basic Energy Sciences Program. (Pacific Northwest National Laboratory is operated by Battelle for the US DOE.)

(CL1) Molecular imaging as applied to cancer and radiation therapy. Patricia M. Price. University of Manchester, Manchester, United Kingdom.

In vivo molecular imaging is an emerging discipline of importance to the development of anti-cancer therapies. Technologies include optical, magnetic resonance and nuclear medicine techniques. Positron emission tomography (PET) is the most sensitive and specific technique for imaging molecular pathways *in vivo* in humans. PET uses positron emitting radionuclides to label molecules, which can then be imaged *in vivo*. The inherent sensitivity & specificity of PET is a major strength. PET can image molecular interactions and pathways, providing quantitative kinetic information down to sub-picomolar levels. It is emerging as a valuable tool for tumour diagnosis, disease staging, treatment monitoring & drug development and in the radiotherapy treatment process. PET in Drug Development: Molecular imaging can provide pharmacokinetic, pharmacodynamic and mechanistic information. Use of the technique in early clinical trials can: (1) provide information on optimum biological dose & PK/PD relationships; (2) identify tumours containing specific molecular targets; and (3) provide *in vivo* pharmacodynamic evaluation of compounds. Its use can also be extended to general physiological questions regarding vascular physiology and *in vivo* pharmacokinetics. PET in radiotherapy: In radiotherapy molecular imaging has potential for quantitating the biological phenotype and can provide additional biological information to improve disease staging and Tumour Volume Definition. PET studies may allow specific biologic subvolumes of tumour tissue to be identified, enabling targeted dose-modulated radiotherapy techniques. Investigations of radiotherapy response/resistance by PET will help to define Biological Target Volumes and optimise patient management. PET will increase our understanding of key tumour biological parameters such as metabolism, hypoxia, proliferation and blood flow. Initial studies suggest such new information could be used to select patients for specific therapy. Further research and development of PET methods will contribute to an increased understanding of tumour biology and the complex biological processes that contribute to chemo & radiotherapy resistance.

(CL2) Low energy electron driven processes and their radiation chemical effects. Leon Sanche. Sherbrooke University, Sherbrooke, PQ, Canada.

Low-energy electrons (LEEs) are produced in large quantities in any type of material irradiated by high-energy particles. In biological media, these electrons can fragment molecules and lead to the formation of highly reactive radicals and ions.[1] The immediate species produced by LEEs and some immediate reactions of these species have been investigated in simple (O_2, H_2O) and large (DNA, peptides) condensed biomolecules. The target usually consist of a multilayer molecular film deposited on a metal substrate bombarded by a LEE beam (0–45 eV) under ultra high vacuum (UHV) conditions. It is possible to measure neutral fragments or ions emanating from these films. The products remaining in the films can be analyzed *in situ* by X-ray photoelectron and electron energy loss spectroscopies; they can also be removed from the UHV system and analyzed by HPLC and GC/MS. By comparing various results, we determined fundamental mechanisms, that are involved in bond-breaking within DNA. Such mechanisms involved (1) the formation of transient anions, (2) dipolar dissociation which produces an anion and cation and (3) reactive scattering that generates new species via non-thermal reactions. The transient anions fragment molecules by decaying into dissociative electronically excited states or by dissociating into a stable anion and a neutral radical. These fragments usually initiate other reactions with nearby molecules, causing further chemical damage. It was shown that the amount of damage to DNA generated by 3–30 eV electrons is dependent on environment, topology, type of counter ion, base identity, base sequence and electron energy. Capture of a LEE by a DNA subunit may also be followed by electron transfer to another. Finally, under identical conditions LEE were found to be three times more effective than X rays to produce damage. The results which led to these findings will be presented at the meeting. Corresponding experiments performed with simple biomolecules and single and double stranded DNA, including its basic constituents, will also be described. This research is financed by the Canadian Institutes of Health Research. [1].For a review see L. Sanche, Mass Spectr. Rev. 21, 349 (2002); Eur. Phys. J. D, 35, 367 (2005).

(CL4) The role of phosphorylation in non-homologous end joining. Susan P. Lees-Miller. University of Calgary, Calgary, AB, Canada.

In human cells, the main pathway for the repair of ionizing radiation (IR) induced DNA double strand breaks (DSBs) is non-homologous end joining (NHEJ). Several factors have been shown to be required for NHEJ including the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), the Ku70/80 heterodimer, Artemis, XRCC4, XLF and DNA ligase IV. In addition, other proteins such polynucleotide kinase (PNK), and DNA polymerases mu and lambda are likely involved. The DNA-dependent protein kinase (DNA-PKcs) is a serine/threonine protein kinase that is composed of DNA-PKcs and Ku and requires ends of dsDNA for activity. Moreover, the protein kinase activity of DNA-PK is required for NHEJ. In order to elucidate the role of DNA-PK phosphorylation in NHEJ we have examined the effects of DNA-PK phosphorylation on several NHEJ proteins including Ku70, Ku80, XRCC4, Artemis as well as DNA-PKcs itself. To date we have identified fourteen autophosphorylation sites in DNA-PKcs and shown that at least nine of these are important for function. Our hypothesis is that autophosphorylation of DNA-PKcs induces a conformational change that triggers dissociation of DNA-PKcs from DNA bound Ku. Here we provide evidence that autophosphorylation of DNA-PKcs produces a conformational change that may involve the region of between amino acids 2609 and 2647. We have also examined autophosphorylation of DNA-PKcs in vivo. Using phosphospecific antibodies and/or mass spectrometry, we show that S2056, T2609, S2612, T2620, S2624, T2638, T2647 and T3950 are phosphorylated in irradiated human cells. Moreover, using specific inhibitors of DNA-PK and ATM (NU7441* and KU55933* respectively) we show that IR induced phosphorylation of DNA-PKcs on S2056, T2609, S2612, T2620, S2624, T2638, T2647 and T3950 is DNA-PK dependent in vivo in HEK293 cells. Finally, we have shown that XLF is phosphorylated in vitro by DNA-PK and in vivo. Preliminary data showing the effects of phosphorylation on XLF function will be discussed. *We thank Dr Graeme Smith, KuDos Pharmaceuticals for NU7441 and KU55933.

(CL6) Second cancers following radiation treatment for childhood cancer. Ann C. Mertens[1], Marilyn Stovall[2]. [1]University of Minnesota, Minneapolis, MN, USA, [2]MD Anderson Cancer Center, Houston, TX, USA.

Previous studies have suggested that radiation exposure during childhood cancer treatment can cause serious, life-threatening sequelae in long-term survivors. The Childhood Cancer Survivor Study (CCSS), a 12-year multi-institutional collaboration supported by a grant from the National Cancer Institute, was designed to investigate long-term effects among five-year survivors of childhood and adolescent cancer. The CCSS has successfully followed 14,370 five-year survivors of childhood and adolescent cancer, with length of follow-up extending up to 35 years post primary diagnosis. This cohort is heterogeneous with respect to host, disease, and treatment exposures and allows for accurate determinations of the incidence and risk factors for rare events, such as subsequent cancers. Results from this study will be presented, including cumulative incidence rates of subsequent cancers, and associations between the development of subsequent cancers and radiation exposure. We will also report on two nested case control studies where tumor site-specific dosimetry was undertaken to more precisely characterize the radiation risk of subsequent tumors.

(CL7) DNA damage responses: mechanisms and implications for human disease. Michael B. Kastan. St. Jude Children's Research Hospital, Memphis, TN, USA.

Significant progress has been made in recent years in elucidating the molecular controls of cellular responses to DNA damage in mammalian cells. Much of our understanding of the mechanisms involved in cellular DNA damage response pathways have come from studies of human cancer susceptibility syndromes that are altered in DNA damage responses. ATM, the gene mutated in the disorder, Ataxia-telangiectasia, is a protein kinase that is a central mediator of responses to DNA double strand breaks in cells. Once activated, ATM phosphorylates numerous substrates in the cell that modulate the cell's response to the DNA damage. The p53 and SMC1 proteins appear to be particularly important targets of the ATM kinase, playing critical roles in modulating cell cycle progression, cell survival, and chromosomal stability after DNA damage. We recently developed a novel system to create DNA double strand breaks (DSBs) at defined endogenous sites in the human genome, and used this system to detect protein recruitment and loss at and around these breaks by chromatin immunoprecipitation (ChIP). The detection of human ATM protein at site-specific DSBs required functional NBS1 protein, ATM kinase activity, and ATM autophosphorylation on serine 1981. DSB formation led to the localized disruption of nucleosomes, a process that depended on both functional NBS1 and ATM. These two proteins were also required for efficient recruitment of the repair co-factor XRCC4 to DSBs, and for efficient DSB repair. These results demonstrate the functional importance of ATM kinase activity and phosphorylation in the response to DSBs, and support a model in which ordered chromatin structure changes that occur after DNA breakage and that depend on functional NBS1 and ATM facilitate DNA DSB repair. Insights about these pathways provide us with opportunities to develop new approaches to benefit patients. For example, inhibitors of the ATM pathway have the potential to act as sensitizers to chemotherapy or radiation therapy and could have anti-neoplastic effects on their own. Conversely, activators of ATM could improve responses to cellular stresses such as oxidative damage. As an example, we have demonstrated benefits of a non-toxic ATM activator in disease settings ranging from metabolic syndrome to cancer in pre-clinical models.

(CL8) Radiation damage and formation of trans fatty acids in membranes: biomimetic and in vivo studies. Carla Ferreri. Consiglio Nazionale delle Ricerche, Bologna, Italy.

Introduction: The cis double bond of phospholipid fatty acid chains is an essential requisite for cell membranes. Sulfur-centered radicals are able to isomerize the cis to the corresponding geometrical trans isomers, this transformation being correlated with a tandem lipid-protein damage of biological interest .[1,2] Methods: Liposomes formed by POPC (1-palmitoyl-2-oleoyl phosphatidyl choline) are used as membrane model for lipid isomerization under gamma-irradiation in the presence of cysteine and methionine residues. Hydrogen atoms react selectively with sulfur-containing amino acid residues, thus modelling conditions of reductive stress. The reaction mechanism involves hydrogen atom attack on sulphur moiety, generating diffusible thiyl radicals able to migrate to the lipid compartment and catalyse the fatty acid isomerization. Results: The isomerization of phospholipid vesicles in aqueous suspension in the presence of S-containing substrates (RNase A, amyloid beta-peptide, Met-enkephalin, hydrogen sulfide) occurred under irradiation conditions.[3–6] *Quercus suber* metallothioneins having different metal:protein ratios and sulfide contents have been also investigated, evidencing Cd-QsMT as the best source of isomerising species.[7] In vivo experiments of cell cultures incubated with hydrogen sulfide evidenced a progressive formation of trans lipids in membranes.[8] Conclusions: Trans lipids emerge as markers of radical stress due to the formation of diffusible isomerising species. This underlines the role of lipidomics in the study of radical damages.

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(CL9) Extrapolation of radiation-induced risks from low to very low doses: What does the science tell us? David J. Brenner. Columbia University Center for Radiological Research, New York, NY, USA.

There is convincing epidemiological evidence that doses of ionizing radiation above about 10 to 50 mGy can cause cancer, but at lower doses even the largest epidemiological studies have insufficient power, and so we have to rely on "expert opinion", guided by the best available biology. Our conclusions may have profound effects on the future of areas as diverse as nuclear power and CT-based radiological screening.

The arguments essentially revolve around the claim that different biological processes dominate radiation damage responses at very low doses. (below ~10 mGy), compared with higher doses. For example, the claim is often made that, at these very low doses, essentially all radiation-damaged cells will be eliminated through apoptosis or other mechanisms, while at somewhat higher doses, radiation damage and subsequent misrepair can ultimate result in cancer. We discuss here whether there is indeed plausible evidence for different damage response pathways at very low doses. If there is not strong evidence for efficient damage removal pathways that are unique to very low doses, it would be hard to make the case that cancer risks at very low doses are zero, nor even that the risks per unit dose at low doses are smaller than at higher doses. It is certainly true that inter-cellular signaling could well modify the shape of the dose-risk relation away from linearity at very low doses, but the magnitude or even the direction of any such modifications away from linearity are, as yet, unknown. There is no doubt that the linear (no threshold) approach for extrapolating risks to low doses - which has been adopted by most national and international organizations - can and should be critically examined. The arguments for a linear no-threshold model at very low doses are plausible, but rely on assumptions about single-cells acting autonomously, which are unlikely to be completely correct. However, at this time we don't know if deviations from the predictions of this linear approach will be large or small, nor even whether they will increase or decrease low-dose cancer risk estimates. We are only just beginning to scratch the surface of our understanding of the impact of inter-cellular interactions on very low dose cancer risks, so it is almost certainly premature to be advocating major changes in policy or practice.

(CL10) Integrating molecular therapeutics with radiotherapy. Kian Ang. M. D. Anderson Cancer Center, Houston, TX, USA.

Many phase III clinical trials showed that several altered fractionation (AF) or concurrent radiation-chemotherapy (CRCT) regimens improved local-regional control (LRC) and/or overall survival (OS) over conventionally fractionated radiotherapy alone in patients with locally advanced head and neck squamous cell carcinoma (HNSCC). However, both AF and CRCT intensify acute toxicity and CRCT also increases late morbidity. These findings along with advances in cancer biology inspired the search for selective enhancers of tumor response. Preclinical and correlative biomarker findings showing an association between high EGFR expression with tumor radiation resistance and enhancement of tumor radiation response by antibody (cetuximab) or small molecule inhibitors of EGFR. Therefore, a phase III trial was undertaken to compare the efficacy of radiotherapy plus cetuximab against radiotherapy alone in patients with locally advanced HNSCC, which showed that the radiation plus cetuximab significantly improved LRC and OS without increasing mucositis or other radiation side effects. Cetuximab induced acneiform rash and sporadically hypersensitivity reactions. The cetuximab trial provided thus a critical proof-of-concept that targeting a perturbed signaling pathway can selectively enhance tumor response to radiotherapy and established a new option for treating locally advanced HNSCC. However, the improvement in the LRC and OS rates has been modest (10–15%) and >50% of patients receiving radiotherapy plus cetuximab still experienced local-regional relapse. Consequently, efforts are continuing to explore other strategies such as combinations of CRCT with cetuximab or other molecular therapeutics. Studies are also ongoing to assess the relative merits of modulators of other signaling pathways in distinct subsets of patients with HNSCC having different pattern of failure. Examples

include the role of bevacizumab and vandetanib (ZD6474), both in combination with CRCT, in patients with nasopharyngeal carcinoma and those with high-risk surgical-pathologic features, respectively. The rationale, available preclinical data, and protocol design will be briefly reviewed.

(CL11) Does radiation induce emergent phenomena that contribute to cancer risk? Mary Helen Barcellos-Hoff. Lawrence Berkeley Laboratory, Berkeley, CA, USA.

The challenge in predicting radiation health effects in humans is to understand how cellular responses that occur in a multicellular context are integrated to produce an organismal response. A primary limitation to current models of radiation risk is the tendency to model events as hierarchical, linear responses, rather than combinatorial networks, and to model consequences of radiation in terms of a collection of minimally interacting cells, rather than treating the irradiated tissue/organ/organism as a system. What distinguishes a *complex* system from a merely complicated one is that some behaviors emerge as a result of altered relationships between the elements. We proposed that the cell biology of irradiated tissues reveals a coordinated multicellular damage response program in which the contributions from individual cells are primarily directed towards suppression of carcinogenesis and reestablishment of homeostasis (*Radiat Res* 156:618–627, 2001). We and others (e.g. *Cancer Res* 67:1246–53, 2007) have observed that radiation-induced extracellular signaling can be directed toward re-establishment of homeostasis and lead to the elimination of abnormal cells. However, high dose radiation (>10cGy) may disrupt extracellular signaling, interfere with the elimination of genomically unstable cells, and promote carcinogenesis. Furthermore, radiation exposure can prime non-malignant epithelial cells to undergo phenotypic changes that may contribute to neoplastic progression (e.g. *Proc Natl Acad Sci*, 100:10728–33, 2003) and alter stromal cells to promote epithelial cancer (e.g. *Cancer Res*, 60: 1254–60, 2000; *Cancer Res*, 65:6734–44, 2005). The capacity of radiation to perturb multicellular signaling and networks could give rise to 'emergent phenomena' that contribute to cancer risk at high doses. If low dose or low dose rate radiation exposures do not elicit such phenomenon, then the assumption of linear cancer risk may not be valid. *Supported by DOE Low Dose Program and NASA Specialized Center of Research.*

(CL12) Chromatin remodeling complexes: essential participants in DNA repair. Brendan Price. Dana-Farber Cancer Institute, Boston, MA, USA.

The compact nature of chromatin presents a significant barrier to DNA dependent events such as gene transcription, DNA replication and DNA repair. Several pathways are employed to alter chromatin structure and allow access to specific regions of the chromatin. These pathways utilize both histone modification and chromatin remodeling complexes to alter DNA-nucleosome interactions. Histone modification (e.g. acetylation by Histone acetyltransferases: HATs) can create binding domains for chromatin binding proteins as well as altering the interaction between DNA and histones. Chromatin remodeling complexes can alter the position of nucleosomes (nucleosome sliding), disrupt DNA-histone interactions or facilitate the removal of histones from specific regions of the promoter. HATs and chromatin remodeling complexes play a vital role in DNA double-strand break (DSB) repair. In yeast, the INO80, Swr1 and NuA4 remodeling complexes are recruited to DSBs, where they can unfold the chromatin structure and displace nucleosomes from damaged sites prior to processing of the DSBs. The Tip60 HAT is a key player in chromatin remodeling associated with the repair of DSBs, as well as being required for activation of the ATM and p53 DNA damage signaling pathways. Tip60 participates in this diverse range of DNA damage responses due to its ability to associate with 2 discrete protein complexes: the ATM-Tip60 complex and the NuA4-Tip60 chromatin remodeling complex. Following generation of DNA strand breaks, the histone aectyltransferase activity of Tip60 is increased. For the NuA4-Tip60 complex, this activation of Tip60′s HAT activity leads to hyperacetylation of histones adjacent to DNA strand breaks, and unwinding of the chromatin structure. Additional components of the NuA4-Tip60 complex, including the p400 SWI/SNF ATPase, are essential for this process. For the ATM-Tip60 complex, Tip60 directly acetylates ATM, activating its kinase activity. The Tip60 HAT therefore provides the crucial step in linking the detection of DSBs to the activation of both ATM signaling pathways and NuA4 dependent chromatin remodeling events. Tip60 therefore represents a novel DNA damage response protein involved in regulating multiple aspects of the cells response to DSBs.

(CL14) Early epigenetic and genetic events in carcinogenesis. Thea D. Tlsty. University of California at San Francisco, San Francisco, CA, USA.

One of the major problems with treating cancer is that it is detected too late. Early lesions and transitions often occur ten to fifteen years prior to our ability to detect the disease by palpation or imaging. By the time many cancers are detected they have long since acquired the ability to generate micrometasases or populations of tumor cells that can develop drug resistance. Our best approach to treating or preventing cancer is early detection and an understanding of the earliest steps in the transition of normal cells to malignant ones. Our ignorance about the earliest molecular changes prevents studies that would identify individuals at risk or identify targets for prevention strategies. An in vitro model that mimics the transitions that occur as normal epithelial cells become malignant would provide major advances in several aspects of dealing with the disease. Early detection of lesions could be used for risk assessment and diagnosis of the disease at early stages. Additionally, and perhaps, the most significant advance would be the insights a model system may provide to the initiation of lesions and the transition of these lesions to malignancy allowing the translation of that information into preventive agents. It is for these reasons that we began studying human epithelial cells in vitro, focusing on mammary epithelial cells in particular. Recently, studies of human epithelial cells and fibroblasts from healthy individuals have been providing novel insights into how early epigenetic and genetic events affect genomic integrity and fuel carcinogenesis. Key epigenetic changes, such as the hypermethylation of the p16 promoter sequence, create a previously unappreciated pre-clonal phase of tumorigenesis in which a subpopulation of epithelial cells is positioned for progression to malignancy (Nature 409:636, 2001). These key changes generate epigenetic and genetic mosaicism, precede the clonal outgrowth of pre-malignant lesions and occur frequently in healthy, disease-free individuals (Caner Cell 5:263, 2004). Prior work from our laboratory has identified biomarkers that may be useful for risk assessment as well as provide targets for the elimination of these cells. Understanding more about these early events should provide novel molecular candidates for prevention and therapy of cancer.

(CL15) Combining ionizing radiation with immunotherapy: preclinical and clinical results. Silvia C. Formenti. New York Univ School of Medicine, New York, NY, USA.

The application of ionizing radiation to cancer therapy has empirically selected dose fractionation regimens that avoid brisk inflammation to achieve over time local tumor control. However, radiation triggers a wide range of additional cellular and stromal effects in addition to cell killing. During the past decade the type of death cancer cells undergo has shown to be a relevant element to the immune system, with cancer cells killed in vivo by radiation therapy serving as a good source of antigens for APC to present to T cells. Radiation can modify the permeability of solid tumors to immune cells by increasing the expression of endothelial adhesion molecules such as ICAM-1, E-selectin, and P-selectin in the tumor vasculature. In addition, radiation markedly enhances homing of the activated T cells to the tumors, an effect associated with trafficking to the tumor of tumor-specific T cells via up-regulation of VCAM-1 on tumor vessels. The ability of radiation to increase expression of MHC class I molecules to levels that enhance the recognition and killing of

tumor cells by CTL has important therapeutic implications. In a mouse model of invasive intracranial glioma we showed that whole brain radiation therapy (WBRT) leads to markedly increased expression of MHC class I on the invading glioma cells. Moreover, when mice with intracranial gliomas detectable by CT scanning were treated with WBRT in combination with a GM-CSF producing autologous tumor cell vaccine given peripherally, 60% of the mice survived long-term, versus none survived in the groups treated with WBRT or vaccination alone. Other examples confirm that in vivo irradiation of tumors can be successfully combined with immunotherapy. Administration of oligodeoxynucleotides containing CpG motifs into irradiated tumors induced a protective anti-tumor response . Similarly, we have shown that effective anti-tumor T cell responses are triggered against a non-immunogenic tumor when tumor radiation is combined with antibody-mediated CTLA-4 blockade, an intervention that prevents T cell tolerance associated with suboptimal APC function.

Radiation-induced effects on tumor immunity remain to be fully elucidated, but these preliminary lines of evidence encourage the experimental combination of radiation with immunotherapy.

(CL16) From cellular to high-throughput predictive assays: going nowhere faster? Soren M. Bentzen. Department of Human Oncology, Madison, WI, USA.

New powerful assays facilitate the simultaneous collection of many thousand biological data items from a single biopsy or a blood sample from an individual. These assays are being widely introduced in for example cancer susceptibility and pharmacogenomics studies and are also being pioneered as discovery tools and potential predictive assays in experimental and clinical radiation research. On one hand, the introduction of these assays opens intriguing possibilities for a comprehensive systems biology approach to radiation effects in tumors and normal tissues. On the other hand, further development and validation of this technology in clinical applications pose a number of challenges in terms of study design and analysis. Researchers, who lived through the initial high hope and subsequent considerable frustration when research into cellular radiobiological assays failed to produce clinically useful predictors of radiation response, may rightly feel that we should try to learn from our past when entering this new field. Seen in hindsight, the vast majority of studies on cellular predictive assays suffered from three problems: (1) the studies were statistically under-powered relative to what would be required to show a clinically meaningful predictive effect, but the fact that the studies were conducted without an a priori scientific hypothesis meant that most of them produced P-values that were significant at the 5% level but subsequently turned out to be false-positive; (2) many studies did not address a clearly defined clinical problem, did not control for dosimetric and other relevant confounding factors and did not use appropriate clinical endpoints; (3) early hypothesis-generating studies should have been followed by rigorously designed hypothesis-testing studies, but this research strategy was only applied in very few cases. At present, high-throughput predictive assay studies in radiation research suffer from all of the above weaknesses, further compounded by the fact that these studies often by design lack an a priori hypothesis and have unsolved problems with a high false-positive rate. In the lecture, I will discuss the great opportunities and the methodological challenges in this research field and discuss the design of the next generation of studies required to move forward in a rational way.

(CL17) Stem cell therapy to reduce radiation-induced normal tissue damage. Robert P. Coppes. Universiy Medical Center Groningen, Groningen, The Netherlands.

Irradiation of normal tissues can result in organ failure and hence seriously can limit the treatment dose used for radiotherapy. Since radiation sterilises stem cells, their replacement by donor stem cells may prevent damage or restore function, similar to bone marrow transplantations. Recently, it became apparent that tissue specific stem cell can be isolated from many other tissues and have potential to rescue diseased tissues. To test the potential of stem cells to reduce radiation-induced damage to normal tissues, we use the salivary gland as a model. Radiation-induced damage to salivary glands may lead to xerostomia (dry mouth syndrome) and severely compromise the quality of life of cancer patients. Therefore, we developed a clinically applicable method for restoration of radiation-impaired salivary gland function using salivary gland stem cell transplantation. Mice submandibular glands were enzymatically dispersed and the cells were grown in vitro using a defined medium containing a number of growth factors. From 24 hrs on spheres were found that expressed salivary gland duct markers and stem cell markers. These 'salispheres' were found to be able to differentiate in vitro into amylase producing acinar cells in liquid culture and into salivary gland like structures in 3D culture. Transplantation of selected cells form these salispheres, either through direct intraglandular or through retrograde ductal injection resulted in the development of donor-derived ductal and acinar structures and restoration of salivary gland function. This approach is therefore the first proof for the potential use of stem cell transplantation to functionally rescue of radiation-induced solid organ deficiency.

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(CL19) Glioma cancer stem cells promote tumor radioresistance and angiogenesis. Jeremy N. Rich, Shideng Bao, Qiulian Wu, Roger E. McLendon, Sith Sathornsumetee, Zhizhong Li, Mark Dewhirst, Darell D. Bigner, Anita B. Hjelmeland. Duke University, Durham, NC, USA.

The mechanisms underlying tumor radioresistance remain elusive. We recently demonstrated that cancer stem cells contribute to glioma radioresistance through preferential activation of the DNA damage checkpoint response and increased DNA repair capacity (*Nature*, 2006). The fraction of tumor cells expressing CD133 (prominin-1), a marker for both neural stem cells and brain cancer stem cells, was enriched after radiation in gliomas. CD133+ glioma cells survive ionizing radiation at increased rates relative to the majority of tumor cells, which are CD133 negative. CD133+ tumor cells preferentially activate the DNA damage checkpoint in response to radiation and repair radiation-induced DNA damage more effectively than CD133- tumor cells. Furthermore, the radioresistance of CD133+ glioma stem cells can be reversed with a specific inhibitor of the Chk1/Chk2 checkpoint kinases. These results suggest that CD133+ tumor cells represent the cellular population conferring glioma radioresistance and could be the source of tumor recurrence after radiation. Targeting DNA damage checkpoint response in cancer stem cells may overcome this radioresistance and provide a novel therapeutic paradigm for malignant brain cancers. In additional studies, we examined the potential of CD133+ glioma cells to support tumor angiogenesis (*Cancer Research*, 2006). Tumors derived from CD133+ cells were morphologically distinguishable from CD133- tumor populations by widespread tumor angiogenesis, necrosis and hemorrhage. In comparison to matched CD133- populations, CD133+ cells consistently secreted markedly elevated levels of vascular endothelial growth factor (VEGF), which were further induced by hypoxia. The pro-angiogenic effects of CD133+ glioma cells were specifically abolished by the anti-VEGF neutralizing antibody bevacizumab (*Avastin*), but bevacizumab had limited efficacy against matched CD133- populations. Together these data indicate that stem cell-like tumor cells can be a crucial source of key angiogenic factors in cancers and that targeting pro-angiogenic factors from stem cell-like tumor populations may be critical for patient therapy.

(CL20) Repair of radiation induced DNA double strand breaks during the mammalian cell cycle. Markus Lobrich. Universitat des Saarlandes, Homburg/Saar, Germany.

DNA double strand breaks (DSBs) represent major lethal lesions generated by ionising radiation (IR) which can be repaired by either non homologous endjoining (NHEJ) or homologous

recombination (HR). NHEJ allows fast but error-prone repair during the entire cell cycle independent of sequence homologies whereas the slower but error-free HR pathway is restricted to S and G2 and requires homologous sequences. We have recently described a DSB repair pathway dependent upon ATM, Artemis and proteins locating to gH2AX foci which is required for the repair of approximately 10% of IR-induced DSBs in G0/G1 cells.

To investigate ATM/Artemis dependent DSB repair in different cell cycle phases and to uncover a potential role of ATM and Artemis in HR, we analysed DSB repair kinetics in different NHEJ and HR mutants using gH2AX foci analysis in combination with cell cycle markers. Rad51 foci formation served as a measure for the proportion of DSBs repaired by HR. Epistasis-like studies were performed by using specific inhibitors for ATM and DNA-PKcs, or by employing siRNA mediated down-regulation of different NHEJ and HR proteins.

We demonstrate that NHEJ represents the major repair pathway in cycling G1 cells and also contributes substantially to DSB repair in G2. HR mutants show a repair defect in G2 which is smaller than that of NHEJ defective cells. ATM and Artemis deficient cells reveal a diminished repair capacity both in G1 and G2. The repair defect in ATM and Artemis deficient G2 cells resembles the defect observed in HR mutants raising the possibility that ATM and Artemis represent components of HR. Studies with specific inhibitors for ATM and the NHEJ component, DNA-PKcs, support the notion that Artemis and ATM contribute to HR-directed repair in G2. Data obtained after siRNA mediated down-regulation of ATM/Artemis and different NHEJ or HR components and the observation that ATM deficient G2 cells show reduced Rad51 foci formation consolidate this finding. Collectively, we demonstrate that Artemis and ATM contribute to HR-directed repair in G2.

In conclusion, NHEJ constitutes the major DSB repair pathway during the mammalian cell cycle whereas HR repairs a subset of IR induced DSBs in G2 cells. Strikingly, ATM and Artemis represent components of NHEJ in G1 but are involved in HR-directed repair during G2.

(CL21) Radiation chemistry of DNA in cells. Jean Cadet, Thierry Douki, Jean-Luc Ravanat. CEA/Grenoble, Grenoble, France.

Emphasis has been placed in the last decade on the elucidation of the main degradation pathways of DNA model compounds mediated by $^{\bullet}OH$ radical and one-electron oxidation reactions as the result of indirect and direct effects of ionizing radiation respectively. This has led to the isolation and characterization of almost 100 oxidized purine and pyrimidine nucleosides if hydroperoxide precursors and diastereomers are considered. However, far less information is currently available on the mechanisms of radiation-induced degradation of bases in cellular DNA mostly due partly to analytical difficulties. It may be reminded that the measurement of oxidized nucleosides and bases in nuclear DNA is still a challenging issue which until recently has been hampered by the use of inappropriate methods such as the GC-MS that have led to overestimated values of the lesions by factors varying between 1 and 3 orders of magnitude. At the present, using the accurate and sensitive HPLC/MS/MS assay, 11 single modified nucleosides and bases were found to be generated in cellular DNA upon exposure to gamma rays and heavy ions. This validates several of the $^{\bullet}OH$-mediated oxidation pathways of thymine, guanine and adenine that were previously inferred from model studies. The concomitant decrease in the yields of oxidized bases with the increase in the LET of heavy ions is accounted for by the preponderance of indirect effects in the damaging action of ionizing radiation on DNA. Further evidence for the major role played by $^{\bullet}OH$ radical was provided by the results of irradiation of cells with high intensity 266 nm laser pulses. Under these conditions 8-oxo-7,8-dihydroguanine is mostly produced as the result of biphotonic ionization of DNA nucleobases and subsequent hole migration to guanine bases. It is likely that some of the oxidized bases that have been isolated as single lesions are in fact involved in clustered damage. Interestingly it was recently shown that a single oxidation hit is capable of generating complex lesions in DNA. Thus $^{\bullet}OH$-mediated abstraction at C4 of the 2-deoxyribose moiety gives rise to DNA strand cleavage together with the formation of a highly reactive aldehyde that undergoes an addition reaction to the amino group of a proximate cytosine, leading to 4 diastereomeric cycloadducts as components of likely interstrand cross-links.

(CL22) Late effects of radiation. Mike E. Robbins. Wake Forest University School of Medicine, Winston-Salem, NC, USA.

With 62% of adult and 77% of pediatric cancer patients surviving beyond 5 years in the United States as a result of continuing improvements in cancer therapy and health care, the long-term consequences of the primary therapeutic interventions have emerged as a significant risk. Study of these late effects has been targeted by the National Cancer Institute as one of the new areas of public health emphasis, particularly studying late effects (http://plan.cancer.gov). In radiation biology, the injury to normal tissue that develops in long-term survivors resulting from irradiation is now a primary area of cancer research. Our views on the pathogenesis of radiation-induced late normal tissue injury have changed markedly. The classic model of specific target cell clonogenic death leading to progressive and non-treatable reductions in organ function has been replaced with a new paradigm. Rather than simply a loss of normal cellular components, radiation-induced late effects are viewed now as a combination of loss of normal cellular function as well as an orchestrated response to injury that involves interactions between multiple cell types within a particular organ. The normal cellular response to injury may in fact initiate a chronic active process that ultimately leads to progressive damage. Most importantly, however, evidence is emerging to suggest that this process can be modulated. Following irradiation of normal tissues, an acute inflammatory response is observed, involving activation of stress-sensitive kinases, transcription factors and increased production of inflammatory cytokines. A chronic inflammatory/wound healing response developing over months to years is then responsible for persistent vascular and parenchymal cell dysfunction and cell loss. Recognition that the associated chronic overproduction of cytokines and growth factors, results in fibrosis and/or necrosis offers a novel approach to radiation-induced normal tissue morbidity. Recent findings support the hypothesis that radiation-induced injury can be modulated by therapies directed at mitigating the cascade of events resulting from normal tissue injury. However, the mechanisms responsible for the clinical expression and progression of late, radiation-induced normal tissue injury, remain poorly understood.

(CL23) Radiation-induced bystander effects: the good, the bad and the ugly. Carmel Mothersill. McMaster Univ, Hamilton, ON, Canada.

This lecture will review current research on bystander effects with particular emphasis on the consequences of these phenomena for radiation protection and radiotherapy. The lecture will consider data suggesting radiation-induced bystander effects may be inducible, adaptive and therefore protective mechanisms following low dose, scatter dose or chronic exposures and will then review the data suggesting that they may in fact have harmful consequences such as mutagenic or carcinogenic effects. Finally the lecture will review some of the many confusing, contradictory or obscure results in the literature before attempting to reach a synthesis.

(CL24) Influence of angiogenesis on cancer treatment. Gillian Mary Tozer. The University of Sheffield, Sheffield, United Kingdom.

The influence of tumour angiogenesis on conventional and novel cancer treatments will be addressed followed by presentation of recent data focusing on vascular-targeted treatment. Recognition that angiogenesis is a crucial step in tumour progression has led to novel cancer treatments. *Anti-angiogenic* approaches aim to prevent the neo-vascularization processes in tumours, whereas *anti-vascular* or *vascular disrupting* approaches aim to cause a selective shut-down of existing tumour vasculature, leading to secondary tumour

cell death. Endothelial cell proliferation and migration are prime targets for current anti-angiogenic compounds. However, the later maturation phase of angiogenesis, which is defective in tumours, may provide novel treatment targets. Vascular disrupting agents (VDAs) exploit this characteristic of tumours. The tubulin binding agent, combretastatin A-4-P (CA-4-P), is the prototype VDA, which is currently in Phase II clinical trial in combination with conventional therapy. Tumour endothelial cells are exposed to high levels of vascular endothelial growth factor (VEGF or VEGF-A). We have developed fibrosarcoma cell lines expressing only single isoforms of VEGF (VEGF120, 164 and 188). In VEGF188 tumours, virtually 100% of blood vessels stained positively for α-smooth muscle actin, denoting maturity of the vessel walls. Corresponding values were 25% for VEGF120 and VEGF164 tumours and 40% for wild-type (w/t) tumours. VEGF120 tumours were also characterized by haemorrhage and poor network development, whereas VEGF188 tumours represented the other extreme, with narrower vessels and no haemorrhage. The VEGF120 and VEGF164 tumours were significantly more sensitive to CA-4-P than the VEGF188 or w/t tumours. Pre-treatment with the VEGF receptor inhibitor, SU5416, caused significant vascular re-modelling in the VEGF120 tumours that protected them against CA-4-P treatment. Thus, tumour response to VDAs is dependent upon the maturation status of the tumour blood vessels, which is uniquely influenced by VEGF188. This approach may also be useful for investigating the influence of angiogenic maturation on response to conventional therapy. *Supported by Cancer Research UK*.

(S1.1) Tumor imaging with immunohistochemistry. Albert J. van der Kogel, Jan Bussink, Hans Kaanders. Radboud University, Nijmegen, The Netherlands.

After the widespread introduction of high-precision radiotherapy technologies such as IMRT the next logical step has been the combination with biological imaging (image guided radiotherapy - IGRT), and today all forms of non-invasive imaging (MR, PET, US) are being implemented in radiotherapy departments. The radiation response of tumors has traditionally been linked to three factors: proliferation, intrinsic radiosensitivity and hypoxia. To identify the relative importance and spatial heterogeneity of these factors various PET tracers and new MR technologies are being introduced in the clinic. However, the value of these markers needs validation at the tissue, cellular and molecular level, and immunohistochemistry-based imaging can provide this link. Initially studies on tumor characteristics regarded as important for outcome in radiation treatment were limited to proliferation (Ki67 or BrdU/IdUrd labeling), or vascular density as a surrogate for tissue oxygenation. In the last decade this has rapidly changed with the availability of highly specific antibodies to all main functional cellular compartments, signaling pathways, and DNA damage/repair related proteins. Studies in our laboratory have focused on a quantitative immunohistochemical assessment of factors with proven impact on outcome of radiation treatment - proliferation, hypoxia and intrinsic radiosensitivity. These studies are performed on frozen tumor biopsies of patients or in xenografted tumors. Characteristic phenotypic differences between individual tumors are also reflected in differences in the downstream signaling pathways (AKT, MAPK) and the response to irradiation and blockade of growth factor receptors.

Our results show that for a prediction of response pretreatment characteristics should be compared with early changes in receptor expression/distribution, as well as key markers of downstream signaling pathways. The future of prediction of therapy outcome and individualization will not only rely on a pretreatment biological profile, but also on insight in the early response at a molecular and cellular/tissue level.

(S1.2) Reporter gene imaging for tumor characterization and radio-gene therapy. Gloria C. Li. Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

Tumor hypoxia, often found in many human tumors, leads to radio- and chemo-resistance, aggressive phenotype, and is an important prognostic factor of treatment outcome. We initiated non-invasive imaging studies, and developed an image-guided strategy, combining radiation and gene therapy, to overcome such radioresistance. Recently, we established a preclinical model for noninvasive imaging of hypoxia-induced gene expression. Colorectal cancer cells were stably transfected with a dual reporter gene (HSV1-tk and eGFP fusion gene), under the control of a hypoxia-inducible promoter (HRE). Individual stable colonies (HCT8-HRE and HT29-HRE) were isolated. The hypoxia inducibility of the *tkeGFP* fusion gene was evaluated *in vitro* by fluorescent microscopy, flow cytometry, and ^{14}C-FIAU accumulation. *In vivo*, microPET showed readily detectable ^{124}I-FIAU uptake in tumors containing *tkeGFP*, with no accumulation in the parental tumors. Furthermore, the intratumoral distributions of ^{124}I-FIAU and ^{18}F-FMISO microPET images were similar. In tumor sections, we compared radioactivity distributions (digital autoradiography) with immunohistological staining using various markers. The results demonstrated similarity between the spatial distributions of ^{124}I-FIAU, ^{18}F-FMISO, pimonidazole, eGFP and CA9. This system provides a link between hypoxia-induced molecular events and the use of exogenous and endogenous tumor hypoxia markers. We have also initiated studies to develop a therapeutic strategy combining radiotherapy and gene therapy, for possible future application targeting hypoxic/radioresistant cancers. This involves the imaging and localization of tumor hypoxia, image-guided delivery of adenoviral vectors that disrupt DNA repair, verification of gene delivery by molecular imaging, and irradiation to eradicate the tumors. Specifically, we showed that down-regulation of Ku70 via adenovirus-mediated gene transfer radiosensitized human tumor cells *in vitro* (cell culture studies) and *in vivo* (animal model). Our vectors, expressing Ku70 antisense or dominant negative Ku70 gene fragment, radiosensitize *in vitro* and *in vivo* models, without toxic effect from the transduced genes. Thus, this approach has potential for future clinical trials involving hypoxic/radioresistant disease sites.

(S1.3) Magnetic resonance imaging of prostate cancer patients receiving radiation therapy. John Kurhanewicz. UCSF, San Francisco, CA, USA.

Introduction: Published studies suggest that a multi-parametric magnetic resonance imaging exam involving the acquisition of high spatial resolution anatomic images (T_2 weighted MRI), diffusion tensor images (DTI) and magnetic resonance spectroscopic imaging data (MRSI) is a useful adjunct to PSA and other clinical data for radiation treatment planning and follow-up. Purpose: To demonstrate the current ability of a combined MRI/MRSI/DTI exam for improved radiation treatment planning and follow-up. Experimental Procedures: Commercial combined MRI/MRSI exams are currently available on standard MR scanners. DTI sequences are also commercially available and can be added to the MRI/MRSI exam in a matter of minutes resulting in an hour MR exam. Results/Discussion: Prostate cancer can be discriminated from benign tissues based on a combination of reduced signal intensity on T2 MRI, decreased mean diffusion coefficient on DTI, increased choline and reduced citrate and polyamines on MRSI. After therapy, there is a decrease in signal intensity on T2, a early reduction of citrate and polyamine levels, a later reduction in choline and creatine levels, and an increase in the apparent diffusion coefficient (ADC) of water. The loss of all metaboliites (metabolic atrophy) has been associated with effective therapy, while residual prostate cancer was identified based on the presence of 3 or more voxels having Choline/Creatine > 1.5 with an accuracy of 80%. On DTI, residual prostate cancer has a lower ADC than surrounding regions of benign and atrophic tissue. A reduction in serum PSA levels paralleled reductions in prostate metabolism; however the amount of residual metabolism within the prostate did not correlate with serum PSA in individual patients, and the resolution of metabolic abnormalities occurred significantly earlier than PSA nadir. Studies to date indicate that complete metabolic atrophy and three or more contiguous voxels of elevated choline to creatine on MRSI may serve as early biomarkers of successful and unsuccessful radiation therapy. Additionally, combining DTI findings with MRSI findings may improve the overall accuracy of detecting residual cancer and provide an improved assessment of the extent of disease. Ongoing 10-year clinical outcomes are necessary to confirm these findings.

(S1.4) Imaging of the tumor microenvironment in radiation therapy. Zaver M. Bhujwalla. Johns Hopkins Univ School of Medicine, Baltimore, MD, USA.

Unlike other diseases where hypoxia induces severe damage, cancer cells have the remarkable ability to adapt, survive, and disseminate in physiological environments characterized by hypoxia and extracellular acidosis. The hypoxic environment of solid tumors has also proven to be a major obstacle for successful radiation and chemotherapy of cancer for several decades. Because of this remarkable ability of cancer cells to adapt and survive, finding effective treatments against cancer depends upon identifying and attacking targets critically important for the cancer cell to survive. While a decade ago the focus in cancer research was primarily on genetic alterations, it is now apparent that the tumor physiological microenvironment, the interaction between host cells including stem cells and cancer cells, the extracellular matrix, and a multitude of secreted factors and cytokines influence progression, aggressiveness and response of the disease to treatment. Using combined MR and optical imaging of human breast and prostate cancer xenografts engineered to express GFP under hypoxia, we have obtained useful insights into the dynamics between the tumor extracellular matrix (ECM), vascularization, interstitial fluid transport, and metabolism. MRI and MRSI were used to obtain co-localized maps of vascular volume, permeability, interstitial fluid transport, total choline and lactate/lipid, while optical imaging was used to obtain co-localized maps of hypoxia and ECM fiber distribution. Multi-modality and

multi-parametric molecular and functional imaging provide unprecedented opportunities for identifying and imaging key common pathways specific to cancer cells, and imaging the effectiveness and outcome of therapies targeting these pathways.

(S2.3) Induction of DNA damage by heavy ions: lesion clustering and localized cellular response. Gisela Taucher-Scholz. GSI, Darmstadt, Germany.

The biological effects of densely ionizing particle radiation are based on physical parameters like the extremely localized dose deposition. In consequence, multiple lesions are expected to be induced in close proximity on the DNA, leading to impaired repair of this clustered type of lesions, especially of double-strand breaks (DSBs). Experimental evidence for the production of clustered damage at the level of the DNA molecule is given by the analysis of DNA fragments induced after exposure to accelerated particles. Upon irradiation of plasmid DNA, the fragments produced can be measured with a detection limit of 50 nm using Atomic Force Microscopy. In agreement with theoretical calculations, fragment length distributions after carbon ions indicate a higher fraction of small fragments compared to similar X-rays doses, demonstrating induction of nearby DSBs. Here, in contrast to cells, higher order chromatin structure does not play a role. In cells, the localized microscopic dose deposition by ion traversals is directly reflected in form of discrete subnuclear regions of induced DNA damage, visualized by immunostaining of the recruited repair protein foci. Intensity and the kinetics of foci formation are in part influenced by the ion's LET. A better resolution of the spatial distribution of proteins is obtained by analyzing linear ion tracks, after irradiation with the incident beam almost parallel to the cell layer. However, the patterns of protein clusters do not represent individual lesions, but rather the underlying chromatin structure. In connection with lesion processing the loss of protein foci, changes in track morphology with time and a constricted migration of foci are observed. The increased persistence of repair protein foci provides further indirect support for the increased complexity of heavy ion-induced lesions. The new ion-based focal irradiation technologies have thus greatly improved the visualization of clustering of heavy ion induced damage.

(S2.4) Ion irradiation damage in structural materials. Gary S. Was. University of Michigan, Ann Arbor, MI, USA.

Radiation effects research has been conducted with a variety of energetic particles; neutrons, electrons, protons, He ions and heavy ions. The interest in ion irradiation has grown in recent years for several reasons; understanding neutron radiation damage in reactor materials, the avoidance of high residual radioactivity to minimize the cost of handling irradiated materials, and the decline of neutron sources for materials irradiation studies. The damage state and microstructure resulting from irradiation depend upon the particle type. The degree to which irradiation by ions emulates neutron irradiation effects is also a function of the damage state and irradiated microstructure. This talk will examine the damage function, primary recoil spectra and efficiency of defect production for various particle types on both microstructure and microchemistry. The roles of dose, dose rate, and temperature parameters and the constraints on parameter space by each particle source will be discussed and compared against the effects of neutron irradiation.

(S3.1) Multiple parameter biological dosimetry. William F. Blakely, Natalia I. Ossetrova, Ira H. Levine, David J. Sandgren, G David Ledney, Marcy B. Grace, Pataje G S Prasanna. USU/AFRRI, Bethesda, MD, USA.

Effective medical management of suspected radiation exposure incidents requires the measurement of dynamic medical data and physical dosimetry in order to provide diagnostic information to the treating physician and dose assessment for personnel radiation protection records. The accepted generic multiparameter and early-response approach includes observing prodromal signs and symptoms; obtaining complete blood counts with white blood cell differential; measuring radioactivity and monitoring the exposed individual; bioassay sampling, if appropriate, to determine radioactivity contamination; sampling blood for the chromosome-aberration cytogenetic bioassay using the "gold standard" dicentric assay for dose assessment; and using other available dosimetry approaches. Our Biodosimetry Assessment Tool (BAT) is a comprehensive software application developed for recording diagnostic information in suspected radiological exposures. The need to rapidly assess radiation dose in mass casualty and population-monitoring scenarios prompted an evaluation of suitable biomarkers that can provide early diagnostic information after exposure and the development of a complimentary software application, First-responder Radiological Assessment Triage (FRAT), for use on hand-held personal digital assistant devices. FRAT provides data collection templates for analysis of clinical signs and symptoms, lymphocyte counts, physical dosimetry, radioactivity, and location-based dose estimates. We recently investigated the combined utility of serum amylase activity, blood protein biomarkers (i.e., C-reactive proteins or CRP, etc.), and blood cell count changes as prognostic indicators of severe radiation exposure using a non-human primate radiation model. Rhesus macaques exposed to whole-body irradiation of 6.5 Gy ^{60}Co-gamma rays (40 cGy/min) showed 12- and 2.5-fold of day zero samples increases in serum amylase activity, ~150- and 70-fold of day zero samples increases in CRP, and lymphocyte cell counts depleted ($\leq$20 % of day zero samples) one- and two-days after radiation exposure. These results demonstrate that blood protein biomarkers along with hematological counts provide early and enhanced triage discrimination of individuals with severe radiation exposure and injury.

(S3.2) Network for cytogenetic biodosimetry in Japan. Mitsuaki A. Yoshida[1], Isamu Hayata[1], Hiroyuki Tateno[2], Kimio Tanaka[3], Shinichi Sonta[4], Seiji Kodama[5], Yoshiaki Kodama[6], Masao S. Sasaki[7]. [1]National Institute of Radiological Sciences, Chiba-shi, Japan, [2]Asahikawa Medical College, Asahikawa-shi, Japan, [3]Institute for Environmental Sciences, Rokkasyo-mura, Japan, [4]Human Service Center, Nagoya-shi, Japan, [5]Osaka Prefecture University, Sakai-shi, Osaka, Japan, [6]Radiation Effect Research Foundation, Hiroshima-shi, Japan, [7]Kyoto University, Kyoto-shi, Japan.

Chromosome analysis is more effective technique to estimate doses of exposed persons in the radiological accident. In Japan, the chromosome network system for biological dosimetry was organized in 2001 just after Tokai-mura criticality accident. One of the purposes in this network is to integrate the biodosimetry work to be ready for a large-scaled radiation accident. It consists of 7 laboratories which covers entire areas in Japan.Those laboratories have the same metaphase finder and CCD camera system for the chromosome analysis. Recently, the peripheral blood lymphocytes were irradiated at doses of seven points; 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 Gy by using ^{137}Cs gamma ray and the images including false positive one detected by a metaphase finder from the prepared slides of each points were archived into the disk. The stored images were distributed to seven laboratories participated in this network in order to construct the standard dose response curve. The criteria for the chromosome analysis is being discussed and established in a series of this study. The inter-laboratory comparison has also been preformed by using the chromosome figures at doses of two points; 1.0 and 5.0 Gy. The selected metaphases from each dose points were compared between these members. The concordance rate was 80–90% in the selection of the analyzable metaphases. The estimated doses from the frequency of chromosome aberrations were in line with the actual dose given to the blood samples. This study suggests that more important process in the chromosome analysis for the biodosimetry may be the selection of the metaphases suitable for the analysis in the slides. Furthermore, the chromosome network is developing the soft ware for the training of successors in the biodosimetry work. The images from the samples exposed at the seven doses of ^{137}Cs gamma ray were installed into the training program. Thus, the chromosome network has been established in Japan in preparedness for a radiological emergency.

(S3.3) Electron paramagnetic resonance radiation dosimetry. François Trompier[1], Harold Swartz[2], Alexandr Romanyukha[3]. [1]Institut de Radioprotection et de Sûreté Nucléaire, Fontenay-aux-Roses, France, [2]Dartmouth Medical School, Hannover, NH, USA, [3]Uniformed Services University of the Health Sciences, Bethesda, MD, USA.

Over the past decade, the threat of malevolent uses of radioactive materials has grown and some of the scenarios could lead to the exposure to ionizing radiation of a larger number of people than in classical situation encountered for past radiation accidents. In this case, dose estimations should be carried out in individuals rapidly and with sufficient accuracy to enable effective medical triage. In addition of existing triage methods (haematology, clinical and biological dosimetry), there are recent developments in emerging biodosimetric technologies for acute external exposure, such as Electron Paramagnetic Resonance spectrometry (EPR), that can enhance triage and dose assessment strategies. The use of EPR for dosimetry is based on the capability of the technique specifically and sensitively to measure unpaired electron species and the fact that ionizing radiation creates such species in exact proportion to dose. While the lifetimes of these species are very short in aqueous systems (nanoseconds life times), the radiation-induced signals can be extremely stable in non-aqueous media, including teeth and bone, and also sugars, fingernails, and hair for example. EPR measurements of tooth enamel or bone biopsies, although currently used in classical radiation accident situation, are obviously out of scope for medical triage, because an invasive sampling is needed. In order to overcome this difficulty there are two approaches using EPR that seems to be suitable for emergency purposes: in vivo measurements of teeth and ex vivo measurements of fingernail or toenail clippings, which are non-invasive or minimally invasive. This presentation will give an overview of the last developments in EPR methodology as triage tools. Even if a large and intensive work remains to perform to fully establish this method, recent developments are promising. Thus, it could be, in addition of established methods, a tool of choice in the medical management of victims of a large-scale radiation accident. This technique of retrospective dosimetry could at the end allow fast dose estimation without any delay. The dose sensitivity, according to the most recent works, should be sufficient for most of the scenarios of accidents.

(S3.4) Triage biological dosimetry using the dicentric assay. Ruth Wilkins. Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, ON, Canada.

In the event of a large scale radiological/nuclear emergency, biological dosimetry is an essential tool for providing timely assessment of radiation exposure for the general population and to identify first responders who must be restricted from further exposure. The frequency of radiation-induced dicentric and ring chromosomes in lymphocytes can be converted to dose estimates using the dicentric assay. Traditionally 1000 metaphases per sample are analysed, allowing detection of exposures as low as 0.15 Gy. However, when turn around time is critical, such as with a large number of samples following a radiation accident or terrorist attack, the detection threshold can be raised to 1 Gy for initial triage, thus reducing the number of metaphases to be analysed to 50. More metaphases can be analysed later to refine these dose estimates. Capacity for the dicentric assay is still limited by the speed with which samples can be scored. To address this issue, Canada has developed a National Biological Dosimetry Response Plan (NBDRP) which is able to respond to national needs in a nuclear or radiological event. In the initial phase of development, four core reference laboratories were established that developed standard operating procedures and reference curves for the dicentric assay and are the Canadian biodosimetry experts. These four laboratories participated in two exercises to demonstrate their ability to provide accurate and timely triage quality dose estimates. In order to extend this network, clinical laboratories across the country were recruited to assess their ability to expand our current capacity. To test the ability of these laboratories to score dicentric chromosomes each laboratory was provided with blinded slides. Results demonstrated the expertise of clinical cytogenetic laboratories and the potential for a greatly enhanced response capacity in the event of a nuclear/radiological emergency. The NBDRP provides a network of laboratories across Canada ready to respond to radiological/nuclear emergencies with the capability of providing rapid radiation dose estimates to large populations of potentially exposed individuals. (Funded by the Chemical, Biological, Radiological and Nuclear Research and Technology Initiative).

(S4.1) DNA polymerase beta: role in repair and survival after ionizing radiation. Conchita Vens, Christie Vermeulen, Sari Neijenhuis, Manon Verwijs-Janssen, Ingrid Hofland, Adrian Begg. The Netherlands Cancer Institute, Amsterdam, The Netherlands.

Several types of DNA lesion are induced after ionizing radiation of which double strand breaks (DSB) are thought to be the most lethal, although single strand breaks (SSB) and DNA base damages are quantitatively in the majority. Proteins of the base excision repair (BER) pathway repair these numerous lesions. DNA polymerase β (polβ) has been identified as a crucial enzyme in BER and single strand break repair (SSBR) and therefore thought to be implicated in damages induced by ionizing radiation (IR). BER/SSBR intermediates, left unrepaired, are expected to result in additional lethal DSBs. However, the lack of DNA polymerase β has been shown to have no effect on radiosensitivity, implying the existence of potent redundant pathways. We have previously shown that expression of a dominant negative to polβ (polβDN) resulted in radiosensitisation, confirming it's proposed critical role in repair of IR induced DNA damage. Here we show that mouse embryonic fibroblasts deficient in DNA polymerase β are considerably more sensitive to ionizing radiation than wild-type cells, but only when the fraction of S-phase cells is reduced either by confluence or by cell cycle synchronization. Further analysis by alkaline comet assays confirmed SSBR deficiency. Our data show that cell survival after IR in G0 and G1-phase of the cell cycle is strongly dependent on polβ. In addition, these studies revealed a second polβ-independent replication associated repair. Radiosensitisation of proliferating polβ-proficient and deficient cells by the polβDN suggests additional inhibition of this redundant repair. We speculate that the increased cytotoxicity when lacking polβ results from the formation of additional DSBs from unrepaired BER/SSBR intermediates. In deed, inhibition of BER after IR by the expression of the polβDN showed an increased induction of chromosome aberrations after IR, thus demonstrating the formation of additional secondary DSBs.Further studies excluded direct involvement in DSB repair and revealed increased dependency on proficient DSB repair. These data confirm the critical role of efficient and successful BER/SSBR after ionizing radiation for cell survival. They also have potential implications for the clinic, since a significant proportion of tumors present altered polβ that resemble the polβDN used in our study.

(S4.2) The roles of MDC1 in response to DNA double strand breaks via regulation RPA: inhibition of replication and promotion of homologous recombination. Junran Zhang, Wei Shi, Simon Powell. Department of Radiation Oncology, Washington University School of Medicine, St. Louis, MO, USA.

The inhibition of DNA replication and the subsequent facilitation of DNA repair are important mechanisms to minimize genetic alterations that arise after various types of cellular stress. Replication protein A (RPA) is involved in both DNA replication and DNA repair. Here, we show that RPA protein can be degraded by the ubiquitin-proteasome pathway in response to either ionizing radiation or hydroxyurea. In addition, RPA2 becomes phosphorylated and colocalizes with Rad51 in nuclear foci, suggesting a role for phosphorylated RPA in homologous recombination. Knockdown of RPA2 resulted in a decreased frequency of homology directed repair (HDR), and substituting wild-type rather than phospho-defective RPA2 led to greater correction of this defect. MDC1 is colocalized with phosphorylated RPA2 foci in response to DNA damage. Interestingly, knockdown of MDC1 led to decreased stress-induced ubiquitylation of RPA and therefore increased levels of RPA2. Furthermore, MDC1 does not localize to RPA-associated sites of DNA replication, whereas MDC1 showed nearly complete

colocalization with BRCA1 and γ-H2AX foci, representing replication-associated DNA repair. Together, these results suggest that MDC1 facilitates the degradation of RPA to inhibit DNA replication and promotes HDR via association with phosphorylated RPA.

(S4.3) Maintenance of replication fork progression on damaged and undamaged DNA. Eva K. Petermann[1], Apolinar Maya-Mendoza[2], Dean A. Jackson[2], George Zachos[3], David A. F. Gillespie[3], Shunichi Takeda[4], Keith W. Caldecott[1]. [1]Genome Damage and Stability Centre, University of Sussex, Brighton, United Kingdom, [2]Manchester Interdisciplinary Biocentre (MIB), University of Manchester, Manchester, United Kingdom, [3]Beatson Institute for Cancer Research, Glasgow, United Kingdom, [4]Departments of Radiation Genetics, Kyoto University Graduate School of Medicine, Kyoto, Japan.

We employed DNA fibre labelling to analyse changes in replication fork progression after DNA damage or in different genetic backgrounds in vertebrate cells. Cells are sequentially labelled with BrdU and IdU, chromatin fibres are spread on microscope slides and stained with antibodies to distinguish the two labels by immunofluorescence, thus allowing analysis of replication dynamics at the level of individual forks. We show that replication forks slow down after treatment with the crosslinking agent cisplatin. This slowing down is dependent on DNA repair and does not occur in cells lacking XRCC3, which is involved in homologous recombination repair of stalled replication forks and double strand breaks, or PARP-1, which is involved in the repair of single and double strand breaks. Fork slowing in the presence of cisplatin is not the result of checkpoint signalling, as forks still slow down in the absence of the checkpoint kinases Chk1 and Chk2. However, Chk1, the main effector kinase in the vertebrate ATR pathway, is required to maintain high global replication fork rates during normal S phase in untreated cells. Average replication fork rates in chicken DT40 or human cells depleted of Chk1 are 50–60% of those observed in wild-type cells. *Chk1*$^{-/-}$ DT40 cells display increased origin firing, prolonged fork stalling or collapse and high levels of single-stranded DNA. HeLa cells depleted of Claspin, which interacts with both Chk1 and replication forks, display similarly reduced fork rates as Chk1-depleted cells. Double knockdown of Claspin and Chk1 shows that both proteins act in the same pathway for maintenance of high fork rates. Claspin has been reported to be required for phosphorylation of Chk1 by ATR after replication inhibition in Xenopus egg extracts and several human cell lines. However, in Hela cells, Claspin is not required for Chk1 phosphorylation after Aphidicolin treatment. Claspin has shown to be a Chk1 substrate and might therefore act downstream of Chk1. In conclusion, our data demonstrate that homologous recombination is involved in fork slowing after cisplatin damage, whereas components of the ATR-Chk1 signalling pathway maintain replication fork progression in undamaged cells, presumably by preventing aberrant origin firing and stabilising replication forks.

(S4.4) Phosphorylation of RPA in replication and repair. Kathleen Dixon. University of Arizona, Tucson, AZ, USA.

RPA (Replication Protein A) is the major single-stranded DNA-binding protein in mammalian cells. This protein complex, composed of three subunits (p70, p34, and p14), is highly conserved from yeast to human and is essential for DNA replication and DNA repair. The RPA-p70 subunit is the major DNA-binding component; RPA-p34 is thought to mediate protein/protein interactions. RPA-p34 exists in multiple phosphorylation states in the cell and the N-terminus contains 9 confirmed sites for phosphorylation. A limited number of these sites are phosphorylated in S and M phases of the cell cycle and additional sites are phosphorylated in response to replication fork stalling or DNA damage. To learn more about the induction of RPA phosphorylation by DNA damage and the associated changes in subcellular localization and protein/protein interactions, we have used RPA-p34 phospho-specific antibodies with immunofluorescence microscopy of cultured human cells and a variety of biochemical techniques. After treatment of human cells with agents that cause replication fork stalling or DNA strand breaks, highly phosphorylated RPA localizes to nuclear foci and becomes tightly bound to chromatin. The co-localization with other repair proteins depends on the phase of the cell cycle and the type of DNA damage. Our studies have focused on the interaction of RPA with the MRN complex (Mre11, Rad50, and Nbs1) that participates in double strand break repair. After inhibition of DNA replication fork progression with hydroxyurea, phospho-RPA and MRN co-localize in nuclear foci. In contrast, after etoposide treatment that induces DNA breaks, phospho-RPA-p34 foci are found in some cells and not others and there is an inverse correlation between the presence of RPA foci and MRN foci. Interestingly, in some cases phospho-RPA-p34 is present in the cell but not localized in foci. After UV radiation, the induction of phospho-RPA-p34 occurs primarily in the S-phase of the cell cycle and correlates with arrest of the cells in S-phase and the loss of *in vitro* DNA replication activity. After UV, phospho-RPA-p34 and MRN co-localize in foci, presumably at stalled replication forks. All of these data suggest that RPA functions both in DNA damage signaling and in repair at stalled replication forks and DNA strand breaks. *Supported by NS34782*

(S5.1) Evaluation of the bioreductive activity of AQ4N, from lab to clinic. Stephanie R. McKeown[1], Rebecca Gallagher[2], Mark R. Albertella[3], Alvin Wong[4], Paul M. Loadman[5], Roger M. Phillips[5], Laurence H. Patterson[5]. [1]University of Ulster, Coleraine, United Kingdom, [2]Queen's University, Belfast, United Kingdom, [3]KuDOS Pharmaceuticals Ltd., Cambridge, United Kingdom, [4]Novacea Inc, South San Francisco, CA, USA, [5]University of Bradford, Bradford, United Kingdom.

The tumor-selective bioreductive prodrug AQ4N is an alkylaminoanthraquinone containing two aliphatic N-oxide side chains that inhibit binding to DNA. In hypoxic tumor cells, both N-oxide groups undergo 2-electron reduction to form AQ4, a stable cation at physiological pH. AQ4N has poor DNA binding affinity and a short plasma half-life; in contrast AQ4 is a potent topoisomerase II inhibitor and can non-covalently bind to DNA with high affinity, resulting in a long tumor residence time. The activation of AQ4N only occurs in low oxygen environments and is catalysed by a number of cytochrome P450s including 1A1, 2B6 and 3A4, enzymes which frequently show increased activity in tumors. It is therefore proposed that AQ4N has ideal properties for selective toxicity to hypoxic tumor cells. There is a growing body of preclinical evidence that demonstrate AQ4N can permeate throughout solid tumors *in vivo* and, where hypoxia occurs, there is rapid and selective formation of AQ4. Furthermore, treatment with AQ4N can complement and enhance the activity of radiation and chemotherapy which target better oxygenated tumor cells. In early clinical studies, anti-tumor efficacy has been demonstrated when AQ4N is combined with radiation or cisplatin without evidence of any potentiation of toxicities. As predicted from its prodrug mode of action, AQ4N is well-tolerated in patients and induces minimal systemic toxicities. Phase 1 trials have shown that AQ4N can be safely tolerated at doses up to 768 mg/m^2 when administered on a weekly basis or 447 mg/m^2 when combined with fractionated radiation. A Phase 1 pharmacodynamic study has been carried out in which AQ4N was administered to patients 24 hours prior to tumor excision. Analysis of tumor and normal tissues has revealed selective accumulation of the activated AQ4 metabolite within hypoxic tumor regions at concentrations that exceed the therapeutic levels predicted from preclinical studies; in contrast, AQ4 was virtually undetectable in normal tissues. In conclusion, laboratory and clinical studies provide proof of concept of AQ4N as a bioreductive drug that selectively targets hypoxic tumor cells. Current clinical trials are investigating the therapeutic benefit of AQ4N in combination with radiation and chemotherapy in patients with advanced malignancies.

(S5.2) Development of the novel hypoxia-activated bifunctional alkylating agent PR-104. William R. Wilson, Adam V. Patterson. University of Auckland, Auckland, New Zealand.

The development of a clinically effective strategy for eliminating hypoxic cells has been an important goal in radiation oncology for many decades. First-generation hypoxia-activated prodrugs (HAP) such as the quinone porfiromycin, the nitro-imidazoles RSU-1069/RB-6145/CI-1010 and the benzotriazine dioxide tirapazamine (TPZ) have demonstrated promising activity in preclinical models, but have identified two general limitations. One is the difficulty of designing HAP that can penetrate into hypoxic regions efficiently; this has led to development of tools for measuring tissue diffusion properties of prodrugs and their modification by drug design through control of reduction potential, logP and H-bonding. The other limitation is that moderate hypoxia occurs in a number of critical normal tissues; in response to this we have sought HAP that are activated only under severe hypoxia (expected to be more specific for tumors), using chemistries that provide stable cytotoxic effectors able to diffuse locally to kill partially radioresistant cells at higher oxygen tensions. PR-104, developed through a rational lead optimization programme from the early 2,4-dinitrobenzamide-5-mustard SN 23862, embodies these concepts. PR-104 is a water-soluble phosphate ester that is converted readily, in preclinical species and humans, to the corresponding alcohol PR-104A. The latter 3,5-dinitrobenzamide-2-mustard is readily reduced by one-electron reductases (cytochrome P450 reductase and other flavoenzymes) under hypoxia to the corresponding 5-hydroxylamine and 5-amine, but this requires 10-fold lower oxygen concentrations than for TPZ. This reduction activates the pre-positioned mustard moiety to effect DNA cross-linking selectively in hypoxic cells. The hypoxic selectivity and potency of PR-104A is no greater that for tirapazamine *in vitro*, but PR-104 shows clear superiority over TPZ in multiple human tumor xenograft models both as a single agent and in combination with radiotherapy or chemotherapy. Pharmacokinetic/pharmacodynamic modeling, based on diffusion of PR-104A in multicellular layer cultures, argues that this notable activity is due to a potent bystander effect resulting from diffusion of activated metabolites from severely hypoxic regions. PR-104 is currently in phase I/II clinical trials.

(S5.3) Anti-metastatic opportunities in targeting tumour hypoxia. Kaye J. Williams. University of Manchester, Manchester, United Kingdom.

Hypoxia-selective bioreductive drugs have been developed to complement standard treatment approaches that are less effective against hypoxic cells. However tumour hypoxia is not only associated with treatment resistance but also with an aggressive disease phenotype and an increased propensity for metastasis. The association between the level of tumour hypoxia and the likelihood of metastatic progression suggests that targeting primary tumour hypoxia may influence subsequent metastatic dissemination. Furthermore hypoxia may be an important physiological stimulus in early stage disseminated disease suggesting that hypoxia-selective agents may be useful adjuvants to standard therapies in controlling tumour growth at secondary sites. We have used the murine KHT tumour model to investigate whether bioreductive agents can be used to successfully control metastases. Three different bioreductive agents have been investigated; the nitro-imidazole based drugs NLCQ-1 (NSC 709257) and RB6145 and the aromatic-N-oxide tirapazamine (TPZ). The bioreductive drugs were administered in fractionated protocols pre- or post- single high dose radiotherapy and effects both on local control and the presentation of lung metastases were assessed. These studies were complimented with analysis of hypoxic fraction both in primary tumours and secondary tumours to confirm the presence of a viable target population. Data will be presented that strongly support the utility of bioreductive drugs as anti-metastatic agents and that the most appropriate application of each individual drug is related to their discrete pharmacodynamic/oxygen dependent properties.

(S6.1) Chemoradiotherapy: biology of drug - radiation interactions and improvement strategies. Luka Milas. MD Anderson Cancer Center, Houston, TX, USA.

The use of chemotherapeutic drugs in combination with radiotherapy has become a common strategy for cancer treatment. Solid evidence exists showing that chemotherapy administered during the course of radiotherapy increases both local tumor control and patient survival, and organ preservation rates in a number of cancer sites. These therapy improvements have been achieved by using standard chemotherapeutic agents, most commonly cisplatin-based chemotherapy, but they were achieved at the expense of considerable toxicity. Chemotherapeutic agents augment the cytotoxic action of radiation and offer the prospect of spatial cooperation, whereby the local effect of radiation is complemented by the systemic effect of drugs on disseminated disease. In spite of these improvements, local-regional recurrence still remains a major concern. To further improve chemoradiotherapy, there have been extensive explorations of the potential of newer chemotherapeutic agents including taxanes, nucleoside analogues, and drug-polymeric macromolecules conjugates. Preclinical studies on these drugs in combination with radiation have provided not only a biological rationale for using these drugs in combination with radiotherapy but generated information critical for designing effective treatment schedules in clinical settings. The therapeutic efficacy of newer drug-radiation combinations have been tested clinically, and provided encouraging results. Recent advances in molecular biology have exposed many potential targets for augmentation of radio (or chemo) response, including EGFR, COX-2, dysregulated cyclin-dependent kinases and angiogenic molecules. Agents that selectively inhibit these molecules are becoming available at a rapid rate, and many of them have been shown in preclinical testing to be highly effective in improving tumor radioresponse or chemo-response without affecting normal tissues. Some of them, such as cetuximab, have been effective in clinical treatment settings.

(S6.2) Clinical trials on chemotherapy enhanced radiation therapy in europe and their translational research. Harry Bartelink. The Netherlands Cancer Institute, Amsterdam, The Netherlands.

The results from fundamental research to the discovery of pathways involved in radiation damage and the search for new targets and drugs to enhance tumor cell kill with radiation, has led to a rapid increase of the amount of phase I - III clinical trials in Europe. These trials are exploiting the concomitant use of chemotherapy and radiotherapy, mostly with drugs involved in DNA damage repair or apoptosis. Trials are ongoing with drugs inhibiting tumor cell repopulation or angiogenesis during irradiation. Mechanism involved these interactions will be summarized and presented in relation to the results of clinical trials that are carried out in brain, lung, head and neck and rectal cancer. These trials are more and more paralleled by the search towards predicting treatment efficacy and/or prognosis. Typical examples will be presented: for example the increased formation of cisplatin DNA adducts in head and neck cancer is related to a better treatment outcome after the concomitant use of cisplatin and irradiation. The search for gene profiles with micro-arrays to predict prognosis in these patients will be shown. A benefit is observed from adjuvant cisplatin based chemotherapy in patients with lung tumors and a negative expression of the ERCC1 DNA repair protein. Patients with glioblastoma containing a methylated MGMT promoter benefited from temozolomide if given together and with irradiation.

(S6.3) Chemotherapy enhanced radiation therapy: U.S. clinical trials and their influence on the standard of care. Patricia J. Eifel. MD Anderson Cancer Center, Houston, TX, USA.

For more than four decades, radiation oncologists and biologists have searched for ways to enhance the effectiveness of radiation therapy by combining it in various ways with chemotherapy. During the past 15 years, studies of these combinations have dominated radiation oncology clinical research in the United States, with large phase III trials demonstrating the efficacy of combined treatment in patients with cancers of the lung, esophagus, rectum, anus, stomach, bladder, cervix, head and neck and other sites. Although sequential administration appears to be of benefit in some

sites, the greatest benefit has usually been seen with concurrent administration of drugs and radiation. The results of these trials have increased the efficacy as well as the complexity and cost of routine cancer treatment in the U.S. In the U.S., cisplatin has been a dominant element in most chemo-RT regimens. However, the dose, schedule, and pairing with other drugs has varied widely between sites and studies. Cisplatin has been given successfully in 3-week intervals, weekly, or daily. The total dose during a course of radiation has ranged from $<$100 mg/m2 to $>$ 200 mg/m2. Other platinum analogues have received little attention; however, cisplatin has been given alone or paired with other drugs, including 5-FU, docetaxel, paclitaxel, and etoposide. Although investigators have sometimes borrowed from successes in other disease sites, preferred treatment regimens have tended to evolve within site-specific interest groups. Although choices have sometimes been informed by preclinical studies, results in other sites, and site-specific normal tissue considerations, tradition and investigator preference have also played important roles. Schedules made popular in different sites have rarely been compared head-to-head. Although pre-clinical studies are occasionally cited in the overall rationale for concurrent chemoradiation, specific schedules are rarely justified in terms of scientific principles, pharmacology, or comparative tumor biology. Although the costs, convenience, and toxicities of schedules differ greatly, little is known about the relative benefits of popular chemoradiation regimens. This knowledge gap demonstrates the need for detailed collaborative studies of the mechanisms and pharmacology of chemoradiation.

(S6.4) Current status of development of various chemo-radiotherapeutic approaches, with reference to industrialized and limited-resource settings. Jolyon H. Hendry. International Atomic Energy Agency, Vienna, Austria.

Chemotherapy is often used in addition to radiotherapy for treating particular types and stages of cancer. The cost of chemotherapy is often greater than that of radiotherapy, and this is more important in countries with limited resources. The International Atomic Energy Agency has considered combined treatments in its programme on nuclear applications. To address the potential of agents under development, not those well established and in use, a consultants meeting discussed a selection of agents at various stages of development from pre-clinical studies to clinical trials. These included agents interfering with repair of radiation injury, antibodies that interfere with molecules in cell signalling pathways, drugs that target tumor hypoxia and vasculature, and molecular antisense and gene therapy strategies. All the drugs discussed have previously been shown to act as tumor cell radiosensitisers or to kill hypoxic cells present in tumors. Multi-centre trials organised by IAEA in countries with limited resources have shown no major influence of mitomycin-c on loco-regional tumor control, survival or morbidity after primary radiotherapy in stage III-IV head and neck cancer, but a significant increase in loco-regional tumor control and survival with no increase in toxicity using radiotherapy and a nitrotriazole hypoxic cell sensitiser for advanced cervix cancer. Several new agents were identified by the consultants as suitable for further such trials. These included pentoxyfylline in combination with chemoradiotherapy, drugs that target the epidermal growth factor receptor and cyclooxygenase-2, and nimorazole in sites where tumor hypoxia has been shown to influence outcome after radiotherapy. In addition, more preclinical work was recommended with various approaches including manipulation of deoxycytidine kinase activity, the use of alkyl-phosphocholines, bioreductive N-oxide series drugs, combretastatin and fractionated radiation, as well as the molecular strategies of antisense against bcl-2 and related anti-apoptotic proteins or other resistance molecules, gene-directed enzyme prodrug therapy, and radiation-activated molecular-switch concepts. Consultants: MR Horsman, L Bohm, GP Margison, L Milas, JF Rosier, G Safrany, E Selzer, M Verheij.

(S7.1) Imaging cell proliferation, apoptosis, and necrosis with positron emission tomography. Robert H. Mach. Washington University, St. Louis, MO, USA.

Cellular homeostasis is controlled by a delicate balance between cell proliferation and programmed cell death (i.e., apoptosis). The transformation of a normal cell into cancer is caused by the inability of a cycling cell to enter a natural quiescent state. When cellular proliferation exceeds cell death via apoptosis, there is a net accumulation of malignant cells that produces the lethality of cancer. Successful treatment of cancer consists of a strategy that results in both a reduction in cell proliferation and increase in apoptosis to the extent that cell death exceeds cell proliferation. An alternative pathway by which chemotherapeutics and radiation can kill tumor cells is by causing necrosis. Research in our group has focused on the development of novel imaging agents that are capable of providing accurate measurements of proliferation, and the different mechanisms by which chemotherapeutics and radiation can kill tumor cells, apoptosis and necrosis. The molecular targets for our imaging studies are: 1) the sigma-2 receptor, which is a receptor-based biomarker of cellular proliferation; 2) caspase-3, an executioner caspase that is activated once the cell has committed to undergo programmed cell death; and 3) PARP-1, which becomes hyperactivated under conditions of severe oxidative stress and kills cells via necrosis. This talk will highlight the advantages of our approach for imaging cell proliferation, apoptosis, and necrosis, and contrast them with alternative methods currently being used to study these processes.

(S7.2) Image registration and segmentation in functional IGRT. Thomas Guerrero. Univ of Texas M D Anderson Cancer Center, Houston, TX, USA.

This presentation will review image registration and segmentation methods and their application to image-guided radiotherapy (IGRT). Deformable image registration (DIR) is becoming an enabling image analysis tool in radiotherapy with applications to multi-modality image fusion, image analysis, semi-automated image segmentation, 4D dose estimation, and 4D IGRT. The DIR algorithms provide a spatial transformation from the content of one image to another, with a vector displacement for each voxel. Two categories of DIR algorithms exist, this dichotomy arises from the data on which the algorithms operate, image content (e.g. image features, landmark-based, segmentation-based, etc.) or voxel properties (e.g. grey level as in optical flow). IGRT utilizes intra-session registration for respiratory motion related effects and inter-session registration for changes that occur over a multi-week course. Image segmentation is the process of delineating the spatial location of normal tissues and cancer targets. Image segmentation has long been a component of the planning process. The generation of the dose volume histograms (DVH) requires a segmented CT image volume on which to compute the radiation dose, the DVH is then derived from the two using histogram processing. Automated anatomic segmentation which has relied on voxel properties, such as grey level and connectivity with a seed point, has been limited to the few organs with high contrast borders (e.g. lungs or brain). More recently, anatomic segmentation tools based on anatomic models have begun to appear in commercial treatment planning systems. Recent developments in the use of molecular images for image guided targeting to define the spatial location of clinical targets for dose intensification will also be discussed. This lecture will provide an overview of image registration, image segmentation, and strategies using these methods to integrate functional imaging into IGRT.

(S7.3) Use of PET/CT for molecular image guided radiotherapy. Karin M. Haustermans. Leuven Cancer Institute, Leuven, Belgium.

Molecular imaging allows molecular events to be tracked in a living organism. PET is a powerful imaging technology for which specific tracers have been developed for tumor cell proliferation, metabolism, apoptosis, angiogenesis, receptor and gene expression. The long term goal of these studies is to use PET to stage the disease, to select the treatment for the patient (treatment individualisation), to plan treatment volumes that compensate for volumes of radioresistant disease, and to evaluate treatment efficacy

(early response prediction and early detection of recurrent disease). In disease staging, PET has been proven to have high accuracy in detecting unsuspected but pathological lymph nodes and other metastases, and this has been further improved with the use of integrated PET/CT systems. Precise and accurate target delineation is the first step in delivering curative doses of radiation while sparing surrounding normal tissue. Images from specific tracers can assist normal treatment planning and allow dose painting of radioresistant foci to improve biological dose conformality. In addition to selectively targeting subregions within the tumor with higher doses, tumor specific therapies may be incorporated into treatment. This approach is currently being pioneered using specific tracers to image hypoxia, but has broader implications, such as targeting rapidly proliferating areas within tumors or areas expressing other forms of molecular heterogeneity. As a response indicator, volume measurement is known to lack specificity and significance. PET/CT of functional parameters can assist is assessing outcome and can also help differentiate viable tumor from radiation-induced effects such as fibrosis, atelectasis, and radiopneumonitis. The best tracers and optimal timing of these PET exams before, during and after treatment is still under experimental investigation and before PET/CT imaging enters into the clinical routine of the radiotherapy department, several methodological issues need to be addressed. For example, PET-based target volume definition using different PET tracers needs to be addressed. Finally there is an urgent need for controlled studies to establish the impact of PET/CT on the final outcome of patients treated with image guided radiotherapy.

(S8.1) Intercellular induction of apoptosis - role of reactive oxygen species and low dose radiation. Georg Bauer. Universität Freiburg, Freiburg, Germany.

Transformed target cells are subject to efficient apoptosis induction exerted by nontransformed effector cells. This reactive oxygen (ROS)-mediated process has been termed intercellular induction of apoptosis and has been shown to be relevant for a variety of effector and target cell systems. In addition to intercellular induction of apoptosis, transformed cells can also be eliminated by ROS-mediated autocrine selfdestruction. In this process, the effector molecules are generated by the transformed target cells themselves and establish ROS-mediated signaling between transformed cells. Sensitivity to intercellular induction of apoptosis and autocrine selfdestruction are strictly correlated to the expression of the transformed state. Selectivity as well as efficiency of intercellular induction of apoptosis are based on reactive oxygen and nitrogen species. Extracellular superoxide anions generated by transformed target cells represent the central element in this signaling system. They allow and control four different signaling pathways, based on HOCl/hydroxyl radicals, NO/peroxynitrite, nitrylchloride and on the metal-catalyzed Haber-Weiss reaction. Dependent on the spatial situation of the cells and on the concentrations of the various players in this system, these four signaling pathways may act synergistically or may show negative interference. The central elements of this extracellular signaling chemistry have been elucidated through the use of specific scavengers and through small interfering RNA (siRNA)-mediated genetic knock-down.

Tumor formation and progression in vivo seem to depend on the selection of transformed cells that are resistant against intercellular induction of apoptosis and autocrine selfdestruction. Intercellular induction of apoptosis and autocrine apoptotic self-destruction are discussed as a hitherto unrecognized control system during oncogenesis. Interestingly, low dose radiation enhances intercellular induction of apoptosis through stimulation of signaling functions of nontransformed effector cells as well as transformed target cells. These findings may be relevant for the understanding of the complex picture of low dose radiation effects in vivo.

(S8.2) Induction of apoptotic signaling cascades by aldehyde by-products of lipid peroxidation**.** Diana A. Averill-Bates. Université du Québec à Montréal, Montréal, PQ, Canada.

The aim of the study is to determine mechanisms of action of the toxic aldehyde, acrolein. Lipid peroxidation is a deleterious chain reaction occurring mainly in biological membranes, resulting from oxidative stress. Several aldehyde by-products are generated including acrolein, malondialdehyde and 4-hydroxy-2-nonenal. Acrolein has been implicated as a possible mediator of oxidative damage to cells and tissues in a wide variety of disease states, including atherosclerosis, neurodegenerative and pulmonary diseases. Our findings show that acrolein can induce apoptotic cell death in several mammalian cell types, as indicated by cleavage of inhibitor of caspase activated DNase (ICAD) and condensation of nuclear chromatin. Investigation of the signalling mechanisms showed that acrolein activated both the Fas death receptor and mitochondrial pathways of apoptosis. Acrolein caused translocation of adaptor protein Fas-associated with death domain (FADD) to the cytoplasmic membrane, caspase-8 activation and cleavage of Bid, which activated the crosstalk pathway between the death receptor and mitochondrial pathways. Activation of the mitochondrial pathway by acrolein was confirmed by cytochrome c release from mitochondria and caspase-9 activation. Acrolein-induced apoptosis was mediated by the mitogen-activated protein kinases (MAPK). The inhibition of the MAPKs, extracellular signal-regulated kinase (ERK) and p38 kinase, decreased acrolein-induced chromatin condensation, ICAD cleavage, and activation of caspases-7 and –9. The c-jun N-terminal kinase (JNK) and AKT/protein kinase B both appeared to be implicated in survival pathways against acrolein insult. Acrolein also induced phosphorylation of the pro-apoptotic factor p53. These findings show that the reactive aldehyde acrolein can cause apoptotic signalling and activation of MAPK signalling cascades. These findings could have widespread implications in multiple disease states which appear to be mediated by oxidative stress and lipid peroxidation.

(S8.3) Main route of radiation-carcinogenesis is dna damage-independent pathway. Masami Watanabe[1], Hanako Yoshii[1], Kimiko Watanabe[1], Keiji Suzuki[2], Seiji Kodama[3], Jun Kumagai[4]. [1]Research Reactor Institute, Kyoto University, Sennan, Osaka 590-0494, Japan, [2]Nagasaki University, Nagasaki 852-8521, Japan, [3]Osaka Prefecture University, Sakai 599-8570, Japan, [4]Nagoya University, Nagoya 464-8603, Japan.

It has been believed that the first target of radiation carcinogenesis is DNA. However, this expectation is not proved directly yet. We analyzed results of research of malignant cell transformation by radiation for 30 years generally and came to strongly believe that a radiation cancer-causing primary target was not DNA itself. One example is that transformation frequency in Syrian hamster embryo (SHE) cells irradiated with X-rays is 500–1,000 folds higher than that for somatic mutation. This contradicts "the multistage mutation theory" which carcinogenesis produces in accumulation of 3–5 independent mutations. This thought was recently developed by results of research of a radiation hereditary effect reduction effect by vitamin C. Because more than 80% for cellular constitution are aqueous, it is likely that large quantities of OH radicals are produced by radiation exposure. Activity of OH radical is strong extremely and it is thought to be a direct cause of DNA breakdown. However, recently we discovered that stable protein radical (Long-lived radicals; LLR) was present in normal temperature. Surprisingly, this radical does not attack direct DNA, but causes carcinogenesis. Vitamin C catches the LLR effectively and suppresses carcinogenesis. From these results, we speculate that nongenetic damage induced by LLR plays an important role in an initial process of cellular malignant transformation. Therefore, we are searching for an intracellular target of LLR related to carcinogenesis in SHE cells and human embryo cells. As a result, we found that the intracellular LLR level rises with an intracellular oxidation level by high-density culture and radiation exposure. And LLR attack telomere-related protein and centrosome, and destroy their structure. In those cell culture, structural aberration of chromosome does not occur, but aneuploid is seen in high frequency. These results suggest a possibility that a main target of radiation carcinogenesis is not DNA, but is centrosome that is the proteins to constitute chromosomal homeostasis maintenance mechanism. If our experiment results are right, "mutation theory of carcinogenesis" is to be wrong. I will suggest a hypothesis of new radiation carcinogenesis by this presentation.

(S8.4) A sirt3 / foxo3a / mnsod signaling model for mitochondrial superoxide levels. David R. Gius. RadiationOncology Branch, Bethesda, MD, USA.

Cellular longevity regulation is a complex processes relevant to age-related diseases including cancer, diabetes, and metabolic syndromes. The accumulation of mitochondrial damage has been clearly implicated in the aging process. Humans have seven homologs of the yeast histone deacetylase *SIR2* (sirtuins), that localize to the nucleus, cytoplasm, and mitochondria. Sirtuins may serve as guardian genes for critical cellular functions. We hypothesize that SIRT3 monitors intramitochondrial conditions and initiates intracellular signals serving to optimize and/or repair mitochondrial function. We propose a model whereby a signaling cascade initiated in the mitochondria results in an induction of a nuclear transcription factor and subsequent expression of a nuclear encoded mitochondrial protein. This connects three well established longevity genes: (1) SIRT3, a mitochondrial sirtuin gene; (2) daf-16/foxo family; and (3) MnSOD proteins. To address this, HCT116 cells, which express low levels of SIRT3, were genetically altered to overexpress either wild-type or dominant negative mutant SIRT3. Using mitochondrial isolation followed by Co-IP, and western analysis we demonstrate for the first time that FOXO3a is localizes to the mitochondrial and physically interacts with SIRT3. Chromatin-IP (ChIP) assays demonstrated several fold increase in FOXO3a DNA binding to the MnSOD promoter region containing a FOXO site in the SIRT3 overexpression cells. Transient co-transfection of a nuclear constitutively active FOXO3a expression vector increased luciferase activity of a MnSOD reporter plasmid. MnSOD gene expression and activity are increased in the cell lines overexpressing the SIRT3 wt cells. Biochemical characterization demonstrated a 2.5 fold increase in superoxide levels in the sirt3 deacetylation mutant vs a 30% decrease in the SIRT3-wt cells, as compared to cells expressing a control empty vector. A model is proposed whereby SIRT3 plays a central role in the regulation of mitochondrial function that serves as a rheostat to regulate intracellular superoxide levels.

(S9.1) Removing carcinogenic nitrosamines from waters using radicals. Stephen P. Mezyk[1], Nicholas A. Landsman[1], Katy L. Swancutt[1], Casandra R. Cox[1], Keith P. Madden[2], James J. Kiddle[3]. [1]California State University, Long Beach, CA, USA, [2]Radiation Laboratory, Notre Dame, IN, USA, [3]Western Michigan University, Kalamazoo, MI, USA.

Nitrosamines (R^1R^2N-NO) are a class of mutagenic, teratogenic, and carcinogenic chemicals that exist in the environment as by-products of various manufacturing, agricultural, and natural processes. There are at least 300 known carcinogenic nitrosamines, which have been shown to induce tumors in over thirty animal species. Nitrosoamines, particularly *N*-nitrosodimethylamine (NDMA) are also present in foods and beverages that contain nitrite, or which have been exposed to nitrous oxides. NDMA has also been shown to form in waters undergoing disinfection, by the reactions of added monochloramine with dimethylamine, unsymmetrical dimethylhydrazines, or natural organic matter. The California Department of Health Services currently has set notification levels of 10 ng L^{-1} for NDMA in drinking water. To achieve such low concentrations, new technologies such as Advanced Oxidation Processes (AOPs) which utilize oxidation via the hydroxyl radical ($^{\bullet}OH$), and/or reduction by hydrated electrons (e^-_{aq}) and hydrogen atoms ($^{\bullet}H$), are being investigated. For AOP treatments to occur efficiently and quantitatively, a full understanding of the chemistry involved under the conditions of use is essential. A critical component for the modeling of any free radical based technology is a full description of the reaction mechanisms and kinetics of the reactions of all the organic compounds involved. In this work we report on our determination of quantitative rate constants, reaction mechanisms, and specific degradation efficiencies for the free-radical-based destruction of various nitrosamines in water. Combining commercially-available and synthesized nitrosamine moieties, and utilizing LINAC electron pulse radiolysis, ^{60}Co-steady-state radiolysis, and UV-visible absorption/electron paramagnetic resonance detection techniques, along with gas chromatography/mass spectrometry stable product measurements, we have correlated these radical reactions with the specific chemical structure of these nitrosamines, particularly on how the oxidations and reductions depend upon their alkyl and aryl substituents. We have also established the importance of some of the reactions for the formed nitrosamine radicals, particularly the formation of nitrosamine peroxyl radicals and self-fragmentation pathways.

(S9.2) Hydroxyl radical removal efficiencies of model organic contaminants in different quality wastewaters. Julie R. Peller[1], Stephen P. Mezyk[2], William J. Cooper[3]. [1]Indiana University Northwest, Gary, IN, USA, [2]California State University at Long Beach, Long Beach, CA, USA, [3]University of California, Irvine, Irvine, CA, USA.

Water treatment facilities are challenged with the difficult task of remediating a wide variety of organic contaminants which conventional treatments are not fully capable of removing. The incorporation of advanced oxidation processes into wastewater treatment plants provides a feasible means for the remediation of organic contaminants, namely by the hydroxyl radical, in waters intended for reuse or discharge. The effectiveness of organic contaminant removal by the generated hydroxyl radical is dependent upon many factors, including TOC (total organic carbon), carbonates, nitrates/nitrites and pH. Radiation chemistry techniques allow for detailed analysis of hydroxyl radical reactions and were utilized to assess the ability of the hydroxyl radical to remediate organic contaminants in pure water and in treated wastewaters. Additionally, the toxicity of the stable intermediates formed in the oxidation must be assessed since they can be a greater health concern than the parent compounds. Four organic compounds were selected as model contaminants: bisphenol A, caffeine and two sulfa drugs, all of which react rapidly with the hydroxyl radical ($1–8 \times 10^9$ $M^{-1}s^{-1}$). Solutions of these organic compounds were prepared in deionized water and various treated wastewaters, and subjected to variable doses of gamma rays from a cobalt-60 source. The changes in concentration of the parent compounds and the formation of prominent intermediates were followed using HPLC. Removal constants were determined for the model compounds, and in all cases, the values decreased with a decline in water quality. The water quality parameter, TOC, shows the most correlation to the removal constants. Other details of the hydroxyl radical-mediated oxidations, such as extent of intermediate formation/degradation and variations in intermediates also vary according to water quality, especially in the hydroxyl radical oxidation of bisphenol A. Prominent intermediates have been identified using mass spectrometry techniques.

(S9.3) Radiation chemistry of water: lab scale to pilot plant. William J. Cooper[1], Julie R. Peller[2], Stephen P. Mezyk[3]. [1]University of California, Irvine, Irvine, CA, USA, [2]Indiana University, Northwest, Gary, IN, USA, [3]California State University at Long Beach, Long Beach, CA, USA.

Advanced oxidation processes (AOPs) are widely used in water treatment. Advanced oxidation processes are defined as those that use they hydroxyl radical ($^{\bullet}OH$) as the primary reactive species. The treatment of water in this context is any water (industrial or domestic), from any source (ground or surface water) that is contaminated with organic compounds that for one reason or another are present in the water and must be removed. There are a number of AOPs that concomitantly form reducing species, for example hydrogen atom ($H^{\bullet}$) or the solvated electron (e^-_{aq}) and these might be referred to as advanced oxidation/reduction processes (AORPs). Radiation chemistry and the tools of radiation chemists provide the experimental approaches to addressing the fundamental chemistry of AORPs. The general approach that we have adopted is to determine bimolecular reaction rate constants, elucidate destruction mechanisms, develop kinetic models and then apply the models to larger scale studies. The models can be thought of as three series of differential equations that describe 1) the formation of the reactive species, 2) destruction mechanism, and 3) the fluid dynamics of the treatment process. These models can also be used to assist in designing large scale studies and for preliminary estimates of treatment costs. We have applied these models to a

number of compounds that fall into two categories, emerging pollutants of concern and disinfection by-products. The recent focus of our studies has been for the treatment of waters intended for water reuse, where water reuse may be either for irrigation or as an indirect source for drinking water. This paper will describe how the tools of radiation chemistry are used to provide a fundamental understanding of AOPs and also serve to potentially tie these studies to health effects.

(S10.1) Structural aspects of the recognition of oxidized purines by the Formamidopyrimidine-DNA glycosylase, Fpg. Bertrand Castaing[1], Matthias Ober[2], Thomas Carell[2], Franck Coste[1]. [1]Centre de Biophysique Moléculaire, CNRS, Orléans, France, [2]Fakultät für Chemie und Pharmazie der Ludwig-Maximilians, Universität München, Müchen, Germany.

The bacterial Fpg protein (the functional homologue of the eukaryote Ogg1) catalyses the removal of oxidized purines such as imidazole-ring opened purines (Fapy) and the major oxidized product of purines, the 8-oxoguanine (8-oxoG) [1,2]. Both these lesions can be generated in DNA by irradiation (UV and gamma) and are associated with replication arrests (Fapy residues) and G to T transversions (8-oxoG) [3,4]. Fpg cleaves the N-glycosidic bond between the damaged base and its associated sugar (DNA glycosylase) thus generating an AP site in DNA. The enzyme is also associated with an AP lyase activity which eliminates the resulting AP site by successive cleavages at 3' and 5' sides by a beta/delta-elimination reaction [5]. The challenge of DNA glycosylases, such as Fpg, is to detect in DNA and excise one lesion among million of normal bases, all the more this lesion does not disturbed the global DNA structure at the damaged site. Crystal structures of Fpg bound to damaged DNA is one of the approaches which permitted to document at the atomic level the molecular mechanism by which this enzyme recognizes and removes the damaged nucleobase. We will present how we have initiated this structural study through the design and synthesis of uncleavable substrate analogues or inhibitors-containing DNA [6,7,8].

(S10.2) Visualizing a replicative polymerase encounter unrepaired free radical DNA lesions. Sylvie Doublie, Pierre Aller, Matthew Hogg, Karl Zahn, Susan S. Wallace. University of Vermont, Burlington, VT, USA.

Modifications in the DNA genome can have profound consequences: DNA polymerases, which otherwise faithfully replicate DNA, stumble when they encounter oxidative DNA lesions. Polymerases either will be blocked at the site of lesion, or bypass it. The latter case, referred to as translesion synthesis, may initiate an oncogenic process if the wrong base is inserted opposite the lesion. Uncovering the fundamental mechanisms underpinning translesion synthesis is paramount to understand the initial events of mutagenesis. We aim to elucidate at the atomic level the factors that influence the interactions between a replicative polymerase and DNA lesions. We recently solved the crystal structure of a replicative DNA polymerase of the B family (RB69 gp43) encountering an abasic site. This structure revealed that the DNA did not translocate after incorporation of dAMP across the abasic site, illustrating why a site of base loss is such a strong block. Furthermore, in our crystals, we captured four different conformations of the enzyme bound to DNA. In addition to its primer extension activity, RB69 gp43 also harbors a proofreading activity. When proofreading is invoked, the DNA strand to be edited must travel about 40 Å to reach the proofreading site. All four molecules in our crystal exhibit conformations that differ from the previously published structures of the polymerase with undamaged DNA and may represent steps along the active site switching pathway. This crystal structure paved the way for additional lesion complexes, including thymine glycol. Thymine glycol (Tg) is a common product of oxidation and ionizing radiation, including that used for cancer treatment. Although Tg is a poor mutagenic lesion, it has been shown to present a strong block to most DNA polymerases. Our structure of RB69 gp43 with Tg-containing DNA shows that the templating Tg is intrahelical and forms a regular Watson-Crick base pair with the incorporated A. The C5 methyl group protrudes axially from the ring of the damaged pyrimidine and hinders stacking of the adjacent 5' template guanine. The position of the displaced 5' template guanine is such that the next incoming nucleotide cannot be incorporated into the growing primer strand, which illustrates why primer extension past the lesion is prohibited.

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(S10.4) Recombinational DNA repair: biochemistry and visualization at the single-molecule level. Stephen C. Kowalczykowski. University of California, Davis, CA, USA.

Breaks in either one or both strands of double-stranded DNA can be repaired by a variety of recombination-dependent replicational repair processes. The biochemical mechanism of this process and its visualization at the single-molecule level will be presented.

Individual molecules of the bipolar DNA repair helicase, RecBCD, have been seen acting on single molecules of DNA. Detection involves optical trapping of a polystyrene bead to which fluorescently-tagged dsDNA or enzyme is attached. Translocation is monitored using fluorescence microscopy. Visualization of translocation by the chromatin-remodeling protein, Rad54, has been also achieved, and it will be shown that Rad54 is a rapid and processive translocase. The implications for chromatin-remodeling will be discussed. Finally, assembly of RecA and Rad51 on single DNA molecules has recently been visualized. Implication for DNA repair and cancer progression will be discussed.

(S11.2) Regulation of protein folding during hypoxia. Marianne Koritzinsky[1], Kasper M. Rouschop[1], Ineke Braakman[2], Bradly G. Wouters[1]. [1]Maastricht University, Maastricht, The Netherlands, [2]Utrecht University, Utrecht, The Netherlands.

We and others have shown that hypoxia rapidly activates the endoplasmic reticulum (ER) kinase PERK, resulting in differential gene expression regulated both at the level of transcription and mRNA translation. Activation of PERK is known to occur following accumulation of misfolded proteins in the ER, and the resulting induction of many chaperone proteins that aid in protein folding is termed the unfolded protein response (UPR). The ER serves as a unique oxidizing cellular compartment that allows formation of disulfide bonds and hence covalent protein folding of secreted and membrane-targeted proteins. Recent studies in yeast have indicated that molecular oxygen can act as the final electron acceptor in the chain of redox reactions necessary for disulfide bond formation in the ER. During folding, electrons are believed to flow from the client protein via protein disulfide isomerase (PDI) and endoplasmic reticulum oxidase (Ero1) to molecular oxygen. These findings led us to hypothesize that hypoxia activates the UPR by inhibiting disulfide bond formation and protein folding in the ER, due to the lack of molecular oxygen as the terminal electron acceptor. To address this directly, we have characterized the folding properties of a model protein, influenza virus hemagglutinin (flu-HA), under hypoxic conditions. Flu-HA contains six intrachain disulfide bonds that are formed in a known and well-defined order following its synthesis. Consistent with this hypothesis, our results indicate a significant reduction in folding of this protein during hypoxia. In order to address the importance of Ero1-Lα and PDI in protein folding efficiency during hypoxia we have utilized a flp-in recombination model to create isogenic cell lines where Ero1-Lα is either overexpressed or knocked down with shRNAi from a tetracycline-inducible promoter.

Many important hypoxia-induced proteins are either membrane-embedded or secreted (CA9, VEGF), and therefore have to successfully fold in and pass through the ER. It remains an interesting question as to whether these proteins have evolved specific affinity for the ER folding machinery, and by which molecular mechanisms this process is regulated. The approach presented here for a model protein can be readily adapted to study the folding pathways of these endogenous hypoxia induced proteins.

(S11.3) Hypoxia-induced upr activation regulates ca9 expression and sensitizes tumor cells to er stress-inducing agents. Constantinos Koumenis[1], Meixia Bi[1], Diane Fels[2], Jiangbin Ye[1]. [1]University of Pennsylvania, Philadelphia, PA, USA, [2]Wake Forest University, Winston-Salem, NC, USA.

We and others have previously shown that tumor cells can activate an endoplasmic reticulum (ER) stress program called the Unfolded Protein Response (UPR) as an adaptive mechanism under hypoxic conditions. A critical role for the UPR components PERK and eIF2α was shown by the inability of cells harboring inactivating deletions/mutations in these gene products to support tumor cell survival under hypoxic conditions and to inhibit tumor growth. The expression of ATF4, a downstream effector of the UPR was found to be upregulated in the hypoxic areas of human tumor specimens, and to be required for hypoxic cell survival *in vitro*. We have performed microarray gene expression analysis of $ATF4^{+/+}$ and $ATF4^{-/-}$ cells exposed to normoxia and hypoxia. Several gene products involved in cell survival and ER stress responses were found to be regulated by hypoxia in an ATF4-dependent manner. One of these was the mRNA for the HIF target CA9 (CAIX, carbonic anhydrase IX), which plays a critical role in the export of CO_2 and maintaining intracellular pH homeostasis. Induction of CA9 mRNA by hypoxia was significantly atenuated in the absence of ATF4 expression and siRNA against ATF4 inhibited induction of CA9 in HeLa cells. Unlike CA9, the expression of other HIF targets including VEGF and Glut-1 was not affected by ATF4 status. Overexpression of ATF4 resulted in higher levels of CA9 induction under hypoxia. These findings suggest that the UPR and HIF pathways may interact downstream of HIF stabilization and indicate a potential mechanism by which ATF4 upregulation may contribute to hypoxia resistance and tumor growth. We also report that hypoxic tumor cells are highly sensitive to pharmacologic agents that induce additional ER stress including thapsigargin, and the FDA-approved agent PS-341 (Velcade). This increased cytotoxicity correlates with an increase in UPR targets, suggesting an overactivation of UPR. Interestingly, the increased cytotoxicity did not result in increased apoptosis, but did produce an increase in markers of autophagy, such as acridine orange staining, autophagosome formation and induction of LC3. Altogether, our data suggest that hypoxia-induced UPR activation may provide a unique opportunity to preferentially kill hypoxic tumor cells with ER stress-inducing agents. *This work was supported by NIH grant CA94214.*

(S11.4) A translationally controlled angiogenic switch in breast cancer. Robert J. Schneider, Ksenia Karpisheva, Steve Braunstein, Carolina Pola, Judith Goldberg, Silvia C. Formenti. NYU School of Medicine, New York, NY, USA.

Tumors critically require the development of a vasculature to thrive, which they acquire through the process known as angiogenesis, which is activated by hypoxia (decreased oxygen). We demonstrate that in the majority of large tumors of the breast known as locally advanced breast cancers, angiogenesis is regulated by a translationally controlled switch which acts through the overexpression of translation regulatory protein 4E-BP1 and initiation factor eIF4G. 4E-BP1 is shown to orchestrate a hypoxia-activated switch that drives tumor angiogenesis and growth at the level of selective translation of mRNAs that contain internal ribosome entry sites (IRESs). These mRNAs include those encoding vascular endothelial growth factor (VEGF) which drives angiogenesis, HIF1α which orchestrates the hypoxia response, and Bcl2 which protects against apoptosis, among others. All of these mRNAs also have capped 5' ends and can translate through conventional cap-dependent mRNA translation under normoxic conditions. Model animal and cell systems demonstrate that the elevated levels of 4E-BP1 serve to trigger hypoxia inhibition of cap-dependent mRNA translation at high levels of oxygen, and in conjunction with eIF4G, to increase selective translation of cap-independent (IRES) mRNAs to promote angiogenesis, hypoxia responses and prevent cell death. Overexpression of 4E-BP1 was found to more than double tumor growth and triple angiogenesis in animal models, and may be linked to suppression of metastasis. This work adds to our understanding of the importance of translational control in breast cancer development and progression. Our findings support two molecular scenarios for translational control in breast cancer. (1) Overexpression of eIF4E and increased cap-dependent mRNA translation, associated with formation of small tumors with higher metastatic potential, achieved in part by mutational uncoupling of hypoxia inhibition translation; and (2) Overexpression of 4E-BP1 and eIF4G that stimulate cap-independent mRNA translation of select mRNAs that drive tumor angiogenesis and survival under conditions of only moderate hypoxia, associated with formation of large locally advanced breast cancers with lower metastatic potential.

(S12.1) Traditional chinese medicine for cancer: the road to china. Lorenzo Cohen[1], Zhiqiang Meng[2], Zhongxing Liao[1], Luming Liu[2]. [1]MD Anderson Cancer Center, Houston, TX, USA, [2]Fudan University Cancer Hospital, Shanghai, China.

This session will describe the formation of the NCI-funded International Center of Traditional Chinese Medicine (TCM) for Cancer. The international collaboration between The University of Texas M. D. Anderson Cancer Center and the Fudan University Cancer Hospital, Shanghai, China focuses on examining three of the main areas of TCM for cancer: herbal/natural products, acupuncture, and qigong. Each of the project areas will be described including a phase I trial of huachansu and the planned follow-up phase II trial; a study of acupuncture to prevent prolonged postoperative ileus and the planned follow-up trials of acupuncture for treating nausea and xerostomia; and an ongoing study of qigong for women with breast cancer undergoing radiation treatment. The collaborative exchange and training, an important part of the partnership, will also be described.

(S12.2) Combined chemotherapy, radiation therapy and traditional chinese medicine treatment for pancreatic cancer. Luming Liu. Fudan University Cancer Hospital, Shanghai, China.

Objectives

To investigate the effects of QingReXiaoJi herbal decoction (QRHJ) plus regional transcatheter arterial infusion chemotherapy or embolization (TAI or TAE), and 3-dimensional conformal radiotherapy (3DCRT) in treatment of stage III/IV pancreatic cancer. Patients and Methods: Total 134 patients with pathological proven Stage III/IV pancreatic cancer received multidisciplinary treatment between 5/2000–12/2005 at Fudan University Cancer Hospital. Among them, 31 patients had liver metastases and 42 were enrolled in a randomized clinical trial to determine the efficacy and tolerability of QRHJ plus TAI or TAE chemotherapy and 3DRT. Results: The median survival time (MST) for the entire group was 5.7 months (mo) and overal survival (OS) rates at 6-mo, 1-, 2-, and 3-year were 46.8%, 21.0%, 8.9% and 8.9%, respectively. Cox proportional hazard model and multivariate analyses suggested a favorable correlation between OS and treatment with QRHJ (HR=0.56) and radiotherapy (HR=0.62), and an adverse correlation between survival and serum CA19–9 $>$ 1000U/ml (HR= 2.77) and jaundice (HR=1.77). Of the 31 patients with liver metastases, 15 received TAI and 16 received TAI plus 3DCRT. There were 12.9% partial response, 51.6% stable disease, and 35.5% progression of disease. The MST was 5.4 mo with the 6- mo, 1-, and 2-year OS rates of 46.9%, 23.4%, 7.8%, respectively. Further analysis showed a better response rate of 25% in TAI plus 3DRCT compared with TAI group (P=0.04). The OS rates at 6-mo, 1-, and 2-years were 61.9%, 38.7%, 15.5% vs 30.5%, 7.6%, 0%, in TAI plus 3DRCT vs TAI only group, respectively. The MST were 11.6 mo vs 4.2 mo (p=0.0095) in TAI plus 3DCRT vs TAI group, respectively. Of 42 patients enrolled to the clinical trial, 21 received 3DCRT 36–40 Gy 2 weeks after first cycle of TAI of gemcitabine, and cisplatin or Oxaliplatin. The other 21 received same TAI plus 3DCRT plus QRHJ. Overall MST was 6.1 mo in QRHJ and 4.3 mo in the control groups, respectively. Six-months and 1-year OS rates were 50.7% and 16.4% vs 26.8% and 0% in QRHI and control groups, respectively (p=0.062). Conclusion: QRHJ combined with TAI/TAE chemotherapy and 3DCRT may be a valuable and effective treatment for stage III/IV pancreatic cancer.

(S12.3) Ku Mai Cai and radiation lung injury: rationale and preliminary data. Zhongxing Liao. Department of Radiation Oncology, Houston, TX, USA.

Lung cancer is expected to cause more than 160,000 deaths in 2004. Preventing therapeutic success in unresectable cancers is radiation pneumonitis, which occurs in about 25% to 30% of cases. Besides the disabling effects of pneumonia, lung fibrosis, pain, cough, and labored breathing with which it is associated, it is also thought responsible for therapy abandonment, which has kept long-term survival rates from improving. Agents that improve lung function during therapeutic assault and agents that protect normal lung tissue from inflammation and thus enhance the therapeutic ratio are desperately needed. Ku mai cai may do both Ku mai cai is the whole plant of Ixeris chinensis. It has been used in TCM for thousands of years. The young shoots, when properly prepared, are edible and consumed as a bitter but appetizing vegetable in Asia. The plant has analgesic, antipyretic, and anti-inflammatory effects. It has been used as a folk remedy for everyday pain and fever and for such life-threatening maladies as tuberculosis, infections of the lung, abscess of the abdomen, appendicitis, and hepatitis, and for malignant growths with unknown primaries and those of the lung, breast, and skin. The central constituents of I. chinensis have been well investigated and documented. It contains sesquiterpene lactones, isoflavonoids, triterpenoids, and other components, including sitosterol and organic acids In vivo, ku mai cai inhibited arachidonic acid-induced edema, and in vitro, it significantly increased 13-HODE levels in human lung cancer A549 cells in a concentration-dependent manner. The level of 13-HODE in A549 cells treated with 1 mg/ml was fivefold that of the control. In other tests, we selected human lung cancer cells A549 (high expression of COX-2) and H1299 (low expression of COX-2) as the target cell lines. Our preliminary studies showed that ku mai cai inhibited the proliferation of both A549 and H1299 cells with IC50 of 0.81 and 1.13 mg/ml, respectively. Therefore, our preliminary data show that ku mai cai inhibits the proliferation of human lung cancer cells in vitro and that this effect may be mediated through upregulation or restoration of 15-LOX-1 in these cancer cells. The rationale, potential, and challenges of using Ku Mai Cai as a therapeutic agent for radiation lung injury will be discussed.

(S12.4) A study of chinese herbal medicine qingxue granule on radioprotection of lung. Yufei Yang[1], Wang Luhua[2], Xiao zhen WANG[2], Xian wen WU[2]. [1]Oncology Department of Xi Yuan Hospital, China Academy of Traditional Chinese Medicine Science, Beijng, China, [2]Department of Radiation Oncology, Cancer Hospital of Chinese Academy of Medical Sciences, Beijng, China.

Background: Qingxue Granule (QXG) is a Chinese herbal formulary consisting of seven main ingredients, and has been used in Chinese Traditional Medicine (TCM) to reduce stasis, improve blood circulation, and enhance immunologic function of erythrocyte and the T-lymphocyte. We hypothesized that QXG has a protective effect on radiation lung injury in mice, and utilized QXG in 15 lung cancer patients who received radiotherapy. Methods and materials: One hundred and twenty-eight BALB/C mice were divided into control (C), radiation (RT), radiation plus QXG (RT+QXG) once daily for two months. Whole thorax radiation was delivered with a single ventral-dorsal field with 6 MV X-ray to 12Gy. Animals in groups C and RT received 21 days of 0.5 ml saline feeding. Mice were sacrificed at 1, 2, 4 or 6 months (mo) after radiation. Macrophage cell count of lung lavage fluid and hydroxyproline content of whole lung were assayed, and the lung fibrosis was scored according to the Ashcroft's criteria. The plasma interleukin-6 (IL-6), transforming growth factor-β1 (TGF-β1) and vascular endothelial growth factor (VEGF) concentration were assayed with ELISA method. The One-way ANOVA was used to test the significance of differences between groups at each time point. A p-value <0.05 was considered statistically significant. Results: The number of macrophage in lung lavage was significantly lower in RT+QXG compared with of RT ($p<0.05$) groups, and no significant difference between RT+QXG and C groups. The hydroxyproline content in RT+QXG was significantly lower than RT ($p<0.05$), higher than C ($p<0.05$) groups at 1- and 6mo after radiation. The fibrosis scores in RT+QXG were significantly lower than RT groups at 2-, 4-, and 6-mo ($p<0.05$). The IL-6 concentration in RT+QXG was significantly lower than that in RT, and not significantly higher than that in C groups at 2mo. The VEGF level was significantly higher in RT+QXG compared with RT ($p<0.05$), and RT compared with C ($p<0.05$) groups at 2- and 6-mo. In 15 patients treated with QXG and radiation, no grade$\geq$2 any toxicity was observed. Conclusion: Oral QXG was associated with decreased macrophage, hydroxyproline, and fibrosis score in mice lungs, and decreased IL-6 in plasma. There was no correlation between plasma VEGF level and degree of lung fibrosis. It is safe to use QXG clinically.

(S13.1) Predicting DNA radiation damage by a Monte-Carlo simulation method based on molecular structures of DNA or DNA-protein complexes. Marie Davidkova[1], Viktorie Štísová[1], Stephane Goffinont[2], Natalie Gillard[2], Melanie Spotheim-Maurizot[2]. [1]Nuclear Physics Institute AS CR, Prague 8, Czech Republic, [2]Centre de Biophysique Moléculaire CNRS, Orléans, France.

Since living matter contains a high proportion of water, the effect of ionising radiation occurs preferentially via the reactive species generated by water radiolysis. Computational methods can be used to calculate yields and distributions of radiation damage in cellular targets at the molecular level. The stochastic model RADACK allows to predict probabilities of radiolytic damages caused by $OH^{\cdot}$ and $H^{\cdot}$ radical attack within different biomolecules (Begusova *et al.* 2001, J. Biomol. Struct. Dyn. 19, 141). The calculations can provide an assessment of sequence-, structure- and ligand-dependent modulation of damages within DNA (strand breaks and base damages), proteins (peptide chain breaks and modified amino-acids side chains) and their complexes (Begusova *et al.* Radiat. Phys. Chem. 72, 265, 2005, Davidkova *et al.* Radiat. Prot. Dosim., doi:10.1093/rpd/ncl442, 2007). The theoretical model RADACK based on Monte Carlo technique will be described and applied to DNA-protein complexes involved in the regulation of gene expression, in the hormone signal transduction or in the repair of oxidative lesions of DNA. The structure of the molecules and of the complexes is either obtained by molecular modeling or is extracted from structural data banks (NMR- or crystallography-based structures). The predicted DNA damage patterns are consistent with the experimentally observed distributions of lesions within the partners of the complex. The modulation of damage along the molecules will be discussed in terms of the mutual protection of the partners within the complexes and of their binding-induced conformational changes.

(S13.2) How is radiation affecting the protein-bodyguard of DNA ? Melanie Spotheim-Maurizot[1], Nathalie Gillard[1], Stephane Goffinont[1], Bertrand Castaing[1], Francoise Culard[1], Corinne Bure[1], Martine Cadene[1], Marie Davidkova[2]. [1]Centre de Biophysique Moleculaire, CNRS, Orléans, France, [2]Nuclear Physics Institute, Prague, Czech Republic.

The binding of some proteins to a specific DNA sequences is a key step in the regulation of gene expression, DNA structuring and DNA repair. We have previously shown that DNA-binding proteins are acting as efficient bodyguards against the attack of hydroxyl radicals produced by water radiolysis. They protect their binding sites on DNA by shielding and by radicals scavenging. They also modify the conformation of DNA (compaction, bending) thereby rendering DNA more resistant to radiolysis. But protein-bodyguards are also vulnerable and get damaged under irradiation. The progressive accumulation of damages on the protein (side chain modifications, peptide chain breaks) firstly affects the configuration of the DNA-protein couple and finally renders the protein unable to play its bodyguard role : the protein loses its ability to bind to its the cognate DNA sequence. We have studied the effect of irradiation on three systems: 1) the E. coli lactose operator-repressor complex, 2) the complex between MC1, a DNA structuring (histone-like) protein of Methanosarcina, and its specific DNA sequence and 3) the complex between a DNA bearing an analogue of an abasic site and the repair protein Fpg of Lactococcus Lactis. At low doses all these proteins protect their specific binding site on DNA. At high doses, the three studied complexes are disrupted mainly due to the

damage to the proteins. A transient situation is observed at intermediary doses : the configuration of the complexes is modified. CD data show that upon irradiation, the conformation of the protein changes. Fluorescence measurements reveal the destruction of tyrosine residues. Mass spectrometry data complemented by RADACK calculations allow to identify the oxidised amino-acids in the DNA binding domains of the proteins. Most of the oxidised amino-acids are essential for the DNA-protein interaction as revealed by the analysis of the NMR- or crystallography-based structures of the complexes. Thus, through the oxidation of amino-acids and the subsequent conformational changes, irradiation critically affects proteins properties and thereby modifies/hinders their binding to DNA.

(S13.4) Modeling radiation-induced chromosome aberrations. Francesca Ballarini, Andrea Ottolenghi. University of Pavia -DFNT and INFN, Pavia, Italy.

Living cells exposed to ionising radiation can show different types of chromosome aberrations (CAs). CAs represent a particularly important biological endpoint, since they can lead both to cell death and to cell conversion to malignancy. Although it is generally accepted that aberrations arise from breakage of the DNA double helix and subsequent mis-rejoining of the chromatin free ends, some aspects of the mechanisms underlying CA induction are still not fully understood. In particular, it is still not clear whether any double-strand break type can potentially lead to aberrations, or if only severe (typically clustered) DNA breaks are involved. Also the role played by interphase chromatin organization claims for further investigation. Theoretical models can help to interpret CA experimental data and to provide estimates where the data are not available, e.g. at low doses. Furthermore, such models can be extended to bridge the gap between chromosome aberrations and important cellular endpoints such as cell death and conversion to malignancy. In the present paper, examples of modeling approaches simulating the induction of CAs in human cells will be discussed. In particular, a mechanistic model and a Monte Carlo code developed at the University of Pavia will be presented, mainly based on the assumption that clustered DNA damage plays a fundamental role in the processes leading to CA formation. The current version of the model, in which interphase chromosome territories are described as the union of adjacent cubic boxes, can predict dose-response curves for the main aberration types following irradiation with low- and high-LET radiation, as well as with mixed fields. Both Giemsa and FISH scoring can be simulated. The very good agreement with literature data provides a validation of the model in terms of both the adopted assumptions and the simulation techniques. As an application in the field of low-dose radiation protection, estimates of Chronic Myeloid Leukemia risk have been performed, as well as CA induction in astronauts' lymphocytes. The implementation of heavy ions is in progress, and preliminary results will be presented. Acknowledgments: This work was partially supported by the European Commission (EC Contracts RISCRAD and NOTE) and by the Italian Space Agency (ASI Contract Mo-Ma).

(S14.1) Applications of SHIELD-HIT, MCNPX and GEANT4 for ion transport calculations in radiation therapy and space. Irena Gudowska. Karolinska Institute and Stockholm University, Stockholm, Sweden.

There is an increased worldwide interest in radiation therapy with proton and heavier ions and several clinical facilities are in use. Accurate evaluation of the dose delivered to tissue and estimation of biological effects in ion beams, require correct knowledge of the physics of ion interaction with matter. Such studies are also of importance for evaluation of biologically equivalent doses delivered to astronauts in long-term manned interplanetary missions. Heavy charged ions produce secondary radiation from beam fragmentation processes and nucleus-nucleus interactions in the structural materials of accelerator or space station, as well as in the human body. These secondaries consist of neutrons, protons, heavier ions, photons and electrons within energy ranges up to several GeV. They are characterized by a wide range of LET and can be a source of significant biological doses to healthy tissues. Due to the very complex interaction pathways of high energy ions in the shielding materials and the human body, the 3-D Monte Carlo (MC) transport codes provide unique and very useful tool in simulating therapeutic beams and evaluations of radiation environment around spacecraft. The capability and accuracy of any MC hadron transport code depend critically upon the model used to describe elastic and inelastic nuclear interactions of light and heavy ions, and on the cross sections for production of secondary particles. Recently, several MC hadron transport codes have significantly improved the implemented nuclear models for secondary particle production in the ion energy range 0 - 1 GeV/A. In these studies a capability of the SHIELD-HIT, MCNPX and GEANT4 MC codes to simulate radiation field around proton/ion facility and space station will be discussed. The code features will be presented and implemented nuclear models discussed. MC calculations using these codes will be compared with experiments and evaluations using other MC techniques. Some deficiencies in the descriptions of nuclear inelastic interactions of high energy ions with tissue materials are still noticed for all analyzed codes. Generally better agreement for heavier target material is observed. There is a need both for extensive theoretical studies to validate the physical models implemented in the hadron MC codes and for further development of these codes.

(S14.2) The ICRP Task Group on international guidelines on radiological protection for space missions. Guenther Dietze. PTB, Braunschweig, Germany.

The International Committee on Radiological Protection (ICRP) has established a Task Group No. 67 (Radiation Protection in Space) dealing with the problems of radiation protection of astronauts in long term space missions. The presentation will present some discussions within this Task Group about the problems specific for this situation in space. The ICRP has nearly finished the preparation of its new general recommendations following ICRP Publication 60 (1991). It does, however, not deal with the situation in space. The specific radiation field with its high content of protons and heavy ions of high energies is strongly different from usual radiation fields on earth and the dose rate is much higher than environmental exposures on earth. Therefore, the problem of defining appropriate radiation protection quantities for estimating risks due to radiation exposure and their measurement is still not finally solved. An application of all quantities used in radiation protection practice on earth is not appropriate, e. g. a single radiation weighting factor of 20 for heavy ions of all types and energies. A more realistic approach of using the quality factor concept in the definition of an "effective dose" is mostly applied in actual space missions. Individual dosimetry is a further subject of discussion. The operational dose equivalent quantities generally in use on earth are not appropriate in radiation fields with a high content of high energy heavy ions. The requirements for individual dosimetry and what an individual dosemeter is really measuring in an environment inside or outside of a space station are still under debate.

(S14.3) Late effects of heavy-ion exposures. Eleanor A. Blakely. Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

Cancer patients treated with hadron therapy, or space travelers exposed to galactic cosmic radiation and solar particle events are both at risk of physiological changes appearing months or many years after exposure to the charged particles. Although the particle radiotherapy patients receive high doses of a single radiation type at a high dose rate to specific tumor sites usually in a 5-day per week daily regime over the course of several weeks, normal tissues surrounding the tumor can receive lower absorbed doses. In contrast, astronauts and cosmonauts usually receive whole-body absorbed doses of mixed radiation types and ionization qualities totaling $<<$ 1 Gy protracted over several years. Late effects include increased risk of cancer and cataract, and other chronic multifactorial conditions, such as cardiovascular disease, and changes in function of the immune, endocrine, and central nervous systems.

This symposium address will present a summary of available pertinent information about these late effects acquired from space travelers, accelerator-based clinical radiotherapy and laboratory studies, and from studies of atomic bomb survivors, radiation workers and radiation accident survivors. What is known about the tissue-type, dose-level and dose-rate-dependence of the radiation effects will be reviewed, as well as what can be done to ameliorate the effects. Common and unique mechanisms of action linked in many cases to the individual responses of tissue stem cells, will be discussed, as well as the limitations of any competing risks that may compromise conclusions.

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(S14.4) Heavy ion effects on the central nervous system. Gregory A. Nelson. Loma Linda University, Loma Linda, CA, USA.

The presentation will begin with a brief review of the overall sensitivity of the central nervous system (CNS) to ionizing radiation. The dose-time responses for neurologic and cognitive impairment in humans and animals will be identified followed by a summary of the general pathogenesis of lesions for different regions of the CNS. The competing hypotheses of vascular versus glial cell dysfunction as primary causes for CNS deterioration will be described. Then the radiation responses for the different cellular compartments will be summarized; these include stem cells, neurons, glia and vasculature. The features of the responses will be discussed in terms of cell kinetics, functional output and alterations in tissue architecture. Examples of functional changes include loss of incorporation of neural precursor cells into hippocampal circuits, blood-brain barrier breakdown and reduction in synaptic efficacy. Where data are available, high LET radiation effects will be compared and contrasted to low LET effects. Results from low dose experiments comparable to anticipated space mission exposures will be emphasized. Similarly, *in vivo* experiments will be emphasized with only selected discussions of results from cell culture systems. Following the general discourse several special topics will be addressed. These include the role of inflammation and oxidative stress in the CNS response to radiation, the potential for charged particle tracks to interact in unique ways with highly ordered CNS structures and the utility of the microlesion concept.

(S15.1) Cancer incidence in the atomic bomb surivors: the new incidence report. Kiyohiko Mabuchi[1], Dale L. Preston[2], Elaine Ron[1], Shoji Tokuoka[3], Sachiyo Funamoto[3], Nobuo Nishi[3], Midori Soda[4], Kazunori Kodama[3]. [1]National Cancer Institute, Rockville, MD, USA, [2]Hiroshoft International, Eureka, CA, USA, [3]Radiation Effects Research Foundation, Hiroshima, Japan, [4]Radiation Effects Research Foundation, Nagasaki, Japan.

The second general report on solid cancer incidence risks in the Life Span Study cohort of atomic bomb survivors in Hiroshima and Nagasaki includes additional eleven years of follow-up and uses new DS02 dose estimates. From 1958 through 1998, 17,488 first primary cancers were diagnosed among 105,427 cohort members. About 850 of these solid cancers were estimated to be radiation-related, representing 11% of all solid cancer cases with colon doses > 0.005 Gy. For all solid cancers combined, there was a linear dose response over the 0 to 2 Gy dose range. The excess relative risk per Gy decreased with increasing age at exposure and also with increasing attained age. The excess absolute rates, however, were increased throughout the follow-up period, thus suggesting that the elevated cancer risk may continue throughout life regardless of age at exposure. Based on the limited follow-up in early years, there was a significant, and apparently large, radiation-related excess risk of cancer occurring in adolescent and young childhood. The excess absolute rates were higher for women than men for all solid cancers but not after excluding gender specific cancers. Significant radiation-related excess risks were found for cancers of the oral cavity, esophagus, stomach, colon, liver, lung, non-melanocytic skin, breast, ovary, bladder, nervous system and thyroid. Dose responses for cancers of the pancreas, prostate, and kidney were statistically insignificant but consistent with that for all solid cancers combined while dose responses for cancers of the rectum, gallbladder and uterus appeared to be weaker compared with that for all solid cancers. There was an indication that childhood exposure may increase the risk of cancer of the uterine corpus. Radiation-related increased risks were seen for all of the five broadly classified histological groups: squamous cell carcinomas, adenocarcinomas, other carcinomas, sarcomas and other non-epithelial malignancies. While a great deal has been learned about radiation risks from the current Life Span Study data, there currently is appreciable uncertainty regarding the lifetime radiation-related cancer risk because most of the persons who were exposed at < 20 years are still alive. It is important to continue to follow the atomic bomb survivors for another 15–20 years.

(S15.2) Cancer risks from radiation exposure in the former Soviet Union. Elaine Ron. NCI, NIH, Bethesda, MD, USA.

The health effects of exposure to radiation at low doses and low-dose rates are uncertain; however, investigations in the former Soviet Union are now contributing important new data on these exposures, as well as on plutonium and ^{131}I. In Russia, several populations have experienced substantial occupational and environmental exposures as a result of inadequate radiation protection measures and accidents at the Mayak nuclear weapons facility and in Ukraine, Belarus and parts of Russia, hundreds of thousands of people were exposed to radiation from the Chornobyl accident. As a result of operations at Mayak, three cohorts are under study: Mayak workers; villagers chronically exposed to radioactive wastes discharged by Mayak into the Techa River, and persons living in Ozyorsk, the city next to Mayak, during the years of radioiodine releases from the reactor and radiochemical plants. Among the Mayak workers, statistically significant increases in solid cancer and leukemia mortality were related to increasing doses of gamma radiation, and cancers of the lung, liver and bone were positively associated with plutonium body burdens. Radiation dose responses also were observed for mortality from all solid cancers combined and from leukemia in the Techa River cohort. A pilot study of Ozyorsk residents indicated that the incidence of thyroid neoplasia is higher among persons exposed during childhood to ^{131}I emissions from Mayak than to non-exposed residents. In April 1986, the worst nuclear accident took place in Ukraine. To date, the major late health consequence of the contamination from the Chornobyl accident appears to be a large increase of thyroid cancer incidence among persons living in contaminated areas who were children or adolescents at the time of the accident. The excess relative risk estimates obtained from analytic studies of Chornobyl are consistent with those found following external radiation to the thyroid gland. Increased risks of thyroid cancer, leukemia and cataracts among Chornobyl clean-up workers, and elevated risks for leukemia and other cancers and diseases among people living near the Chornobyl plant at the time of the accident have been reported, but these results await further confirmation. Results from these studies generally are consistent with those from the atomic bomb survivors.

(S15.3) Cancer risk estimation derived from studies of nuclear workers. Mary K. Schubauer-Berigan, Robert D. Daniels, Sharon R. Silver. National Institute for Occupational Safety and Health, Cincinnati, OH, USA.

Current occupational and environmental dose limits for ionizing radiation are based on studies of atomic bomb survivors and medical cohorts, requiring extrapolation from high- to low-dose populations. The National Academies' Committee on the Biological Effects of Ionizing Radiations noted in its recent report the potential for studies of pooled occupational cohorts to permit the direct estimation of risk associated with chronic, low-dose radiation exposure. Recent large, pooled studies by the International Agency for Research on Cancer and the National Institute for Occupational Safety and Health provide new data on low-dose leukemia radiogenicity and suggest that risks per unit dose in occupational populations are comparable to or higher than those estimated from the atomic bomb survivor cohort. However, precision of the risk estimates remains an issue. This presentation will discuss the latest

findings with respect to low-dose leukemia risk and how these studies may be used as a source of risk coefficients for low-dose radiation exposures. Specifically, a nested case-control study will be described which combines risk estimates for seven cohorts of 157,000 U.S. nuclear workers exposed since 1950 (Table 1). This study, with 168 cases and a projected excess relative risk of 7.1% per 10 mSv, should generate precise estimates of the relation between occupational radiation exposure and leukemia mortality by reduction of dose uncertainty and control of potential confounding by benzene. These risk coefficients should provide highly relevant data for risk assessments of low-level exposures to low-LET radiation.

Occupational sources of risk estimation for pooled nested case-control study

Cohort (hired after 1950)	Base cohort size	No. cases	ERR per 10 mSv (95% CI)
NIOSH Leuk. Case-Control Study	68,333	90	0.17 (−0.0074, 0.73)
Idaho National Laboratory	34,827	52	0.056 (−0.0091, 0.21)
US Nuclear Power Plants	53,698	26	0.057 (−0.026, 0.30)

(S15.4) Cancer risks from diagnostic and screening X-ray examinations. Amy Berrington de Gonzalez. Johns Hopkins University, Baltimore, MD, USA.

Although diagnostic and screening X-ray examinations provide great medical benefits it is generally accepted that their use also involves a small risk of radiation-induced cancer. To fully evaluate these risks directly would require very large scale studies, which are generally considered to be impractical due to the expense and the length of follow-up that is required. It is possible, however, to obtain a more timely assessment of these risks by extrapolating estimates from existing studies, such as the study of the Japanese atomic bomb survivors. Estimates from several such risk assessment exercises will be presented, including: risks from current levels of diagnostic X-ray exposure in the general population, a risk-benefit assessment for mammographic screening for pre-menopausal women, and the risks from lung cancer screening using computed tomography (CT) and the associated work-up procedures. The assumptions that are required and the uncertainties involved in these risk assessment exercises will also be discussed.

(S16.1) Developing new targets: lessons learned from HIF-1. Benjamin J. Moeller[1], Matthew R. Dreher[2], Zahid N. Rabbani[2], Yiting Cao[2], Thies Schroeder[2], Chuan Y. Li[3], Mark W. Dewhirst[2]. [1]MD Anderson Cancer Center, Houston, TX, USA, [2]Duke University, Durham, NC, USA, [3]University of Colorado Health Sciences Center, Auroro, CO, USA.

Interest continues in developing rational molecular targeting strategies for tumor radiosensitization. Successfully bringing such strategies to the clinic depends first and foremost on choosing appropriate targets. With tumor radiosensitivity being a highly complex endpoint, the process of deciding upon a target to investigate is difficult. We discuss here our experience with developing hypoxia-inducible factor-1 (HIF-1) as a molecular target for tumor radiosensitization. In addition to reviewing these results, our goal will be to highlight lessons learned from this work which might guide future efforts at target development. Areas to be addressed include the potential importance of: (1) tumor physiology, (2) intact tumor/host interface, and (3) unanticipated off-target effects. We will speculate, based on these experiences, what an ideal target development strategy might involve.

(S16.2) DNA replication repair: from molecular insights towards new approaches to anti-cancer therapy. Thomas Helleday. University of Oxford, Oxford, United Kingdom.

DNA repair and damage response pathways are activated as a tumour barrier at early stages during cancer development. Here, we describe a direct link between oncogenes that start cancer outgrowth and the tumour barrier. Here we show that oncogene-induced senescence is associated with signs of DNA replication stress, including prematurely terminated DNA replication forks and DNA double strand breaks. The replication lesions caused by oncogenes are tumour specific and indicate that an increase in DNA damage is associated with tumour development. Such DNA lesions are similar to those produced during radiation- or chemotherapy to kill tumour cells. A new concept for cancer therapy is to amplify endogenous tumour-specific DNA lesions, to specifically kill tumour cells. This can be achieved following inhibition of DNA repair.

Based on this concept we report that BRCA2 defective breast cancers can be specifically targeted using inhibitors of Poly(ADP-ribose) polymerase (PARP). We propose that, in the absence of PARP, spontaneous DNA single strand breaks collapse replication forks and trigger homologous recombination repair. We further show that BRCA2 deficient cells, as a result of their recombination deficiency, are acutely sensitive to PARP inhibitors, presumably because resultant collapsed forks are no longer repaired. Thus, PARP activity is essential in recombination deficient BRCA2 mutated cells. We exploit this requirement to specifically kill BRCA2 deficient tumours by PARP inhibition alone. Treatment with PARP inhibitors is likely to be highly tumour specific since only the tumours (which are BRCA2-/-) in the BRCA2+/- patients are completely defective in homologous recombination repair. The use of an inhibitor of a DNA repair enzyme alone, in the absence of an exogenous DNA damaging agent, to selectively kill a tumour represents a new concept in cancer treatment.

(S16.3) Assessment of novel hypoxia response pathways as clinical molecular targets. Bradly G. Wouters, Marianne Koritzinsky, Kasper M. Rouschop, Michael Magagnin, Twan van den Beucken. Maastricht University, Maastricht, The Netherlands.

The majority of solid human tumors have been demonstrated to contain areas that are poorly oxygenated. These hypoxic areas lead to treatment resistance and are implicated in promoting malignancy through changes in metabolism, angiogenesis and metastasis. Oxygen sensing pathways strongly influence both cell behavior and cell survival during hypoxia through their ability to alter gene expression, and have thus received attention as novel targets for therapy. Most research to date has focused on the HIF family of transcription factors and their target genes that are activated during moderate hypoxic conditions. We and others have identified additional oxygen-sensing pathways that affect gene expression and hypoxia tolerance by regulating mRNA translation. Hypoxia results in inhibition of mRNA translation through at least two independent mechanisms. The first occurs as a result of activation of an evolutionarily conserved pathway known as the unfolded protein response (UPR) and the second by antagonizing the activity of the mammalian target of rapamycin (mTOR) kinase on its target 4E-BP1. Each of these newly discovered oxygen-sensing pathways have unique activation parameters and are likely relevant for different oxygenation patterns in tumors. I will discuss recent insight into the mechanistic basis behind the oxygen sensitivity of these pathways, their effects on hypoxia tolerance and gene expression, and their potential as new therapeutic targets alone or in combination with traditional therapies.

(S16.4) Modulating radiation resistance: is Akt the target. Anjali K. Gupta. University of Pennsylvania, Philadelphia, PA, USA.

The critical pathways determining the resistance of tumor cells to ionizing radiation are poorly defined. Because the *ras* oncogene, a gene activated in many human cancers treated with radiotherapy, can induce increased radioresistance, we originally asked which Ras effector pathways are significant in conferring a survival advantage to tumor cells. The phosphoinositide-3-kinase (PI3K) inhibitor LY294002 radiosensitized cells bearing mutant *ras* oncogenes, but the survival of cells with wild-type *ras* was not affected. This and

other experiments including siRNA work led us to believe Akt was a crucial target for radiation resistance. There is, however, no clinically useful inhibitor of Akt available. Insulin resistance and diabetes are recognized side effects of HIV protease inhibitors (HPIs), suggesting that these agents may inhibit Akt signaling. Five first-generation HPIs were subsequently tested and three of the five (amprenavir, nelfinavir, and saquinavir but not ritonavir or indinavir) inhibited Akt phosphorylation at Ser473 at serum concentrations routinely achieved in HIV patients. In both tumor cell colony formation assays and tumor regrowth delay experiments, combinations of drug and radiation exerted synergistic effects compared with either modality alone. But the HPI's affected more than just Akt signaling. We saw that MAPK and JNK were also down-regulated at the same concentrations needed to inhibit Akt signaling. In xenograft models, we had inhibition of angiogenisis yet paradoxically an increase in tumor oxygenation was observed. We believe that HPI's, particularly nelfinavir (Nfv), inhibit proteasome activity. Inhibition of the proteasome leads to endoplasmic reticulum (ER) stress with accumulation of mis-folded proteins, which triggers the unfolded protein response (UPR). We have seen that bortezomib similarly down-regulates Akt. With so many intricate pathways in a cell being affected, we ask the question whether we are certain that Akt is the key target for radiation sensitization strategies. Many pharmaceutical companies are in the process of developing a specific Akt inhibitor that is safe in the clinic. It will be interesting to compare data from these targeted therapies to more general inhibitors such as Nfv.

Symposia

(S17.1) Immunological effects of hyperthermia- multifaceted responses that augment anti-tumor immunity. Elizabeth Repasky. Roswell Park Cancer Institute, Buffalo, NY, USA.

Several recent clinical trials on cancer patients have demonstrated a positive survival benefit from the addition of hyperthermia to radiation and chemotherapy, validating the early promise of hyperthermia as an adjuvant cancer therapy. Several lines of evidence support the hypothesis that at least some of the clinical benefit of modern hyperthermia protocols could be through direct and indirect effects on thermally sensitive parameters associated with the immune system. These data further support the use of hyperthermia in combination with radiation or chemotherapy, and perhaps more importantly with immunotherapy. This presentation will highlight new data from this field as well as our own laboratory data suggesting a direct role of a moderate elevation of body temperature in the regulation of the activation threshold of T cells, NK cells and dendritic cells through thermally sensitive membrane lipid domains associated with specific signaling molecules. Further, the increased infiltration of various leukocyte subsets, including macrophages and neutrophils into tumors following moderate "fever-range" whole body hyperthermia treatments will be described. Indirect effects of hyperthermia on anti-tumor immunity are likely to be related to changes in tumor blood vessel perfusion and interstitial fluid pressure which facilitate the entry of critical immune effectors, chemotherapeutic molecules, and oxygen into tumors. These data on direct and indirect effects of mild whole body heating will be compared to those obtained by ourselves and others using higher temperature, local heating protocols. *Supported by NCI grants R01 CA 71599 and P01 CA94045.*

(S17.2) Progress in non-invasive thermometry for hyperthermia. Peter Wust, Mirko Weihrauch, Jacek Nadobny, Johanna Gellermann. Charite Universitätsmedizin Berlin, Berlin, Germany.

Background: For further improvement in regional hyperthermia we need to access patterns of multi-antenna RF-applicators realtime and in three dimensions. We realized a simultaneous operation of RF-hyperthermia (SIGMA-Eye applicator) in an MR scanner (Siemens Symphony) and evaluated several methods in phantoms and patients to achieve an online characterization of heating patterns (online monitoring). In the next step we attempted to improve the patterns (online optimization). Methods: We started in anthropomorphic phantoms with phase difference methods (gradient echo, i.e. GRE sequences at different echo times, double echo method), and applied this in tumors of the pelvis (rectal recurrences) and extremities (soft tissue sarcomas). A drift correction was performed using either the water bolus or fat tissue employing direct temperature measurements. We developed techniques to monitor heating patterns in the abdomen such as navigated T1 images and flow measurements in the V. portae. In order to separate perfusion and temperature we registered time-resolved sequences of double echo phase difference images (every 10 s) and deduced basal perfusion, perfusion and temperature increase from the MR temperature (deg) time curve. We developed an MR measurement based adaption of base functions to improve agreement between planned and measured deg distributions. Then we employed a model-based optimization of power deposition patterns. Results: In phantoms MR-thermometry agrees well with measured temperature patterns with $\pm$ 0.5 °C. In clinical studies MR temperatures were significantly correlated with response. The analysis of time-resolved MR-temperature sequences suggests a relationship of around 1 deg per 5 ml/100g/min perfusion change, which might be utilized to separate perfusion and deg change (MR temperature). Most impressive improvements of power deposition patterns according to a specified target volume were achieved in phantom measurements using the method of computer-based online optimization. However, monitoring of the abdomen (part body hypertherthermia) still reveals as a great challenge. Conclusion: Suitable GRE sequences are ready for supervision, online control and optimization of hyperthermia patterns in motionless tissues.

(S17.4) Regional hypethermia combined with systemic chemotherapy in the management of locally advanced, high grade soft tissue sarcomas of the extremities, the body wall and the abdomen: A phase III randomized prospective trial (EORTC-ESHO Intergroup Trial). Rolf D. Issels, Lars H. Lindner. University Hospital Medical Center Grosshadern, Munich, Germany.

For high-risk soft tissue sarcomas (HR-STS) of adults, new treatment strategies are needed to improve outcome with regard to local control and overall survival. Systemic chemotherapy has been integrated either after (adjuvant) or before (neoadjuvant) optimal local treatment by surgery and radiotherapy in HR-STS. The presentation summarizes the results of the combination with regional hyperthermia (RHT) as a treatment strategy to open a new therapeutic window. Under the auspices of the European Organization for Research and Treatment of Cancer (EORTC) and the European Society of Hyperthermic Oncology (ESHO) we recently completed a randomized Intergroup phase III trial (EORTC 62961/ESHO RHT-95) of multimodal treatment in patients with primary (S1 group) and recurrent (S2 group) disease or after inadequate surgery (S3 group: resections with positive margins or macroscopic residual tumor) in high-risk STS (tumor size $\geq$ 5 cm + histologic grade of 2 or 3 + deep location + extracompartmental extension). In this trial, all patients with HR-STS to extremity and non-extremity received neoadjuvant systemic chemotherapy (four cycles of the EIA regimen) and were randomized in two arms: chemotherapy alone or combined with RHT, followed by definitive surgery and radiotherapy. Thereafter, in addition, four cycles of the EIA regimen were administered with or without RHT according to the initial randomization. The results in terms of overall outcome for extremity and non-extremity STS will be presented.

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(S18.1) Cancer incidence among atomic bomb survivors exposed in-utero or as young children. Dale L. Preston[1], Harry Cullings[2], Kiyohiko Mabuchi[3], Kazunori Kodama[2]. [1]Hirosoft International, Eureka, CA, USA, [2]Radiation Effects Research

Foundation, Hiroshima, Japan, [3]National Cancer Institute, Bethesda, MD, USA.

It is generally accepted that radiation exposure in-utero greatly increases the risk of childhood cancer. However, little is known about the effect of radiation exposure on the risk of cancers occurring later in life. The Radiation Effects Research Foundation (RERF) cohort of people exposed to atomic bomb radiation in utero provides a unique opportunity to investigate the effect of in-utero radiation exposure on the risk of adult onset cancers. We have recently completed analyses of solid cancer incidence between the ages of 12 and 55 for the period from 1958 through 1999 in the RERF cohorts of atomic bomb survivors who were exposed in-utero or as young children. The study population includes 2,452 people (90 cancer cases) who were in-utero and 15,388 people (649 cancer cases) who were less than 6 years old at the time of the bombs and have individual dose estimates. While the largest dose estimates exceed 3 Gy, only 37% of the in-utero group and 44% of the children received doses in excess of 5 mGy. A statistically significant linear dose response was seen for the both the in-utero and childhood exposure groups. There are some indications, albeit not statistically significant at this time, that the temporal pattern of the excess risk for those exposed in-utero differs from that for people exposed as children. In particular, while the excess relative risk among those exposed in -utero was quite large during the earliest years of follow-up, it may be decreasing more rapidly with increasing age than that for those exposed as children. In terms of excess rates, it appears that excess rates for adult-onset cancers have remained fairly constant over time for those exposed in-utero while excess rates for those exposed as young children have increased. Since follow-up is continuing and since the study cohorts are just reaching ages at which cancer rates increase dramatically, the next 5 to 10 years will provide unique and important information on the nature of the radiation effects on the risk of adult-onset solid cancers among people exposed in-utero.

(S18.2) Chromosome aberration induction following fetal radiation exposure in mice and humans. Nori Nakamura, Yoshiaki Kodama. Radiation Effects Research Foundation, Hiroshima, Japan.

Human fetuses are generally thought to be highly sensitive to radiation exposure because diagnostic, low-dose X-ray exposures have been suggested to substantially increase the risk of childhood leukemia. However, animal studies generally have not demonstrated a high radiosensitivity of fetuses for tumor induction and the underlying causes for the discrepancy remain unanswered. Here we present our recent results on two studies. In one study, atomic bomb survivors exposed *in utero* were examined for translocation frequencies in blood lymphocytes at 40 years of age. Contrary to our expectation, the frequency did not apparently increase with dose but a small hump at low doses (< 0.1 Sv), while the cells from the mothers did show a clear dose response. In the second study, mice were exposed at various ages to X rays, and translocation frequencies were determined with FISH when the animals reached 20 weeks of age (in peripheral blood T cells, spleen cells, and bone marrow cells). We found that the mean translocation frequencies were very low in mice exposed at fetal or early postnatal stages. However, with the increase of the age at the time of irradiation, the frequency observed at 20 weeks old became progressively higher, and reached a plateau level when mice were irradiated at ≥ 6 weeks of age. The major role of *p53*-dependent apoptosis for elimination of aberrant cells was not suggested because irradiated fetuses, regardless of *p53*-/- or *p53*+/-, showed low translocation frequencies when compared to the frequency in the *p53*-/- mother. We interpreted the results as indicating that 1) Fetal cells are generally sensitive to induction of chromosome aberrations as in bone marrow cells of adults but the aberrant cells do not persist because fetal stem cells tend to be aberration free by some unknown mechanisms and their progeny replaces the preexisted cell populations during postnatal growth of the animals. 2) The survivor data indicated a humped response (also reported in a tumorigenesis study in mice), which might provide a biological basis to resolve the long-standing controversy that substantial risk of childhood leukemia and solid cancers is implicated in human fetuses exposed to diagnostic low-dose X rays whereas animal studies composed mainly of high-dose exposures generally do not confirm it.

(S18.3) Early molecular radiation events in embryogenesis. Ohtsura Niwa. National Institute of Radiological Sciences, Chiba, Japan.

It has been documented that the pre-implantation stage embryos are sensitive to radiation for killing, yet the surviving embryos progress to full term without showing the history of radiation exposures such as malformation. In contrast, fetal development is sensitive to radiation induction of malformation with little induction of cancer after birth, and carcinogenesis seems to be the feature of post-partum radiation exposures. The mechanistic insight of these interesting features of stage specific radiosensitivity is still lacking largely. We have been studying the stage specificity of damage response in the preimplantation embryos, from the zygotic stage to implantation. The results demonstrated that utilization of various damage response pathways is dependent on the stage of development and there exist a hierarchy in their use. p53 is the first to function at the zygotic stage and the rest of damage responses seem not operating in this stage. Besides, a p53 dependent S checkpoint is what is operating at this stage among multiple functions of p53. This checkpoint functions at relatively low dose range of up to 2–3 Gy, requires the DNA binding domain of the protein and suppresses the replication fork progression in the presence of DNA damage. The p53 dependent S checkpoint is important in avoiding erroneous replication of damaged DNA and to reduce chromosome mutation. p21 is the second to come at the blastocyst stage. This means that the G1/S and G2/M checkpoints are not operating before this stage. p21 start suppressing cell cycling upon irradiation to arrest cleavage and this somehow prevents delayed chromosome instability. Apoptosis operates even later in inner cell mass to eliminate deleterious cells. Thus, early development of sperm irradiated embryos is protected at least by three layers which are regulated by p53 and by p21. Underlying molecular mechanisms for the stage specific activation of p21 and apoptosis require further analyses.

(S18.4) "Fetal irradiation effects on the brain development" radiation induced apoptosis in the developing brain. Louis D. de Sant-Georges, Joris Verheyde, Mohammed Abderrafi Benotmane. SCK/CEN, Mol, Belgium.

The developing brain is very sensitive to ionizing radiation. The detrimental effect of radiation has typically been attributed to its ability to cause DNA damage. Fortunately cells possess complex and efficient DNA repair mechanisms capable of repairing most genomic damage. However, occasionally, damaged DNA is misrepaired or incompletely repaired. This can result in the triggering of apoptotic cell response.

In the developing nervous system, apoptosis results in naturally occurring cell death, a process that eliminates neurons that have made faulty synapses or have not reached appropriate targets. Apoptosis is also a response to many stimuli like ionizing radiation and factors into many neurodegenerative diseases. The aim of this work is to understand the effect of low dose ionizing radiation on brain development and to estimate the radiation induced apoptotic response. Apoptosis requires the expression of several specific genes among which the Trp53 gene. The analysis of those expressed genes by quantitative PCR and by DNA microchips array will help in unraveling the molecular aspect of the radiation induced apoptosis. We performed qPCR and cDNA microarray analysis at embryonic day E 13 after in utero exposure to 50cGy x-radiation of both wild-type and p53 knock-out mice in irradiated and non-irradiated conditions. Only genes with identified function and a minimal 2 fold amplification were further considered. Our results indicate that genes activated in p53 +/+ and in p53-/- mice appear quite different. It revealed that the main activated pathways in irradiated wild type embryos are involved in the regulation of a p53 mediated pathway that may lead to cell cycle arrest and increased level of apoptosis. To define whether the transcriptional radiation response was solely p53 mediated, we analyzed the

expression of cell cycle regulating genes in a Trp53 null mutant. The modulated expression of cell cycle regulating genes such as cyclins and Cdk genes indicated the induction of a cell cycle arrest, without evidence for the onset of apoptosis. Additional gene expression studies have shown that various E2F transcription factors may be involved in this event. Together these results provide a detailed view of the different p53-related mechanisms that are triggered in response to ionizing radiation in the developing brain.

(S19.1) Formation and biological consequences of peroxide generation on proteins by radiation. Michael J. Davies. The Heart Research Institute, Sydney, Australia.

Proteins are major targets for oxidative damage due to their high abundance and their rapid rates of reaction with a wide range of radicals and excited states (e.g. $^1O_2$). Exposure of proteins to radicals generated by radiation (and other sources, e.g. metal ion/hydroperoxide systems, peroxyl radicals, peroxynitrite, activated white cells) results in the formation of unstable protein-derived radicals. Subsequent reaction of these radicals with O_2 gives rise to the formation of new reactive groups including hydroperoxides and 3,4-dihydroxyphenylalanine. Previous studies have shown that protein hydroperoxides play a key role in the propagation of oxidative chain reactions. These species are long-lived, as they are poorly removed by enzymatic reactions, and can been detected in intact cells. They can also diffuse away from their site of generation, and may mediate secondary (bystander) damage. In contrast to their slow reaction with protective enzymes, reaction with thiols is fast. As a result, we hypothesized that the generation of protein hydroperoxides might inactivate thiol-dependent enzymes and thereby induce cellular dysfunction as a result of the key role played by such enzymes in cell metabolism, signalling, redox maintenance and apoptosis.Recent data supports this hypothesis. Thus GAPDH, glutathione reductase, caspases, cathepsins, Ca^{2+}-ATPases, and protein tyrosine phosphatases (PTPs) are all readily inactivated by hydroperoxides present on oxidised proteins. Inactivation occurs in a concentration-, time- and structure-dependent manner. These reactions involve the consumption of the hydroperoxide with concomitant loss of the thiol group. In some cases the corresponding sulfenic acid intermediate has been detected. In most cases protein hydroperoxides are particularly effective, with inhibition occurring with greater efficacy than with H_2O_2, probably as a result of the longer lifetime of the protein hydroperoxides in cells. Overall, there is now good evidence that hydroperoxides formed on oxidized proteins, may contribute to cellular dysfunction and altered redox signalling in systems subject to oxidative stress by inducing strand breaks and mutagenic lesions in DNA, inhibiting key cellular enzymes, altering cellular redox status and signalling, and depleting antioxidants.

(S19.2) One electron oxidation of peptides: role of oxygen. O. Mozziconnacci, F. Rusconi, K. Bobrowski, Chantal Houee-Levin. Universite Paris Sud, Orsay, France.

Free radical damages on proteins occur in numerous situations. During irradiation by ionizing radiation, it has been shown that they play a crucial role in the recognition of DNA by the nuclear factors, which leads either to activation or to inhibition of gene expression. Moreover, inflammation *i. e*. oxidative stress is involved in the induction and/or development of all diseases. In situations of oxidative stress, peptides and proteins are the first line of defense of cells. Most of the processes initiated by oxidizing free radicals like OH radicals, are known. However some mechanisms that may be very important are still undiscovered. We have studied the one-electron oxidation of Methionine enkephalin, a natural pentapeptidic opiate inhibiting nociceptive signals in oxidative stress that contains Tyrosine and Methionine residues. Azide radicals were the oxidant species. In the absence of oxygen, the main target is the tyrosine residue as expected, that undergoes dimerization. A minor oxidation of methionine to its sulfoxide form is also observed. In the presence of oxygen, the main target is still the Tyr residue, but another compound is formed. If the N-terminal amine function is blocked by a *tert*-butoxycarbonyl group, the peptide is entirely protected from oxidation. Blocking the terminal amine group had thus a key role in protection of the tyrosyl residue, which intriguingly is the biological situation when the pentapeptide is in its /pro form.. This finding might be exploited in the search for new pain inhibitors.

(S19.3) Tryptophan-mediated photolysis of disulfide bonds in proteins and peptides. Ignace Hanssens[1], Ann Vanhooren[1], Bart Devreese[2], Kris De Vriendt[2], Zsuzsa Majer[3], Eszter Illyés[3], Gábor Szilvágyi[3]. [1]Interdisciplinary Research Center, Katholieke Universiteit Leuven Campus Kortrijk, Kortrijk, Belgium, [2]Laboratory for Protein Biochemistry and Protein Engineering, University of Ghent, Ghent, Belgium, [3]Institute of Chemistry, Department of Organic Chemistry, Eötvös Loránd University, Budapest, Hungary.

The high absorption of the indole nucleus makes Trp the prime candidate for mediating protein degradation upon illumination with near-UV light. The fluorescence of Trp is strongly quenched by vicinal disulfide bonds. The accompanying transfer of energy makes disulfide bonds susceptible to cleavage. Goat a-lactalbumin (GLA) contains four Trp and four disulfide bonds. In order to examine the relationship between the photoexcitation of a particular Trp residue and the reduction of disulfide bonds, we constructed four GLA mutants by replacing single Trp residues with Phe. After a fixed time of illumination the amount of free thiol groups in the mutants and in wild-type GLA was measured. We deduced that the sum of the individual contributions by each of the four Trp residues is close to the value for free thiol production of wild-type GLA. This result shows that, in GLA, potential photolytic cleavage of disulfide bonds not mediated by Trp residues is small compared to Trp-mediated cleavage. To obtain more information about the reaction products, the illuminated proteins were carbamidomethylated and digested with trypsin and the fragments were analysed by mass spectrometry. Simple peptide fragments containing a carbamidomethyl group at Cys61, at Cys91 and at Cys120, respectively, were detected. In addition we observed two complex fragments which were created by a new combination of elementary peptides: the peptides were linked by Cys-Lys bonds. The creation of new Cys-Lys bonds suggests that, in addition to Trp groups, Lys groups may interfere in the cleavage of disulfide bonds. To further explore the impact of Lys groups in Trp mediated photolysis of disulfide bonds the following series of peptides containing internally cross-linked Cys residues were constructed: Ac-(CWAKC)-NH_2, H-(CWAGC)-NH_2, H-(CVAKC)-NH_2 and H-(CVAGC)-NH_2. Markedly larger amounts of free thiol were created upon illumination of cyclic Ac-(CWAKC)-NH_2 with near-UV light, than upon illumination of cyclic H-(CWAGC)-NH_2. Upon illumination of cyclic H-(CVAKC)-NH_2 and of cyclic H-(CVAGC)-NH_2 no measurable amount of free thiol was created. These results confirm the idea that Lys groups can interfere in the cleavage of disulfide bonds after initiation by Trp-mediated photoexcitation.

(S19.4) Selective oxidative modification of proteins in vivo: mechanisms and functional consequences. Christian Schoneich. University of Kansas, Lawrence, KS, USA.

Protein oxidation is a hallmark of oxidative stress and disease. In order to correlate protein oxidation with physiological dysfunction, accurate information needs to be obtained regarding the target proteins and target sites within these proteins. One of the features of protein oxidation *in vivo* is the surprising selectivity to a small subset of proteins, frequently independent on the abundance of these proteins. There are many potential reasons for this, such as the chemistry of the oxidizing species, protein-protein interactions, neighboring group effects, selective repair and turnover etc. In this lecture, several examples will be presented for the selective modification of some target proteins, which show *in vivo* oxidation patterns different to what is expected based on corresponding *in vitro* oxidation reactions or from the inspection of protein structure. Some of this selectivity will be rationalized through specific radical reaction cascades involving electron transfer and hydrogen transfer mechanisms.

(S20.1) Cardiovascular disease in the atomic bomb survivors:-mortality, morbidity and laboratory findings and its association with atomic bomb radiation. Kazunori Kodama. Radiation Effects Research Foundation, Hiroshima, Japan.

The Radiation Effects Research Foundation has been conducting a mortality study since 1950 on population of about 120,000 subjects including atomic bomb survivors and their controls (Life Span Study or LSS). The Adult Health Study (AHS), which is a clinical follow-up, has also been conducted since 1958 on a subset of LSS of about 20,000 subjects. In the LSS, an excess of the non-cancer disease mortality with radiation dose was first suggested for the period of 1950–85. This was reconfirmed and strengthened by the subsequent mortality analyses. In these analyses, statistically significant association were seen for diseases of the cardiovascular, digestive and respiratory systems. Some of these diseases may have a dose threshold in occurrence or upward curvature in the dose response. In addition, there was considerable uncertainty about dose response in the low-dose range, in particular the excess of mortality below 0.5 Gy was unclear. In the most recent analysis for the period of 1950–2003, excess mortality was again observed for CVD as a group (ERR/Gy = 0.11, 95%CI 0.05, 0.17), cerebrovascular disease (ERR/Gy = 0.09, 95% CI 0.01, 0.17) and heart disease (ERR/Gy = 0.13, 95% CI 0.05, 0.21). However, by dividing into subtype of diseases, increases of the risk was uncertain for coronary heart disease (CHD). The risk of CHD appeared to be increased only in the higher dose categories. Contrary to this, stronger associations with radiation were observed in hypertensive heart disease (ERR/Gy = 0.37, 95% CI 0.08, 0.72), and rheumatic heart disease (ERR/Gy = 0.86, 95% CI 0.25, 1.72). In the AHS, a significant quadratic relationship was evident for incidence of myocardial infarction among survivors exposed at younger than 40 years of age. An analysis of total cholesterol (TC) showed that TC levels among irradiated subjects were higher than those among. A trend of increased blood pressure among the younger exposed subjects was also observed. Induction by radiation of CVD has enormous implications for those exposed to radiation and to government and private agencies all over the world, with large economic and political consequences, so that it is essential to clarify this issue. By continuing to collect an increased number of cases we should be able to evaluate risk more accurately and to clarify issues such as the shape of the dose response curve.

(S20.2) Cardiovascular disease following radiotherapy for breast cancer. Sarah C. Darby. CTSU, University of Oxford, United Kingdom.

Each year over a million women worldwide are diagnosed with breast cancer. In the majority of cases, the disease is diagnosed sufficiently early for surgery to be appropriate. For women with node-positive disease who receive mastectomy, and for women with either node-positive or node-negative disease who receive breast-conserving surgery, adjuvant radiotherapy has been shown to decrease the risk of dying from breast cancer (1). Despite the beneficial effect of radiotherapy on breast cancer mortality, there was in the past no net benefit of radiotherapy on mortality from all causes, as the beneficial effect was often more than offset by the risk from radiotherapy. The principal component of this risk was an increased risk of death from cardiovascular disease (1). In recent years radiotherapy techniques have changed, and in many countries exposure to the heart is lower now than in the past and risks may now also be lower (2). It is, however, likely that many of the regimens that are in use today still carry some risk, although there is little information on the risk associated with any particular regimen. This creates a difficult situation for those planning radiotherapy treatments. The talk will summarize the evidence that is presently available regarding the risks of mortality from cardiovascular disease following radiotherapy for breast cancer. The talk will also describe work that is currently underway to enable the risk associated with any particular regimen to be characterized. *References:* (1) Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005; 365: 1687–1717. (2) Darby SC, McGale P, Taylor CW and Peto R. Long-term mortality from heart disease and lung cancer after radiotherapy for early breast cancer: prospective study of about 300 000 women in US SEER cancer registries. Lancet Oncol 2005; 6: 557–65.

(S20.4) The biology of radiation-induced atherosclerosis and cerebrovascular damage. Fiona A. Stewart[1], Saske Hoving[1], Nicola S. Russell[1], Marion Gijbels[2], Sylvia Heeneman[2], Mat Daemen[2]. [1]The Netherlands Cancer Institute, Amsterdam, The Netherlands, [2]CARIM, Maastricht, The Netherlands.

Background: Recent evidence has shown that radiation is an independent risk factor in vascular disease after radiotherapy for Hodgkin's disease, breast and head and neck cancer, but it is not clear how this occurs or whether the resulting lesions have a different phenotype from age related atherosclerosis. We propose that radiation induces inflammatory vascular and endothelial cell damage, which initiates atherosclerosis in susceptible subjects and predispose to development of unstable, thrombotic lesions prone to rupture. Methods: We established a mouse model for studying radiation-induced atherosclerosis in the carotid arteries. Atherosclerosis prone ApoE-/- mice were irradiated with 1×8–14 Gy, 20×2 Gy, or sham treatments to the neck. At various times after irradiation, blood samples were taken and the arterial tree was removed for histological examination. In parallel clinical studies, resection material of mid-sized irradiated and unirradiated arteries was collected from head and neck and breast cancer patients undergoing reconstructive surgery after previous radiotherapy or surgery. Results: Atherosclerotic lesions developed earlier and were more numerous in irradiated arteries of ApoE-/- mice. Lesions were macrophage-rich, with a remarkable influx of inflammatory cells. Atypical endothelial cells, intraplaque hemorrhage and erythrocyte containing macrophages were common in lesions of irradiated arteries but not in age-matched controls. Clinical resection material of the head and neck cancer patients also showed increased ratios for intima-media thickness (IMT), and more inflammatory cells in the irradiated arteries than in the unirradiated arteries. Conclusions: Irradiation accelerates the development of macrophage-rich, inflammatory atherosclerotic lesions, prone to intra-plaque hemorrhage. Specific anti-inflammatory intervention could help to prevent this.

(S21.1) Characterization of structurally distinct, isoform-selective PI3-kinase inhibitors as radiosensitizing agents in the treatment of human gliomas. Daphne A. Haas-Kogan, Jack Chen, Linda Zhou, David Stokoe. University of California, San Francisco, San Francisco, CA, USA.

The phosphoinositide 3'-kinase (PI3K)-mediated signaling pathway plays an important role in fundamental cellular functions such as proliferation and survival. Deregulated signaling through this pathway due to mutations in PTEN has been well established to contribute to the development of gliomas and their resistance to treatment by radiotherapy and chemotherapy. Targeting the PI3K signaling pathway has thus emerged as a promising approach to improving treatment of gliomas. We assessed the radiosensitizing potential of four small molecule inhibitors of the 110 kDa catalytic subunit of PI3K (p110). These structurally distinct inhibitors, known as PI-103, PIK-75, PIK-90 and PIK-108, differ in their p110 isoform specificities. p110α inhibitors blocked phosphorylation of both PKB/Akt and S6 in all cell lines examined, and were effective inhibitors of proliferation. The p110β inhibitor PIK-108 inhibited PKB/Akt phosphorylation only in some cell lines and did not block S6 phosphorylation. PIK-108 had no effect on cell viability either alone or in combination with either rapamycin or radiation. The p110α inhibitors PI-103 and PIK-90 are more effective in combination with radiation in a panel of PTEN mutant glioma cell lines as compared to PTEN wild type cell lines. PIK-103 is also an effective radiosensitizer of the PTEN mutant cell line U251 in vitro and in vivo. In conclusion, PI3K inhibitors are promising agents in the treatment of PTEN mutant GBM, especially when used in combination with ionizing radiation.

(S21.2) Combining molecular targeting agents with radiation: multicenter clinical trials. Paul M. Harari. University of Wisconsin Hospital, Madison, WI, USA.

The purpose of this presentation is to examine selected multicenter clinical trials that combine molecular targeted agents with radiation. Head and neck (H&N) cancer studies are used as the primary teaching example. Indeed, the first Phase III trial to identify a survival advantage when combining a molecular targeting agent with radiation has recently emerged in H&N cancer (NEJM 354:567–78, 2006). This study randomized 424 advanced stage H&N cancer patients to curative-intent radiation alone +/- weekly cetuximab (anti-EGFR monoclonal antibody). There was a statistically significant improvement in locoregional disease control and overall survival favoring the experimental arm. Cetuximab treatment was generally well tolerated without significant enhancement in classic radiation toxicity profiles. However, not all patients benefited from the addition of cetuximab. The identification of those patients most likely to derive benefit from EGFR inhibitor therapies remains an active arena of research. A series of additional clinical trials that combine EGFR inhibitors with radiation are underway. Through active collaborative interaction between preclinical and clinical researchers, strategies to further optimize the application of EGFR inhibitors in combination with radiation in cancer treatment will continue to advance.

(S21.3) Preclinical evaluation of molecular targeted drugs in the specific context of radiotherapy. Michael Baumann. Dept. of Radiation Oncology and OncoRay Center for Radiation Research in Oncology, Medical Faculty, University of Technology, Dresden, Dresden, Germany.

Combination of molecular targeting drugs with radiotherapy has become an important field of preclinical and clinical cancer research. The large number of novel substances requires extensive preclinical evaluation to select the most promising strategies for further translation into clinical trials and to investigate mechanisms of action. The presentation gives an overview of preclinical research methodology and results with focus on the effect of molecular targeted drugs on experimental tumor models in vivo. Interaction of irradiation and drug action requires specific experiments for defining the potential of a new drug for combination with radiotherapy. Proof-of-principle experiments have shown efficacy of a variety of molecular targeted approaches (e.g. EGFR and VEGFR inhibition, antibody linked chemotherapy) combined with irradiation. Different experimental endpoints may reveal different results. Evaluation of tumor regression, tumor volume and growth delay, particularly when performed with low radiation doses and at only one dose level, may significantly overestimate the efficacy of combined treatments. Local tumor control measures inactivation of tumor stem cells and is more relevant for curative radiotherapy. However, tumor control assays are expensive and slow, which limits their use and calls for supplementation with surrogate markers, e.g. by using biological imaging. As specific radiobiological mechanisms (e.g. repopulation, reoxyenation) may be targeted by combined treatment approaches, it is important to select adequately characterized tumor models and relevant treatment schedules for the experiments. In conclusion, molecular targeting combined with radiotherapy has demonstrated effectiveness on all steps of the translational research chain. The validity of preclinical in vivo experiments depends critically on the appropriateness of tumor models, experimental design and endpoints.

(S22.1) Damage recognition, cell-cycle checkpoints and cell death: identifying the mechanism of HRS. Brian Marples[1], Sarah A. Krueger[2], George D. Wilson[1], Michael C. Joiner[2]. [1]William Beaumont Hospital, Royal Oak, MI, USA, [2]Wayne State University, Detroit, MI, USA.

Over the past two decades a range of *in vivo* and *in vitro* studies have established that low-dose hyper-radiosensitivity (HRS) is an important component of the survival response of tissues and cells exposed to low doses of ionizing radiation. While the definitive molecular basis of HRS still remains to be discovered, significant progress has been made recently delineating some of the important pathways. For example, apoptosis has been identified as the mode of cell killing most closely linked with HRS. In addition, it has been demonstrated that the activation of ATM (ataxia telangiectasia mutated) Ser1981 activity alone is not the key determinant for overcoming the HRS in response to increasing levels of radiation damage, while the recognition of DNA double strand breaks by γH2AX was shown to occur equally in cell lines that do and do not exhibit HRS. More recently, it was established that the ATM-dependent early G2-phase checkpoint was essential for overcoming HRS, an observation that supports the concept that HRS is a response almost certainly restricted to cells in the G2-phase of the cell cycle at the time of irradiation. The aim of this presentation is to review the historical observations that established the existence of the HRS response, and reconsider these data with regard to the latest molecular studies of HRS in order to provide a fresh insight into the biological mechanics of HRS. The available data will be compiled into a working model to provide the basis for future experimentation.

(S22.2) Molecular basis for cellular responses to low dose radiation. Louise Enns, Michael Weinfeld. Cross Cancer Institute, Edmonton, AB, Canada.

Many human cell lines exhibit a hyper-radiosensitivity (HRS) to radiation doses < 20 cGy, which manifests as a significant deviation from the clonogenic survival response predicted by a linear-quadratic fit to higher doses. This is followed by increased radioresistance (IRR) at slightly higher doses. Our examination of HRS indicated that cell death at low doses is due to caspase-3 regulated apoptosis. We further showed, using the p53 inhibitor, pifithrin, and cells with inactive p53, that this apoptotic response is dependent on p53. Normal human fibroblasts and those derived from individuals with Ataxia telangiectasia (AT) display HRS, but AT cells do not show IRR. Analysis of ATM autophosphorylation of serine 1981 indicated that ATM-positive cell lines fail to show a measurable increase in the number of cells with phosphorylated ATM after 10 cGy up to 4 h post-irradiation, but a 3–4-fold increase in the number of cells with phosphorylated ATM after 25 cGy. Similarly, we observed that dephosphorylation of histone H2AX over 24 h is more proficient after 25 cGy than after 10 cGy, indicating that double-strand break (DSB) repair is more efficient during IRR than HRS. In AT cells the activated caspase-3 levels do not decrease at IRR doses and are elevated for longer periods, reaching maximal levels at ~7 days post-irradiation. One possible explanation for the AT response may be related to proteasome function. Proteasomes are required to degrade many proteins (repair proteins and caspases among others) and their activity is inhibited by radiation. We speculate that in AT cells proteasomes are aberrantly regulated resulting in prolonged caspase-3 activation, and thus a failure to end HRS and commence IRR. We tested this possibility by using a proteasome inhibitor, MG123. When normal cells were treated with MG132 we observed that they lose their IRR response, but the drug has no effect on the survival of AT cells. Furthermore, the pattern of caspase-3 activation in the irradiated MG132-treated normal cells mirrors the caspase-3 activity pattern of AT cells. This evidence indicates the requirement for both ATM and proteasome activity in the IRR response. It also suggests that proteosome inhibitors may be useful radiosensitizers at low radiation doses.

(S22.3) Chemopotentiating effects of low-dose hypersensitivity make headway in cancer therapy. Mansoor M. Ahmed[1], Seema Gupta[1], Susanne M. Arnold[2], Paul M. Spring[3], William F. Regine[4], Mohammed Mohiuddin[5]. [1]Weis Center for Research, Geisinger Clinic, Danville, PA, USA, [2]Division of Hematology-Oncology, Department of Internal Medicine, University of Kentucky, KY, USA, [3]Department of Otolaryngology, University of Arkansas Medical, Little Rock, AR, USA, [4]Department of Radiation Oncology, University of Maryland, Baltimore, MD, USA, [5]Geisinger-Fox Chase Cancer Center, Geisinger Clinic, Wilkes-Barre, PA, USA.

Extensive pre-clinical research has demonstrated that combined chemotherapy and radiation eliminate malignant cells by the induction of apoptosis as well as by "mitotic death". Joiner and colleagues revolutionized thinking about low doses of radiation (<100 cGy) by demonstrating an initial phase of hyper-radiation sensitivity (HRS) to radiation doses below 1 Gy. Interestingly, the degree of HRS response varies in different tumor cell lines and this was independent of cell type, radiation-induced cell cycle arrest and p53 functional status. An alternative approach that exploits the enhanced cell killing with multiple low dose fractions (<1 Gy) would be to use as an adjuvant with systemic chemotherapy to enhance the effects of chemotherapy itself. We analyzed this novel strategy combining low dose radiation fractions as an "enhancer" of full dose chemotherapy to circumvent the development of resistance found with standard clinical doses of radiation and chemotherapy. Our extensive biologic studies indicated that chemotherapy followed by low dose fractionated radiation (LDFRT: four fractions of 0.5 Gy at 8 hr. interval) caused significant potentiation of cell killing with both chemotherapy drugs and biological modifiers in various cancer cell lines. The chemo-potentiation mechanism of action was conferred (a) by mitigating NFkappaB /NF-YA / ERE activity, (b) down-regulating MDR-1 gene expression and (c) by direct induction of bax, translocation of bax into mitochondria with increased Cytochrome C release. These compelling *in-vitro* and *in-vivo* data led to the conductance of induction clinical trials in patients with advanced head and neck cancer, lung cancer and pancreas cancer. The results from these trials demonstrated a complete tolerance with no to minimal toxicity, greater radiographic response and a higher rate of primary site complete response. In summary, the findings of *in-vitro*, *in-vivo* and clinical trial studies strongly suggest that the use of HRS causing low dose radiation in multiple fractions with a chemotherapeutic agent or biological modifying agent, is a novel approach to exploit the observed HRS phenomenon in order to achieve significant chemo-potentiation, eliminate "induced radiation resistance" and improve overall survival in solid tumors.

(S22.4) Low dose total body irradiation (ldtbi) in patients of advanced non-hodgkin's lymphoma: a preliminary clinical and radiobiological evaluation. Pranshu Mohindra[1], Mary Ann Muckaden[1], Siddhartha Laskar[1], Amit Kumar[2], Haladhar Dev Sharma[2], Badri Narain Pandey[2], Shyam Kishore Shrivastava[1], Ketayun Ardeshir Dinshaw[1], Kaushala Prasad Mishra[3]. [1]Tata Memorial Hospital, Mumbai, India, [2]Bhabha Atomic Research Centre, Mumbai, India, [3]Research Institute for Radiation Biology and Medicine, Hiroshima, Japan.

Aim: The study aim was to examine the therapeutic outcome of Low Dose Total Body Irradiation (LDTBI) in advanced NHL patients, with relapse post chemotherapy & design radiobiological studies to understand the underlying mechanism. Materials and Methods: LDTBI (200cGy/ 10–20 fractions @ 5 fractions/week) followed by boost for the bulky sites was scheduled for 13 prospectively accrued patients (including four on treatment). Blood cell counts, serum liver/renal function, 2 D-Echocardiography, pulmonary function & whole body PET scan were noted. Serum anti-oxidants; *catalase & superoxide dismutase* and peripheral blood lymphocyte parameters; *apoptotic index, intracellular ROS & mitochondrial membrane potential* were measured pre-LDTBI, at 100 cGy, 200 cGy & at follow up. Results: No acute, moderate/ severe, non-haematological toxicity was noted. The haematological nadir (predominantly thrombocytopenia) occurred by 4- 8 weeks with recovery in 6 of 9 patients. One patient achieved a complete response. Three partial responses, two < 50% regressions but good response to boost, three stable/ progressive diseases were noted. The differential WBC counts showed an interesting *Crab's jaw* shaped pattern in 5 of 8 eligible patients characterized by initial rise in relative polymorph count & a fall in relative lymphocyte count. Subsequently the counts crossed twice as LDTBI approached completion and during recovery. Serum catalase levels fell by first 100 cGy, with subsequent stabilization. A significant increase was seen in apoptotic index with initial 100 cGy dose in the two patients who responded well to LDTBI. No trends, however, could be observed at this point in measurement of other parameters. Conclusions: Good clinical response with acceptable toxicity in well selected, advanced NHL patients reinforce use of LDTBI as a definite treatment option. The ***'LDTBI Crab's jaw'*** pattern & an initial rise followed by fall seen in polymorphs & platelet counts suggest immune-modulatory and apoptotic response to LDTBI. Sixty patients of low grade NHL will be accrued in the next three years for a prospective clinical response evaluation study with continued radiobiological experiments to understand mechanism & develop possible predictive assays allowing selection of patients likely to respond to LDTBI.

(S23.1) Genome wide screen of genes affecting response from yeast to radiation. James A. Brown[1], Nicola M. Burrows[1], Marsha S. Willamson[2], John C. Games[2], J. Martin Brown[1]. [1]Stanford University, Stanford, CA, USA, [2]Lawrence Berkeley Laboratory, Berkeley, CA, USA.

Efforts to comprehensively identify all of the genes and pathways required for DNA damage repair have met with limited success. We have shown that transcriptional profiling in yeast following DNA damage to be a very poor predicator of the gene products that are important to the cell for protecting against DNA damage. We are therefore exploiting the utility of a collection of strains carrying the deletion of every open reading frame in yeast to systematically determine the importance of the various DNA damage response pathways and the physiological role of genes whose function remains elusive. This collection has enabled us to screen for novel genes required for survival to many traditional and novel DNA damaging agents. Many of the genes conferring resistance to DNA damage in the yeast *Saccharomyces cerevisiae* have been well characterized but we have uncovered novel genes which may function in under-appreciated mechanisms of genotoxic resistance. These include a Rad6 dependent pathway involving Bre1, Dot1, Rtf1 and Lge1 which function in histone ubiquitination and methylation. We have employed hierarchical clustering to the spectrum of these sensitivities to over 70 different agents with the hypothesis that genes that act in the same pathway would form functional clusters. By combing these clusters for significant enrichment for gene ontological terms, we can connect functional pathways with phenotypic clusters. We show that this is a more powerful method of predicting the function of genes in yeast than protein-protein interactions, expression profiling and synthetic lethality. All of the genes we have identified have clear human orthologs within highly conserved pathways.

(S23.2) Genome-wide analysis of genomic instability in C. elegans. Marcel Tijsterman. Hubrecht Laboratory, Utrecht, The Netherlands.

A principal challenge currently facing biologists is how to connect the complete DNA sequence of an organism to its development and behaviour. With the recent advent of RNA interference (RNAi), we are now able to analyse function on a genomic scale. We use the nematode *C. elegans* to systematically screen for genes that are required to keep genomes stable, and when mutated can predispose humans to cancer. We use genome-wide RNAi on recently developed transgenic animal systems, which allow visualization of specific types of DNA repair (*e.g.* micro-satellite instability, double-strand break repair and G4-DNA instability). We have used the same protocol to identify genes that protect the genome from radiation-inflicted DNA double strand breaks, alkylating agents (MMS) and intrastrand crosslinks (CisPt). Candidate genes are further characterized in *C. elegans*, in human siRNA-knockdowns, and in patient material, using both classical as well as genomics orientated strategies, such as Comparative Genome Hybridization (CGH) developed to visualize specific types of DNA instability. We have also started to use our screening setup to unravel genetic interactions in metazoan DNA damage response networks: parallel genome-wide screens are performed with animals that have defects in various DNA repair/signalling pathways. Ongoing efforts and results will be presented.

(S23.3) Systematic modeling of breast cancers using a diverse cell line panel. Paul T. Spellman. Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

Breast cancers are a heterogeneous in nearly every aspect. We have catalogued changes in genome copy number and gene expression for a diverse collection of breast cancer and breast cancer cell lines. We show that the aberrations in breast cancers are recapitulated in breast cancer cell lines. We have attempted to model several aspects of breast cancer cell lines to predict responses of corresponding tumors. We used the Pathway Logic modeling system to generate signaling network models for the cell lines Pathway Logic is designed to build discrete, logical models of biological systems. Logical models are fundamentally related to schematic diagrams that show relationships among genes, proteins, and other cellular components, and are easily interpretable in biological context. These models are therefore well designed for making predictions about key signaling events. We used mRNA and protein abundance data to populate a unique network model for each cell line. Genes or proteins that are differentially expressed across the cell lines were considered present in some cell lines and absent in others. With this approach, we were able to model the diversity of signaling across the panel of cell lines, as well as the features common to them. We found that the initial states of our models showed only a small amount of variation: of 286 components, only 13% varied across the cell lines. Importantly, even with this small amount of variation in the initial state, we were able to capture many of the key features used to classify these cell lines. Furthermore, the network models are vastly different: of the reactions predicted to be activated, over half of them varied across the cell lines. We used these network models to generate (and verify) two biological predictions about signaling in our cell lines. Our first prediction was that cell lines that express CAV1 and Integrin B1 have an alternate route for the activation of the MAPK cascade. Second, we have identified PAK1 as a key regulator of the RAF-MEK-ERK pathway in our cell lines. We have also examined the responses of the breast cancer cell lines to a substantial number of FDA approved therapeutic agents. This has allowed us to identify predictive signatures of response that, at least in one case, have been externally validated on tumor response data.

(S24.1) Radiation effect of carbon nanotubes in aqueous system. Jing Peng, Hai Bo Yu, Mao Lin Zhai, Jiu Qiang Li, Gen Shuan Wei. Peking University, Beijing, China.

Recently, interest has focused on producing the small carbon nanotubes (CNTs) since they have potential use in hydrogen storage, molecular sieves and biological engineering such as a carrier for drug release and a biosensor for early diagnosis of cancer cells. The purpose of this work is to produce the short CNTs via radiation-induced degradation and study the interaction between CNTs and active species produced by laser photolysis of aqueous solution. The multi-walled carbon nanotubes (MWCNTs) was irradiated at room temperature with the absorbed dose in the range up to100 kGy at a dose rate of 30 Gy/min in the presence of dilute sulfuric acid. Finally, water-soluble and short MWCNTs (~200nm) could be prepared. Both X-ray diffraction (XRD) analysis and Raman spectra indicated that no obvious change in structure of short MWCNTs compared to raw MWCNTs was found, except that several polar groups such as carboxyl group were present on the surface of short MWCNTs. Laser photolysis of MWCNTs in pure water or $K_2S_2O_8$ aqueous solutions was carried out by using a NL303HT: YAG laser as excitation source (266nm with pulse width of 6ns). The reactivity of MWCNTs towards·OH and·SO_4^- radicals were investigated. Comparing with transient absorption spectra of pure water, several new absorptions could be found for the case of pure water with dispersed MWCNTs, in addition, the amount of transient species increased with the length of MWCNTs. The length of MWCNTs has significant influence on the decay kinetics of ·SO_4^- at the length of less than 450nm. In conclusion, the gamma irradiation is a feasible method to modify MWCNTs with slight change in their structure. Furthermore, some transient active species could be formed on the surface of MWCNTs in aqueous system, which could be employed to cut or functionalize MWCNTs.

(S24.2) Barrier-controlled hole migration in duplex DNA to minor-groove binding bisbenzimidazole ligands. Robert F. Anderson[1], Sujata S. Shinde[1], Johnathan M. White[2], Pavel N. Lobachevsky[3], Roger F. Martin[3]. [1]University of Auckland, Auckland, New Zealand, [2]University of Melbourne, Melbourne, Australia, [3]Peter MacCallum Cancer Centre, Melbourne, Australia.

We have used pulse radiolysis to investigate the mechanism of hole transfer in DNA in a process initiated by extraction of an electron from a guanine-cytosine base-pair (GC), using the selenite radical anion as the oxidant. Hole transfer to bisbenzimidazole ligands, tightly bound to discrete sites in the minor groove, was monitored by time-resolved spectrophotometry. The rate of ligand oxidation increased as the average distance between the hole (fixed at approximately 1 per 1000 base pairs, bp) and the nearest bound ligand is decreased, by increasing binding ratio (r ligands per bp) from 0.005 to 0.05. The experimental design also allows estimation of the maximum range of hole transfer (up to *ca*. 30 bp) and use of a set of ligands, with different one-electron reduction potentials of their radicals (0.90 - 0.99 V) measured for the DNA-bound ligands. Both the rate (at a given r) and maximum range of hole migration were found to increase with the free energy change between reactants. Linear plots of ln rate of hole transfer *versus* r are characterised by an intercept at $r = 0$, of *ca*. 7×10^3 s^{-1} at 25°C, which is essentially independent of ligand. We interpret this intercept as reflecting a barrier to hole transfer. Studies at different temperatures show that the intercept, but not the rates of hole transfer, is temperature-dependant, and allow an estimation of the free energy of activation for this barrier. The value obtained, 50 kJ mol^{-1} (0.52 eV per molecule), is close to the difference in the calculated vertical ionisation potentials for GC and AT base pairs. We propose that this is the energy required for the hole to surmount the barrier represented by the AT base pair adjacent to the initially oxidized GC base pair, and that subsequent charge transfer is driven by the thermodynamic potential difference between the hole and the DNA-bound ligands acting as reductants.

(S24.3) Nucleation, growth and properties of metal clusters studied by radiation chemistry. Jacqueline D. Belloni. CNRS, Orsay, France.

Metal nanoclusters are very small objects made of a few atoms, which exhibit specific physical and chemical properties distinct from the bulk metal and depending on the number of atoms they contain.[1] Radiation chemistry methods have been proven to be of high potentiality to induce small and size-monodispersed metal clusters, as nanocolloids or supported on various materials. Pulse radiolysis provides the means to study the dynamics of electron transfer, that is nucleation and growth of clusters, mono- and bi-metallic, from the monomers to the stable nanoparticle, and to observe directly their reactivity, specially to determine, during the growth, their nuclearity-dependent properties, such as the redox potential.[2] These are of crucial importance for the understanding of the mechanism of the cluster growth itself, in the radiation-induced as well as in the chemical or photochemical reduction processes, and also of the mechanism of certain catalytic reactions.[3] This understanding permitted first to guide the choice of conditions for their radiation-induced synthesis and to control their final size. Quite numerous systems based on mono- and multimetallic clusters, such as alloyed or layered clusters of gold, silver, platinum, palladium, ruthenium, nickel, copper, tin. . .. have been studied, with their action in some applications in nanosciences. Clusters attract indeed a quite high interest because of their role in various important fields, such as phase transition, catalysis, electronics, non linear optics,[4] photography.[5]

(S25.1) Insights on apoptotic cell death following radiation exposure. Kathryn D. Held[1], Martin Purschke[1], Asima Chakraborty[1], Tetyana Antyushyna[2], Kevin M. Prise[2]. [1]Massachusetts General Hospital, Boston, MA, USA, [2]Gray Cancer Institute, Northwood, United Kingdom.

Traditionally, radiation biologists have assessed cellular effects of radiation primarily in terms of the loss of a cell's ability to divide indefinitely, i.e., to form a colony. The loss of clonogenic ability can occur because of either loss of proliferative capacity (e.g., cell cycle arrest, quiescence, senescence or terminal differentiation) or cell death (apoptosis, necrosis, autophagy and mitotic death/catastrophe). This talk will briefly summarize the main characteristics of those modes of cell death, then discuss recent findings on mechanisms of radiation-induced apoptosis.

The extent of apoptosis and the pathways involved depend on cell type and radiation dose. For example, it is well appreciated that normal fibroblasts typically show little radiation-induced apoptosis, but if they are transformed with certain oncogenes, e.g., myc, they become more prone to apoptosis. In contrast, many cells of hematopoietic lineage are prone to radiation-induced apoptosis, but that may depend on p53 status, radiation dose and time of damage assessment after irradiation. We showed previously that when some cell types (e.g., HL-60 human leukemia cells) are exposed to high doses of radiation, they undergo rapid (within 5 h), pre-mitotic, mitochondria-dependent, caspase 3-independent apoptosis, but after lower doses, they undergo apoptosis more slowly (maximum at 3–4 days) after one or more cell divisions. That later death is dependent on both mitochondria and caspase 3. A few years ago, Dewey and colleagues performed elegant studies using computerized time-lapse microscopy (CTLM) to demonstrate that radiation-induced apoptosis can occur one to four cell divisions after irradiation. We have undertaken a series of studies, some using CTLM, in myc-transfected rat fibroblasts and WTK1 human lymphoblastoid cells to investigate: whether cells undergoing apoptosis pre or post-mitosis use the same apoptotic pathways and what the pathway(s) are; how the appearance of apoptosis relates quantitatively to loss of clonogenicity; whether non-nuclear irradiation can induce apoptosis; and if the pathway for apoptosis induction is the same in directly irradiated cells and their non-irradiated neighbors, bystander cells. These studies will be discussed.

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(S25.2) Prx1 in radiation-induced cell death. Young-Mee Park, Yun-Jeong Kim, Soo-Yeon Park, Sun-Hee Baek, Xiaofei Yu, Jonah Riddell. Roswell Park Cancer Inst., Buffalo, NY, USA.

Peroxiredoxin 1 (Prx1) has been observed in several human cancers, including lung cancer. Recently, we cloned and characterized human *prx1* promoter, and reported that the elevated expression of Prx1 may be explained in part by the activation of Nrf2 in a hypoxic and unstable oxygenation climate of a tumor (1). Although the antioxidant function of Prx1 is expected to affect the radio-sensitivity of cancer cells, the physiological significance of the ROS-removing activity of Prx1 in irradiated cells is unclear because the catalytic site cysteine, Cys^{52}-SH, is easily inactivated by ROS as a result of its over-oxidation to sulfinic or sulfonic acid. We undertook a study to investigate the role of Prx1 in radiation sensitivity of lung cancer cells, with special emphasis on the redox status of the catalytic Cys^{52} (2). We found that over-expression of Prx1 enhances the clonogenic survival of irradiated lung cancer cells, and suppresses radiation-induced JNK activation and apoptosis. The antioxidant activity of Prx1, however, is not essential for inhibiting JNK activation. The latter effect is mediated through its association with the GSTpi-JNK complex, thereby preventing JNK release from the complex. Reduced JNK activation is observed when the antioxidant activity of Prx1 is compromised by over-oxidation of the catalytic Cys^{52} or in the presence of the Cys^{52} to Ser^{52} mutant Prx1 (Prx1C52S) that lacks ROS-removing activity. These results provide new insight into the anti-apoptotic function of Prx1, and suggest that cancer cells with higher Prx1 levels may be resistant not only to radiation but also to multiple agents targeting the JNK pathways. Our recent study demonstrating the prognostic value of Prx1 in predicting the clinical outcome of lung cancer is consistent with this hypothesis (3). Based on the above information, we suggest that Prx1 may serve as a new therapeutic target in the management of lung cancer and other malignancies.

(S25.3) Non-targeted insights on cell death following radiation exposure. Kevin M. Prise[1], Tetyana Antyushyna[2], Laurence Tartier[2], Melvyn Folkard[2], Giuseppe Schettino[2], Kathryn D. Held[3]. [1]Queen's University Belfast, Belfast, United Kingdom, [2]Gray Cancer Institute, Northwood, United Kingdom, [3]Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA.

A key aspect of our understanding of mechanisms of radiation action is the delineation of the processes leading to cell death. This impacts on two important areas. Firstly, understanding cell death mechanisms can lead to improvements in the efficacy of using radiation in a therapeutic environment, i.e. in tumour cell kill. Secondly, in the process of radiation-induced carcinogenesis, the overall level of risk is related not only to the induction of mutational change in the genome, but also to the level of surviving cells. Cells can die by three well characterised processes; apoptosis (or programmed cell death), necrosis or reproductive (or mitotic) death. The standard paradigm for radiation effects, including those leading to cell death has been based on the direct link between energy deposited in the nucleus of a cell and the downstream biological consequences. Central to this has been the primacy of the DNA damage response especially involving DNA dsb and clustered damage, with a cells ability to correctly repair this damage determining the overall level of survival. In some circumstances, cells have the choice via a p53 dependent mechanism of not repairing damage but initiating apoptosis. In general it has been thought that cells ability to live or die after irradiation will be determined within 1 or 2 cell cycle times of the initial insult. The direct DNA model has been questioned with the realisation that cell to cell communication mechanisms can influence response to radiation exposure and that these can contribute to overall effect *in vitro* and potentially *in vivo*. Novel targeted radiation approaches have directly shown that radiation exposure of the cell cytoplasm is capable of leading to the formation of damaging reactive oxygen or nitrogen species, which can indirectly lead to genotoxic damage. Cell type specific cytokines are capable of transmitting these signals to non-irradiated neighbours and recent studies have now shown that these non-targeted responses can also trigger apoptosis. In many circumstances delayed responses may lead to cell death many generations after exposure due to genomic instability. Overall, we are shifting from a single-cell based understanding of basic mechanisms of radiation-induced cell killing to a multicellular / tissue signalling model of more relevance to the *in vivo* situation.

(S25.4) Regulation of radiation induced cell death by PKCdelta. Yun-Sil Lee, Yoon-Jin Lee, Eon-Ho Kim. Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea.

Although conventional clinical and pathological factors have been helpful in identifying risk and guiding clinical decision-making for both local and systemic management, there is clearly a need to identify additional prognostic markers, which can aid in refining our treatment strategies and improving outcomes. A substantial amount of research efforts have been devoted to identifying molecular markers from prognostic and therapeutic strategies. Heat shock proteins (HSP) are a large family of proteins with different molecular weights and different intracellular localizations and HSP25/HSP27 belongs to the small heat shock proteins. Recently HSP25/27 reported that it acts as emerging anti-apoptotic molecules. In the present study, we described molecular signaling of HSP27/25 in predictor of radiation or chemo sensitivity, especially focused on inhibition of PKCdelta-mediated apoptosis. HSP27 overexpressed in human lung tissues and treatment of NCI-H1299 cells which show high expression of HSP27 with small interference RNA (si-RNA) targeted for HSP27 abolished the resistance of radiation or cisplatin, suggesting that HSP27 is responsible for radio- and chemo-resistance in lung cancer cells. Furthermore, PKCdelta activation is involved in sensitization of cancer cells, and carboxy terminus of PKCdelta V5 region, interacted directly with HSP27, resulting in inhibition of PKCdelta activity and PKCdelta-mediated cell death. Therefore, we prepared various deletion mutants of V5 region and found that peptide sequence spanning residues 668–674 of V5 region was the binding site that interacted with HSP27. Treatment of NCI-H1299 cells with biotin labeled heptapeptide of residues p668–674 (E-F-Q-F-L-D-I)

efficiently interacted with HSP27 and dramatically increased radiation induced cell death, while PKCdelta activity inhibited by HSP27 overexpression was restored. *In vivo* nude mice grafting data also suggested that NCI-H1299 cells were sensitized by this heptapeptide treatment. The above data strongly demonstrate that heptapeptide of PKCdelta-V5 region sensitized human cancer cells induced by variety of death stimuli through it's interaction with HSP27, thereby sequestering HSP27, and delineate a novel strategy for the selective neutralization of HSP27.

(S26.1) Immune cells in the tumor microenvironment: friend or foe? Theresa L. Whiteside. University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA.

Most human tumors are infiltrated by immune cells, and the role of these cells in tumor progression has been debated for years. Numerous studies have linked infiltrations of tumors with lymphocytes or dendritic cells (DC) to improved patient survival, while others have claimed that immune cells promote tumor growth. More current cellular and molecular findings emphasize the dominant role of the tumor in orchestrating its own microenvironment in parallel with genetic changes taking place in tumor cells over a period of time. The nature of interactions between infiltrating immune cells and the tumor also changes. From the initially tumor-growth promoting, pro-inflammatory type milieu rich in TNF-α, IL-6, IL-8 and VEGF, the tumor environment becomes increasingly immunosuppressive, attracting regulatory T cells (CD3+CD4+Foxp3+), which produce IL-10 and/or TGF-β, and $TCR\zeta^{dim}$ memory T cells, which are functionally aberrant. The tumor subverts the host immune system from the start by diverse mechanisms aimed at corrupting immune cells to, at first, promote tumor growth and, later, to facilitate its escape. Functional attributes of infiltrating immune cells and molecular mechanisms contributing to their survival or death in the tumor microenvironment are clinically important endpoints, which influence disease outcome in chronic inflammatory conditions, including cancer. Effects of radiation therapy are likely to profoundly alter interactions of immune cells with tissue and tumor cells. By impacting on immune functions, radiation therapy represents a proverbial "double sword" that should be wielded with great care and especially carefully timed when combined immune and radiation therapies are considered.

(S26.2) Irradiated tumors recruit immune cells. Sandra Demaria. New York Univ Medical Center, New York, NY, USA.

The goal of tumor immunotherapy is to empower the immune system to control tumor growth. Most cancers express tumor associated antigens (TAA) that can be recognized by anti-tumor T cell. However, suboptimal function of antigen-presenting cells in tumor-bearing hosts leads to defective priming of anti-tumor T cells. Therefore, many immunotherapy strategies have focused on improving priming by vaccination with TAA. Vaccination can be successful in boosting pre-existing or inducing de novo immune responses. However, this rarely translates into significant tumor regression, indicating that the defects at the effector phase of the anti-tumor immune response are important and need to be overcome to obtain true clinical benefits. Many barriers to the recruitment and function of immune cells with anti-tumor activity have been identified within the tumor microenvironment. Ionizing radiation therapy (RT), in addition to its cytocidal effects, alters the phenotype of the surviving neoplastic and stromal cells. We and others have shown in preclinical syngeneic models of cancer that local RT used in combination with different immunotherapy approaches promotes immune-mediated tumor destruction. Evidence is accumulating that at least some of the radiation-induced changes in the tumor microenvironment facilitate tumor rejection. We have identified the radiation-induced up-regulation of chemotactic factors that attract effector T cells to the tumor as one critical component. The concept that RT could be used to enhance the effectiveness of immunotherapy by overcoming the barriers to homing of effector T cells to solid tumors has important implications for cancer treatment.

(S26.3) Radiation and immunity in the tumor microenvironment: role of cytokines. Edith Lord. Univ of Rochester Medical Center, Rochester, NY, USA.

Nonmaligant host cells including stromal and immune cells play an important role in establishing the tumor microenvironment and affecting tumor growth. This microenvironment is highly dynamic and is often markedly heterogeneous in cellular composition and in respect to physical parameters such as hypoxia and pH. Tumor cells are able to influence and respond to the changing microenvironment by producing factors that alter vascular formation and the extracellular matrix. These factors may also alter the infiltration of host immune cells and functions such as cytokine production. Many studies, including those in which tumor cells are genetically modified to produce cytokines, have demonstrated the importance of the cytokine milieu and the potential for altering tumor outcome by individual cytokines. For example, IL-12, a cytokine produced by antigen presenting cells and known for its ability to drive Th1 cell differentiation and inflammatory responses has been shown by this method to limit tumor growth by stimulating immune cells and altering vessel formation within tumors. Moreover, the recruited immune cells can further alter the cytokine mix within the tumor microenvironment. The molecular mechanisms for the action of this and other downstream cytokines are being elucidated and illustrate the importance of the interplay between infiltrating host cells and tumor cells. Radiation therapy, which is highly effective in treating many tumor types, is often viewed as being highly immunosuppressive. However its cytocidal effects may also provide antigenic stimulation for immune cells and result in changes that facilitate the generation and function of immune cells. For example, other investigators have shown that radiation can up-regulate chemotactic factors that attract effector cells. We have demonstrated that radiation enhances the expression of vascular cell adhesion molecule (VCAM-1) on intratumor vessels and increases the number of infiltrating host immune cells. Thus radiation may facilitate the ability of immune cells to gain access to tumors, even under situations where the radiotherapy is not curative. Results such as these emphasize the potential for combining conventional radiotherapy with immunotherapy and the need to develop appropriate protocols to prevent destruction of immune effectors.

(S26.4) Radiation and its impact on immunity in the tumor microenvironment. William McBride. UCLA, Los Angeles, CA, USA.

The battle between the tumor and host takes place in an ever-changing microenvironmental landscape. The disruption is typified by disorganized angiogenesis and poor vascular structure that has metabolic consequences and creates variable areas of high interstitial fluid pressure, low oxygen tension (hypoxia), low extracellular pH and low nutrient levels. The abnormal microenvironment generates an inflammatory response with infiltration by macrophages, neutrophils, lymphocytes and fibroblasts. The tumor site comes to resemble a chronic wound with a mosaic of pro-inflammatory and anti-inflammatory forces with high levels of cytokines, growth factors, and hormones that become a force to be reckoned with systemically as well as locally. Tumors therefore are continually reinvented phenotypically by the extreme physiological environment and by the host assault while the host co-evolves with changes in tumor phenotype and is also continually redefined. This interactive tumor-host microenvironment provides a platform that is as critical for malignant development as the malignant transformation process itself. Radiation therapy dramatically impacts this relationship. The impact it has will depend upon the nature tumor-host relationship but it is very likely to affect the likelihood of tumor cure. Our understanding how best to exploit the temporary imbalance in the tumor-host relationship following radiation therapy to allow the host to gain the upper hand and eliminate the tumor is still rudimentary, but there are models that show this is possible

even with non-immunogenic tumors and that light the way to further progress in combining radiation therapy with immunotherapy.

(S27.1) Can individual risk of adverse radiotherapy effects be predicted from genetic profiles? Christian Nicolaj Andreassen[1], Jan Alsner[2], Jens Overgaard[2]. [1]Dept. of Oncology, Aarhus, Denmark, [2]Dept. of Experimental Clinical Oncology, Aarhus, Denmark.

Over the last decade, increasing efforts have been taken to establish associations between various genetic germline alterations and risk of normal tissue complications after radiotherapy. Though the studies have been relatively small and methodologically heterogeneous, preliminary indications have been provided that single nucleotide polymorphisms in the genes TGFB1 and ATM may modulate risk of particularly late toxicity. In addition, rare ATM alterations may enhance complication susceptibility. Nevertheless, we are still far from having an exhaustive understanding of the genetics that may underlie differences in clinical normal tissue radiosensitivity. Recent technical advances and emerging insights to the structure of inter-individual genetic variation open up unprecedented opportunities to dissect the molecular and genetic basis of normal tissue radiosensitivity. However, to fully exploit these new possibilities well-planed large-scale clinical studies are mandatory. Currently, international initiatives are taken to establish the bio banks and databases needed for this task.

(S27.2) Need for large bio/outcome databanks to predict normal tissue radiosensitivity - the GENEPI-ENTB project. Tobias Hoelscher. Dep. of Radiation Oncology, TUD, Dresden, Germany.

To assess the genetic basis of the variability in the response to irradiation and to allow a validation of the results very large sample sizes are warranted. Such large cohorts can only be collected in multicenter efforts. The requirements for such multicenter research infrastructures are presented at the example of the European GENEPI-ENTB project. Aspects to be discussed: Selection and recruitment of patients, Common informed consent procedure and ethics counseling, Processing and handling of material, Assessment of patient, tumor, therapy related data, Assessment of radiation late effects and outcome, Database standards and security measures, Quality assessment procedures for stored data and tissue, Relevant tools for clinical users and researchers, Maintenance and long term availability, GENEPI-ENTB has been initiated 2003 as a pan European biobank and database to facilitate research in the field of radiation research and radiation protection. It is dedicated to research in the genetic determinants of the variation in individual radiation sensitivity. With more than 10.000 tissues of 5800 patients registered, GENEPI is currently among the largest dedicated infrastructures in this field. Funded by EURATOM / EC (FP6–036437)

(S27.3) Multiple genetic variants associated with risk of adverse skin reactions following radiotherapy in breast cancer patients. Takashi Imai, Mayumi Iwakawa. National Institute of Radiological Sciences, Chiba, Japan.

Radiotherapy for breast cancer patients occasionally induces adverse effects. The adverse reactions to radiotherapy are caused by multiple factors, including individual genetic differences. The heterogeneity in normal tissue reactions may result from the combined effect of several different genetic alterations. Single nucleotide polymorphisms (SNPs) and derived haplotypes within multiple genes may be used to detect the genetic alterations related to the heterogeneity in normal tissue reactions. In our previous multi-institutional study, nongenetic risk factors, such as operative procedure, magnitude of photon energy, and the use of multi-leaf collimeters, for adverse skin reaction (ASR) to radiotherapy among breast cancer patients were tested, and in the current study, only patients who were eligible for genetic analysis were enrolled. DNA samples were collected from 399 breast cancer patients. Using the NCI-CTC scoring system, the patients were grouped according to ASR within 3 months of starting radiotherapy (grade $\leq$ 1, n = 290 and $\geq$ 2, n = 109). The candidate genes for genotyping were selected by our gene expression analysis of mouse strains with different radiosensitivity, expression analysis of human cell lines with varied radiosensitivity, and bibliographic search. In total, 486 SNP sites on 99 genes which were polymorphic (allele frequency >5%) were analyzed. Variations in the candidate genes were considered as haplotypes because the statistical power of association tests using phased data is likely to increase and because analysis of haplotype frequencies enables the detection of predisposing haplotypes, even without typing the true functional SNP. Linkage disequilibrium maps were constructed and haplo-tag SNPs were selected for each locus. Global haplotype analysis using the Haplo.stats program (Schaid DJ et al.) indicated that estimated haplotypes in 6 loci, PTTG1, MAD2L, REV3, LIG3, RAD9A and CD44, were associated with ASR risk. In conclusion, Individual radiosensitivity may be partly determined by combinations of these haplotypes in multiple loci. This would provide an understanding of the mechanisms underlying the genetic variation in radiation sensitivity or resistance among the population, and would show the possibility of prediction of the risk of ASR prior to radiation therapy.

(S28.2) Microenvironmental factors impact the radiation response of neural precursor cells. John R. Fike[1], Radoslaw Rola[1], Yani Zou[2], Ting-Ting Huang[2], Kelly Fishman[1], Jennifer Baure[1], Susanna Rosi[1], Heather Milliken[1], Charles L. Limoli[3]. [1]University of California, San Francisco, San Francisco, CA, USA, [2]Stanford University, Palo Alto, CA, USA, [3]University of California, Irvine, Irvin, CA, USA.

Ionizing irradiation results in significant alterations in hippocampal neurogenesis that are associated with cognitive impairments. Such effects are influenced, in part, by alterations in the microenvironment within which the neurogenic cells exist; inflammation and oxidative stress are 2 of these factors. Oxidative stress is a biochemical mechanism that has been shown to regulate the fate of neural precursor cells, and we initiated a study to determine if and how the extracellular isoform of superoxide dismutase (SOD3, EC-SOD) mediated radiation-induced alterations in neurogenic cells. Wild type (WT) and EC-SOD knock out (KO) mice were irradiated with 5 Gy and acute (8–48 hr) cellular changes and long-term changes in neurogenesis were quantified. Acute radiation responses were not different between genotypes suggesting that the absence of EC-SOD did not influence mechanisms responsible for acute cell death after irradiation. On the other hand, the extent of neurogenesis was decreased by 39% in non-irradiated KO mice relative to WT controls. In contrast, while neurogenesis was decreased by nearly 85% in WT mice after irradiation, virtually no reduction in neurogenesis was observed in KO mice. Surprisingly there were more activated microglia in the irradiated KO than WT suggesting that such cells may have a beneficial effect when acting in an environment of increased oxidative stress. These findings show that after irradiation, an environment lacking EC-SOD is much more permissive in the context of hippocampal neurogenesis. This finding may have a major impact in developing strategies to reduce cognitive impairment after cranial irradiation. Supported in part by: NIH grants R01 NS46051 and AG24400, NASCOR grant NNJ04HC90G, and ACS grant RSG-00-036-04-CNE.

(S28.3) Sensing radiosensitivity of human epidermal stem cells. Michele T. Martin. CEA, Evry, France.

Radiosensitivity of stem cells is a matter of debate, as both radiosensitive and radioresistant stem cell populations have been described. We addressed in the present work the question of radiosensitivity of epithelial stem cells of human epidermis. The deepest layer of human epidermis contains two main types of keratinocytes. One is the rare and quiescent stem cell, responsible

for the long term renewal of human skin, the other one is its direct progeny, the keratinocyte progenitors, which are responsible for the short term renewal of epidermis (28 days). In this study, we used α6 integrin and CD71 to isolate by flow cytometry these two basal keratinocyte populations. Short-term cell survival was measured with the XTT assay and the long-term cell survival was evaluated with a colony forming assay. Both assays showed that the stem cells were radioresistant whereas the progenitors were radiosensitive. We made the hypothesis that upstream DNA damage signalling might be different in the stem cells ans tested this hypothesis using microarrays. The stem cells exhibited a much more reduced gene response than the progenitors, as we found that 1.5% of the spotted genes were regulated in the stem cells and 19% in the progenitors. Radiation exposure induced very specific pathways in the stem cells, notably cytokines and growth factors pathways, suggesting that the induction of the signal transduction of growth factors might be directly related to stem cell radioresistance. Secondly, we postulated that the repair of DNA damage itself might be different in the two types of cells. We used the γH2AX assay to evaluate the number of double strand breaks. At 5 minutes, similar numbers of foci were found in both cell types, suggesting that the difference in radiosensitivity was not related to variations in the initial numbers of induced DSBs. Later, foci were rapidly lost in stem cells and totally at 24 h. On the contrary, the foci were slowly lost in progenitors and a mean of 8 foci per nucleus persisted at 24 h. This result suggests that the repair of DSB was more efficient in stem cells than in progenitors. Taken together, these results show for the first time that keratinocyte stem cells from human epidermis are a radioresistant cell population, which has important implications for skin regeneration after radiotherapy.

(S28.4) Antioxidant gene therapy treatment of the irradiated organ microenvironment enhances stem cell engraftment. Joel S. Greenberger. University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA.

In addition to inducing apoptosis of resident stem cells and differentiated progeny, ionizing irradiation induces radical oxygen species (ROS) production by cells of the organ-specific microenvironment (including the esophagus, lung, and bone marrow). Bone marrow derived hematopoietic stem cell and progenitor engraftment during repair of radiation damage is enhanced by antioxidant transgene over expression. MnSOD-Plasmid Liposome administration intraorally or swallowed, facilitates decreased ROS, lipid peroxidation, cytokine elevation after irradiation and enhances resident stem cell recovery as well as bone marrow derived circulating cell progenitor engraftment of the oral cavity and esophagus. Bone marrow derived progenitors of esophageal squamous epithelium increase reconstitution of the irradiation damaged esophagus and side population cells removed from first generation recipient esophagus, transplanted I.V. to MnSOD-PL intra-esophageally treated second generation irradiated mice resulted in enhanced progenitor cell engraftment to the irradiated esophagus. Inhalation MnSOD-PL administration during single fraction or fractionated irradiation of the lung enhances bone marrow derived pulmonary stem cell progenitor migration. MnSOD-PL administration intravenously to total body irradiated mice enhances marrow stem cell engraftment. Thus, protection of the organ specific microenvironment from oxidative stress following irradiation, can facilitate resident stem cell repair as well as engraftment of bone marrow derived stem cell progenitors.

(S29.1) Electron microbeam studies of radiation induced bystander effects and adaptive responses. Marianne B. Sowa[1], Wilfried Goetz[2], Yijun Li[2], Janet Baulch[2], William F. Morgan[2]. [1]PNNL, Richland, WA, USA, [2]University of Maryland, Baltimore, MD, USA.

Historically low-LET bystander effects have been studied using indirect methods involving media transfer. These studies indicate that cells do not have to be traversed (hit) by radiation, or even in a radiation environment to elicit detrimental effects usually associated with radiation exposure. Using targeted irradiation protocols, we have been examining the role of the radiation environment in bystander and adaptive responses initiated by low-LET radiation. Using a variable energy electron source to mimic low-LET radiation, we have studied bystander and adaptive responses for gap junction positive and negative cells (AG1522 and RKO36, respectively). Various endpoints, including clonogenic survival, micronuclei formation, induced genomic instability and γ-H2AX foci formation, have been studied. Using the electron microbeam in a broad beam configuration, we have measured cell survival for both cell line lines following exposure to 50 keV electrons. Irradiations were performed at 37° C exposing 100% of the cell population. Identical dishes were irradiated with X-rays to provide a positive controls. Comparing this data to the dose response for x-rays, we found that more cells survive exposure to 50 keV electrons relative to the same dose of x-rays. Bystander experiments were performed using the electron microbeam to irradiate 100, 10, or 1% of each cell population with a lethal dose of 50 Gy. Following irradiation, cells were left in the irradiation medium for various times to allow diffusion of any soluble bystander factors. Cells were then trypsinized, re-plated and scored for clonogenic survival. Since the directly irradiated cells were lethally irradiated, all cells scored were bystanders or their progeny. Samples were monitored for both direct effects of being bystanders in a radiation environment and indirect effects where medium transfer recipient cells were never exposed to a radiation environment. Based on evaluation of cell survival in these direct irradiation or medium transfer experiments, we observe no evidence of a low-LET irradiation induced bystander effect for either cell line studied. Adaptive response experiments are currently underway.

(S29.2) Photon microbeams. Melvyn Folkard[1], David Hart[1], Alan Michette[2], Giuseppe Schettino[3], Giselle Flaccavento[1], Kevin Prise[3], Slawka Pfauntsch[2], Borivoj Vojnovic[1]. [1]Gray Cancer Institute, University of Oxford, Northwood, United Kingdom, [2]King's College London, London, United Kingdom, [3]Centre for Cancer Research and Cell Biology, Belfast, United Kingdom.

Microbeam methods are now seen as an important tool in the study of non-targeted effects (such as the bystander effect) and in other biological responses to low dose exposures. Most microbeams make use of focussed or collimated particles, however there are compelling reasons to also develop and exploit microbeams that use low-energy X-rays. An X-ray microbeam is more appropriate to study low LET effects and has the potential to form the finest probes (low energy X-rays interact almost entirely by the photoelectric effect and are therefore not scattered). It is also possible to develop 'tabletop' sources that are relatively compact compared to particle facilities. We have designed and built an X-ray microprobe that makes use of X-rays generated by bombarding a target with a focussed beam of electrons. The X-rays are focussed using a zone-plate diffraction lens. The targets are interchangeable between carbon, aluminium and titanium to allow the generation of 0.278 keV C_K X-rays, 1.48 keV Al_K X-rays, or 4.5 keV Ti_K X-rays. Preliminary biological experiments have begun using C_K X-rays. Initial measurements show the X-ray spot size to be about 2 μm. The measured photon count rate at the X-ray focus is ~5000 photon s^{-1} with the source operated at 10 kV, 300 μA. This allows doses to be delivered to a cell in less than one second for most studies. Although the facility is compact, synchrotron-based X-ray microbeams sources still have the advantage of being tuneable and of producing higher fluxes. To address this, a new project will develop and exploit novel 'smart' X-ray focussing methods, based on reflective optics. Such devices will be capable of high efficiencies, not least because they are achromatic, and therefore do not require monoenergetic radiation sources. These methods are being developed primarily at King's College London, in collaboration with the Universities of Edinburgh and Birmingham, while GCI is designing and building a new X-ray source capable of fully exploiting the new devices.

(S29.3) Imaging modes with the columbia university microbeam. Alan White Bigelow, Oleksandra Victorovna Lyulko, Gregory John Ross, Gerhard Randers-Pehrson, Charles Ray Geard,

David Jonathan Brenner. Columbia University - Radiological Research Accelerator Facility, Irvington, NY, USA.

At the Columbia University single-cell single-particle microbeam, technical innovations integrate multiphoton microscopy and phase-based, non-stain cell imaging to enable 1) imaging within tissue samples, 2) observing post-irradiation cell dynamics, and 3) targeting unstained cells during irradiation experiments. Microbeam experiments at Columbia University's Radiological Research Accelerator Facility (RARAF) have typically involved stained mammalian cells plated on a polypropylene substrate and imaged with fluorescence microscopy. While results from cell-culture experiments have contributed to studies on low-dose radiation effects, such as the bystander effect, radiation studies using tissue samples have the potential to represent cellular response within organisms. For observing post-irradiation cellular dynamics within tissue, multiphoton microscopy offers optical sectioning compatible with live samples and can image at depths of several hundred micrometers; multiphoton excitation of fluorochromes occurs at the focal point of a tunable, mode-locked Titanium Sapphire laser source. At RARAF, a custom multiphoton microscope design conforms to the geometrical constraints of an irradiation endstation with an available Nikon Eclipse 600FN microscope at the end of a vertical ion beam. To avoid UV illumination and potential cytotoxic additives for cell imaging during single-cell irradiation experiments, phase-based techniques using visible light have been investigated at RARAF for imaging unstained cells: immersion Mirau interferometry (IMI) and quantitative phase microscopy (QPm). IMI was developed at RARAF to image cells in medium; the region between the spot mirror and the beam splitter is backfilled with an immersion fluid that matches the index of refraction of cell-growth medium. IMI images are acquired using a series of interferograms with incremental ¼-wavelength path-length differences. With QPm, three reflected-light images are obtained: in focus and slightly above and below the sample plane. These images are then used to solve the light transport equation using Fourier transform-based software from Iatia. Combined, these developments enhance irradiation protocols to accommodate bulk samples and no-UV / no-stain imaging.

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(S29.4) A single-ion microbeam facility for cell irradiation: the experience at infn-laboratori nazionali di legnaro. Silvia Gerardi, Roberto Cherubini. INFN-Laboratori Nazionali di Legnaro, Legnaro (Padova), Italy.

Charged particle microbeam facilities are nowadays a powerful tool which allows the targeting of single cells and the analysis of the induced damage on a cell-by-cell basis, by contributing to investigate the single-particle effects characterizing the low-dose exposures. At INFN - Laboratori Nazionali di Legnaro a single-ion single-cell microbeam facility has been installed and dedicated to radiobiological studies related to low-dose effects. A mechanical collimator in air has been adopted to reduce the beam to micrometer section, comparable to cell size. Different materials (titanium, tantalum, silicon) and geometries (slits, pinhole, square aperture) have been tested to optimize the microcollimated beam quality in air by reducing the percentage of scattered particles and the beam spot size and maximizing the full-energy particle component. A Monte Carlo code for the transport of light ions along the facility has been also developed for such a study as a complementary tool to the measurements. A tantalum pinhole of 2 or 5 um diameter has proved to be the best solution in the current set-up. Before each irradiation experiment the microcollimator is properly aligned in terms of beam spectrometry and particle spatial distribution by using, respectively, a silicon detector and a cooled-CCD camera as high-granularity pixel detector. Cell recognition is performed with a phase contrast microscope coupled with appropriate LabView software. No additional fluorescent staining is used for cell visualization, the sample is scanned automatically under the microscope and cells are localized by the operator. In order to speed up this procedure, software for automatic recognition of unstained cells is under development. After cell coordinates are logged, the sample is moved from the horizontal position under the microscope to the vertical one in front of the beam. Cell coordinates are automatically recalled one-by-one and every cell is irradiated. During irradiation a silicon detector is placed downstream of the Petri dish: the number and energy of particles hitting each cell are monitored on-line and a trigger signal is sent to the electrostatic deflector to shut-off the beam, when the pre-set number of ions has been delivered.

The main features and performances of the facility are presented and discussed in the presentation.

(S30.2) Medical device industry perspective on collaborations with academia. Richard K. Morse. Calypso Medical Technologies, Inc., Seattle, WA, USA.

Academic scientists and industrial employees have a common interest in advancing the state of the art in a field of oncology. A successful collaboration between them may result in invention and/or publication using their combined talents and resources. There is also the academic dilemma of publishing early vs. patenting quietly. In either case, industry can provide funds, as well as proprietary materials, software or hardware to advance an idea. While some resources may be granted or loaned for philanthropic reasons, most are tied to a hoped benefit of a new or improved device that can be sold profitably. Improvements can include new information from clinical trial or lab work that can enhance the utility of the device to improve sales. Some examples from a large mature device company will be contrasted with those from a start-up company with fewer and more impatient owners. Both types of companies use similar research agreements with similar benefits and protections for the company and the institution.

(S30.3) Pharmaceutical industry perspective on collaborations with academia. David J. Chaplin. Oxigene Inc, Oxford, United Kingdom.

Biotech companies, particularly the small to mid size ones, can encounter serious challenges in obtaining the resources needed to rapidly build strong research and development capabilities. Indeed, accessing the variety of technologies associated with identifying and developing new therapeutics is an expensive, time consuming process fraught with uncertainties. This has led many biopharmaceutical companies to develop relationships with suppliers of these resources. A widely used strategy is for companies to develop links with universities. Links with leading researchers and universities serve other important purposes such as improving a firm's reputation and increasing its access to key sources of innovation. From a university perspective, links can provide access to emerging technologies including small molecules and biologics. Links with business can also generate funds needed to pursue other R&D projects. In spite of positive outcomes, collaborations can be problematic in terms of quality control and the fact that university scientists often have priorities that conflict with strict industry schedules. Incompatibilities between cultures such as secrecy versus free dissemination of knowledge can also become a major issue. In this presentation, I will highlight how the collaborations with academia have been central to the discovery and development of OXiGENEs two lead clinical compounds CA4P and OXi4503

(S31.1) Advances in the treatment of whole body and localized irradiation. Patrick Gourmelon. Institut de Radioprotection et de Sûreté Nucléaire, Fontenay-aux-roses, France.

Whole body irradiation presents unique characteristics which explain why healthcare management is so complex and why it is difficult to harmonise and standardise the methods of diagnostic and treatment. In order to face the threat of terrorist event, a consensus conference on European preparedness for haematological and other medical management of mass radiation accidents has been established by a working group of 65 physicians from the 25 European Union countries. The medical strategy of this consensus is a new original approach taking into account both the new paradigm of the radio-induced multiple organ failure (MOF) and the

heterogeneous characteristics of accidental irradiation which have a major impact on the indication of the haematopoietic stem cell transplantation and cytokine treatment. Localized irradiation at high exposure could induce severe radiation burns characterized by the occurrence of unpredictable successive inflammatory waves leading to the extension in surface and depth of necrotic processes. The medical management of these severe radiations burns remains today a challenging issue unresolved with the classical therapeutical approach derived from the management of thermal or electrical burns. In the framework of two recent radiological accidents where two victims had been overexposed to Iridium gammagraphy radioactive source we used innovative therapeutic strategies combining dosimetry-guided surgery lesion excision and cellular therapy with Mesenchymal Stem Cell (MSC). Thus, a very severe radiation burn was widely excised using a physical and anatomical dose reconstruction in order to better define the limit of the surgical excision in apparent healthy tissues. This surgery was associated to a local cellular therapy using autologous expanded MSCs as source of trophic factors to promote tissue regeneration. Bone marrow-derived MSCs were expanded using closed culture devices and serum-free medium. The clinical evolution was remarkable without recurrence of radiation inflammatory waves. This novel multi-disciplinary therapeutic approach combining physical techniques, surgical procedures and cellular therapy should be of clinical relevance for the management of severe localized irradiations and could open new prospects in the field of radiotherapy complications.

(S31.2) Major modification of radiation-induced damage by diet. Jennifer A. Lemon, C. David Rollo, Douglas R. Boreham. McMaster University, Hamilton, ON, Canada.

Dietary supplements, particularly antioxidants, have been studied extensively as radioprotectants with little success. We wanted to determine if a properly designed dietary supplement could provide significant protection from acute doses of ionizing radiation in normal and transgenic mice with elevated levels of endogenous free radicals. The *in vivo* protective effects of the diet supplement were tested on a range of endpoints. In bone marrow, we measured chromosome aberrations in all cell types using spectral karyotyping. In the G0/G1 components of the cell population, we measured DNA damage and repair by monitoring γH2AX. Oxidative base damage was determined by levels of 8-OHdG. Apoptosis in lymphocytes was measured using a flow based Annexin/7-AAD assay. Also, PET and SPECT scanning were used to study brain function.

When the mice were gamma-irradiated with 2Gy, 46.6±5.3% of bone marrow cells had chromosome aberrations, whereas only 8.8±2.5% of cells from supplemented animals contained aberrations. There was a reduction of greater than 130% in both γH2AX (corresponding to number of foci) and 8-OHdG fluorescence showing that DNA damage was significantly reduced in diet supplemented mice. Lymphocyte apoptosis decreased from 40% apoptotic cells post-irradiation in unsupplemented animals to 18% apoptosis in diet supplemented mice. PET and SPECT scans revealed that the diet supplement completely protected against the loss of basic brain functions. Effectively, the diet supplement prevented the destruction of greater than 56% of brain cells by chronic oxidative stress-associated processes. Given that our supplement targets several free radical-based processes; our data suggests that the dietary supplement is likely to provide significant protection from the degenerative late effects of radiotherapy. Most of these complications including deterioration in cognitive function and lung fibrosis are known to be driven by late oxidative processes.

(S31.4) Cytokines and radiation treatment: mitigators or mitigating targets? Jacqueline P. Williams, Eric Hernady, Carl Johnston, Christina Reed, Jacob N. Finkelstein. University of Rochester Medical Center, Rochester, NY, USA.

Agents that counteract the effects of radiation following an accident or terrorism incident fall into a number of different potential treatment categories depending on the target population. For example, radioprotectors are used by first responders and military personnel moving into an incident area; for those who have been exposed to a radiation exposure, agents fall into either treatment or mitigation roles, depending on the dose and time post-exposure. Secondarily, but of importance, it would be hoped that many of the agents that are developed also could play a role in cancer treatment. A limited number of preclinical studies have investigated the use of cytokines as radioprotectors, such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α, although the main focus of such studies has been on the use of cytokines and growth factors in the mitigation of the acute syndrome, with some degree of success. Clinically, granulocyte colony stimulating factor (G-CSF), GM-CSF, and IL-11 have FDA approval in the area of myelosuppression and, therefore, may find use in the treatment of that component of the acute radiation syndrome. Examples of other potential candidates that may find broader utility are IL-1, IL-4, and interferon (IFN)-γ, all of which have been shown to restore hematopoietic cells in animal models. However, over the past decade, a number of investigators have demonstrated that cytokines and growth factors play a major role in the body's response to radiation injury, not only during the acute response period, but in particular when the development and progression of late effects are brought into consideration. Many of these same cytokines also have been shown to play pivotal roles both in the maintenance of normal tissue homeostasis and during wound repair, which has led some to suggest that it is cytokine dysregulation that underlies the development of radiation-induced late effects. This finding therefore begs the question as to whether cytokines should be used as therapeutic agents or themselves be the targets of manipulation. The role of cytokines in the response to radiation injury will be reviewed in the context of past and current findings, their choice as targets rather than agents will be discussed, as well as their potential as surrogates for response. Supported by funding from NIAID/NCI (U19 AI067733).

(S32.1) Experimental determination of track structure in ion beam irradiated DNA. Michael D. Sevilla[1], David Becker[1], John Zimbrick[2], Michael Bowman[3]. [1]Oakland University, Rochester, MI, USA, [2]Colorado State University, Fort Collins, CO, USA, [3]University of Alabama, Tuscaloosa, AL, USA.

Track structure calculations have progressed substantially but have been limited by the few experimental determinations of track structure especially at the level of the distribution of the initial radical species formed. In this talk results are presented from a series of experiments using ESR (electron spin resonance) and pulsed electron paramagnetic double resonance (PELDOR or DEER) techniques that have given information about the spatial properties of trapped radicals produced in heavy-ion-irradiated solid DNA at 77 K . These are compared to earlier studies on the size of the spur in γ irradiated solid DNA. Hydrated DNA samples at 77 K were irradiated with argon-ion beams (^{36}Ar or ^{40}Ar beam at energies between 60 and 100 MeV/nucleon and LET ranging from 300 to 800 keV/mm). The individual free radicals formed were identified and their yields were found using ESR spectroscopy (Radiat. Res. 160. 174 (2003)). Argon-ion irradiation resulted in lower yields of base ion radicals and higher yields of neutral radicals than γ irradiation. The lower *G* values and *k* values for ion radicals and the higher fraction of neutral radicals found for argon-ion-irradiated DNA are directly related to differences in track structure inherent in the two radiations. PELDOR measurements were made using a refocused echo detection sequence that allows dipolar interaction between trapped radicals to be observed (Radiat. Res. 163, 447 (2005)). The EPR spectrum is attributed to electron loss/gain DNA base radicals and neutral carbon-centered radicals that likely arise from sugar damage. We find a radical concentration of 1.4x 10^{19} spins/cm^3 in the track core and a track core radius of 6.79 nm. The cross section of these tracks is 144 nm^2, yielding a lineal radical density of 2.6 radicals/nm. This radical density is far higher than the overall radical density. These values are compared to those expected from track structure calculations. These measurements of radical density and spatial extent provide the first direct experimental determination of track characteristics in irradiated DNA. Supported by the Structural Biology Program of the OBER, US DOE and by NIH grants R01 CA45424, CA80211, and GM61904.

(S32.2) DNA damage and repair in human cells following exposure to charged particles. Maria Antonella Tabocchini. Istituto Superiore di Sanità, Rome, Italy.

Reduction of uncertainties associated with radiation health risk during long term space flights, as well as development of effective countermeasures, require increasing knowledge of the biological effects of HZE particles. Although less abundant than protons, these are more effective in damaging biological systems. It is thought that this is due to the production of spatially correlated and/or clustered DNA damage, in particular double strand breaks (DSB), that being difficult to repair accurately can lead to severe consequences for the cell.

Various approaches have been undertaken to characterize DNA breakage and to follow the kinetics of DSB repair in human cells irradiated with charged particles. Most measurements rely on pulsed-field gel electrophoresis as the technique of choice. DNA fragmentation studies have shown that both the yield and the spatial distribution of DSB are influenced by radiation quality as well as the rejoining kinetics are. The spatial correlation of DSB, in particular, seems to reflect the differences in biological effectiveness for cellular damage among different radiation types. Experiments aimed to evaluate the efficiency of different shields have shown that protection depends not only on their type and thickness but also on the beam energy. Recently, immunofluorescence techniques have been developed based on antibodies against proteins involved in DNA damage response. They have been applied to analyze the morphology of the fluorescent foci to be used as an indicator of the DNA damage severity, and to study the time course of DBS induction and processing. In particular, detection of phosphorylation of the variant histone H2AX (γ-H2AX) has provided a valuable and highly sensitive method to monitor DSB formation, and persistence of γ-H2AX has been suggested as potential predictor of cell radiosensitivity. Although the role of H2AX phoshorylation and dephosphorylation in DSB repair is not completely understood, this functional technique is at the present the only one that allows investigation of DNA damage and repair in single cells at doses as low as those released by one particle traversal, and in situations where the contribution of both targeted and non-targeted effects is relevant for the biological response of the overall cell population.

(S32.3) Charged particle mutagenesis and genomic instability in vitro and in vivo. Amy Kronenberg[1], Hiroko Sudo[1], James Garbe[1], Martha Stampfer[1], Mary Helen Barcellos-Hoff[1], Stacey Gauny[1], Ely Kwoh[1], Cristian Dan[1], Mitchell Turker[2]. [1]Lawrence Berkeley National Lab, Berkeley, CA, USA, [2]Oregon Health and Science University, Portland, OR, USA.

Cancer is a major concern for humans exposed to galactic cosmic radiation (GCR). Our research considers two aspects that impact carcinogenesis: mutagenesis at specific loci and the induction of genomic instability. Cancer results from the accumulation of mutations and other heritable changes in susceptible cells. Most mutations involved in carcinogenesis occur on autosomes. In studies with 1 GeV/amu Fe ions, we considered mutagenesis in human lymphoid cells or mouse kidney epithelial cells. We studied two autosomal loci: the *TK1* locus in human cells , on chromosome 17q, and the *APRT* locus on mouse chromosome 8. Mutations at these loci can include intragenic changes, deletions up to at least 64 cM of DNA (*TK1*) or 67 cM of DNA (*APRT*), and loss of heterozygosity by allelic recombination. Most autosomal mutations (>90%) seen after Fe ion exposure involved loss of heterozygosity that frequently extends over tens of cM of DNA. Similar patterns of mutation were seen in mouse kidney epithelial cells exposed *in vitro* or *in vivo*. Another hallmark of cancer is genomic instability. We monitored genomic instability in human mammary epithelial cells (HMEC) with a finite life-span since the breast is susceptible to radiogenic cancer and instability is observed early in breast cancer progression. We collected clones that survived Fe ion exposure and monitored centrosome hyperamplification and karyotypic instability many generations post-exposure. We observed a dose-dependent increase in centrosome hyperamplification in these HMEC. More than 60% of irradiated colonies had increased proportions of cells with supernumerary centrosomes. Severe karyotypic instability was noted in 20% of Fe exposed clones but not in control colonies. Thus, destabilization of the genome is a very common event following Fe exposure and is likely driven by epigenetic changes. In sum, densely ionizing Fe ions elicit large scale loss of heterozygosity on autosomes in human and mouse models, which may lead to tumor suppressor gene inactivation. Fe ion exposure also results in a persistent state of instability in a high proportion of surviving cells. Both processes are likely to contribute to the carcinogenic potential of this important component of the GCR. Supported by NASA grant T-903X (A. Kronenberg) and the NSCOR in Radiation Health (M.H. Barcellos-Hoff).

(S32.4) Charged particle radiation effects in neural cells and tissues. Charles Limoli[1], Atefeh Izadi[1], Erich Giedzinski[1], Radoslaw Rola[2], John Fike[2]. [1]University of California, Irvine, Irvine, CA, USA, [2]University of California, San Francisco, San Francisco, CA, USA.

The mammalian brain contains pools of neural precursor cells concentrated in the subventricular and hippocampal dentate subgranular zones, that retain their ability to proliferate, migrate and differentiate in response to multiple stimuli throughout the lifespan of an organism. We have shown that high and low LET ionizing radiation elicits damage that triggers a stress response involving the onset of oxidative stress, apoptosis, cell cycle perturbations, mitochondrial alterations, changes in radiosensitivity, neuroinflammation, and disruptions to the phenotypic composition of differentiated neural precursor cell progeny. These observations correlate to the inhibition of neurogenesis and onset of cognitive decrements found after irradiation, suggesting that these cells play pivotal roles in the repair of the irradiated CNS. We have isolated neural precursor cells to elucidate the mechanisms that might regulate the response of these cells to charged particle and photon irradiation. Our data have shown that gamma rays, protons and iron ions elicit oxidative stress over acute and chronic times post-irradiation. Depending on dose and post-irradiation time, exposure to 56Fe ions generally elicits 3–4 times more reactive oxygen and nitrogen species than lower LET radiations. However, neural precursor cells subjected to 56Fe-irradiation show significant and dose-responsive increases in oxidative stress that occur in the low dose range of 1–10 cGy. Importantly, low doses of 56Fe ions induce a very persistent oxidative stress, that can be as high as 30-fold over unirradiated controls 2 months after prior exposure to 10 cGy. The marked persistence in radiation-induced oxidative stress shows no signs of abatement, and can in part be accounted for by increased superoxide output from the mitochondria. Neural precursor cells are also sensitive to changes in the microenvironmental redox state that impact their radiosensitivity and ability to differentiate into neurons. Based on these observations, we maintain that radiation-induced oxidative stress is a critical biochemical mechanism regulating the function of neural precursor cells. Our data also suggests that antioxidants may be useful neuroprotective countermeasures for astronauts, and data will be presented in support of this idea.

(S33.1) Gene networks and pathways induced in the CNS of mice exposed to low-dose ionizing radiation. Andrew J. Wyrobek, Sanchita Bhattacharya, Francesco Marchetti. Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

Whole-body exposure of male mice to low-LET radiation altered the transcript expression in their central nervous system (CNS), with distinct time- and dose-dependent clusters and identified low-dose unique gene sets (Yin et al. 2003). In the current study, we applied bioinformatics to identify the major gene networks and biochemical pathways that were uniquely associated with low-dose versus high-dose exposures as well as pathways shared across doses. A set of 395 genes was modulated (>1.5 fold) at 4 hours after low-dose exposure (0.1 Gy) and 406 genes after high dose (2 Gy). Of these, 123 and 134 were unique to low dose and high dose, respectively, while 272 were in common. For the low-dose genes, we identified two major networks, each populated with 35 genes that were modulated in irradiated brain tissue ($p<10^{-50}$). These networks contained nodes such as Jun, Grin1, Ywhaz, and Ywhay, with several examples of genes known to be involved in human neurological diseases. For example, Grin1, a

glutamate receptor whose expression was down regulated after low-dose radiation, is known to be associated with schizophrenia. Among the low-dose genes, there was significant pathway enrichment for genes associated with integrin, Huntington disease, and calcium signaling, as well as, for oxidative phosphorylation, synaptic long-term depression and potentiation, and ubiquinone biosynthesis. Among these pathways, synaptic long-term depression and potentiation pathways were significantly enriched after low dose, but not high doses. Q-PCR and western blotting are being used to validate the genes in these networks and pathways in irradiated brain regions. Our results support the model that brain tissue exposed to low-dose radiation employs unique molecular response pathways not observed after high doses, which underscore the problems that will be encountered when using high-dose data to infer low-dose mechanisms and to assess low-dose CNS radiation risks. Supported by the DOE Low Dose program.

(S33.2) Adaptive response to extremely low conditioning doses of low LET radiation. Pamela J. Sykes. Flinders University and Medical Centre, Bedford Park, South Australia, Australia.

There is a large body of literature demonstrating chromosomal damage and detrimental health effects after high dose radiation exposure. However, the vast majority of the population is only likely to be exposed to very low doses of diagnostic or occupational radiation exposure. It is now clear that cells respond to low doses of radiation in a different manner to that of high doses. High doses induce "SOS"-type responses. Low doses (conditioning doses) induce stress responses that likely regulate homeostatic control mechanisms with overcompensation leading to overall health benefits, including a reduction in tumorigenicity. This latter response is termed an adaptive response. We have been studying the effect of extremely low doses of radiation on chromosomal inversion in the pKZ1 transgenic mouse assay. In this assay, mice are treated with X-radiation and the tissues are examined by histochemical staining for cells in which inversion of the *E.coli lacZ* transgene has occurred resulting in expression of *E.coli* β-galactosidase. We have demonstrated that single acute whole body doses of X-rays as low as 1 μGy can induce an adaptive chromosomal inversion response in pKZ1 spleen and prostate. The adaptive response protects from inversions normally caused by higher doses of radiation as well as from endogenous inversions. These low doses are relevant to radiation exposure received by the general public. The magnitude of the adaptive response is similar for conditioning doses ranging over several orders of magnitude of dose suggesting an on/off response. A low conditioning dose prevents inversions induced by a high challenge dose whether administered a few hours before or after a high challenge dose, demonstrating that the adaptive response is an earlier temporal response than the resolution of chromosomal inversions. The signals responsible for the adaptive response act as natural radioprotectors. Identification of dose thresholds below which natural radioprotectors are operating will provide a more rational approach to radiation protection compared to the linear no-threshold model, and utilisation of natural radioprotectors may provide novel anti-cancer agents. This research was funded by the Low Dose Radiation Research Program (BER), U.S. DOE, grants # DE-FG02-01ER63227 and # DE-FG02-05ER64104.

(S33.3) Combined effects of low dose/dose-rate irradiation and a carcinogenic agent. Kazuo Sakai[1], Toshiyasu Iwasaki[2], Yuko Hoshi[2], Takaharu Nomura[2], Hiroshi Tanooka[1], Shizuko Kakinuma[1], Yoshiko Amasaki[1], Kazumi Yamauchi[1], Mayumi Nishimura[1], Tatsuhiko Imaoka[1], Yoshiya Shimada[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Central Research Institute of Electric Power Industry, Tokyo, Japan.

A group of 5-week-old female C57BL/6N mice, 35 in each group, was irradiated with gamma-rays at 1.2, 0.65, or 0.35 mGy/hr for 35 days. The mice were then injected in the groin with 0.05 mg of 20-methylcholanthrene (MC) dissolved in olive oil and irradiation was continued. A statistically significant suppression of cumulative tumor incidences up to 200 days after MC injection was observed in the group irradiated at 1.2 mGy/hr. TD_{50} technique revealed that the ability for tumor rejection was increased in the irradiated mice. The incidence of thymic lymphoma induced by *N*-ethyl-*N*-nitrosourea (ENU) in B6C3F1 mice was synergistically increased by 4 weekly X-irradiation with 0.8 or 1.0 Gy given prior to the ENU administration. When the weekly dose was reduced to 0.2 or 0.4 Gy, on the other hand, the suppression of tumor incidence was observed. These results indicated that the low dose/dose-rate irradiation may suppress the process of chemical carcinogenesis, while larger doses resulted in its enhancement.

(S33.4) Exploring the mechanisms of the radioadaptive response at chernobyl. Brenda E. Rodgers[1], Jeffrey K. Wickliffe[2], Kristen M. Holmes[3], Adam, D. Brown[4]. [1]Texas Tech University, Lubbock, TX, USA, [2]University of Texas Medical Branch, Galveston, TX, USA, [3]M.D. Anderson Cancer Center, Houston, TX, USA, [4]The University of Texas Health Science Center, San Antonio, TX, USA.

The genetic consequences resulting from environmental exposure to ionizing radiation have a significant impact on both radiation regulatory policies and the comprehension of the human health risks associated with radiation exposure. The Chernobyl environment is composed of low-LET, low dose-rate ionizing radiation (IR), primarily γ radiation from ^{137}Cs and ^{90}Sr and is unique as a natural laboratory in which environmental exposures to IR can be assessed. Previous studies conducted by our research team in Chernobyl, utilizing a variety of endpoints, have demonstrated no increase in mutagenesis in either native species or inbred strains of laboratory mice experimentally exposed to the Chernobyl environment. Rather than deleterious effects from exposure, a radioadaptive, or protective response has been documented in all cases. The lack of detectable mutations is likely the result of physiological alterations that mitigate the deleterious effects of ionizing radiation. To gain insight into the mechanism of the radioadaptive response, additional experiments were conducted examining transcriptional responses in genes thought to play a role in radioadaptation. Transcriptomics (quantification of mRNA levels) using oligonucleotide genearrays encompassing virtually the entire mouse genome was employed to provide the broadest possible view of potential responses. Genes expected to respond to insult from IR with altered transcription levels include those crucial to oxidative stress, DNA repair, cell cycle regulation, oxygen radical scavenging and apoptosis pathways. Ingenuity Pathway Analysis ® ver 4.0 was employed to assess relationships between genomic networks and to determine involvement in the cellular processes in question. Only a single DNA repair gene was differentially expressed in irradiated animals versus controls. No genes known to directly interact with primary DNA repair proteins exhibited altered levels of transcription. Altered transcription levels were observed in a significant portion of the machinery of the apoptosis pathway.

(S34.1) Independent generation of reactive and metastable intermediates for elucidating the effects of ionizing radiation on DNA. Marc M. Greenberg. Johns Hopkins University, Baltimore, MD, USA.

Ionizing radiation damages DNA via the formation of radical and radical ion intermediates. Reactive intermediates produced include those resulting for formal addition to nucleobases and hydrogen atom abstraction. These reactive intermediates give rise to direct strand breaks, cross-links, and nucleotide modifications (lesions). Elucidating the chemical and biological roles of any one of these reactive intermediates or lesions is complicated by the indiscriminate damage to DNA caused by ionizing radiation. Organic chemistry is a powerful tool for elucidating the roles of individual reactive intermediates by enabling their independent generation at defined sites within DNA from chemically synthesized photolabile nucleotides. This approach has revealed unrecognized DNA damage processes, such as interstrand cross-link formation, and helped resolve mechanistic controversies, including the controversial subject of electron transfer in DNA. In addition, the ability to synthesize oligonucleotides containing lesions, some of

which are chemically unstable (such as oxidized abasic lesions), facilitates in vitro characterization of their interactions with polymerase and repair enzymes, as well as determination of their effects on replication in cells. Studies using oligonucleotides containing site specifically incorporated DNA lesions have unveiled surprising secrets, such as the formation of DNA-protein cross-links between lesions and repair enzymes. Vector experiments using plasmids containing DNA lesions have revealed unexpected and biologically significant effects on replication. Recent results obtained using this approach will be presented.

(S34.2) DNA damage by decay of auger emitters I-123, I-124 and I-125. Pavel Lobachevsky, Roger Martin. Trescowthick Research Laboratories, Peter MacCallum Cancer Centre, East Melbourne, Australia.

Purpose: The purpose of our work was to study DNA damage arising from decay of Auger electron emitting radionuclides in the context of their potential use in cancer therapy and imaging. Decay of DNA-associated ^{125}I induces cytotoxic DNA damage, and can be targeted to DNA of tumour cells using labelled DNA ligands conjugated to tumour specific antibodies. The relatively long half-life of ^{125}I (60 days) have prompted investigation of shorter-lived isotopes, namely ^{123}I and ^{124}I, with half-lives of 13 h and 4.2 d, which however are known to have a "weaker" Auger cascade compared to ^{125}I. ^{124}I is an Auger emitter of particular interest since it is also a positron emitter, enabling tumour targeting to be monitored by Positron Emission Tomography (PET) imaging. Our main objectives were to compare the efficiencies of DNA strand break induction by decay of radionuclides ^{123}I, ^{124}I and ^{125}I, to study the effect of the isotope position relative to DNA using different labelled DNA ligands, and to investigate mechanisms leading to DNA breakage. *Experimental procedures:* We studied DNA single-stranded breaks (SSB)and double-stranded breaks (DSB in the plasmid DNA assay system, in which DNA breaks are detected as the conversion of the native supercoil plasmid molecule to relaxed and linear species. To target Auger decay to DNA, we used DNA binding ligands (*meta-)* iodo Hoechst 33258 and *para*-iodo Hoechst labelled with radionuclides ^{123}I, ^{124}I and ^{125}I. *Results:* Our results using radiolabelled iodo Hoechst 33258 demonstrated that for ^{124}I, the probability of DNA DSB induction (plasmid linearization) per decay is 0.54, compared to 0.62 for ^{123}I and 0.82 for ^{125}I. Probabilities of DNA DSB induction per decay with the use of *para*-iodo Hoechst were 0.58 and 0.85 for ^{124}I and ^{125}I respectively. *Conclusions:* Although the breakage efficacy is lower for ^{123}I and ^{124}I compared to ^{125}I, it is high enough to consider potential therapeutic applications. In the case of ^{124}I, the additional feature of positron emission, introducing the possibility of combining PET imaging with the therapeutic (Auger) modality, has obvious advantages. Our results also indicated that *para*-iodo Hoechst might be more efficient than iodo Hoechst 33258 vehicle for Auger emitters.

(S34.3) Sequence selectivity of guanine oxidation by biological oxidants. Yelena Margolin[1], Vladimir Shafirovich[2], Nicholas E. Geacintov[2], Peter Dedon[1]. [1]MIT, Cambridge, MA, USA, [2]New York University, New York, NY, USA.

Selective oxidation of guanine runs in double-stranded DNA has been previously noted for a variety of oxidizing agents, including photoactive Rh(II)-phenalthrolines, anthraquinones, naphthalimides, pterins, and riboflavin; metal-based systems such as Cu(I)/benzoyl peroxide and Ni(II) in the presence of SH compounds; as well as direct laser irradiation. This generalized sequence selectivity has been interpreted in the context of charge transfer process, in which an electron hole created on a guanine base in the initial oxidation step travels through the π-stack and is trapped at sites of lowest ionization potential, resulting in a guanine oxidation product. We will describe our studies with biological oxidants that do not conform to the rules of charge transfer and display unexpected sequence selectivities: nitrosoperoxycarbonate and hydroxyl radicals. The results will be discussed in the context of chemical mechanisms of initial guanine oxidation steps and subsequent oxidation product formation.

(S34.4) Spectrum of radiation-induced DNA damage in cells. Thierry Douki, Olivier Falletti, Jean-Luc Ravanat, Jean Cadet. CEA, Grenoble, France.

Several pathways lead to the formation of DNA damage upon exposure of cells to ionizing radiation. Two of them involve the interaction between matter and the incident photon or particle. This process leads to either direct ionization of DNA or radiolysis of water into reactive species such as the hydroxyl radical that may then induce damage to the 2-deoxribose and base moieties of DNA. Using specific measurements of modified bases, we found that the indirect effect is predominant, even with high LET particles such as heavy ions. The yield of radiation-induced oxidized bases was found to be quite low, in agreement with a major role played by complex clustered damage in the deleterious effects of ionizing radiation. These reactions taking place along the incident track may not be the only ones to consider in order to getting a complete view of the radiation-induced insults to the genome. First, evidence has been obtained for the occurrence of an oxidative stress long after the end of the irradiation. Deleterious processes associated with the bystander effect have also been shown to be mediated by an oxidative stress. The reactive oxygen species produced under these conditions might have different mode of DNA degradation than those involved in the indirect effect, giving rise to a different damage distribution with a likely lack of clustered lesions. For instance, we have studied the oxidative properties of peroxyl radicals that behave as one-electron oxidant and mostly induce guanine damage. Moreover, DNA is not the only cellular target of radiation. Genotoxic pathways involving degradation products of other biomolecules have also been proposed. These include protein hydroperoxides or breakdown-products of lipid peroxidation. Among the latter, 4-hydroxynonenal has been extensively studied and proposed to be a second messenger of oxidative stress. In summary, growing amounts of information suggest that interaction between incident radiations and nuclei may not be the only source of genotoxic processes. Further data are needed to link other aspects of oxidative stress to radiation-induced DNA damage.

(S35.4) Radiation chemistry of supercritical water. Yosuke Katsumura. University of Tokyo, Tokyo, Japan.

The chemical condition of the coolant water in light water reactors at around 300°C is controlled by the radiolysis. In order to keep the integrity of the reactor and to predict of the distribution of water decomposition products in the reactor, the understanding of water radiolysis at elevated temperature is inevitably important. Recently, a new concept of the reactor taking supercritical water (>374°C, >22.1MPa) as a coolant, so called, supercritical water-cooled reactor (SCWR) has been proposed [1] and we started the pulse radiolysis study of supercritical water to obtain the basic data for the development of the SCWR. We have detected the hydrated electron in supercritical water for the first time [2]. The absorption band of the hydrated electron shifts significantly to longer wavelength with increasing the temperature and absorption coefficient as a function temperature is not known. Then, the yield is measured after the electron is converted to the secondary radical by using the suitable scavengers. We have determined the $G(e^-_{aq})$ up to 400°C by using methylviologen as a scavenger [3]. The yield of the sum of $G(e^-_{aq})$+G(H)+ G(OH) was also determined in the presence of ethanol. It was found the yield is strongly dependent not only on temperature but also on the density of water and the value is larger at lower densities. For the measurement above 400°C we introduced 4,4'bipyridyl as a scavenger and we succeeded the measurement up to 500°C. The pressure dependence of $G(e^-_{aq})$ is becoming smaller with further increase of the temperature and almost disappears at 500°C.

(S36.1) Molecular mechanisms of radiation induced intestinal inflammatory damage. Meritxell Molla[1], Julian Panes[2]. [1]Centro Medico Teknon, Barcelona, Spain, [2]Hospital Clinic, Barcelona, Spain.

Radiation induces an important inflammatory response in the irradiated organs, characterized by leukocyte infiltration, that is regarded as one of the main determinants of radiation-induced organ damage. Recently, a considerable investigative effort has beed directed at determining the molecular mechanisms by which radiation mediates leukocyte infiltration in order to create strategies to prevent intestinal inflammatory damage. Studies using in vivo intravital microscopy have that inflammatory cell infiltration into the intestine venules occurs as early as2 hours after irradiation, with a marked increase 24 hours after. This inflammatory response depends on radiation characteristics as total dose, dose-rate and time elapsed after radiation. Radiation induces inflammation in the intestine by upregulating the expression of various adhesion molecules including intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells. Mice genetically engineered to lack the ICAM-1 gene (Knockout mice) and treatment with anti-ICAM-1 antibodies significantly reduce leukocyte adhesion and prevent the increase in vascular permeability at early time points after irradiation indicating that ICAM-1 is a key molecular determinant in the firsts steps of inflammatory radiation response. The upregulation of VCAM-1 expression and VCAM-1 immunoneutralization occurs 14 days after irradiation, suggesting that VCAM-1 is the main molecular determinant of leukocyte recruitment in late time points. Proinflammatory mediators have also been proposed to contribute to the pathogenesis of radiation-induced intestinal damage. Radiation induces an increase in leukotriene B4 production, and evidence has been provided showing that blockade of LTB4 synthesis markedly attenuates leukocyte adhesion. Basic research has thus contributed to the identification of molecular targets to prevent radiation-induced intestinal damage that should be tested in the clinical setting.

(S36.2) Radiation induced telangiectasia. Jacqueline JCM Kruse, Debbie Sprong, Nicola S. Russell, Fiona A. Stewart. The Netherlands Cancer Institute, Amsterdam, The Netherlands.

Improvements in treatment of cancer have lead to increased numbers of long-term survivors at risk for developing treatment related tissue damage and mortality. Late effects that develop in normal tissues after radiotherapy (RT) can seriously reduce the quality of life of cancer survivors. A late complication of RT for carcinoma of the rectum or prostate cancer is severe rectal bleeding, often requiring abdominoperineal resection (APR). Rectal bleeding occurs as a result of radiation-induced telangiectasia, which is characterised by abnormal dilated capillaries that create focal lesions in mucous membranes. Mechanisms whereby telangiectasia develop are not understood. Telangiectasia is not unique to radiation injury. Similar processes occur in diseases, such as hereditary haemorhagic telangiectasia (HHT) and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Mutations in ALK1, endoglin and Notch were found to segregate with the presence of the syndrome in HHT and CADASIL families. These divergent diseases are characterised by dysregulation of vessel branching/ maturation; processes for which proper Transforming Growth Factor-beta (TGFß) and Notch signaling are required. The purpose of the study is to investigate the influence of radiation on TGFß and Notch signaling pathways in human microvascular endothelial cells (HMVECs) in vitro. Irradiation of HMVECs resulted in a reduction of ALK1 signaling with decreased production of its downstream target Id1 and stimulation of ALK5 signaling with increased production of PAI1. Radiation induced PAI1 was abolished in the presence of the ALK5 inhibitor SB-431542, indicating that PAI1 is at least partly dependent on ALK5 signaling. Radiation induced increases in Notch ligand Jagged1 and downstream target Herp3 were observed. These changes suggest an important role in the downregulation of Id1. The functional consequences of radiation induced changes in signaling were determined by studying the migratory behavior of HMVECs using a scratch assay. Ionizing radiation reduced the number of cells migrating into the scratch area. In conclusion, radiation disturbs TGFß/Notch signaling in HMVECs in vitro. These changes might be implicated in EC functional defects and subsequent development of telangiectasia.

(S36.3) Cytokine cascades and radiation-induced fibrosis. Marie-Catherine Vozenin-Brotons. IRSN/IGR, Villejuif, France.

Delayed radiation enteropathy in radiotherapy patients is characterized by a transmural fibrosis. This pathological and excessive deposition of extracellular matrix, that impairs intestinal functions, is achieved by the resident mesenchymal cells (subepithelial myofibroblasts, fibroblasts, smooth muscle cells) chronically differentiated into secretory cells. The local release of cytokines and growth factors that occurs immediately after irradiation is responsible for the onset of the fibrogenic differentiation and while the initial steps of normal wound healing and fibrogenesis are thought to be similar, fibrosis is characterized by a self-perpetuating cycle of cytokine generation and secretion that causes chronicle cell differentiation. Today, TGF-β1 is known as the primary inductor of the fibrogenic process and its fibrogenic signals are mediated through two TGF-specific Ser/Thr kinase receptors leading to downstream activation of the transcription factors Smad 3 and 4. This pathway triggers direct transactivation of fibrosis-related genes including the α-sm actin, connective tissue growth factor and extracellular matrix (collagens, fibronectin). But while the role of TGF-β1/Smad3/4 in the initial steps of the fibrogenic differentiation is clear, studies performed in our laboratory and others with various models of established fibrosis (radiation enteropathy, radiation skin fibrosis, scleroderma) questioned it's relevance during the phase of maintenance. Recently, activations of alternative pathways including Rho/ROCK, Smad1 and ERK1/2 have been proposed. These novel findings have significant implications for the understanding of the long-term persistence of fibrosis and are likely to impact the development of anti-fibrotic treatments.

(S36.4) Endothelial dysfunction: key to the chronicity of intestinal radiation fibrosis. Martin Hauer-Jensen. Arkansas Cancer Research Center, Little Rock, AR, USA.

An increasing body of evidence supports the notion that dysfunction of the vascular endothelium plays a pivotal role in the development of early (inflammatory) and delayed (fibrotic) radiation responses in the intestine and other normal tissues. Clinical and preclinical data from our laboratory has demonstrated that exposure to ionizing radiation causes rapid loss and sustained deficiency of endothelial thrombomodulin (TM), an endothelial cell glycoprotein with potent anticoagulant and anti-inflammatory properties. Deficient levels of TM result in insufficient activation of Protein C. This leads to increased thrombin formation and thrombin receptor activation, resulting in a plethora of inflammatory, mitogenic, and fibroproliferative effects. This lecture will 1) review clinical and preclinical evidence supporting the involvement of endothelial dysfunction in development of early and delayed intestinal radiation responses; 2) discuss the various mechanisms by which ionizing radiation influences endothelial TM expression levels and TM function; and 3) present preclinical evidence in support of pharmacological interventions that ameliorate intestinal radiation toxicity by preventing and/or reversing post-radiation endothelial dysfunction.

(S37.1) Radiation-induced adaptive response and genomic instability. Takeo Ohnishi[1], Akihisa Takahashi[1], Hideki Matsumoto[2]. [1]Nara Medical University School of Medicine, Nara, Japan, [2]University of Fukui, Fukui, Japan.

A classical paradigm of radiation biology asserts that all radiation effects on cells, tissues and organisms are due to the direct action of radiations. However, there has been a recent growth of interest in the indirect actions of radiation including the radiation-induced adaptive response, the bystander effect and genomic

instability, which are specific modes of stress exhibited in response to low-dose or low-dose rate radiation. Although many researchers have investigated these molecular mechanisms, they remain obscure because of their complex signaling pathways and the involvement of various proteins. We have reported that conditioning exposure to low doses or at a low dose-rate blunted radiation-induced *p53*-dependent apoptosis. The conditioning exposure is also reported to suppress p53 function through radiation-induced signaling and/or p53 stability. It is well known that the loss of p53 function enhances genomic instability. We have focused on identifying the molecular mechanism underlying the adaptive response, the bystander effect and the genomic instability. The p53 is regulated by its interaction with HDM2, which serves as an ubiquitin ligase to target p53 for degradation. Therefore, we show that radiation-induced HDM2 contributes to the induction of radioresistance to a subsequent high dose using RITA (a specific inhibitor of the interaction between p53 and HDM2, and p53 ubiquitination). The induction of radioresistance was not observed in the presence of RITA. In addition, we show that radiation-induced nitric oxide (NO) radicals contribute to the induction of radioadaptive response. The induction of radioresistance was not observed in the presence of aminoguanidine (a specific inhibitor of iNOS) or c-PTIO (a specific scavenger of NO radiacals). Moreover, radioresistance was obtained when cultures were treated with isosorbide dinitrate (a NO radical generating agent) alone. Since p53 accumulated after irradiation can attenuate the iNOS, these findings suggest that NO radicals are an initiator of the radioadaptive response through the activation of Hdm2 and depresses p53 accumulation. In this symposium, we will discuss our recent proposal of radiation-induced adaptive response, the bystander effect and genomic instability.

(S37.2) Targeted and non-targeted irradiations using an electron microbeam and their effects on radiation-induced genomic instability. William F. Morgan. University of Maryland, Baltimore, MD, USA.

Cellular exposure to DNA damaging agents like ionizing radiation can result in mutations, gene amplifications, chromosomal rearrangements, carcinogenesis, and even cell death. The paradigm for understanding how induced damage results in these cellular endpoints dictates that cellular responses to the induced damage, e.g., DNA repair, and cell cycle arrest "fix" the damage and thereby dictate the fate of the irradiated cell. This presentation will focus on delayed genetic effects occurring in the progeny of cells after exposure to ionizing radiation, including delayed chromosomal rearrangements, and recombination/mutation events as determined by a plasmid based assay system based on green fluorescence protein. We will present new data on how the cellular microenvironment can perpetuate instability in clonally expanded populations of cells surviving irradiation after exposure to very low doses of radiation after targeting a subset of a cell population using our unique electron microbeam. These results will be discussed in terms of non-targeted "bystander" like effects where by cells that themselves were not irradiated exhibit many of the same detrimental effects as irradiated cells and what implication these effects may have for radiation therapy.

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(S37.3) Nhej and the double-strand break response in igh class switch recombination and translocations. Frederick W. Alt[1], Ali Zarrin[1], Sonia Franco[1], Catherine Yan[1], Monica Gostissa[1], Jing Wang[1], Abhishek Datta[1], John P. Manis[2], Klaus Rajewsky[3]. [1]Howard Hughes Medical Institute, Children's Hospital, CBR Institute for Biomedical Research, and Department of Genetics, Harvard University Medical School, Boston, MA, USA, [2]Joint Program in Transfusion Medicine and Department of Laboratory Medicine, Children's Hospital Boston, Department of Pathology, Harvard Medical School, Boston, MA, USA, [3]CBR Institute for Biomedical Research, Boston, MA, USA.

IgH class switch recombination (CSR) joins Sm to downstream S regions. To elucidate S region function, we deleted the 10kb mouse Sg1 region and replaced it with an ISceI endonuclease site. In B cells, inhibition of CSR on the mutant allele was rescued by retroviral introduction of ISceI. CSR fused Sm into the cleaved ISceI site 100kb downstream, indicating that S regions are specialized structures to generate DNA double-stand break (DSBs). Non-homologous end-joining (NHEJ) repairs general DSBs and V(D)J recombination DSBs. To examine NHEJ in CSR, we analyzed XRCC4 NHEJ factor-deficient B cells generated via knock-in IgH and IgL V(D)J rearrangements. XRCC4-deficiency reduced CSR, but significant residual CSR still occurred via a micro-homology mediated end-joining pathway. We previously implicated chromatin components in holding DSB ends in proximity for rejoining via NHEJ and proposed DSB response factors (e.g. H2AX, 53BP1 and MDC-1) function in a complex that prevents premature DSB separation. We cytogenetically analyzed the IgH locus in NHEJ or DSBR-response factor deficient B cells activated for CSR and found, during CSR, that these factors prevent IgH locus DSBs from separating and causing chromosomal breaks and translocations. Correspondingly, conditional inactivation of NHEJ or DSB-response factors in p53-deficient mature B cells led to mature B lymphomas or plasmacytomas with translocations that fused S regions directly to the c-Myc and, in certain tumors, Igl locus translocations that join Igl to different oncogenes and appear to result from aberrant editingb.

(S37.4) Fetal irradiation induced genomic instability in mouse hemopoietic stem cells. Uma Devi Pathirissery. JN Cancer Hospital & research centre, Trivandrum, India.

Genomic instability is expressed as delayed cell death, chromosomal aberrations and mutations. The purpose of the present study was to trace the radiation induced genomic instability in the mouse hemopoietic stem cells in vivo from the fetal liver to adult bone marrow. Swiss albino mice were exposed on day 14 of gestation to 0 - 1.5Gy of ^{60}Co gamma radiation. Some of the mice were sacrificed by cervical dislocation at 24h after exposure and the fetal liver cells were injected into bone marrow ablated adult mice. Spleen colonies formed from these cells were used to study the genomic instability in the short-term repopulating stem cells (STRSC). Other mice were left to deliver their young. Chromosomal aberrations were scored in the bone marrow from 3 months to 18 months postpartum (pp) to evaluate the genomic instability in the long-term repopulating stem cells (LTRSC). Irradiation produced a dose dependent increase in cell death in the fetal liver. The STRSC that suffered severe chromosomal damage were eliminated by reproductive cell death as seen from a dose-dependent decrease in the spleen colonies. The surviving cells did not show any increase in chromosomal aberrations compared to control. Genomic instability in the LTRSC was expressed as delayed chromosomal aberrations in the adult bone marrow. There was no increase in aberrations compared to control at 3–6 months pp. But unstable chromosomal aberrations appeared de novo in the irradiated groups from 9 months pp and the percent aberrant cells and aberrations per cell increased with age. There was also a significant increase in aneuploidy in the bone marrow of the in utero irradiated offspring, which showed a positive correlation with abnormal increases in the blood leucocytes suggestive of a pre-leukemic state. This indicates a possible link between fetal irradiation induced genomic instability in the surviving LTRSC and postnatal leukemogenesis. Treatment with the antioxidant phytochemicals, orientin and vicenin before 1Gy fetal irradiation significantly reduced the chromosomal aberrations in the bone marrow of adult offspring, suggesting that pretreatment with chemical radioprotectors can protect against fetal irradiation induced genomic instability and its delayed effects.

(S38.1) The tumour microenvironment and metastasis. Richard P. Hill[1], Anthony Fyles[2], Sarah-Jane Lunt[1], Naz Chaudry[1], Li Zhang[1], Patrick Subarsky[1], Michael Milosevic[2]. [1]Ontario Cancer Institute/Princess Margaret Hospital, Toronto, ON, Canada, [2]Princess Margaret Hospital, Toronto, ON, Canada.

Objective: Both hypoxia and interstitial fluid pressure (IFP) are predictive for treatment outcome in cervix cancers and hypoxia has also been associated with treatment outcome in a number of other tumor types. We are investigating mechanisms for these effects using tumor model systems, with emphasis on metastatic development. Materials/Methods; Cells from human tumors in culture or grown as xenografts have been exposed to different levels of chronic or cyclic hypoxia or increased pressure and tested for invasive and metastatic ability. QRT-PCR analysis has been performed on treated cells obtained from both in vitro and in vivo exposures to examine changes in the expression levels of specific genes believed to be associated with metastasis. Results: Individual rodent and human tumors demonstrate a wide range of IFP levels (10–50 mm Hg) but these levels do not obviously correlate with spontaneous metastases. Reduction of IFP levels in vivo using drugs also does not modify metastasis levels. In contrast exposure of orthotopic ME180 and Casci cervix cancer xenografts to cyclic hypoxia in vivo causes increased lymphnode metastasis and we have observed upregulation of a number of genes in tumors and their metastases exposed to such conditions; notably uPAR, MDM-2, CXCR4 and VEGF-C. These genes are also upregulated in the cells growing in culture when exposed to chronic (24hr) hypoxia induced by gassing with $< 2\%$ oxygen or by exposure to cyclic hypoxia over a 24 hr period. We are currently examining whether these genes are also overexpressed in human cervix cancers. Both HT-1080 human fibrosarcoma and MDA-231 human mammary cancer cells have been exposed to different oxygen concentrations in vitro (0–5%O2) and tested for invasive capacity or metastatic ability. For both cell lines the results indicate that intermediate O2 concentrations (0.2–4%) induce the largest increases in these properties. Conclusions: Although the clinical data indicate a correlation between high IFP levels in human cervix cancers and metastatic development we have not observed this effect in animal models. In contrast intermediate levels of hypoxia do affect metastatic behaviour in a number of different animal tumor models. Gene expression changes associated with such exposure are currently being examined in primary cervix cancers.

(S38.2) The role of hypoxia-induced lysyl oxidase in metastasis. Janine T. Erler, Kevin L. Bennewith, Christine Ham, Quynh-Thu Le, Amato J. Giaccia. Stanford University, Stanford, CA, USA.

Metastasis is a multistep process responsible for most cancer deaths, and it can be influenced by both the immediate microenvironment (cell-cell or cell-matrix interactions) and the extended tumour microenvironment (for example vascularization). Hypoxia (low oxygen) is clinically associated with metastasis and poor patient outcome. We recently reported that the expression of lysyl oxidase (LOX) is elevated in hypoxic human tumour cells. We further showed that LOX expression is regulated by hypoxia-inducible factor (HIF) and is associated with hypoxia in human breast and head & neck tumors. Patients with high LOX expressing tumors have poor distant metastasis-free and overall survivals. Inhibition of LOX significantly reduced metastasis in mice with orthotopically grown breast cancer tumors. Mechanistically, secreted LOX is responsible for the invasive properties of hypoxic human cancer cells through focal adhesion kinase activity and cell to matrix adhesion.

We now demonstrate that LOX secreted by hypoxic breast tumor cells co-operates with fibronectin (FN) in recruiting bone marrow-derived cells (BMDCs) and creating a permissive niche for incoming metastatic tumor cells. Secreted LOX found at pre-metastatic sites acts as a chemo-attractant for circulating BMDCs, enhancing their invasiveness. LOX inhibition through genetic, chemical or antibody means, disrupts premetastatic niche formation and metastatic growth. In order to investigate effectiveness of anti-LOX therapy against advanced-stage metastasis, we performed in vivo imaging of mice bearing orthotopic lung tumors expressing luciferase . LOX inhibition was highly effective against already established metastatic tumors, resulting in a significant reduction in bone and liver metastases together with a significant increase in survival. Similar findings were observed in a model of pancreatic cancer. These data strongly support LOX as a good therapeutic target for the treatment and prevention of metastatic disease.

(S38.3) Hypoxia and remodeling of tumor microenvironment. Zhong Yun. Yale University School of Medicine, New Haven, CT, USA.

Tumor hypoxia does not only affect tumor cell behavior, but also exerts profound influence on stromal cells. In breast cancers, for example, tumor-associated stroma contains densely populated fibroblasts and/or myofibroblasts. In contrast, normal mammary tissue is rich in mature adipocytes. This observation suggests that tumor microenvironment undergoes significant remodeling and acquires new stromal characteristics. We hypothesize that hypoxia has the potential to alter the differentiation or cell-fate decision of mesenchymal fibroblastic progenitor cells and consequently to exert significant impact on remodeling of tumor-associated stroma. Consistent with this hypothesis, we have found that hypoxia strongly inhibits adipogenic differentiation. Hypoxia-inducible factor 1 (HIF-1) plays a critical role in the regulation of adipogenesis. Interestingly, hypoxia prevents progenitor cells from responding to adipogenic stimuli and arrests progenitor cells in an undifferentiated state. In contrast to adipogenic differentiation, myogenic differentiation is able to adapt to moderate hypoxia (0.5% - 2% O2). Nevertheless, severe hypoxia ($<0.01\%$ O2) can completely block myogenic differentiation. Consistent with adipogenic precursor cells, myoblasts remain undifferentiated under hypoxia and maintain their myogenic potential. Our data demonstrate that hypoxia can contribute to the fibroblast-rich phenotype of tumor-associated stroma via regulation of the cell fate decision of mesenchymal progenitor cells. Interactions between altered stromal cells and tumor cells in a hypoxic microenvironment may play a significant role in promoting malignant tumor progression.

(S38.4) Influence of HIF-1 functionality on tumour growth and metastatic dissemination. Ian J. Stratford. University of Manchester, Manchester, United Kingdom.

Hypoxia is known to promote the expression of genes involved in the metastatic process. The regulation of these genes is controlled by the transcription factor HIF-1. We have explored the consequences of knocking out HIF-1 activity on the growth of primary tumours and subsequent metastatic dissemination. To do this we have engineered HCT116 colon cancer cells to express a dominant negative form of HIF-1. These cells show no increased expression of downstream HIF-1 targets such as Glut-1, when exposed to hypoxia. Wild type HCT116 cells injected into the the spleen will metastasize to the liver. However, this process is almost eliminated in those cells carrying the dominant negative HIF-1. To further evaluate the importance of hypoxia and HIF-1 in the metastatic process we have engineered cells to express an HRE-driven firefly luciferase together with renilla luciferase under the control of a constitutative promoter. This allows us to use Xenogen optical technology to follow the location of tumour cells, in real time, when in injected into mice. Hence we can follow the formation of primary tumour (via renilla-luc), the development of hypoxia in the primary tumour (via HRE firefly-luc) and the subsequent development of micro-metastases (via renilla-luc), simply by giving mice the appropriate luciferase substrate at various times after tumour implantation. Preliminary experiments show this to be a suitable method for following, non-invasively, tumour growth and metastatic spread.

(S39.2) Wnt/β-catenin signaling in intestinal and mammary cancer stemness. Riccardo Fodde. Erasmus MC, Rotterdam, The Netherlands.

Breast and colon cancers are generally thought to arise from normal epithelial cells through a stepwise accumulation of genetic alterations in oncogenes and tumor suppressor genes. However, this genetic model does not take into account other essential characteristics of human cancers, namely their vast intratumor cellular heterogeneity and the role played by a minority of cells, the cancer stem cells (CSCs), in determining invasion into surrounding tissues and distant organ sites. The Wnt/β-catenin signal transduction pathway is known to play a central role in self-renewal and

differentiation during embryonic development and in the maintenance of many stem cell niches in adulthood. To study how different dosages of Wnt signaling activation may trigger multi-organ tumorigenesis, we have generated several hypomorphic alleles of the *Apc* tumor suppressor gene by gene targeting. Notably, while both in man and mouse *Apc* mutations result in intestinal cancer, we have generated specific allelic variants associated with tumor susceptibility in organs other than the GI tract, namely in the mammary gland, skin and liver. Notably, in cancers resulting from *Apc* mutations intracellular β-catenin accumulation, the earmark of canonical Wnt signaling activation, is found to be heterogeneous within the tumor mass. Here, I will present experimental data indicating that intracellular β-catenin accumulation earmarks cancer stem cells capable of self-renewal and differentiation. The results also indicate that the specific Wnt signaling dosages encoded by different *Apc* mutations differentially affects homeostasis of adult stem cell compartments and triggers tumor initiation, progression, and metastasis in a organ-specific fashion.

(S40.1) Exploiting the molecular network involving NFBD1/MDC1 in response to DNA damages. Phang-Lang Chen. UC Irvine, Irvine, CA, USA.

DNA surveillance mechanisms have evolved to monitor and maintain the integrity of the human genome by arresting cell cycle progression and activating DNA repair upon genotoxic stress, defined as UV-damage, g-irradiation, aberrant replication intermediates, and pathogenic intrusion. It is postulated that efficient repair of DNA double strand breaks (DSB) depends on an intact signaling cascade comprised of molecules involved in DNA damage signaling pathways and checkpoints. Defective surveillance and/or inactivation of the DNA repair system can result in increased mutagenesis, genome instability, apoptosis and ultimately cancer. NFBD1/MDC1, together with p53 binding protein 1 (53BP1), plays a partially redundant role in regulating phosphorylation of the downstream effector protein, Chk2. We establish several inducible knockdown clones of human U2OS cells and to examine the phenotype upon depletion of NFBD1, or 53BP1 to examine their phenotypes upon DNA damages. Our results placed NFBD1 parallel to 53BP1 in regulating Chk2 implying their redundant activity in activating ATM. Consistent with this postulate, we found that ATM activation and ATM dependent phosphorylation of H2AX is impaired in cells missing both NFBD1/MDC1 and 53BP1, confirming that both NFBD1/MDC1 and 53BP1 play critical role in activating ATM kinase. Together our results support a novel model that NFBD1 and 53BP1 serve as structural scaffolds to retain and amplify the ATM dependent signal transduction at DNA damage sites, underlining a new regulatory mechanism for DNA surveillance.

(S40.2) Chromatin remodeling in checkpoints and repair. Sang Eun Lee. UTHSCSA, san Antonio, TX, USA.

Repair of DNA double-strand breaks (DSBs) protects cells and organisms, as well as their genome integrity. As DSB repair occurs in the context of chromatin, chromatin must be modified to prevent it from inhibiting DSB repair. Evidence supports the role of histone modifications and ATP-dependent chromatin remodeling in repair and signaling of chromosome DSBs. Previously, we have initiated a genome wide screen for novel yeast non-homologous end joining (NHEJ) genes using an NHEJ assay with a chromosomal DSB. This screen has identified two new DSB repair genes, *RSC30* and *RSC8*, which encode the components of the chromatin-remodeling complex called RSC. RSC is an essential multi-subunit protein complex that functions in the ATP-dependent chromatin remodeling in yeast. RSC has been implicated in a variety of cellular activities including regulation of gene expression in response to stress and cell cycle progression through its ability to mobilize nucleosomes. We and one other group showed that RSC is targeted to the DSB created *in vivo* and involved in repair of DSBs by NHEJ and homologous recombination. The role of RSC in DSB repair is further supported by its physical and genetic interactions with several key DSB repair proteins, including yKu, and Mre11. We also uncovered the RSC- and DSB-dependent chromatin changes at the DSB that facilitate end resection, damage-induced phosphorylation of histone H2AX, and targeting of repair factors to the damaged site for an efficient NHEJ and recombinational repair. Currently, we are investigating the role of RSC in damage-induced cell cycle checkpoint and suppression of genome instability. Since human and mice both contain two distinct Swi/Snf ATP-dependent remodeling complexes, one of which is a homolog of RSC, the study will provide the conceptual framework needed to dissect the role of human chromatin remodeling complexes in an equivalent process.

(S40.3) The role of ATM and ATR in hypoxia induced replication arrest and recovery. Ester M. Hammond, Amato J. Giaccia. Stanford University, Stanford, CA, USA.

Hypoxia occurs in virtually all solid tumors and is an important factor in tumor progression. The level of tumor hypoxia has been shown to predict for poor patient prognosis in a number of tumor types. Oxygen concentrations within hypoxic regions vary and can reach almost anoxic levels. Due to the nature of tumor vasculature, hypoxic regions can result from temporary vessel blockage and can undergo rapid reoxygenation when the blockage is alleviated. Recently, we have described a role for ATM in response to the DNA-damage induced by reoxygenation. This includes the induction of a reoxygenation-induced cell cycle arrest in both the G1 and G2 phases, dependent on p53 and Chk 2 respectively. The importance of ATM-mediated signaling in response to hypoxia/reoxygenation has been highlighted using survival assays, which indicate that loss of ATM results in increased sensitivity to hypoxia/reoxygenation. Despite a lack of detectable hypoxia induced DNA-damage, we have observed rapid and robust phosphorylation of ATM at serine 1981 during hypoxia. Both the ATM and ATR response occurs in cells derived from both ATLD and NBS patients, indicating that the MRN complex is not required for hypoxia induced activation of these damage inducible signaling proteins. The mechanism and consequences of hypoxia-induced ATM phosphorylation will be discussed further.

(S40.4) Activation of the DNA damage checkpoint in the absence of DNA damage. David Toczyski. University of California, San Francisco, San Francisco, CA, USA.

DNA damage, such as double stranded breaks, activate a signal transduction pathway called the DNA damage checkpoint. Activation of this pathway blocks cell cycle progression and promotes resistance to damaging agents. Genetics has identified several proteins important for this response. Two of the central players are a two subunit kinase (mec1/Ddc2) related to PI3 kinases and a heterotrimeric complex (the 9-1-1 complex) related to PCNA. Work in our lab has shown that these two complexes are independently recruited to sites of DNA damage. We proposed that their co-localization at damage sites leads to checkpoint activation. To test this hypothesis, we have fused checkpoint components to the bacterial DNA binding protein LacI. This allows their recruitment to a the LacI binding site, LacO, when tandem arrays of LacO are integrated into a chromosome. With this system, we have artificially co-localized the two complexes and observed checkpoint activation, as measured by several downstream events, in the absence of actual damage. Checkpoint activation requires not just the co-localization of these two components, but also requires their localization to chromatin containing histone modifications known to be important for checkpoint signaling.

(S41.1) Are multiply damaged sites converted to double strand breaks in cells? Lynn Harrison. LSU Health Sciences Center at Shreveport, Shreveport, LA, USA.

Multiply damaged sites (MDSs) are defined as $\geq$ 2 lesions within ~15 base pairs and can contain oxidative base damage, abasic (AP) sites and single strand breaks (SSBs) when generated in DNA by ionizing radiation. We have developed assays to determine whether synthetic MDSs are converted to double strand breaks (DSBs) in bacteria or mammalian cells. Both assays utilize a firefly luciferase reporter with the MDS positioned within the luciferase coding region. The DNA is transferred into cells and luciferase activity measured following DNA repair in the absence of DNA replication. DSB formation dramatically reduces activity. In bacteria we have determined that while two closely opposed 8-oxo-7,8 dihydroguanine lesions result in an enhanced mutation frequency and not a DSB, two closely opposed uracils or tetrahydrofurans (furans) dramatically decrease luciferase activity and result in destruction of the plasmid. Repair involves exonuclease III and endonuclease IV and not nucleotide excision repair. Studies using uracil in both HeLa cells and mouse embryonic fibroblasts (MEFs) have demonstrated that two closely opposed uracils are not converted to a DSB. A major difference between *E. coli* and mammalian cells is the ability to repair DSBs by nonhomologous end-joining (NHEJ). Ku has been found to inhibit *in vitro* cleavage at a base damage opposite a SSB. We have therefore generated *E. coli* that express the *Mycobacterium tuberculosis* Ku (Mt-Ku) and/or Ligase (Mt-LigD) and plan to determine whether Mt-NHEJ can stop the formation of a DSB from clustered uracils or furans. To determine whether NHEJ prevented the formation of a DSB from uracils in mammalian cells, we performed our assay in MEFs null for DNA PKcs, Ku70 and Ku80. In these cells DSB formation was not detected even in the presence of a PARP inhibitor. We therefore tested two closely opposed furans. Initial studies indicate that two furans can be converted to a DSB in wild-type as well as $Ku80^{-/-}$ MEFs. The expression level of uracil DNA glycosylase, which is required to convert a uracil into an AP site, may therefore be limiting in the MEFs. The physiological expression level of each enzyme, as well as its ability to cleave at MDSs, may therefore be key to the formation of DSBs. MDSs can also occur at termini of DSBs. Repair of damaged termini by Mt-NHEJ in *E. coli* will also be discussed.

(S41.2) Mechanisms of processing clustered DNA damage including 'dirty' DSB. Peter O'Neill, Sophie Bellon, Siobhan Cunniffe, Martine Lomax, Tracey Dobbs. MRC Radiation & Genome Stability Unit, Harwell, Oxfordshire, United Kingdom.

Clustered DNA damage, in which two or more lesions are formed within a few helical turns of DNA by a single radiation track, are induced in cellular DNA by ionising radiation. Radiation-induced double strand breaks (DSB) are often characterised by the presence of base lesions in close proximity to the break termini ('dirty' DSB) and are believed to be a major cause of the biological effects of ionising radiation. The complexity of clustered damage sites and 'dirty' (DSB) increases as the ionisation density of the radiation increases and evidence exists that they present a challenge to the repair machinery. For instance, these clustered damage sites may be highly mutagenic, as a result of an increased probability of lesions being present at replication in cycling cells, or can be converted to DSB which if not repaired may be lethal. Evidence will be presented showing that bistranded clustered damage sites containing thymine glycol (Tg), a polymerase blocking lesion, and 8-oxo-7,8-dihydroguanine (8-oxoG) are sequentially processed, thus avoiding DSB formation in contrast to the formation of a DSB with bistranded clustered sites containing an abasic site and Tg. The clustered sites containing 8-oxoG/Tg are highly mutagenic relative to that of the individual lesions. MutY, a post-replicative glycosylase, is most important in protecting against the formation of predominantly G:C→T:A transversion at the position of the 8-oxoG lesion, reflecting the consequence of clustered sites if present at replication. That the mutation frequencies of the corresponding bistranded clustered damage containing either 8-oxoG/AP site or 8-oxoG/dihydrothymine are similar to those for the 8-oxoG/Tg cluster, is consistent with the notion that Tg is preferentially excised from the 8-oxoG/Tg cluster. Evidence will also be presented showing that the presence of 8-oxoG close to DSB termini, a complex DSB, reduces the efficiency of DSB rejoining by cell extracts and that only about 50% of the DSB were re-joined faithfully. The role of glycosylases/endonucleases in orchestrating the processing of clustered sites will be discussed. Overall, the results demonstrate the importance of the effect of 'dirty' DSB and clustered damage to understanding the mechanisms of the biological consequences of DNA damage induced by ionising radiation.

(S41.3) Chemical detection and mechanisms of formation of tandem DNA damage. Jean-Luc Ravanat, Peggy Regulus, Jean Cadet, Alain Favier. CEA Grenoble, Grenoble, France.

Radiation-induced formation of DNA lesions has been studied in details during the last three decades and about 70 DNA lesions have been identified so far. However, using a new strategy we have recently detected four new DNA lesions upon treatment of aerated aqueous solution of isolated DNA by gamma irradiation. Among them one was found to be generated at the cellular level and efforts have been made to characterize the newly detected DNA lesion. Interestingly, our work allows us to demonstrate that the lesion could be considered as a clustered DNA damage requiring a single radical event, i.e. hydrogen abstraction at the C4' position of 2'-deoxyribose. The mechanism of formation of the detected clustered lesion involves, subsequently to C4' hydrogen abstraction, the formation of a modified sugar residue exhibiting a reactive α,β-unsaturated aldehydic group that could react with a proximate cytosine base by a mechanism similar to that described for 1,4-dioxo-2-butene. Thus formation of the lesions involves a strand break together with a cross-link between the modified sugar residue and a cytosine base, most probably located on the complementary strand. The lesion thus produced is detected subsequently to DNA extraction and digestion by HPLC coupled to tandem mass spectrometry as the four diastereomers of a novel oxadiazabicyclooctaimine adduct of 2'-deoxycytosine identified as 6-(2-deoxy-ß-D-erythro-pentofuranosyl)-2-hydroxy-3(3-hydroxy-2-oxopropyl)-2,6-dihydroimidazo[1,2-c]-pyrimidin-5(3H)-one. We have also shown that bleomycin, an antitumoral therapeutic radiomimetic acting mostly on DNA by hydrogen abstraction at the C4' position could also induce the formation of the lesion in cellular DNA. Moreover, the lesion was detected in freshly isolated human lymphocytes, at a level around 0.01 lesion per million nucleosides, indicating that the clustered lesion could have an endogenous origin. For the first time, our work demonstrates that a single radical event could induce the formation of a clustered DNA lesion within cellular DNA. The difficulty of repairing such a DNA lesion may explain, at least partly, in addition to double strand breaks formation, the origin of bleomycin and ionizing-radiation toxicity.

(S41.4) Repair of clustered DNA damages induced by low radiation doses in human cells and skin. Betsy M. Sutherland, Paula V. Bennett. Brookhaven National Laboratory, Upton, NY, USA.

We have asked how human cells and tissues exposed to low radiation doses corresponding to high survival deal with complex clustered damages. Clustered DNA damages–two or more closely-opposed abasic sites, oxidized bases or strand breaks within about one helical turn–are thought to be difficult to repair and thus potentially persistent, mutagenic and /or lethal, radiation-induced DNA alterations. They are hypothesized to be cleaved simultaneously by cellular repair enzymes, producing double strand breaks. Indeed cells exposed to supra-lethal radiation doses do create de novo DSBs during repair. To evaluate the fate of clusters in cells exposed to low radiation doses, human 28SC monocytes were exposed to 10 cGy X-rays, incubated at 37°C, and sequential samples harvested at times from 2 min to 24 hr. DNAs were isolated, and four classes of clusters measured: double strand breaks, Fpg-OxyPurine clusters, Nth-OxyPyrimidine clusters and Nfo-Abasic clusters. Little if any increase in DSBs was detected; however, the levels of OxyPurine-, OxyPyrimidine- and Abasic clusters increased substantially at early times after irradiation. The levels of these cluster classes then decreased. The kinetics of repair in these cells shows an ordered and sequential strategy of repair, and implicates specific repair-intermediate cluster species in cluster processing in these cells. We have also asked how human skin exposed to low doses of X-rays deals with complex clustered

damages. Cluster repair in intact skin tissue showed a similar repair strategy: double strand breaks were rapidly rejoined without evidence of de novo DSBs, whereas all three classes of clustered damages increased shortly after irradiation and were then resolved rapidly. The kinetics of repair strongly suggest effective repair modes that circumvent double strand break induction in human cells and in human tissue exposed to low radiation doses corresponding to high survival levels.

(S43.1) Reversible regression of tumor blood vessels after inhibition of VEGF signaling. Donald M. McDonald. University of California San Francisco, San Francisco, CA, USA.

Abnormalities of tumor blood vessels are being exploited in the diagnosis and treatment of cancer. These abnormalities, which involve all components of blood vessel walls including endothelial cells, pericytes (mural cells), and the vascular basement membrane, impair blood flow and efficient delivery of drugs to tumors. Endothelial cells of tumor vessels undergo sprouting and proliferation, have a defective barrier function, may be dependent on VEGF for survival, and have abnormal gene expression, including unusually high levels of VEGFR-2, VEGFR-3, and alpha5beta1 integrin. Pericytes of tumor vessels also have prominent abnormalities in structure and function, and the vascular basement membrane has redundant layers that reflect the dynamic nature of the tumor vasculature. Inhibitors of VEGF signaling have multiple actions on the vasculature of tumors. In mouse tumor models, VEGF inhibitors cause robust and rapid changes in endothelial cells, pericytes, and vascular basement membrane. The inhibitors block angiogenesis, cause regression of some tumor vessels, and reduce the abnormality of tumor vessels that survive. Within 24 hours, vascular sprouting is suppressed, endothelial fenestrations disappear, vessel patency is lost, and blood flow ceases in some tumor vessels. By 7 days, as many as 80% of tumor vessels may regress. Surviving tumor vessels acquire more normal structural characteristics. Empty sleeves of vascular basement membrane persist, providing a record of regressing blood vessels, sites for growth factor binding, and a scaffold for vascular regrowth after cessation of therapy. Many pericytes also persist. One day after drug withdrawal, endothelial sprouts begin to grow into empty sleeves of basement membrane. Patent tumor vessels connected to the bloodstream become abundant within a few days, and by 7 days, tumors are fully revascularized. Importantly, the regrown vasculature regresses as much during a second treatment as it does in the first. These results suggest that empty sleeves of basement membrane and accompanying pericytes that do not regress provide a scaffold for rapid revascularization of tumors after removal of anti-VEGF therapy and highlight their importance as targets in cancer therapy.

(S43.2) Optimization of the treatment modality combining ionizing radiation with inhibitors of angiogenesis. Martin Pruschy, Christoph Oehler, Melanie Wergin, Oliver Riesterer. University Hospital Zurich, Zurich, Switzerland.

On the preclinical level the combined treatment modality of ionizing radiation with inhibitors of angiogenesis has been demonstrated to be highly effective, but only a small number of clinical trials exists combining these different treatment modalities. Preclinical optimization strategies for this treatment combination currently focus on the identification of novel targets on the molecular and cellular level, on the application of multitarget inhibitors and on the evaluation of different treatment regimens. Using invasive and complementary non-invasive methods we analyzed in ectopic and orthotopic murine tumor models the treatment response of different inhibitors of angiogenesis (in particular with clinically-relevant receptor tyrosine kinase inhibitors against the VEGF- and ErbB-receptors) alone and in combination with ionizing radiation on the tumor microenvironment, on the integrity of the tumor vasculature and on tumor hypoxia. Using small animal hypoxia positron emission tomography we determined that inhibition of angiogenesis with the VEGF-receptor tyrosine kinase inhibitor increases overall and local tumor hypoxia. Combined treatment with fractionated irradiation results in extended tumor growth delay, tumor cell apoptosis but no increase in tumor hypoxia. These results demonstrate that irradiation antagonizes the increase of hypoxia by VEGF-receptor tyrosine kinase inhibition and abrogates the potential negative effect on tumor hypoxia. Furthermore we analyzed the sequence of events induced by the different treatment modalities on the level of the tumor vasculature and the microenvironment and identified the treatment-regulated proliferative status as a major determinant for the treatment response. Interestingly hypoxia-dependent regulation of cytokine-expression (e.g. erythropoietins) co-determine the radiosensitivity and as such contribute to the interdependent, multilayered stress response to ionizing radiation and inhibitors of angiogenesis. Our results are discussed in a general context towards understanding the dynamic treatment response to this combined treatment modality.

(S43.3) bFGF and VEGF inhibit radiation-induced apoptosis of endothelial cells by inhibiting acid sphingomyelinase activity. Jean-Philip Truman, Dolares Hambardzumyan, Monica Garcia-Barros, Michael Chan, Richard Kolesnick, Zvi Fuks, Adriana Haimovitz-Friedman. Memorial Sloan Kettering Cancer Center, New York, NY, USA.

Previous studies (Haimovitz-Friedman et al., 1994) showed that ionizing radiation (RT) induces rapid sphingomyelin (SM) hydrolysis to generate ceramide and apoptosis in enucleated bovine aortic endothelial cells (BAEC). These studies indicated that the cellular membrane is the primary target of ionizing radiation in these cells. We also showed that basic fibroblast growth factor (bFGF) inhibits RT-induced apoptosis in these endothelial cells. The goal of the current study was to explore the mechanism by which bFGF inhibits RT-induced apoptosis in BAEC and whether other survival factors, such as VEGF, act similarly. The response of these cells to RT in the presence or absence of bFGF and VEGF was investigated using plateau-phase BAEC cultures. Both growth factors inhibited RT-induced apoptosis in these cells. Further, ceramide levels were significantly elevated at 266.4pmol/106 cells 60 seconds post-irradiation ($p<0.001$), and were reduced to basal levels upon pretreatment with bFGF (129.4pmol/106 cells in bFGF-pretreated irradiated cells vs. 138.44pmol/106 cells in untreated control BAEC). In resting cells, acid sphingomyelinase (ASMase) is localized to the inner plasma membrane and upon activation translocated onto the exoplasmic leaflet, where its substrate SM is preferentially concentrated. bFGF inhibited RT-induced ASMase translocation, and decreased ASMase activity. Further, exogenous long chain C16-ceramide, at concentrations alone insufficient for apoptosis induction, bypassed bFGF and VEGF inhibition and restored radiation-induced apoptosis. These results show for the first time that the principle anti-apoptotic effect of bFGF and VEGF on endothelium is to inhibit ASMase-mediated ceramide generation.

(PS1001) Neurobiological risks due to tritiated water exposure accessed against bulk tritium release from nuclear industry. Narendra Jain[1], Arvind Bhatia[2]. [1]Department of Zoology, Government College, Jhunjhunu, Rajasthan., India, [2]Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India.

With the introduction of nuclear energy, an additional consideration outside the radiation protection framework has appeared i.e. the need to access the radioecological and radiobiological impact of radionuclides of long half lives existing in the environment for lengthier durations. Tritium, a radioactive by-product of power reactors also is one of such radionuclides of concern. Besides having longer life it disperses more rapidly and represents a significant risk to the population exposed. As the nuclear fusion technology is approaching fast, there is now, a growing emphasis on tritium release and its behaviour in the environment. There is apprehension that the recent controversy concerning the health and environmental impact of tritium may end up as worldwide contaminants in the final analysis. Our investigations clearly reflect that low levels of tritiated water (HTO) exposure impart significant deleterious effects on the cerebellum of Swiss albino mice during its prenatal, neonatal and postnatal developmental stages which are manifested by a reduction in the size and weight of the organ together with many histopathological lesions and reduced number of cells. A dose dependent behaviour so established shows that a continuous HTO-exposure at a dose level 11.1 kBq (0.3 μCi), though does not render an appreciable damage both qualitatively and quantitatively, it gets intensified and causes lasting impairments in some of the studied parameters at a higher dose of 111.0 kBq (3.0 μCi)/ml. drinking water. In cerebellum, where the cell renewal system is lacking, major cytoarchitectural changes occur mainly during the first three weeks after birth. This accounts for its high radio vulnerability vis-à-vis a capability to repair and recover from the rendered damage during the first half (1 week to 3 week) of postnatal development, whereas during the second half (4 week to 6 week of age) a tendency towards radioresistance is achieved. Radiotoxic effects of tritium on cerebellum are consistent and apparently higher with those expected from an equivalent absorbed dose from external X-irradiation. Therefore, the possibility of a higher RBE for 3H with regard to brain and especially cerebellum can not be ruled out which warrants the need for the formulation of new rules for tritium release from nuclear industry.

(PS1002) Simulation study of sea-level cosmic radiation in a human body phantom and shielding effects. Pushpa Wijesinghe, Xiaochun He. Georgia State University, Atlanta, GA, USA.

Secondary cosmic ray particles are constantly present at the surface of the earth. On average, at sea level one muon particle hits per square centimeter per minute. These particles impart a yearly dose of 26 millirems to a human. Most of the sea-level cosmic ray particles are muons in the energy range of 100 MeV up to several hundred GeV or more. Some of these muons at the lower end of the energy range stop and decay inside the bio-system. As they carry a considerable mass energy, they generate a large number of secondary electrons in biological tissues leading to trails of ionizations. They contribute to the fragmentation of small molecules and to single and double strand breaks in DNA. A Gant4-based simulation model of a human body phantom has been employed to study sea-level muon interactions in the bio-system. The Geant4 simulation toolkit, which was first developed for studying particle interaction in high energy nuclear and particle detectors has been recently applied to medical and space radiation studies. In this study, a human body phantom that includes body materials such as tissue, muscle, bone and organs was irradiated with simulated cosmic ray particle fluxes. Concrete shieldings in different thicknesses were then placed above the phantom for studying radiation doses imparted by cosmic ray muons. The preliminary result for the annual dose from this simulation in the body was about 22 millirems, which is consistent with the average annual radiation dose from cosmic rays. This study found that, contrary to commonly available literature, the dose received by the human body when it was shielded by a thin (1–2 meters) concrete slab was higher than the dose received by an unshielded human on the street. The highest dose simulated was 33.7 millirems when a 1.5 meter concrete slab was present. When the thickness of the concrete slab gradually increased, the dose received by the phantom started decreasing after 2 meters and the muons were completely blocked by an 8-meter concrete slab. The reason for the shielded human body to receive a higher dose of the radiation is that the concrete slab slowed down the fast muons, and therefore more muons tended to decay inside the body. Detailed simulation results including dose distributions and track structures are presented in the paper.

(PS1003) The experience of FISH technique application for reconstruction of individual radiation doses in Chernobyl liquidators. Sergey S. Dybskiy, Maria A. Pilinskaya. Research Centre for radiation medicine, Kiev, Ukraine.

In 1999 for the first time in Ukraine within framework of Ukrainian-American project "Studies of Leukemia and Related Haematological Disorders in the Ukrainian-US Study of Cleanup Workers following the Chornobyl Accident" method FISH-WCP have been successfully introduced in cytogenetic laboratory of RCRM and applied for retrospective dose reconstruction in Chernobyl liquidators. 247 persons have been examined. Results received in matched control groups as a whole corresponded to the main regularities of spontaneous chromosome mutagenesis determined in labs of different countries and typical for persons in age before and after 50 years. Mean-group spontaneous frequencies of stable chromosome aberrations in control groups (0,009 + 0,001 and 0,013 + 0,002 per cell per genome-equivalent, correspondingly) exceed the age norm, estimated in Russia, West Europe and USA that probably can be connected with action of unknown "confounding" factors. In most part of liquidators individual FISH doses significantly differed from official dose records. In civil liquidators the cases with overestimated individual official doses have been predominated (mean-group doses - 600 and 450 mGy, accordingly) as well as in military liquidators the cases with underestimation of official doses in comparison with FISH doses had been prevailed (mean-group doses - 340 and 430 mGy, accordingly). In majority of exposed persons individual doses elevated permissible for radiation accident (250 mGy) and confirmed their participation in accidental activity in nearest terms following Chernobyl disaster. Thus FISH-WCP technique permit to conduct enough proper estimation of biologically-equivalent individual radiation doses in human within the range of ~ 0.2 - 2.0 Gy.

(PS1004) Estimation of RBE values for carbon beams at high dose region using multicellular spheroids of HMV-I cells. Yoshitaka Matsumoto[1], Daisuke Shimao[2], Mizuho Aoki[1], Ryoichi Hirayama[1], Nobuo Kubota[2], Koichi Ando[1], Hirohiko Tsujii[1], Yoshiya Furusawa[1]. [1]National Institute of Radiological Science, Chiba-shi, Japan, [2]Ibaraki Prefectural University of Health Sciences, Ami-machi, Japan.

(Introduction) Heavy-ion beams have good dose distribution for tumor. Carbon beams, accelerated by HIMAC in National Institute of Radiological Sciences (NIRS), is a candidate for use in hypofractionated radiotherapy of tumors included malignant melanoma of choroid, non-small cell lung cancer or hepatocellular carcinoma. In this case, relative biological effectiveness (RBE) at high dose region (low survival level) must be considered however, the RBE is calculated physically and not verified biologically. (Purpose) The purpose of this study is to estimate RBE at high dose range as well as low dose range using monolayer cells and multicellular spheroids. (Materials and Methods) One human malignant melanoma cell line, HMV-I was used for this study. We used the 96 wells spheroid plates (SUMILOM MS-0096S) for making the multicellular spheroids. Cells were irradiated with 290 MeV/u carbon ions, 490 MeV/u silicon ions or 500 MeV/u argon ions with a dose-averaged about 13, 35, 100 and 300keV/μm LET and X-rays. (Results) Surviving fractions of cells exposed to carbon beams and X-rays at a lower dose range (0 - 8 Gy) and a higher range (8 - 15 Gy) were obtained using monolayer cells and multicellular spherooids, respectively. The lenear-quadratic (LQ) equation fits well in survival data of monolayer cultures after both

radiation at lower dose region. The multi-target single-hit (MT) equation fits well in those of spheroids at higher dose region. In addition, we could fit with a formula combined MT equation and initial slope part of LQ equation as well as multi-process (MP) equation, to survival data. This equation has good fitting for survival data in wide dose range when both monolayer and spheroid data was combined. (Conclusion) We could estimate RBE values of carbon beams from low to high dose range by converting the surviving fractions of spheroids into monolayer cells' using the MP equation. RBE values at high dose range converged on the fixed values at 1.1–1.4, but varied in LET values at very low dose range (0 - 4 Gy).

(PS1005) Biomarkers of radioresistance in cervical cancer cells. Richard A. Britten, Angela Johnson, Richard Drake. Eastern Virginia Medical School, Norfolk, VA, USA.

A pre-requisite to individualizing cancer treatment (where patients receive treatment based upon their specific tumor characteristics) is the identification of reliable biomarkers of response to therapeutic agents. With regard to radiation response, cervical cancer (CaCx) is the only tumor site where cellular radiosensitivity (SF2 values) predicts for clinical radioresponsiveness. In 1996 we established isogenic clones from a CaCx biopsy that had SF2 values that corresponded to no, moderate or good levels of local control and 5 year survival. These isogenic clones are thus an ideal system to identify biomarkers of radioresistance. To this end we have used 2 dimensional gel electrophoresis coupled with MALDI-TOF/TOF based protein sequencing to identify proteins that are associated with a radioresistant or sensitive pheontype in the HT137 CaCx cell line. We have now identified several of the ~ 100 proteins that are uniquely expressed in the radioresistant variants. Of particular interest is a subset of proteins whose expression is typically regulated in an oxygen tension dependent manner: e.g., Aldolase A and IGFBP3 (both HIF responsive genes). Interestingly the cells have not been exposed to hypoxic conditions, which raises questions as to why these HIF responsive genes are up-regulated and whether their expression lead to an enhance ability of the cells to deal with stress. We also observe an upregulation of proteins that are involved in redox status detection, namely Protein Disulphide isomerase and peroxiredoxin II. In addition these proteins appear to play an important role in DNA-nuclear matrix anchoring. NM23 and Prohibitin are also preferentially expressed in the radioresistant variants. These proteins have many functions but NM23 has recently been shown to be involved in DNA repair, while prohibitin impacts upon chromatin acetylation status (and hence spatial organization, gene transcription and repair). The high percentage of identified proteins that are involved in redox status sensing and response, including DNA remodeling leads us to hypothesize that inherent radioresistance is a consequence of an increased capacity of tumors to sense and respond to changes in cellular redox stress, which leaves the cells in a highly "primed" status to deal with oxidative damage induced following irradiation.

(PS1006) In-vivo dose verification and beam flattening in radiobiology laboratory. Natalya V. Morrow, Vladimir A. Semenenko, X. Allen Li. Center for Medical Countermeasures Against Radiological Terrorism and Department of Radiation Oncology, Medical College of Wisconsin, Milwaukee, WI, USA.

Previously illustrated establishment of dosimetry procedures for a radiobiology laboratory led to significant improvement of accuracy of radiation delivery. As the next step towards improving accuracy, we show that in-vivo dosimetry is a useful tool in verification of dose delivery. Further, we show the improvement of dose uniformity when using flattening filters. The radiobiology lab is equipped with Pantak HF320 orthovoltage system. Custom flattening filters manufactured of either tissue equivalent material or aluminum can be added to the system. The field profiles with and without the filters were measured at the surface and at 3 cm depth using an ionization chamber. The in-vivo dosimetry was preformed using LiF thermoluminescent dosimeters (TLDs). The TLDs were placed on the skin for the duration of irradiation. The known doses to midline of the animals were used to calculate the expected skin doses, using previously acquired beam data. The TLDs were read out by Harshaw 3500 TLD reader. Since in the orthovoltage energy range the response of the TLDs becomes non-linear, careful calibration procedures were developed in order to obtain reliable and reproducible absolute dose values. TLD measurement of absolute doses delivered during irradiations was preformed with and without the flattening filters. The measurements at the center of the field for rat, dog, and ferret irradiations were within the clinically acceptable 5% uncertainty range of the calculated skin dose. Further, TLD measurements were used to verify the efficacy of a custom head shield for the ferret irradiation, and were consistent with ion chamber measurements. The dose uniformity was improved significantly with the use of a flattening filter. The results for off-axis TLDs were in agreement with the corresponding beam profiles, thus demonstrating the efficacy of the flattening filter and the accuracy of TLD measurement. Using carefully calibrated TLDs for in-vivo dosimetry is shown to be a useful tool in the verification of dose delivery. The use of flattening filter increases dose uniformity across the field. The efforts improve accuracy of radiation dose delivery and documentation in radiobiological lab, facilitating data exchange and reproducibility of the results.

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(PS1007) The heamatological effects of radar on human blood. Shamsi Shekari, Hamid Samavat. Hamedan University of Medical Science, Hamedan, Iran (Islamic Republic of).

Radio frequency (RF) non-ionizing radiation occupies the range from 300 HZ to 300 GHZ in the electromagnetic spectrum which the range from 1–100 GHZ is called radar. The term radar covers a wide range of applications from low power Doppler systems to large air and space surveillance system.

According to most investigators high-intensity microwave radiation(Radar) may cause pathological changes in bio-organisms, but with little agreement on effects of low power density radiation on human health.

In order to assess effects from low level microwave radiation, a hematological observation of technicians and engineers occupationally exposed to radar wave has been under taken.

The results of hematological studies show a lower number of thrombocyte, leukocyte and also erythrocytes counts in exposed group in comparison with controls. There was no significant differences in number of lymphocyte and reticulocyte counts.

In conclusion there wasn't enough evidence to support the changes in peripheral blood is the result of exposure to microwave radiation.

(PS1008) Study of individual responses to combined injuries in non human primate : investigation for relevant neuro-immune biomarkers of prognosis. Martigne P., Peinnequin A., Mathieu J., Vivier M., Clarençon D. CRSSA, La Tronche, France.

Former biological studies highlighted the importance of the peripheral inflammatory syndrome in rodents and nonhuman primate following radiation injury. Investigation to determine relevant prognosis markers of inflammatory response at the transcriptionnal level was the continuation of these experiments. In the event of accidental irradiation, lymphocyte chromosomal abnormalities do not allow to predict the clinical follow-up due to the dose heterogeneity. Moreover, individual variability of radiation response is a critical element to take into account. The intensity of the inflammatory reaction depends indeed on the genetic profile and the physiological status. The objective of our studies to evaluate biological markers of individual susceptibility of both the peripheral innate immunity (cytokine profiles) and the central nervous system (EEG, behavior) in order to characterize the host reaction profile to combined injuries. In the futue, one of the main objectives is the evaluation of the predictive relevance of a functional test carried out *ex vivo* using a whole blood assay. For each primate, a study of the individual profile of innate immunity and central response is carried

out *in vivo* before and after baterial lipopolysaccharide (LPS) administration. We have chosen to evaluate two cytokines and two membrane antigens involved in inflammatory process (TNFα, IL10 CD14 and CD66). After a one-month washing up following the first LPS administration, a partial irradiation (cephalic) was carried out. A second LPS administration was then performed 5 days after radiation exposure. Blood mRNA analysis (TNFα, IL10 CD14 and CD66) showed in the first hand animal-dependent response profiles to LPS and on the other hand radiation-induced modification of individual response to LPS. Ongoing neurobiological studies will be analyzed to develop an integrative experimental model.

(PS1009) Enhanced yield of chromosome aberrations after CT examinations in paediatric patients. Ursula Oestreicher[1], Guenther Stephan[2], Linda Walsh[3], Werner Panzer[4], Karl Schneider[5]. [1]Federal Office for Radiatoin Protection, Oberschleissheim, Germany, [2]Federal Office for Radiation Protection, Oberschleissheim, Germany, [3]GSF-National Research Center for Environment and Health, Neuherberg, Germany, [4]GSF-National Research Center for Environment and Health, Neuherberg, Germany, [5]University of Munich, Munich, Germany.

Purpose: To determine whether computed tomography (CT) could enhance the chromosome aberration yields in paediatric patients.

Material and Method: Blood samples were taken before and after CT scans from 10 children for whom the medical justifications for CT examinations were accidental injuries and not diseases as investigated in earlier studies. Chromosome analysis was carried out in lymphocytes by fluorescence plus Giemsa (FPG) staining exclusively in metaphases of the first cell cycle in vitro.

Results: The mean blood dose of the 10 children was about 12.9 mGy which was determined by a newly developed dose estimation. Based on more than 20,000 analysed cells it was found that after CT examination the frequencies of dicentrics and excess acentric fragments in lymphocytes were significantly increased. By subdividing the children into two age groups, those with an age from 0.4 years to 9 years and from 10 years to 15 years, it became obvious that the observed increase in chromosome aberrations was mainly contributed by the younger age group. In this group the frequency of dicentrics was significantly increased whereas in the older group the observed increase was not significant.

Conclusion: Our results demonstrate that CT examinations enhance the dicentrics yields in peripheral lymphocytes of children aged up to 15 years. Since in particular significantly increased dicentric yields could be observed in children with an age from 0.4 years to 9 years, it can be assumed that children younger than 10 years may be more radiation sensitive than older subjects.

(PS1010) Radiation dose estimation by tooth enamel ESR dosimetry for Nagasaki atomic bomb survivors. Tatsuya Shimasaki[1], Mariko MINE[2], Yutaka OKUMURA[2], Eihichi MIYAMOTO[1], Seiji OKADA[1]. [1]Kumamoto University, Kumamoto, Japan, [2]Nagasaki University, Nagasaki, Japan.

Tooth enamel is the only tissue in the human body that retains indefinitely the history of radiation exposure of Nagasaki Atomic bomb. Tooth enamel has not become widely used for determining radiation exposure of atomic bomb survivors, because any medical or dental diagnostic X rays of the teeth will confuse the measurement of their radiation exposure for atomic bomb. We present that the diagnostic x rays dose can be determined separately from radiation exposure of Nagasaki atomic bomb survivors. We began to request donations of extracted teeth from Adult Health Study participants of the Nagasaki ESR project in 1986. Since that time, 500 teeth have been collected, but only about 35% were found to be suitable for enamel separation and subsequent ESR measurement. To assess dental x-ray dose, each tooth is divided in half-one half from the inside of the mouth and the other from the outside. Separation of the signal of radiation effect from the signal of organic radical is necessary to obtain the accurate intensity. We turned our attention to the problem of separating the contribution due to diagnostic X rays. This technique can be readily achieved by the measurement of two dentalived 17 pieces of extracted human tooth. ESR-estimated doses in their buccal parts plotted against that in their lingual parts. Nearly 12 teeth showed considerably larger ESR-estimated doses in their buccal parts than in their lingual parts, which first seemed to indicate a considerably large contribution from any medical or dental diagnostic X rays exposure. We assessed that the dose ranging 0–170 cGy would be due to medical diagnostic X-ray and to natural radiation and the dental X rays effect. Using ESR dosimetry of tooth enamel to estimate Atomic-bomb gamma ray dose received, we have recently examined 27 teeth donated from Nagasaki A-bomb survivors. All teeth showed considerably smaller ESR-estimated doses than DS86 doses, but most of these discrepant cases were incisors and canines. The results are likely attributable to solar light exposure. In contrast, buccal and lingual doses of molars gave very similar results.We have found that the diagnostic x rays dose can be determined separately from radiation exposure of Nagasaki atomic bomb survivors.

(PS1011) Acute and chronic effects of whole body gamma irradiation on heart contractility and coronary flow. Tatsiana Suvorava[1,2], Klavdiya Bulanova[3], Leonid Lobanok[1,2]. [1]Institute of Radiobiology, National Academy of Sciences of Belarus, Gomel, Belarus, [2]Belarusian State Medical University, Minsk, Belarus, [3]International Sakharov Environmental University, Minsk, Belarus.

Epidemiological and experimental studies revealed the connection between radiation exposure and cardiovascular diseases. We sought to investigate time- and dose-rate depending effects of ionizing radiation on heart contractility and coronary flow, their peripheral neurohormonal and NO-mediated regulation. To accomplish this rats were exposed to whole body acute (^{137}Cs, $9x10^{-4}$ Gy/s, 18 min) and chronic (^{137}Cs, $2.8x10^{-7}$ Gy/s, 41 days) 1 Gy irradiation. Hearts were perfused by Langendorff and left ventricular pressure, maximal rates of pressure development, coronary flow and heart rate were measured on the 3rd, 10th, 30th and 90th days after exposure. Acute irradiation resulted in heart hyperperfusion as evident by NO-mediated increase of coronary flow (9.4 ± 0.3 vs 7.0 ± 0.1 ml/min) and 20 % reduction of the inotropic function on day 3rd. Prolonged irradiation attenuated cardiac contractility only in the late post-radiation (90th day). It was acompanied by a significant reduction of coronary flow (5.6 ± 0.3 vs 6.8 ± 0.2 ml/min) caused by an impairment of endogenous NO-synthesis. Both acute and prolonged radiation significantly decreased cholino- and adrenoreactivity of the heart. Acute radiation attenuated the positive inotropic effects of noradrenaline (NA, 10^{-9}-10^{-5} M) in early terms while low-intensity radiation resulted in the increasing dysfunction with maximal effect on day 90th. NA-mediated relaxation of coronary vessels was attenuated on days 30th and 90th. Similary, hearts of irradiated rats exhibited a significantly lower response to muscarinic stimulation (carbachol, CCh, 10^{-11}-10^{-6}) on days 10th and 30th after acute irradiation and on days 30th and 90th after prolonged one. Moreover, endothelium-dependent CCh-induced NO-synthesis was strongly inhibited in the early and late terms after acute irradiation and for the entire post-radiation after the chronic exposure. Thus, 1 Gy irradiation influences heart contarctility, coronary flow and their neurohormonal regulation in dose-rate dependent manner. Short-term exposure to high intensity radiation causes transient changes while low intensity irradiation results in long-term concequences and may contribute to the increased cardiovascular morbidity and mortality of population living in radioactively contaminated environment.

(PS1012) The changes of human peripheral blood B cell subpopulations and subsets after in vitro irradiation. Zuzana Rehakova[1], Jiri Sinkora[2], Marcela Vlková[3], Doris Vokurkova[4]. [1]Univerzita Obrany, Hradec Kralove, Czech Republic, [2]DAKO, Brno, Czech Republic, [3]Masaryk University, Brno, Czech Republic, [4]Charles University in Prague, Hradec Kralove, Czech Republic.

Biodosimetric markers or biodosimeters are body-born materials capable of reporting on the irradiation dose. In addition to their accessibility, sensitivity and reliability biodosimeters should provide required data as fast as possible since time is a critical factor

for successful treatment of accidentally irradiated individuals. The aim of our work was to evaluate the potential of human peripheral blood B-cell subpopulations and subsets as fast and reliable biodosimetric markers.

Anticoagulant-treated full blood and peripheral blood mononuclear cells (PBMC) were irradiated (3, 6 or 10 Gy) or sham-treated *in vitro*, cultivated for 16 hr and immunophenotyped using cocktails of fluorochrome-labeled monoclonal antibodies and three- or four-color flow cytometry. CD19-positive ($CD19^+$) cell population with light scatter characteristics of intact small lymphocytes was analyzed for the presence of subpopulations and subsets defined by surface expression of one and two additional surface markers, respectively. The "irradiated versus non-irradiated ratio" (IVNIR) for all subpopulations and subsets was calculated by dividing their relative proportion in the irradiated sample by its respective number in the control.

When B-cell subpopulations and subsets characterized by surface expression of CD21, CD27 and CD38 differentiation markers were compared in terms of their relative radiosensitivity, several biodosimetric candidates were revealed. The $CD27^+$ and $CD21^-$ B-cell subpopulations appeared most radiosensitive when the dose of 10 Gy was used. In addition, several B-cell subsets characterized by the expression of two differentiation markers have been shown to bear biodosimetric potential. Among them, the cells with the $CD19^+CD27^+CD38^-$, $CD19^+CD21^+CD27^+$ and $CD19^+CD21^-CD27^-$ phenotypes appear most promising in terms of radiosensitivity, subset size and data variation. The CD19+CD27+ lymphocyte subpopulation has been chosen and tested successfully as a biodosimetric marker within the range of 0 - 10 Gy.

In conclusion, we have identified sensitive, reliable and fast biodosimetric markers in the peripheral blood B-cell compartment.

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(PS1013) Early alterations in pulmonary interleukin expression and cellular responses after low dose radiation. Jacob N. Finkelstein, Jacqueline P. Williams, Eric Hernady, Christina Reed, Carl Johnston. University of Rochester Medical Center, Rochester, NY, USA.

Rationale: As part of our investigation into the consequences of a radiological terrorism event, we have begun to examine the role of previously identified cytokines of interest at low doses and early time points, determining their potential use biodosimeters and targets for therapeutic intervention. Our hypothesis is that lowering the total dose does not alter the mechanism of activation of cytokine expression, but causes a temporal shift, delaying the onset of physiologic symptoms of tissue damage.

Methods: Groups of 10 C57BL/6 mice received either whole lung or total body irradiation (TBI) at doses ranging from 0 - 10 Gy. Mice were sacrificed between 1 hr and 4 weeks post-irradiation, and histologic, mRNA and protein analyses were performed on bronchoalveolar lavage, serum and lung tissue.

Results: TUNEL positive staining was seen as early as 1 hour post-radiation, with a peak at 6 hours before returning to control levels 24 hours following both types of exposure, with, surprisingly, a more robust response seen following TBI. IL-1β and IL-1RII mRNA abundance were increased 1 hr post irradiation (PI), then declined over the following 24 hours in a dose dependent manner. Again, TBI exposed mice demonstrated a greater induction as compared to whole lung exposure.

Conclusions: Comparisons of response following TBI and whole lung irradiation show similar patterns of induction in both cellular and cytokine response, with a greater induction following TBI exposure suggesting a systemic influence. Early induction of specific interleukin family members correlates with our previous work and suggest that this cytokine family will be useful as biomarkers to identify individuals as well as targets for mitigation.

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(PS1014) Radiation injury during postnatal lung development. Carl Johnston, Jacqueline P. Williams, Eric Hernady, Jacob N. Finkelstein. University of Rochester Medical Center, Rochester, NY, USA.

Rationale: Processes involved in postnatal development appear to be critical variables in the ability of the lung to cope with external stress. As part of our investigation into the consequences of a radiological terrorism event, we and other groups are investigating the role of cytokines following low doses of radiation at early time points with the intent of establishing their potential use as markers of radiation exposure. However with our special interest in the pediatric population, we have begun to examine the respective roles of the same cytokines in adults versus pups at different stages of postnatal development. We contend that early induction of specific pulmonary inflammatory markers detected in the adult post irradiation will not appear during postnatal lung development and hypothesize that these cytokine families will not be useful as biomarkers of radiation exposure during development.

Methods: C57Bl/6 mice, 4, 7, 10, 14 and 56 days of age, received total body irradiation of 5 Gy. Mice were examined 1 and 6 hours post irradiation (PI). Analysis included cytokine and chemokine mRNA abundance, TUNEL staining and histological analysis.

Results: Changes in IL-1ß and IL-1RII were not detected until 14 days of age, which coincided with the end of postnatal lung growth. However TUNEL staining was detected at all ages examined with peak staining occurring 6 hours (PI) suggesting that although inflammatory mediators did not respond during development after radiation exposure, pulmonary injury did occur.

Conclusions: Lack of cytokine induction in early life could subject tissues to a greater injury resulting in long-term alterations in structure and function. Different identification end points and drug treatments may be required to treat children.

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(PS1015) Analyisis of dose record and epidemiology for radiation workers in Korea. Soo Yong Choi[1], Hae Won Chung[2]. [1]Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea, [2]School of Public Health, Seoul National University, Seoul, Republic of Korea.

Purpose

The aim of this study is to analyze the occupational exposure for external radiation and to evaluate radiation effects on Korean radiation workers.

Population and Methods

The National Dose Registry contains radiation exposure records for all monitored radiation workers since its creation in 1984.

We are carrying out epidemiological survey for radiation workers according to structured questionnaire. The items of information included personal identification, employment and dose data. The frequencies of various types of chromosome aberrations in radiation workers were compared with controls.

The data were analyzed according to year, sex, age, duration of occupation, exposure dose, etc. using SPSS statistical package(version 14.0). The goodness-of-fit test for Poisson distribution was done with chromosomal aberrations among study subjects.

Results

The total number of workers registered from 1984 to 2005 was 61,610. The number of workers steadily increased and the accumulated dose somewhat increased. The collective annual dose of radiation workers was 345.823 man Sv and the mean annual dose was 1.34mSv.

The frequencies of chromosome aberrations in 102 workers were compared with those in 42 controls. The frequencies of all types of chromosome aberrations in the exposed subjects were higher than those in the control group. Poisson regression analysis, with both exposed and control included, showed that there was significant association of chromosome aberrations with radiation dose, duration of work, age and alcohol intake.

We started to survey radiation workers in order to evaluate radiation effects, collected epidemiological data for 9,157 workers and analyzed their lifetime radiation exposure doses. Follow-up is

carrying out using the Korean Mortality Data, Cancer Registry and individual investigation.

Conclusions

The data on occupational doses shows that radiation protection in Korea is improving, even though annual doses are still higher than other countries.

The frequencies of all types of chromosome aberrations in the exposed subjects were higher than those in the control group.

The epidemiological follow-up from 2000 is carrying out in order to detect and measure directly the risks of cancer.

(PS1016) What consequences of the prolonged irradiation may be? Alexandra P. Kravets. Institute of cell biology and genetic engeneering, Kiev, Ukraine.

In spite of a growing body of information, the question about biological efficacy of low dose is still under the discussion. Available data show that action of a low-level radiation exposure may be beneficial and may increase vital capacity of organisms, induce hormetic reaction or stimulate adaptation. At the same time there are numerous proofs that additional irradiation with low dose results in the anomalies of development, losses of fertility; to the hypersensitiveness phenomena and genetic instability.

In the conditions of protracted environmental exposure development of effects in time is the major aspect of problem of low doses.

The lecture is devoted to analysis of the current data related to the study of dynamic effects of the prolonged irradiation. On the basis of analysis of literary and own experimental information classification of temporal dependence of radiation response is offered.

The several of biological reactions (output of chromosomal aberration, phenomenon of genetic instability in generation of organisms with a short life span, fertility, fecundility) are analyzed.

It is shown that there are such types of reaction development:

1. Primary stimulation (hormetic reaction) with the subsequent extinction of stimulation and forming of new stable level in by promoted radiostability (effect of radioadaptation).

2. Primary stimulation (hormetic reaction) with the subsequent fading of stimulation and appearance of signs of organism exhaustion.

3. Stimulation with the subsequent transition through maximum hormetic effect and output on the new stationary level of biological efficiency irradiation. Biological efficiency can be both higher (full adaptation) and below (partial adaptation) than that which was observed at the beginning of action of factor.

4. Primary oppressing of reaction with the subsequent increase of radiostability (development of adaptation) and forming of new stable level of radiostability. In this case biological efficiency can be both higher (full adaptation) and below (exhaustion) that was observed at the beginning of irradiation.

Question about a necessity to take into account dynamic nature of reactions of organism on constantly operating factors at prognostication of consequences of the prolonged irradiation will be discussed.

(PS1017) Electron paramagnetic resonance dosimetry investigation for population living in the vicinity of the Semipalatinsk Nuclear Test Site. Kassym S. Zhumadilov[1], A. Ivannikov[2], D. Zharlyganova[1], V. Stepanenko[2], V. Skvortsov[2], K. Apsalikov[3], Zh. Zhumadilov[4], S. Toyoda[5], S. Endo[1], K. Tanaka[1], C. Miyazawa[6] and M. Hoshi[1]. [1]Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima 734-8553, Japan, [2]Medical Radiological Research Center, Korolev str., 4, Obninsk, 249036, Russia, [3]Kazakh Scientific-Research Institute for Radiation Medicine and Ecology, Semipalatinsk, Kazakhstan, [4]Semipalatinsk State Medical Academy, Semipalatinsk, Kazakhstan, [5]Department of Applied Physics Faculty of Science Okayama University of Science, 1–1 Ridai, Okayama, 700-0005, Japan, [6]School of Dentistry, Ohu University, 31–1, Aza-Misumido, Tomita-machi, Koriyama-shi, Fukushima Pref. 963-8611, Japan

The method of electron paramagnetic resonance (EPR) dosimetry was applied to human tooth enamel to obtain individual absorbed doses of residents of settlements in the vicinity of the Semipalatinsk Nuclear Test Site (SNTS), Kazakhstan. Most of settlements (Dolon, Mostik, etc.) are located near the central axis of radioactive fallout trace from the most contaminating surface nuclear test in 29, August 1949. The other settlements located in the radioactive fallout trace as a result of surface nuclear tests in 24, August 1956 (Znamenka) and in 12, August 1953 (Sarzhal). Semipalatinsk city was included to investigation as a biggest city which located close to SNTS.

Tooth samples were extracted according to medical indications in a course of ordinary dental treatment. Kokpekty was chosen as control and was not subjected to any radioactive contamination and located 400 km to the Southeast from SNTS.

It was found that the excess doses obtained after subtraction of the contribution of natural background radiation ranged up to about 450 mGy for residents of Dolon, whose tooth enamel was formed before 1949, and do not exceed 100 mGy for younger residents. For residents of Mostik, excess doses do not exceed 100 mGy for all ages. For residents of Sarzhal village, the maximum of excess dose were determined as 138.2 mGy and for Residents of Znamenka maximum excess dose was about 268 mGy.

(PS1018) Biological evaluation of a dose response in the oral cavity of patients undergoing head and neck radiotherapy. Matthew Coleman[1], Samir Narayan[2], Joerg Lehman[2], Kerry Nolan[1], Andrew T. Vaughan[2], Claus Yang[2], James Purdy[2], Grace Loredo[2], Srinivasan Vijayakumar[2]. [1]Lawrence Livermore National Laboratory, Livermore, CA, USA, [2]University of California, Davis, Sacramento, CA, USA.

In vivo dose verification and correlation of dose with biological changes within humans remains an active area of investigation with implications for radiation safety and treatment. In a collaborative effort between Lawrence Livermore National Laboratory and the University of California Davis Medical Center we developed RNA transcript assays for dose analysis within the oral cavity of head and neck cancers patients treated with radiation therapy. Nine males and 3 females undergoing therapy for head and neck cancer were enrolled on a prospective protocol approved by our institutional review board. Median age was 61 (range 23–79). For buccal sampling four points were chosen in separate quadrants of the patient's oral cavity adjacent to identifiable landmarks along the buccal mucosa. Each patient underwent buccal sampling prior to and 24h following the first radiation treatment at the four selected points using a cytobrush to remove surface cells - preserved in RNAlater for transcript analysis using QPCR. Maximum and average radiation doses received by the sampled sites of a single patient ranged from 0.32 to 2.04 Gy and 0.65 to 2.07 Gy, respectively. A point dose maximum of 2.07Gy (Ptn 6) and a widest range 0.05 - 1.1Gy (Ptn 1) were observed for individual sampling points. Calculated dose distributions were compared to dose at each sample point using MOSFET dosimeters. A total of 16 transcripts were selected for analysis based on microarray studies in cell lines that identified robust ionizing radiation-induced responses. QPCR analysis was focused on only 6 of the patients for which at least one of the four samples received a dose >1 Gy. The QPCR method was able to detect all 16 transcripts tested within each patient and in general showed elevated levels post irradiation. However, there was substantial variation in sample transcript both within the same patient as well as between patients. Transcripts such as ASTN2, PCNA, TNFRSF6, GADD45A and CDKNIA showed the most robust irradiation response across the 6 patients. Further analysis is ongoing to correlate the transcript responses with patient clinical outcomes.

This work was performed under the auspices of the US DOE by the University of California, LLNL under Contract No. W-7405-Eng-48 with funding from the DOE Low Dose Radiation Research Program.

(PS1019) Radiation-induced, nitric oxide-mediated bystander effects contribute to the induction of radioadaptive responses. Hideki Matsumoto[1], Masanori Tomita[2], Kensuke Otsuka[2], Takeo Ohnishi[3]. [1]Division of Oncology, BIRC, University of Fukui, Matsuoka, Fukui, Japan, [2]Low Dose Radiation Research Center, Central Research Institute of Electric Power Industry, Komae, Tokyo, Japan, [3]Department of Biology, Nara Medical University School of Medicine, Kashihara, Nara, Japan.

The reduced biological effects of radiation exposure seen in cells after conditioning exposure to low doses or at a low dose-rate (i.e. the acquisition of resistance against high dose radiation) is called the "radioadaptive response", and many studies concerning this phenomenon have been reported since the 1980s. Radioadaptive responses have been observed using various endpoints, such as chromosomal aberrations, mutation, and clonogenic survival. However, the mechanisms of the radioadaptive response are not fully known. Here we show that radiation-induced nitric oxide (NO) radicals contribute to the induction of radioresistance as determined by cell survival to a subsequent high dose exposure of either low or high LET radiation in human glioblastoma A-172 cells or non-small cell lung carcinoma H1299 cells transfected with wild-type *p53* (H1299/wt*p53* cells). The induction of radioresistance was observed when cells were exposed to X-rays, Carbon (290 MeV/u, 70 keV/μm), Iron (500 MeV/u, 200 keV/μm) or Neon (400 MeV/u, 31 keV/μm) ion beam at a low dose followed by an acute high dose of these radiations, respectively. In addition, the induction of radioresistance was not observed in the presence of aminoguanidine (a specific inhibitor of iNOS) or c-PTIO (a specific scavenger of NO radicals). Moreover, the radioresistance was observed when cultures were treated with isosorbide dinitrate (a NO radical generating agent). These findings suggest that (1) high LET radiation might actually induce radioadaptive responses as is the case of low LET radiation, (2) radiation-induced bystander effects might actually contribute to the induction of radioadaptive responses, and (3) NO radicals are an initiator of radioadaptive response. In other words, cells may respond, not to a pre-exposed radiation dose, but to NO radicals generated after the conditioning radiation dose, and it is these NO radicals which subsequently act as a radio-protective agent.

(PS1020) Mitochondria-depended signaling pathway are involved in the early process of radiation induced bystander effects. Shaopeng Chen[1], Lijun Wu[1], Ye Zhao[1], Wei Han[1], Lingyan Zhu[1], Jun Wang[1], Linzhi Bao[1], Erkang Jiang[1], An Xu[1], Tom K. Hei[2], Zengliang Yu[1]. [1]Key Laboratory of Ion Beam Bioengineering, Chinese Academy of Sciences, Hefei, China, [2]Center for Radiological Research, Columbia University, New York, NY, USA.

The initiation and propagation of the early signaling events in radiation-induced bystander effects induced by low doses of α-particles are very important for understanding the underlying mechanism of the bystander process. In our previous study, we demonstrated that nitric oxide (NO) produced by constitutive nitric oxide synthase (cNOS) in human fibroblasts (AG 1522) acted as an intercellular signaling molecule to initiate and activate the bystander process. In the present study, we used either mtDNA-depleted (ρ0) A_L or normal (ρ+) A_L cells as irradiated donor cells and normal human skin fibroblasts as recipient cells in a series of medium transfer experiments to investigated the role of mitochondria in the bystander process. Our results indicated that donor cells that were depleted of mtDNA (ρ0) had an attenuated DNA double strand break (DSB) inducing activity, based on immunohistochemical staining of gamma-H2AC foci, in recipient human fibroblasts compared with normal (ρ+) A_L cells. Furthermore, inhibition of mitochondrial respiratory chain function decreased the DSB inducing activity (DIA) of conditioned medium from irradiated ρ+ A_L donor cells. Using specific inhibitors of NOS, including that of cNOS, it was found that, consistent with our previous observations, the DIA of conditioned medium derived from ρ+ A_L donor cells was significantly decreased and that radiation could stimulate cellular NO production in irradiated ρ+ A_L cells, but not in ρ0 A_L cells. Moreover, treatment with Ruthenium Red (RR) and calmidazolium chloride, inhibitors of mitochondrial calcium uptake and calmodulin, respectively, significantly decreased the DIA of conditioned medium from irradiated ρ+ A_L donor cells compared with the sham-irradiated controls. These observations, together with the findings that RR treatment significantly reduced the NO levels in irradiated ρ+ A_L cells to background suggest that mitochondria are involved in the early process of radiation-induced bystander effects and that calcium-dependent mtNOS might play an essential role in the process.

(PS1021) Direct ESR measurement of novel slow-releasing radicals those might be responsible for delayed mutation induction in 4 Gy γ-irradiated syrian golden hamster embryonic cells. Jun Kumagai[1], Akira Harada[1], Ryuichi Kanamori[2], Eri Yoshikawa[2], Masayoshi Miyazaki[1], Seiji Kodama[3], Masami Watanabe[4]. [1]Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Nagoya, Japan, [2]Department of Applied Chemistry, School of Engineering, Nagoya University, Nagoya, Japan, [3]Frontier Science Innovation Center, Organization for University-Industry-Government Cooperation, Osaka Prefecture University, Sakai, Japan, [4]Kyoto University Research Reactor Institute, Kumatori-cho, Japan.

Delayed radiation effects as genomic instability or bystander effects have been important topics to be elucidated for the last decade since we have found drastic decrease in hprt- mutation frequency by treatment of ascorbate AFTER irradiation (Koyama et al. *Mutat. Res.* 421, 45–54, 1998.). We have found long-lived radicals which might be related to mutation induction but those could be detected with tremendously high dose radiation to the cells (Kumagai et al. *Radiat. Res.* 160, 95–102, 2003). Now we report that novel slow releasing radicals (SRRs) those levels have increased in several hours after irradiation in SHE cells with a dose of 4 Gy. SHE cells cultured in eleven T175 flasks in confluent condition with Dulbecco's modified eagle MEM were γ-irradiated (4 Gy) and harvested at 1 or 5 h after irradiation. The harvested and centrifuged cells, totally ca. 1×10^8 cells, were put into suprasil quartz ESR tubes and immediately frozen at 77 K. ESR spectra at 77 K were measured at 5 μW of microwave power and accumulated 16 times for each spectrum. Unirradiated cells have radicals as a concentration of 1.6×10^5 spins/cell. Although the species have not definitely been identified yet, they might be semiquinone radicals in mitochondria. For 4 Gy irradiated cells after 1 and 5 h, the concentration increased to 1.9×10^5 (+18 %, $p = 0.135$) and 2.2×10^5 (+36 %, $p = 0.0017$) spins/cell, respectively. Because ESR spectra of control and irradiated cells are not completely similar to each other, different kinds of novel radicals are slowly released in 4 Gy irradiated cells AFTER irradiation, denoted here as Slow Releasing Radicals (SRRs). When 5 mM of ascorbate were added to the cultured medium for 4 Gy irradiated cells at 20 min after irradiation and treated for 2 h, the production of SRRs at 5 h after irradiation were suppressed to 1.9×10^5 (−50 %) spins/cell. It indicates that SRRs may have an important role for delayed radiation effect of mutation induction. Treatment of myxothiazol (0.5 μM), which is an inhibitor of ATP production in mitochondria, during and after irradiation prevented the increase in SRRs at 5 h after irradiation, and H_2O_2 treatment (1 mM) for 30 min induced SRRs. These results suggest that SRRs might be produced near functionally abnormal mitochondria that eject electrons to produce O_2^- as a precursor of H_2O_2.

(PS1022) The role of DNA double strand breaks repair in radiation induced bystander responses. Genro Kashino. Research Reactor Institute, Kyoto University, Osaka, Japan.

Evidence is accumulating that irradiated cells produce some signals which interact with non-exposed cells in the same population. We investigated the role of DNA double strand breaks (DSB) repair in radiation induced bystander responses. DSB repair-deficient cell line, xrs5 (Ku80-deficient) and wild type CHO were used in this study. Results from the studies with targeted soft-X-ray microprobe and medium transfer method suggest that DSB are caused by factors secreted in the medium from irradiated cells. We also analysed the mechanism of bystander signal arising in CHO cells and xrs5 cells, focussing on the relationship between DSB repair capacity and bystander signal arising in irradiated cells. When conditioned medium from irradiated cells was transferred to non-

irradiated xrs5 cells, the level of induction was independent of whether the medium came from irradiated CHO or xrs5 cells. This result suggests that the activation of a bystander signal is independent of the DNA repair capacity of the irradiated cells. Overall the work presented here adds to the understanding that it is the DSB repair phenotype of the cells receiving bystander signals which determines overall response rather than that of the cell producing the bystander signal.

(PS1023) Activation of signalling pathways in cells exposed to medium from irradiated cells. Fiona Lyng[1], Orla Howe[1], Rocky Bo Li[1], Brendan McClean[2]. [1]Dublin Institute of Technology, Dublin, Ireland, [2]St Luke's Hospital, Dublin, Ireland.

Considerable evidence now exists relating to radiation-induced bystander effects but the mechanisms involved in the transduction of the signal are still unclear. Signalling pathways leading to apoptosis, such as calcium, MAP kinase, mitochondrial and reactive oxygen species (ROS) signalling have been shown previously. The aim of this study was to investigate the cellular response and the dynamic events which happen very quickly after exposure of cells to medium from irradiated cells.

Human keratinocyte cells (HaCaT cells) were irradiated using a Cobalt-60 teletherapy source. One hour after irradiation the medium was harvested and filtered. This irradiated cell conditioned medium (ICCM) was then added to parallel HaCaT cells. Key proteins implicated in radiation induced bystander responses, including Bcl-2, bax and c-myc were examined by Western blotting. Apoptotic and membrane signalling and the induction of reactive oxygen and nitrogen species was followed in real time in living cells

Increased expression of key proteins was found within 1 hour of addition of ICCM. Apoptotic and membrane signaling and induction of ROS and RNS were found to occur within minutes of addition of ICCM.

Further investigations of these signaling pathways may aid in the identification of novel therapeutic targets.

(PS1024) Modeling of bystander signaling related to different cellular endpoints. Fakir Hatim[1], Wai Yuan Tan[2], Werner Hofmann[1], Rainer Kurt Sachs[3], Kevin Prise[4]. [1]University of Salzburg, Salzburg, Austria, [2]University of Memphis, Memphis, TN, USA, [3]University of California, Berkeley, CA, USA, [4]Gray Cancer Institute, Middlesex, United Kingdom.

Recent advances in the studies of biological effects of low level exposures to ionizing radiation have demonstrated multiple bystander responses that can cause significant differences in the effects observed compared to the results of direct DNA damage. Such bystander effects may have significant implications for risk estimation.

The main questions are: How relevant are bystander effects for carcinogenesis? And how do they affect the uncertainties in assessing cancer risks associated with low doses and dose rates of ionizing radiation?

In this presentation we propose a multiple pathways stochastic model for radiation carcinogenesis involving a one-stage pathway and a two-stage pathway for cancer initiation. We will develop stochastic equations for the development of preneoplastic lesions and tumors.

We assume that initiation is related to single hit frequencies as well as dose-dependent specific energy distributions. Such microdosimetric quantities are important in bystander modeling because they provide information about the fractions of directly irradiated cells and the number of hits per irradiated cell. We further assume that each directly damaged cell may emit signals to a given number of bystander cells which will respond either by inactivation, apoptosis or induction of malignant phenotypes and the probability to trigger such effects is related to the cellular dose. The response will reach a plateau depending on the endpoint studied, the type of cells and the medium.

The results obtained show a good agreement with different recent experiments and correctly reflect the main features observed for multiple endpoints, including cell killing, micronuclei formation and oncogenic transformation. It can be shown that the present model generalizes previous bystander models.

(PS1025) Bystander effects in 3-dimensional tissue: a quantitative mechanistic model of spatial patterns. Igor Shuryak. Center for Radiological Research, New York, NY, USA.

Non-targeted (bystander) effects of ionizing radiation are caused by intercellular signaling; endpoints include production of DNA damage and alterations in cell fate (e.g., apoptosis, differentiation, senescence, proliferation). Recent experimental results suggest that the range of the bystander effect may be of the order of 1 mm in normal human tissue. Quantitative mechanistic models of these bystander effects are needed for cancer risk estimation at low radiation doses below the epidemiological detection threshold: In particular, understanding the spatial patterns of bystander responses is important because it provides estimates of the number of non-irradiated bystander cells that are affected per directly-irradiated cell.

In a first approach to quantitative modeling of spatial bystander effects in 3-dimensional tissue, we assume the following: (1) The bystander phenomenon results from signaling molecules that rapidly propagate from irradiated cells, and decrease in concentration (exponentially in the case of planar symmetry) as distance increases; the mode of propagation is likely to be analogous to the "fire-diffuse-fire" model of calcium signaling. (2) These signals can convert cells to a long-lived epigenetically "activated" state, e.g., a state of oxidative stress; cells in this state are more prone to DNA damage than normal, and therefore exhibit an increased response for many endpoints (e.g., apoptosis, micronucleation).

These assumptions are implemented by a mathematical formalism and computational algorithms. The model adequately describes recent experimental data on spatial bystander responses in a 3-D artificial human skin system, using a small number of mechanistic parameters, some of which can be estimated from the literature.

(PS1026) Mechanism of radiation induced bystander effects: implication from mitochondrial function. Hongning Zhou, Vladimir Ivanov, Yu-Chin Lien, Alan Bigelow, Tom K. Hei. Center for Radiological Research, Columbia University Medical Center, New York, NY, USA.

Considerable evidence has emerged indicating that non-irradiated bystander cells demonstrate similar cytotoxic and genotoxic responses as directly irradiated cells. However, the precise mechanism of the bystander effect is still unclear. To better understand the mechanisms of radiation induced bystander effect, the signal transduction pathways related to mitochondrial function were investigated using the Columbia University charged particle microbeam. Exponentially growing rho^0 and rho^+ human skin fibroblasts (HSF) were plated in specially designed strip mylar dishes and irradiated with alpha particles upon confluency. After irradiation, cells were incubated in the mylar dishes overnight before being processed for survival, and mutagenesis studies. To further explore the role of mitochondria in radiation induced bystander effect, a microbeam was used to lethally irradiate either rho^0 or rho^+ cells in a mixed, confluent culture and the bystander response was determined in the non-irradiated fraction.

Wild type (rho^+) HSF, in the presence of irradiated counterparts (0.5 Gy alpha particles), showed a bystander hprt mutant fraction that was 2.1 fold higher than the spontaneous level. In contrast, under similar irradiation conditions, rho^0 cells had a bystander mutant fraction that was 4 fold higher than non-irradiated rho^0 cells. Furthermore, nitric oxide scavenger can significantly decrease the bystander mutagenesis in both cell lines. The observation that rho^0HSF showed a higher bystander mutagenic response in confluent monolayers was similarly demonstrated using microbeam when a fraction of the same population were irradiated with lethal doses. Using mixed cultures of rho^0 and rho^+ cells and targeted only one population of cells with a lethal dose of alpha

particles, a decreased bystander mutagenesis was uniformly found in non-irradiated bystander cells, in contrast to data obtained using similar cell types. These results indicated that mitochondrial deficient cells have deficiencies either in delivering or receiving the bystander signals and that the bystander signals are dependent on mitochondrial function in the human fibroblast model.

(PS1027) The role of gap-junction communication in the cellular responses to high and low dose gamma-rays. Manuela Buonanno[1], Zhi Yang[2], Badri N. Pandey[3], Sonia M. de Toledo[2], Andrew L. Harris[2], John B. Little[4], Edouard I. Azzam[2]. [1]University of Medicine and Dentistry of New Jersey-NJMS, Newark, NJ, USA, [2]University of Medicine and Dentistry of New Jersey-NJMS, newark, NJ, USA, [3]Bhabha Atomic Research Centre, Mumbai, India, [4]Department of Genetics and Complex Deseases, Boston, MA, USA.

Coordinated interaction of multiple cellular processes is likely involved in the sensing and repair of ionizing radiation-induced damage. While signaling pathways implicated in theses processes are modulated by biochemical and molecular changes that are directly triggered by radiation in individual cells, they may also be affected by bystander effects involving communication of signaling molecules among irradiated cells. Such interactions may involve secreted factors, junctional channels and or other modalities. Gap junction channels are found between most cells in the body, and the molecular signaling they mediate is crucial for normal development, physiology and response to disease. We and others have shown that such channels mediate the propagation of radiation-induced effects (Radiat. Res. 1998), and the phenomenon is not unique to ionizing radiation. Here, we show that functional gap-junctions have a significant role in modulating the duration of the G1 checkpoint, repair of potentially lethal damage and survival of 137Cs γ-irradiated cells in confluent cultures. Furthermore, gap-junctions composed of different connexins differentially modulate the cellular response to radiation. Whereas connexin 43 and connexin 26 gap-junctions enhance radiation sensitivity, connexin 32 junctions attenuate radiation toxic effects. Loss of functional connexin 43 junctional communication significantly attenuates duration of the radiation-induced G1 delay. In irradiated cells, up-regulated connexin proteins localize in cholesterol-rich areas of the plasma membrane and are associated with functional gap-junction communication. Our preliminary data also support a role of gap-junction communication in expression of radiation-induced adaptive responses. Collectively, these data indicate that connexin channel permeability has a significant role in the response to ionizing radiation. In addition, connexins may also participate in other processes that regulate the radiation response. The regulation of intercellular communication may be used in conjunction with radiotherapy and chemotherapy to manage gap-junction proficient tumors. Supported by grant CA92262-01A1 from the NIH and grant DE-FG02-07ER64344 from the US Department of Energy

(PS1028) Non-targeted effects of ionising radiation - a new european integrated project, 2006–2010. Sisko Salomaa[1], Eric G. Wright[2], Guido Hildebrandt[3], Munira Kadhim[4], Mark P. Little[5], Kevin M. Prise[6], Oleg V. Belyakov[1]. [1]STUK - Radiation and Nuclear Safety Authority, Helsinki, Finland, [2]University of Dundee, Dundee, United Kingdom, [3]University of Leipzig, Leipzig, Germany, [4]MRC Radiation and Genome Stability Unit, Harwell, Didcot, United Kingdom, [5]Imperial College Faculty of Medicine, London, United Kingdom, [6]Gray Cancer Institute, Northwood, London, United Kingdom.

The general objectives of the NOTE project are: (1) to investigate the mechanisms of non-targeted effects, in particular, bystander effects, genomic instability and adaptive response; (2) to investigate if and how non-targeted effects modulate the cancer risk in the low dose region, and whether they relate to protective or harmful functions; (3) to investigate if ionising radiation can cause non-cancer diseases or beneficial effects at low and intermediate doses; (4) to investigate individual susceptibility and other factors modifying non-targeted responses; (5) to assess the relevance of non-targeted effects for radiation protection and to set the scientific basis for a modern, more realistic, radiation safety system; (6) to contribute to the conceptualisation of a new paradigm in radiation biology that would cover both the classical direct (DNA-targeted) and non-targeted (indirect) effects. The NOTE brings together 19 major European and Canadian groups involved in the discovery, characterisation and mechanistic investigation of non-targeted effects of ionising radiation in cellular, tissue and animal models. The NOTE research activities are organised in six work packages. Four work packages (WPs 2–5) are problem-oriented, focussing on major questions relevant for the scientific basis of the system of radiation protection: WP2 Mechanisms of non-targeted effects, WP3 Non-cancer diseases, WP4 Factors modifying non-targeted responses, WP5 Modelling of non-targeted effects. The integration activities provided by WP6 strengthen the collaboration by supporting the access to infrastructures, mobility and training. WP7 provides dissemination and exploitation activities in the form of workshops and a public website. Managerial activities (WP1) ensure the organisation and structures for decision making, monitoring of progress, knowledge management and efficient flow of information and financing. Coordinator of the NOTE project is Prof. Sisko Salomaa, STUK - Radiation and Nuclear Safety Authority, Helsinki, Finland. The project is supported by the European Commission under the Euratom specific programme for research and training on nuclear energy, 6th Framework Programme. Please visit the project website http://www.note-ip.org to obtain more information or contact us by e-mail note@stuk.fi.

(PS1029) Atherosclerotic lesion development in gamma-radiation exposed apolipoprotein $E^{-/-}$ mice. Ron E. Mitchel[1], Stewart C. Whitman[2], Heather Wyatt[1]. [1]Atomic Energy of Canada Ltd., Chalk River, ON, Canada, [2]University of Ottawa Heart Institute, Ottawa, ON, Canada.

Mice that are genetically defective for synthesis of the protein apolipoprotein E (*Apoe*$^{-/-}$) become dyslipidemic, and as a result, are predisposed to the development of atherosclerotic lesions that morphologically mimic the human condition. Using this mouse model of cardiovascular disease, we tested the influence of a γ-radiation exposure on atherosclerotic lesion development and serum lipoprotein distributions. Compared to unexposed controls, exposure of the mice to 2 Gy reduced the average aortic lesion area by about 25%, when the mice were examined 6 months after exposure. No significant difference was seen 3 months after exposure. There was no significant difference in the protective effect between exposure to 2 Gy at high dose rate (0.36 Gy/min) or low dose rate (1 mGy/min, 100 mGy/day). Compared to controls, neither 2 Gy radiation exposure significantly changed total serum cholesterol levels, VLDL, LDL or HDL lipoprotein fraction proportions or lesion frequency. Exposure to 0.5 Gy at high dose rate had no significant effect on lesion area or frequency. The results suggest that γ-radiation exposure of mice that are genetically predisposed to atherosclerosis (*Apoe*$^{-/-}$) does not increase the risk of atherosclerotic heart disease, and further suggest that such genetic predisposition is not linked to any reported increased risk of atherosclerotic heart disease in low LET radiation-exposed human populations. Further investigations, including the role of Tp53 and responses to lower doses given at early and late stage disease are in progress as part of the European Commission NOTE program. https://ssl.note-ip.org/

(PS1030) Production of cytokines by splenocytes and macrophages after single or fractionated low-level irradiations with X-rays. Marek K. Janiak, Aneta Cheda, Ewa M. Nowosielska, Jolanta Wrembel-Wargocka. Military Institute of Hygiene and Epidemiology, Dept. of Radiobiology and Radiation Protection, Warsaw, Poland.

Splenocytes and activated cytotoxic macrophages serve as primary effector cells of the anti-tumor surveillance system. The mechanism of the activity of these cells includes non-specific recognition and induction of the tumor cells' death through the secretion of cytolytic factors and cytokines which either directly

induce apoptotic cell death or stimulate cytotlytic function of other effector cells. In view of this, the aim of the present study was to assess the effects of irradiations with low and higher doses of X-rays on the production of the selected cytokines.

In the study, murine splenocytes, splenic NK lymphocytes and peritoneal macrophages obtained form BALB/c mice were irradiated *in vitro* with 0.1, 0.2, or 1.0 Gy X-rays or collected from animals exposed to single or fractionated X-rays so that the absorbed doses amounted to 0.1, 0.2, or 1.0 Gy. Production of IL-1β, IL-2, IL-12, IFN-γ and TNF-α by these cells was examined using the ELISA assays.

Single and fractionated doses 0.1, 0.2, or 1.0 Gy stimulated production of IL-2 by splenocytes. IFN-γ production by NK cells markedly increased after exposure of mice to all three doses, single or fractionated, of X-rays. Likewise, production of IL-1β, TNF-α, and IL-12 by macrophages markedly increased after single or fractionated exposure of mice to all the three doses of X-rays.

Collectively, these results indicate that both single and fractionated exposures of mice to 0.1, 0.2, or 1.0 Gy X-rays stimulate synthesis of cytokines responsible for anti-tumor activities of splenocytes (IL-2), NK cells (IFN-γ) and macrophages (IL-1β, IL-12 and TNF-α).

(PS1031) Morphological and biomolecular changes induced by UV laser irradiation in silkworm, *Bombyx mori* embryo. Hosaholalu B. Manjunatha, Satyanarayanagouda R. Hosagoudar. Department of Sericulture, Karnatak University, Dharwad, Karnataka, India.

The biological, morphological and biochemical impact of UV laser on silkworm, *Bombyx mori* was investigated by irradiating embryos of varied stages to nano and picosecond pulse laser of 10 mJ of 35 psec with 10 Hz at 355 nm. UV laser irradiation resulted in various structural polymorphisms. Asymmetrical fusion of segments was not confined to larva but persisted throughout pupal and adult stages. Development of extra caudal horn, unequal size and lack of antenna, retarded thoracic legs and variation in larval markings were observed in nanosecond irradiated embryos. Notably, the pupae with transpositioned antennae, pseudo abdominal and caudal legs were produced from 2 and 8 hrs old embryos irradiated for 30 and 50 seconds (picoseconds); missing and underdeveloped antennae and legs in moth produced from 16 hr (60 seconds) and 8 hrs (50 seconds) old embryos irradiated revealed the cytotoxic effect of UV laser on embryonic cells determining larval, pupal and adult traits. The changes in protein pattern were not distinct until the 5th day of embryogenesis as revealed by SDS-PAGE. A 178 kiloDalton (kDa) protein resolved into 198, 184 & 169 kDa polypeptides and 154 kDa new protein band along with other proteins of 110, 45, 41 & 38 kDa were noticed on 6th day in UV laser (nanosecond) irradiated eggs. Concomitantly, 41-kDa new protein and delayed utilization of yolk proteins on 9 and 10 day was obvious in picosecond irradiated embryos. Further, 33, 32, and 6.2 kDa and 24, 25 and 6.2 kDa new protein bands were observed in the haemolymph of 5th instar silkworm larvae derived from UV laser nano and picoseconds irradiated embryos respectively. A significant difference in morphological anomalies between nano and picosecond laser in accordance with the age of the embryo was obvious. The rate of morphological variations (somatic mutation) was 6.2 for picosecond and 1.0 percent for nanosecond at 8 and 16 hrs embryos respectively. UV laser irradiation displayed duration dependent effects causing a maximum rate of morphological variations 9.5 percent at 50 seconds of picosecond and 1.2 percent for 150 seconds of nanosecond.

(PS1032) Hematopoiesis under chronic low dose rate irradiation: Quantitative modeling of blood responses of a large animal model. Thomas M. Seed. Radiation Effects Research Foundation, Hiroshima, Japan.

Few quantitative data are available on longitudinal hematopoietic effects of chronic, low-daily dose, ionizing radiation exposure in mammals- large or small. Purpose: Long-term hematological responses of canines exposed to continuous, low daily doses of gamma rays were evaluated using archived data and modeled, with quantitative estimates made of the dose-rate dependent responses. Methods: Responses of 96 beagles were evaluated over life-span equivalent periods: 77 animals were chronically exposed to low daily doses of whole-body ^{60}Co rays (0.3 - 12.75 cGy d^{-1} 22 h^{-1}), while the remaining 19 were unirradiated (0 cGy d^{-1}). Complete hematological evaluations were carried out periodically accordingly to standard veterinary practices. Time-based CBC values (WBCs, RBCs, platelets) from individual animals, at specific times from defined dose-rate groups, were averaged and plotted against time. Dose-rate specific responses were modeled using 2nd order linear regression analyses. Linear and quadratic coefficients were obtained and used to assess overall response kinetics. Results: Analyses revealed consistent time/dose-rate dependent patterns, characterized by initial, progressive depletion of WBCs and platelets, followed by the presence or absence of sustained subnormal accommodation and subsequent recovery. RBCs, by contrast, initially rose at the lower dose-rates (< 3.75 cGy d^{-1}), but eventually declined, and were sustained at subnormal levels proportional to the exposure rate. At dose-rates of > 3.75 cGy d^{-1}, RBCs progressively declined over time, resulting in intractable anemia. Quantitative modeling of the initial exposure phase revealed the following kinetics (linear coefficient): WBCs, -6.8 uL^{-1} cGy^{-1} d^{-1}; platelets, -275.3 uL^{-1} cGy^{-1} d^{-1}; and RBCs, -1.2×10^6 uL^{-1} cGy^{-1} d^{-1}. Conclusions: Differential responses of CBCs over extended periods of chronic exposure were noted. Responses varied according to cell-type, exposure rate, and time. Dose-rate dependent thresholds for initial suppression were not observed for WBCs and platelets, and contrasted a broad threshold noted for RBCs. Although significant blood responses were noted at the lowest dose-rate tested (0.3 cGy d^{-1}), early, acutely injurious, life-threatening responses were noted only at dose-rates > 3.75 cGy d^{-1}.

(PS1033) Differential induction from x-irradiated human peripheral blood monocytes to dendritic cells. Hironori Yoshino, Kenji Takahashi, Ikuo Kashiwakura. Hirosaki University, Graduate School of Medicine, Hirosaki, Japan.

Dendritic cells (DCs) are antigen-presenting cells which play an essential role in the immune system. Recent immunotherapeutic research has been primarily focused on the use of DCs as potential cellular vaccines against malignant tumors. On the other hand, although DCs are resistant to ionizing radiation, DCs that are exposed to ionizing radiation tend to be functionally weakened. Furthermore, it appears that the DCs derived from the monocytes of cancer patients are phenotypically and functionally inefficient in comparison to the DCs derived from the monocytes of healthy donors. However, whether the differential process for DCs, especially the DCs' precursors, is affected by ionizing radiation remains to be elucidated. In order to clarify the effects of X irradiation on differentiation into DCs, we focused our attention on human peripheral blood monocytes, which are known to be the DC' precursors of myeloid DCs, and thereby investigated whether X-irradiated monocytes can differentiate into DCs. Thereafter, either non-irradiated or X-irradiated monocytes were induced into immature DCs (iDCs) with granulocyte-macrophage colony-stimulating factor plus interleukin-4, and then mature DCs (mDCs) were induced from iDCs with tumor necrosis factor-α. The phenotype of the induced cells from both monocytes showed the expression of DC-specific surface antigens, such as CD80, CD86 and HLA-DR. However, the CD40 expression of the iDCs, and the CD1a and CD80 expression of the mDCs which were derived from 5 Gy-irradiated monocytes were significantly elevated in comparison to those of the DCs which were derived from the non-irradiated monocytes in all of the individuals tested. Furthermore, although no significant differences were observed in the phagocytotic activity, which is a function of iDCs, a significant reduction was detected in the mixed leukocyte reaction, which is a function of mDCs. There were no significant differences in the cytokines detected in the supernatants conditioned by the DCs from the non-irradiated and irradiated monocytes. These results suggest that human monocytes which are exposed to ionizing radiation can therefore differentiate into DCs, but some differences in characteristics are observed between the DCs from non-irradiated controls and those from irradiated monocytes.

(PS1034) Behavior of primitive hematopoietic stem cells and peripheral blood cytokines in radiation adaptive responses. Kensuke Otsuka[1], Masanori Tomita[1], Takao Koana[1], Hiroshi Tauchi[2]. [1]Low Dose Radiation Research Center, Central Research Institute of Electric Power Industry, Tokyo, Japan, [2]Department of Environmental Sciences, Ibaraki University, Ibaraki, Japan.

Mice irradiated with low-dose X-rays prior to lethal dose of radiation show significant radioresistance in terms of survival rate. This is an adaptive response *in vivo*, which is known as Yonezawa effect, and is considered to be an early rescue resulting from activation of hematopoiesis. An endogenous CFU-S, known as a hematopoietic progenitor, becomes more resistant to high-dose irradiation (challenge dose) when mice were preirradiated at a low dose 2 weeks before challenge dose. To study how these progenitors acquire radioresistance, we focused on two factors in hematopoiesis: i.e. the radiation responses in primitive hematopoietic stem cells and changes in levels of peripheral blood cytokines responding to high-dose radiation. Multipotent KSL cells, a subpopulation expressing Sca-1 and c-kit among lineage-depleted bone marrow cells in femurs, decreased in cell number within 4 days after exposure to low-dose (0.5 Gy) X-rays because they differentiated into lineage-positive cells. In contrast to this observation, the number of primitive hematopoietic stem cells in mice preirradiated at 0.5 Gy was relatively increased if it was compared to those in non-preirradiated control mice at the same time point. Because the regulation of hematopoiesis is known to be dependent on a signal transduction through cytokines and their receptors, we assessed peripheral blood cytokine levels using antibody arrays. Many cytokines presented different expression levels depending on timing of challenge dose, whereas some cytokines known as stem cell activating factors were constantly up-regulated in mice preirradiated at low dose (0.5 Gy). These results suggest that the adaptive response in terms of hematopoietic radioresistance *in vivo* might be regulated by liquid factors such as cytokines.

(PS1035) Chromosome aberrations do not persist in the lymphocytes or bone marrow cells of mice irradiated *in utero* or soon after birth. Mimako Nakano[1], Yoshiaki Kodama[1], Kazuo Ohtaki[1], Eiji Nakashima[1], Ohtsura Niwa[2], Megumi Toyoshima[2], Nori Nakamura[1]. [1]Radiation Effects Research Foundation, Hiroshima, Japan, [2]Radiation Biology Center, Kyoto, Japan.

Pregnant mice (15.5th day p.c.) or mice of various ages were exposed to 1 Gy or 2 Gy of X rays, and translocation frequencies in peripheral blood T cells, spleen cells, and bone marrow cells were determined with FISH painting of chromosomes 1 and 3 when the animals reached 20 weeks of age. It was found that the mean translocation frequencies were very low ($\leq$0.8%) in mice irradiated *in utero*, and did not show a clear dose response as we have seen in A-bomb survivors who were exposed *in utero*. Mice exposed soon after birth were also incapable of registering chromosome damage at 20 weeks old. However, with the increase of animal age at the time of irradiation, the frequency observed at 20 weeks old became progressively higher, and reached a plateau level (about 5%) when mice were irradiated at $\geq$ 6 weeks of age. The major role of *p53*-dependent apoptosis for elimination of aberrant cells was not suggested because irradiated fetuses, regardless of the *p53* gene status, showed low translocation frequencies (1.8% in $p53^{-/-}$ and 1.4% in $p53^{+/-}$ mice) when compared to the frequency in the $p53^{-/-}$ mother (7.4%). In contrast, various types of aberrations were seen in spleen and liver cells when neonates were examined shortly after irradiation, similar to what was observed for bone marrow cells following irradiation in adults. We interpreted the results as indicating that fetal cells are generally sensitive to induction of chromosome aberrations but the aberrant cells do not persist because fetal stem cells tend to be aberration free and their progeny replaces the preexisted cell populations during postnatal growth of the animals.

(PS1036) The depressed Th1-like responsein in irradiated mice is associated with an impairment of the NK cells. Hae-Ran Park, Uhee Jung, Sung-Kee Jo. Advanced Radiation Technology Institute, Jeongeup Campus of KAERI, Jeongeup-Si, Jeonbuk, Republic of Korea.

In the WBI mice, the canonical Th1 cytokine, IFN-γ, markedly decreases ultimately, resulting in a Th1/Th2 imbalance. In this study, the influence of NK cells on a balance of the Th1/Th2 immune response in WBI mice was investigated. Although the NK cell is one of the IFN-γ secreting cell types, its activity was very low, even 7 weeks after an irradiation. In the NK cell-depleted mice, the Th1-related cytokine was lower than the control mice resulting in a lower production of IgG2a, but a higher production of IgE and IgG1. These results suggested that the NK cells play an important role in the final differentiation of the Th cells into Th1 cells. An impairment of the NK cells in the WBI mice was confirmed by the observation that, compared to those form normal mice, the NK cells from WBI mice had lower activity to restore the IFN-γ production of NK cell-depleted spleen lymphocytes from the normal mice. Also, the WBI mice transferred with the NK cells obtained from the normal mice produced more IgG2a, IL-12 and IFN-γ. Our results carefully suggest that an impairment of the NK cells is an important factor for a weak Th1-like response in irradiated mice.

(PS1037) Alteration of inflammation molecules as function of time after radiation. Weimin Sun, Shanmin Yang, Hengshan Zhang, Wei Wang, Mei Zhang, Chaomei Liu, Steven Schwartz, Lurong Zhang, Paul Okunieff. University of Rochester Medical Center, Rochester, NY, USA.

Total body exposure to ionizing radiation (IR) is a severe insult that damages cells and triggers a host response leading to changes in circulating proteins. The alteration of circulating inflammation molecules (IM) reflects this complex host response with a characteristic time-dependant pattern. To determine the pattern of IM expression as function of time, C57BL/6 mice were exposed to 10 Gy total-body IR and the plasma were collected at day 2, 4 and 9 after IR (5 mice each group). All animals live at least 9 days after this dose. The 96 IMs in plasma were detected with an antibody membrane array, imaged with FluoChem SP system and analyzed with GenePix Pro 6.0 software. Each sample was assayed in duplicate. The mean dot density of each sample was compared with that of six reference dots that were run simultaneously with the sample in the same membrane and had a consistent density in all membranes. The relative density ratio was compared between the normal mice (not receiving IR) and IR mice. The 96 IMs were classified into 7 categories. The results are shown in the table. The number of IM that significantly increase or decrease are shown with arrows. A change was judge significant for $p<0.05$.

The data demonstrated that **1)** on day 2 after IR, half the IMs had significantly changed, with most changes showing a decrease; **2)** on day 4, there was a rebound with half of all the IM now showing a significant increase, many others showing a decrease, and about 30% unchanged; **3)** on day 9, the dramatic changes in cytokine expression were still evident. There is continued change however in the specific proteins that are increased or decreased. The marrow space is largely empty at this time point, indicating many of these proteins are being elaborated by non-hematopoietic tissue. In conclusion, there are brisk and dramatic changes in IM expression over time after a lethal irradiation exposure. The changes are variable over time with a complex pattern. These changes allow for both an estimate of the time and dose experienced by exposed individuals. Multiple measurements over time can provide increased accuracy of these estimates.

(PS1038) Highly radiosensitive germ cells in Medaka ric1 mutant with abnormal DNA double-strand break repair and apoptosis induction. Hiroshi Mitani[1], Kouichi Aizawa[2], Kanako Yori[1], Chiharu Kaminaga[1], Toshikazu Yashita[1], Kanae Nishino[1], Masayuki Hidaka[1], Masato Kinoshita[3], Shoji Oda[1]. [1]Graduate School of Frontier Sciences, Kashiwa, Japan, [2]Savannah River Ecology Laboratory, University of Georgia, Aiken, SC, USA, [3]Graduate School of Agriculture, Kyoto University, Kyoto, Japan.

Most of radiation sensitive mutants have defects in DNA repair or cell cycle checkpoint regulation system. Use of such DNA repair-deficient mutants helps us to understand the nature of genotoxic effects of radiation. However, most of DNA repair knockout mouse strains are homozygous lethal or short-lived with cancer-prone or immune deficiency. So it is very difficult to use such strains with large number of animals. Medaka has been a useful experimental fish because of its attributes, such as small size of the body, easy breeding, relatively short generation time, a small genome size, and availability of inbred strains. Medaka embryogenesis can be easily observed and lots of experimental data on the effects of radiation have been obtained from studies relating to mutagenesis in germ cells and apoptosis in somatic cells.

We screened ENU-induced mutations in Medaka and identified 3 loci affecting embryonic malformation induced by ionizing radiation (Aizawa et al. 2004). Ric1, a homozygous strain for one of these mutations, has a defect in fast repairing of DNA double strand breaks but induction of p21 and Ku80 gene expression after irradiation was normal. The development of Ric1 germ cells was normal and highly fertile, but both adult and embryonic germ cells showed much higher radiosensitivity than wild-type fish. The normal apoptosis in differentiating spermatogonia at 24 hours after irradiation could not be observed in Ric1 testis and unusual delayed cell death in spermatogonial stem cells and differentiating spermatogonia occurred at 3days after irradiation. Using olvas-GFP gene, which contains the GFP gene fused to the regulatory region of the vasa gene allows us to monitor germ cells by GFP in live specimens, and we found that embryonic germ cells in Ric1 were very radiosensitive. There was no sex difference in germ cell radiosensitivity at 32-cell stage, but germ cells in female showed higher radiosensitivity than male in 4 days embryos. These suggest that the ric1-dependent DNA repair system is very important to protect germ cells from ionizing radiation.

(PS1039) Retrospective study of the influence of anaemia in patients with advanced head and neck cancer received postoperative radiotherapy. Sherif A. Abdelwahab[1], Mohamed M. El-Basiouny[1], Hatem M. Abdalla[1], Hany M. Abdel-Aziz[1], Maha Maha Margerges[1], Ali M. Azmy[1], Branislav Jeremic[2]. [1]Ain Shams University, Cairo, Egypt, [2]Applied Radiation Biology and Radiotherapy Section, IAEA, Vienna, Austria.

INTRODUCTION:

Head and neck carcinoma is comprised of a heterogeneous group of tumours that arise from the epithelial lining of the oral cavity, pharynx, and larynx, and it is considered as a locoregional disease. Despite their heterogeneity, head and neck cancers share many features that render them an ideal model for evaluating the influence of anaemia and the associated tumour hypoxia on the outcomes of postoperative radiation therapy.

OBJECTIVES:

This retrospective study was conducted to investigate the impact of pre-treatment haemoglobin level on the local recurrence and overall survival in patients received postoperative radiation therapy for locally advanced head and neck cancer.

PATIENTS AND METHODS:

331 patients with AJCC stage III, IVA or IVB head and neck cancers were treated with postoperative radiation therapy alone with doses ranging from 60–66 Gy, (2Gy/F). Baseline (pre-radiotherapy) haemoglobin values were recorded and the outcome was measured in terms of locoregional control rates and overall survival according to it.

RESULTS:

The median age was 52.2 years and 29% of patients were women. Mean pre-treatment haemoglobin was 10.7 g/dl (8.3–13.1g/dl). Median follow up of surviving patients was 42 months, and 27% of patients had local recurrence and 52% had died. Two-and five -year loco-regional control for anaemic patients was 71% and 64% respectively, while patients with normal pre-treatment haemoglobin levels had 2- and 5- year locoregional control of 80% and 73% respectively (p= 0.042). Two-and five -year overall survival for anaemic patients was 64% and 48% respectively, while patients with normal pre-treatment haemoglobin levels had 2- and 5-year survival rates of 72% and 57% respectively (p= 0.021). Multivariate analysis revealed risk group and low pre-treatment Hgb to be statistically significant predictors for increased locoregional recurrence and lower survival rates.

CONCLUSION:

The low pre-treatment haemoglobin level below11/dl was correlated with statistically significant adverse treatment outcomes in terms of locoregional control rates as well as survival rates in patients receiving postoperative radiation therapy for advanced head and neck cancers.

(PS1040) Quantitative Analyses of Hypoxia and Vessels in Human Tumors. Sydney M. Evans, Kevin Jenkins, W. Timothy Jenkins, Cameron J. Koch. University of Pennsylvania School of Medicine, Philadelphia, PA, USA.

Abnormalities in vessel morphology and distribution contribute substantially to the development of tumor hypoxia, but the quantitative relationship between hypoxia and blood vessels in human brain tumors has not previously been described. We have developed mathematical algorithms to convert EF5 binding values to tissue pO2 and use this to describe pO2 as a function of distance from blood vessel. Briefly, binary masks of viable tissue are semi-automatically computer produced for tissue (based on Hoechst 33342 staining) and for blood vessels (based on CD31 staining). Using these masks a series of annuli are drawn by the computer around each vessel and the EF5 binding is calculated as a function of distances from each vessel. Although any distance away from a vessel can be chosen, we limited our analysis to five 10Âµ annuli in order to determine whether metabolic or vascular processes determined the vessel-adjacent pO2 values. A series of 6 tumors (2 glioblastomas (GBM), 1 anaplastic oligodendroglioma, 1 oligoastrocytoma and 1 hemangiopericytoma (HPCTA)) were initially examined. Challenges to the automatic generation of the masks included tumors with very dense vasculature (HPCTA) or nuclei (GBM), bleed through of dyes conjugated to the EF5 or CD31, large inter-nuclear distances (Hoechst) and dim staining (CD31). Unique approaches to deal with these factors were developed. In this small group of tumors, we find that the grade 2 and 3 glial tumors and the HPCTA are oxic or mildly hypoxic, supported by vasculature that is normal in conformation and spacing; the primary difference between the vasculature of these 3 tumors is the inter-vascular spacing. GBMs are heterogeneous in their vascular pattern as well as in the perivascular EF5 distribution. Regions of hypoxia surrounding vessels are seen in GBMs and the most severely hypoxic tumor cells in GBMs appear to arise from vascular-related, not metabolic processes. Detailed analyses of the vessel and oxygen levels in 10 GBM tumors will be presented.

(PS1041) Vascular density, hypoxia and tumor-associated macrophages in the irradiated tumors. Ji-Hong Hong[1], Fang-Hsin Chen[2], Chun-Chieh Wang[1], Chien-Sheng Tsai[1], William H. McBride[3], Chi-Shiun Chiang[2]. [1]Chang Gung University and Chang Gung Memorial Hospital, Taoyuan, Taiwan, [2]National Tsing Hua University, Hsinchu, Taiwan, [3]UCLA, LA, CA, USA.

We recently reported that tumor-associated macrophages (TAMs) in the irradiated tumors express higher levels of arginase-1, COX-2 and iNOS than those in un-irradiated tumors (in press, Int. J. Radiat. Oncol. Biol. Phys). The present study further investigated the changes of microvascular density, tumor necrosis and hypoxia status in the irradiated tumors and their relation with the distribution and phenotype of TAMs. TRAMP-C1, a cell line derived from the Transgenic Adenocarcinoma of the Mouse Prostate, was used as a tumor model. Tumors grew intramuscularly to the size of 4 mm, and were either continued to grow or irradiated with single-dose 25 Gy or dose of 60 Gy by 15 fractions in 3 weeks. The microvascular density in irradiated tumors, as measured by the immunohistochemistry (IHC) stain with CD31 for endothelial cells, was progressively decreased to 50% level of that in un-irradiated tumors; this was in agreement with the molecular changes that mRNA levels of some endothelial markers such as CD31, endoglin and TIE were also decreased with time in the irradiated tumors. However, irradiated tumors had less necrotic and hypoxia (measured by antibody to PIMO) regions than un-irradiated tumors.

The microvasculature network was progressively disrupted and a central, dilated vessel surrounded by hypoxic region without microvasculature was found in many areas inside the irradiated tumors. There was a strong association of the TAMs with the hypoxia region in the irradiated tumors. More works in the phenotype of TAMs are being done and will be presented in the meeting. These observations suggested the disruption of tumor microvasculare by irradiation might induce tumor hypoxia, and there were mechanisms to recruit TAMs into the hypoxia region in the irradiated tumors. (This study was supported by Grant NSC 93–2314-B-182–030, CMRPG1001 and CMRPG34025)

(PS1042) Identification and therapeutic targeting of hypoxia in H&N cancer. Lester J. Peters, Danny Rischin, Richard Fisher, June Corry, Rod Hicks. Peter MacCallum Cancer Centre, East Melbourne, Australia.

Purpose: To report the outcome of studies using PET imaging for hypoxia in patients enrolled on trials of the hypoxic cell cytotoxin tirapazamine (TPZ)

Methods: A total of 121 patients from the Peter MacCallum Cancer Centre with locoregionally advanced H&N cancer who were enrolled in Phase I, II and III trials of TPZ between 1996–2005 underwent pre-treatment imaging for hypoxia using F-Miso or FAZA. The prevalence and distribution of imageable hypoxia was analysed in all patients and tumour control in randomized patients was analysed as a function of PET-determined hypoxia status.

Results: Ninety-three (77%) patients had imageable hypoxia in the primary, neck nodes or both. Hypoxia at the primary site correlated significantly with T stage, and nodal hypoxia with N stage, but there was no significant correlation between primary and nodal hypoxia in individual patients. Hypoxia at either the primary and/or nodes was associated with a significantly increased risk of loco-regional failure for patients treated with concurrent cisplatin-based chemotherapy without TPZ, but not for patients receiving cisplatin plus TPZ. This was most evident at the primary site:

Primary Site Failure (n=92, randomized patients)

PET Hypoxia status	Treatment	
	RT + cis/no TPZ	RT + cis/TPZ
Non-hypoxic	2/27 (7.4%)	3/21 (14.3%)
Hypoxic	8/18 (44.4%)	0/26 (0.0%)

Conclusions: These results show that hypoxia is common in advanced H&N tumors and provide proof of principle that hypoxia can be identified by PET imaging and successfully targeted using a specific hypoxic cell cytotoxin.

(PS1043) Measuring oxygen levels in human tumors repeatedly to provide information for optimizing therapy. Benjamin B. Williams[1], Marc S. Ernstoff[2], Bassem Zaki[2], Alan C. Hartford[2], Piotr Lesniewski[1], Harold M. Swartz[1]. [1]Dartmouth Medical School, Hanover, NH, USA, [2]Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA.

This report provides the initial results of the clinical use of *in vivo* EPR oximetry to make repeated non-invasive (after an initial minimally invasive placement) measurements of tumor pO_2. The method uses a unique clinical EPR spectrometer developed at Dartmouth that can accommodate human subjects, with a surface resonator that probes the EPR signal from India ink that has been injected into one or more sites of a tumor within 10 mm of the surface. The pO_2 in the tumor is determined by the effect on the linewidth of the India ink. Each site can be measured independently. Measurements can be made continuously for periods of several hours and repeatedly for as many days or weeks are needed. The response of the pO_2 of the tumor to potentially increased availability of oxygen is determined by having the patient breathe an enriched oxygen mixture. The rate of oxygen consumption by the tumor is reflected in the rate at which the pO_2 returns to the baseline levels. The location of the India ink can be determined by MRI and from histological studies if the tumor is resected.

Tumors of five patients have been studied to date (3 melanomas, 1 squamous cell carcinoma, 1 cutaneous lymphoma). Adequate signal to noise was obtained from all patients. The patients differed markedly in the baseline levels of pO_2 in their tumors and in the response to breathing increased oxygen. A patient receiving chemotherapy had significant changes in tumor oxygen after the therapy.

While these studies are at an early stage in which the goal is to demonstrate clinical feasibility, some tentative conclusions can be drawn: 1) the ability to make repeated measurements of oxygen in tumors of human subjects using EPR has been demonstrated for the first time; 2) The pO_2 of the tumors varied among the patients studied; 3) The tumors varied in the response to breathing increased oxygen; 4) The variation in the response to increased oxygen may indicate that this technique could be used to select patients for effective use of strategies to enhance tumor oxygenation at the time of treatment; 5) the data on pO_2 also potentially could be used to monitor the effects of treatments on tumor pO_2 so that radiation therapy and combined therapies could be more optimally timed to achieve delivery of therapy at the most suitable schedule.

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(PS1044) PAI-1 (plasminogen activator inhibitor type-1) correlates to hypoxia and radiation resistance in squamous cell carcinomas of the head and neck (SCCHN). Christine M. Bayer[1], Joerg Hoetzel[2], Hannes P. Egermann[1], Michael Molls[1]. [1]Department of Radiation Oncology, Munich, Germany, [2]Department of Radiation Oncology, Munich, Germany.

Purpose: This work is part of a multi-institutional research project, investigating the importance of hypoxia and the tumor micromilieu for radiation therapy of SCCHN. SCCHN are difficult to treat due to their exceptionally pronounced hypoxic regions. Additionally, hypoxia can up-regulate components of the plasminogen activation system (PAS) and VEGF. Therefore, our aim was to investigate *in vivo* and *in vitro* the inter-relationship between hypoxia, radiation resistance, PAS components (uPA activity, uPA and PAI-1) and VEGF. **Materials and Methods:** 10 SCCHN cell lines and xenograft tumors were investigated. The local tumor control, TCD_{50} and the Pimonidazole hypoxic fraction in the total tumor area, pHF_{tot} were used as functional endpoints. Immunohistochemistry was performed on adjacent cryosections of xenograft tumors. Tumour lysates were extracted from 5 tumors per tumor line and used for expression studies. For *in vitro* experiments, cells were cultured for 24h under either normoxic or hypoxic (~0.66% O_2) conditions. **Results:** Immunohistochemical studies showed that uPA, PAI-1 and VEGF localize to the cytoplasm of individual tumor cells. In addition, PAI-1 stains stroma cells and the extracellular matrix and VEGF stains endothelial cells, depending on the tumor line. PAI-1 expression in tumor lysates was the only factor to correlate independently to pHF_{tot} ($p = 0.648$, $r = 0.043$) and TCD_{50} ($p = 0.733$, $r = 0.016$). Moreover, the level of PAI-1 was significantly higher ($p = 0.008$) in more hypoxic than in less hypoxic tumors. Interestingly, the tumor line, CAL33 expressed high levels and XF354 low levels of all 3 factors (uPA activity, PAI-1 and VEGF). Additionally, CAL33 was among the most hypoxic and most radiation resistant and XF354 among the least hypoxic and least radiation resistant tumors. PAI-1 secretion was also significantly increased after irradiation with 5 - 20 Gy in XF354 and CAL33 cell lines. The greatest (2.4x) and only significant *in vitro* hypoxic induction in supernatants was for PAI-1. **Conclusions:** PAI-1 correlates independently and significantly to hypoxia and radiation resistance *in vivo* and can be induced by both hypoxia and irradiation *in vitro*. These results could explain why PAI-1 is such a strong prognostic marker for poor outcome in SCCHN.

(PS1045) TNF-mediated cell-death signaling pathway and extra-cellular matrix pathway are activated by concurrent use of cisplatin with radiotherapy in sequential biopsy specimens from patients with cervical cancer. Mayumi Iwakawa, Tatsuya

Ohno, Kaori Imadome, Miyako Nakawatari, Minako Sakai, Takashi Moritake, Etsuko Nakamura, Tomoaki Tamaki, Shingo katoh, Hirohiko Tsujii, Takashi Imai. National Institute of Radiological Sciences, Chiba-shi, Japan.

Objective: To identify changes in gene expression related to the concurrent use of platinum compounds with radiotherapy, in the treatment of cervical cancer.

Patients and Methods: Biopsy specimens were obtained from 53 patients with the uterine carcinoma, before and during fractionated radiotherapy. Twenty-five patients were treated with radiotherapy (RT) alone, while 28 received the same radiotherapy plus concomitant chemotherapy with cisplatin (CRT). Changes in gene expression induced by treatment were investigated using CodeLink single-color oligo-microarrays consisting of 44K human sequences. Paraffin-embedded samples were used to examine apoptosis and the expression of protein related with treatment-responsive genes. Changes in mRNA expression were assessed for these genes by real-time reverse transcriptase-polymerase chain reaction.

Results: We found several tens of genes, including *CDKN1A*, *BAX, TNFSF8, RRM2B,* and *FGF2,* responded to CRT using microarray analysis, and suggested CRT activated TNF-mediated cell death pathway and extra-cellular matrix pathway related with cell death pathway. Apoptotic cells were significantly increased in both groups. In immunohistochemical study, CRT significantly increased the numbers of cases with diffusely distributed CDKN1A-positive cells ($P < 0.002$). Protein expression of ICAD was recognized weakly in nucleus of tumor cells in pre-treatment samples and decreased significantly after CRT ($P < 0.05$). BAK1 increased its intensity in half of cases at mid-treatment. The expression changes of FGF2 were revealed to be significantly different between good responders and poor responders ($P < 0.05$).

Conclusions: CRT produced a homogenous pattern of changes in expression of known radiation-responsive genes. Our data suggest that concurrent use of cisplatin produced a radiosensitizing effect in most of these cervical cancer patients. Microarray analysis appeared a useful tool to get a comprehensive overview of the changes in gene expression after irradiation. In addition, transcriptional profiling of sequential biopsies after irradiation would improve our understanding of the biological effectiveness of radiotherapy, and provide information on potential targets for adjuvant therapy.

(PS1046) Lymphopoiesis of treated oncological patients is a probable source of individual life span's variability. A. Shutko, L. Ekimova, I Shoumski, N. Chizova, L. Yurkova, T. Bochkareva, V. Mus, M. Karamullin. Central Research Innst. of Roentgenology and Radiology, St.-Petersburg, Russian Federation.

Objective: The uniform therapy being applied toward oncological patients with uniform TNM diagnosis leads, nevertheless, to very variable results in terms of individual life span (ILS). The aim this report is to search possible reasons for that puzzle at the level of lymphopoietic system, because the expected ability of matured lymphocytes to suppress a tumor growth is very vague at present and incompatible with cytotoxic nature of conventional treatment.

Methods: The survival among patients with breast cancer (BC), renal cell carcinoma and advanced ovarian carcinoma were analyzed after conventional combine treatment including radiotherapy in terms of mortality rate, individual life span (ILS) and the composition of different cells subsets in a mononuclear fraction of PB before and during the treatment (according BD FACScan cytometry).

Results: Analysis of survival curves on the semi-logarithmic plots showed that all patients could be divided on the types A and B, with periods of half-elimination $T_A \pm \sigma \approx 16 \pm 5$ and $T_B \pm \sigma \approx 220 \pm 40$ months correspondently. The tape A is presented, e.g., at BC stages I, II, III-IY as much as 16, 52 and 80%. The whole ILS for type A could be divided on two parts. First one has the slow progressive overbalancing of the $CD34^+$ and $CD4Leu8^+$cells content as well as a dropping down TdT^+ prolymphocytes along with ILS becomes shorter. Second part is final and characterized by relatively prompt exhaustion of the $CD34^+$, $CD4Leu8^+$ pools against the background of slight insufficient increasing of TdT^+cells. The more an ILS, the more a length of final part that varies from ≈ 6–50 months up to the death. Any regular changes of $CD2^+$, 3^+, 4^+, 8^+, 8^+11b^+, 8^+11b^-, 4^+Leu8^- cells haven't been found.

Conclusion: An ILS correlates with individual ability of poietic system to produce extra quantity of stem and semi- stem cells that are spent to support the renewing of tissues regardless of their natural or malignant origin. The collapse of individual resource of hemopoietic system, its disability to produce appropriate number young lymphocytes has been provoking by opposite processes - tumor's progression and cytotoxic therapy. The usefulness of the last one in the final steps of the life is questionable. Reliable tests for evaluation of stem cells resource seems to be perspective for the accurate prognosis of ILS and treatment.

(PS1047) Diagnostics and therapeutics of ^{111}in-vinorelbine liposomal drug in tumor-bearing animal model. T S. Chou[1], Y Y. Lin[1], J J. Hwang[1], H E. Wang[1], Y L. Tseng[2], S J. Wang[3], J Q. Whang-Peng[4], G Ting[4]. [1]Dept of Biomedical Imaging & Radiological Sciences, National Yang-Ming University, Taipei, Taiwan, [2]Taiwan Liposome Company, Taipei, Taiwan, [3]Department of Nuclear Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [4]National Health Research Institute, Taipei, Taiwan.

Purpose:

To explore the efficacy of multifunctional diagnostics and therapeutics of ^{111}In-vinorelbine liposomal drug with human colorectal adenocarcinoma-luciferase (HT-29/*luc*) bearing mouse model.

Materials and Methods:

To evaluate the diagnostic and therapeutic efficacy of nanoliposome (100 nm in diameter) encaged with 5 mg/kg vinorelbine (VNB) and 70 μCi ^{111}In-oxine on HT-29/*luc*-bearing animal model, HT-29/*luc* tumor cells were transplanted subcutaneously into the male NOD/SCID mice. Four injections, once per week, of ^{111}In-vinorelbine liposome were administered followed by multimodality evaluation, including in vivo tumor volume assay, biodistribution, whole-body autoradiography (WBAR), gamma scintigraphy and bioluminescence imaging (BLI). Histopathology, biochemistry and hematology analysis were also examined for the normal tissue toxicity.

Results:

Biodistribution, gamma scintigraphy and WBAR of ^{111}In-NanoX/VNB-liposomes showed that the maximum drug uptake in the tumor was at 48 hrs postinjection. The prominent uptake was also found in the liver, spleen and kidneys. Moreover, BLI revealed that tumor growth could be completely inhibited by combination therapy (i.e. ^{111}In-VNB-liposomes) ($p<0.01$). Normal tissue toxicity was not found as examined by histopathology, biochemistry and hematology.

Conclusions: Combination of ^{111}In-oxine and VNB showed a synergistic therapeutic effect on tumor growth suppression, and also enhanced the survival. 6% PEG, instead of 0.9%, could reduce the nanoliposome accumulation in reticuloendothelial system (RES)-rich organs, such as spleen.

(PS1048) The role of EGFR related proteins in the response to preoperative chemoradiotherapy in combination with cetuximab in patients with rectal cancer. Annelies Debucquoy[1], Jean-Pascal Machiels[2], Olivier Gevaert[3], Anneleen Daemen[3], Sarah Roels[1], William Mc Bride[4], Karin Haustermans[1]. [1]University Hospitals Leuven, Leuven, Belgium, [2]Cinique Universitaires Saint-Luc, Brussels, Belgium, [3]Catholic University of Leuven, Leuven, Belgium, [4]Univ of California, Los Angeles, Los Angeles, CA, USA.

Objectives: To elucidate the role of different proteins related to the EGFR pathway in the tumor response of patients with rectal cancer to chemoradiotherapy in combination with cetuximab. Molecular profiles that reflect an inter-patient variability and are predictive for response were identified using baseline samples. Moreover, on treatment samples were used to monitor the molecular response to cetuximab.

Material and Methods: Forty patients with advanced rectal cancer were included in a phase I-II study with preoperative capecitabine and radiotherapy in combination with cetuximab. Plasma samples, paraffin embedded and fresh frozen biopsies were taken at 3 time points: baseline samples, after the loading dose of cetuximab and at moment of surgery. The plasma samples were used to study the expression EGF and TGFα by ELISA. On the paraffin embedded biopsies, immunohistochemical stains for EGFR and Ki67 as well as FISH were performed. The fresh frozen biopsies were used to perform cDNA micro-arrays.

Results: For TGFα, a high expression before therapy as well as a high expression after the loading dose of cetuximab seemed to be an indication for a bad response (p=0.05). For EGF no correlation was found with histopathological response. From the micro-array analysis, it was possible to predict the Dworak and Wheeler regression grade with a performance of 70,27% from the gene expression profiles both before treatment and after the loading dose of cetuximab. A significant correlation was found between apoptosis pathways and Dworak regression grade at moment of surgery (p=0.02). The FISH experiments on the paraffin embedded tissue showed that 22% of the patients exhibit a low polysomy. No EGFR amplification was detected. The protein expression of EGFR was low in most tumors and did not correlate with EGFR polysomy. The percentage positivity for Ki67 was significantly lower after the loading dose of Cetuximab as well as after the full treatment (p=0,01) and was predictive for T-downstaging (p=0,05).

Conclusions: We conclude that Ki67 and circulating TGFα, which are strongly influenced by cetuximab treatment, are potential important predictors of response. The micro-array analysis allows us to predict before therapy with 70.27% certainty the regression grade at moment of surgery.

(PS1049) Chemotherapy enhanced radiation therapy: U.S. Clinical trials and their influence on the standard of care. Patricia J. Eifel. Department of Radiation Oncology, Houston, TX, USA.

For more than four decades, radiation oncologists and biologists have searched for ways to enhance the effectiveness of radiation therapy by combining it in various ways with chemotherapy. During the past 15 years, studies of these combinations have dominated radiation oncology clinical research in the United States, with large phase III trials demonstrating the efficacy of combined treatment in patients with cancers of the lung, esophagus, rectum, anus, stomach, bladder, cervix, head and neck and other sites. Although sequential administration appears to be of benefit in some sites, the greatest benefit has usually been seen with concurrent administration of drugs and radiation. The results of these trials have increased the efficacy as well as the complexity and cost of routine cancer treatment in the U.S.

In the U.S., cisplatin has been a dominant element in most chemo-RT regimens. However, the dose, schedule, and pairing with other drugs has varied widely between sites and studies. Cisplatin has been given successfully in 3-week intervals, weekly, or daily. The total dose during a course of radiation has ranged from $<$100 mg/m2 to $>$ 200 mg/m2. Other platinum analogues have received little attention; however, cisplatin has been given alone or paired with other drugs, including 5-FU, docetaxel, paclitaxel, and etoposide,

Although investigators have sometimes borrowed from successes in other disease sites, preferred treatment regimens have tended to evolve within site-specific interest groups. Although choices have sometimes been informed by preclinical studies, results in other sites, and site-specific normal tissue considerations, tradition and investigator preference have also played important roles. Schedules made popular in different sites have rarely been compared head-to-head. Although pre-clinical studies are occasionally cited in the overall rationale for concurrent chemoradiation, specific schedules are rarely justified in terms of scientific principles, pharmacology, or comparative tumor biology. Although the costs, convenience, and toxicities of schedules differ greatly, little is known about the relative benefits of popular chemoradiation regimens. This knowledge gap demonstrates the need for detailed collaborative studies of the mechanisms and pharmacology of chemoradiation.

(PS1050) Impact of oxygen concentration on yields of complex DNA damages caused by ionizing radiation. V. Stepan[1,2], M. Davidkova[2]. [1]Nuclear Physics Institute AS CR, Prague, Czech Republic, [2]Czech Technical University, Prague, Czech Republic.

Local hypoxia-induced radiation resistance is one of the major problems in current radiation therapy of solid tumors. Hypoxic cells are two to three times more resistant to a single fraction of ionizing radiation than those with normal levels of oxygen. Concentration of diluted oxygen is not uniform within tumor volume and changes during the course of radiotherapy. Aim of this work is to evaluate the influence of oxygen concentration on the yield of primary DNA damage caused by ionizing radiation of different quality. Theoretical modeling approach based on Monte Carlo technique was used for this purpose. DNA oligomer was described at the atomic level. Energetically minimized spatial conformations of 100 bp DNA fragments were obtained using Amber 7 molecular dynamics package (Pearlman, D.A. *et al.* 1995, Comp. Phys. Commun. 91, 1). Track structures of the ionizing particles were generated by Monte Carlo code TRIOL (Bigildeev, E.A. and Michalik, V., 1996, Radiat. Phys. Chem. 47, 197). Both unscavengeable and scavengeable damage was followed. Unscavengeable damage was assumed to result from direct energy deposition on DNA macromolecule or into the bound water. Energy depositions outside the bound water region but less than 3 nm apart from nearest atom of the DNA molecule were followed in a step-by-step simulation. Water radiolysis including migration and mutual interactions of created radiolytic species were taken into account. Particular interactions of radical species with atoms of the DNA oligomer resulted in scavengeable DNA damage. Yields of various types of complex DNA damages and the ratio of scavengeable to unscavengeable DNA damage for diluted oxygen concentration ranging from anoxic to normal conditions will be presented for electrons, protons and alpha particles with LET in range 0.41–160 keV/μm.

(PS1051) Analysis of T-cell receptor (TCR) variants and apoptosis induced by beta radiation from tritiated water at low-dose rate in different *p53* status mice. Toshiyuki Umata, Naoki Kunugita, Ryuji Okazaki, Akira Ootsuyama, Toshiyuki Norimura. University of Occupational and Environmental Health, Japan, Kitakyushu, Fukuoka, Japan.

The influence of *Trp53* on the elevation of T-cell receptor (TCR) variant fractions and apoptosis induced by beta ray from tritiated water (HTO) and gamma ray from ^{137}Cs at low-dose rate was examined in splenic T lymphocytes of wild-type $Trp53^{+/+}$, heterozygous $Trp53^{+/-}$ and null $Trp53^{-/-}$ mice. For tritium exposure, 10 MBq of HTO per gram of body weight was given to the mice by a single intraperitoneal injection. Mice were killed humanely at 19th day, and the spleens of these mice were sampled immediately. According to our previous study, the cumulative dose absorbed by spleen was about 3 Gy. On the other hand, for gamma ray exposure, mice were irradiated by ^{137}Cs gamma ray irradiation system, which was designed for the simulation-irradiation of gamma ray when studying the biological effects of internal exposure. The dose rate of this system decreased according to the inverse square of the distance from the source. Simulation-irradiation was started at the dose rate of 0.6 mGy/min. The mice received cumulative dose of 3 Gy for 7 days and were killed humanely at 19th day for sample preparation. The spleen cells were gently dissociated and filtered through a stainless steel mesh. T cells were concentrated using nylon wool columns. Subsequently, 500,000 T cells were stained with PE anti-CD4 and FITC anti-CD3 antibodies and analyzed by an EPICS-XL flow cytometer. Fifty thousand events were counted for each measurement. The TCR variant fraction was defined as the number of events in the $CD3^{-}CD4^{+}$ T-cell window divided by the total number of events in the $CD3^{+}CD4^{+}$ T-cell window. In $Trp53^{+/+}$ mice, the TCR variant fraction was elevated by beta-irradiation, but was not elevated by gamma-irradiation. In $Trp53^{-/-}$ mice, the TCR variant fraction was elevated by both beta- and gamma-irradiation. For apoptosis analysis, mice were killed humanely at 12 and 24 hours after a single intraperitoneal injection of HTO or the beginning of simulation-irradiation of gamma ray. Many apoptosis cells were observed in spleen from $Trp53^{+/+}$ mice compared with $Trp53^{+/-}$ mice in both beta- and gamma-irradiation. Furthermore, apoptosis in splenic cells irradiated by beta ray were much

compared with that irradiated by gamma ray in both *Trp53*$^{+/+}$ and *Trp53*$^{+/-}$ mice. These results suggest that RBE for low-dose rate of beta ray from tritium is larger than 1.

(PS1052) Effect of temperature during irradiation on the level of DNA damage in human peripheral blood lymphocytes exposed to X-rays and neutrons. Andrzej Wojcik[1], Kinga Brzozowska[2], Julian Liniecki[3], Christian Johannes[4], Günter Obe[4], Reinhard Hentschel[5], Wolfgang Sauerwein[6], Andrea Wittig[6], Irena Szumiel[2], Josselin Morand[1], Ray Moss[1]. [1]Institute for Energy - EC Joint Research Centre, Petten, The Netherlands, [2]Institute of Nuclear Chemistry and Technology, Warszawa, Poland, [3]Medical Academy Hospital, Lodz, Poland, [4]University Duisburg-Essen, Essen, Germany, [5]Unversity Hospital Essen, Essen, Germany, [6]University Hospital Essen, Essen, Germany.

Objectives: It has been observed by a number of researchers that the level of cytogenetic damage in human peripheral blood lymphocytes (PBL) is higher when cells are exposed at 37°C than when exposed at 20°C or 0°C. The mechanisms of this temperature effect are not known. The aim of our study was to analyse if the effect was related to the indirect or direct action of radiation. For this purpose PBL were exposed to X-rays at 37°C, 20°C and 0°C in the absence or presence of the radical scavenger DMSO. In addition, PBL were exposed at 37°C and 0°C to fast neutrons.

Materials and methods: PBL (isolated or as whole blood) were kept at 37°C, 20°C and 0°C (ice water) for 20 min and exposed to 2 Gy of 180 kV X-rays. 0.5 M DMSO was added to isolated lymphocytes for 20 min before exposure and cells were kept at 37°C, 20°C and 0°C for the same period of time. Thereafter they were irradiated with a dose of 2 Gy X-rays and micronuclei were scored. For exposure to 6.0 MeV neutrons whole blood was kept at 37°C and 0°C for 20 min and exposed to a dose of 1 Gy. Chromosomal aberrations were scored as the endpoint.

Results: The highest frequency of micronuclei was scored following exposure of PBL at 37°C. No effect of temperature was seen when PBL were exposed in the presence of DMSO. A lack of the temperature effect was observed following exposure to neutrons.

Conclusions: That DMSO abolished the temperature effect indicates that the effect is due to the indirect action of radiation, i.e. via reactive oxygen species, possibly resulting from differences in chromatin conformation at various temperatures. This conclusion is supported by the lack of temperature effect following exposure to neutrons. Our results are of significance for radiobiological studies, where the temperature at which cells are exposed is frequently not controlled. Furthermore, they may also be of significance for the planned boron neutron therapy of liver metastases, where explanted livers are exposed to neutrons at 4°C in the presence of boron. The low temperature may protect the healthy liver tissue which is mainly exposed to the resulting gamma rays. Consequently, the actual tolerance dose to the liver may be higher than anticipated from dose-escalation studies.

(PS1053) Induction of H2AX phosphorylation and apoptosis with radiation treatment and expression of PCNA protein in radiosensitive wasted mouse. Barbara A. Szolc-Kowalska[1], Kaori Nakamura[2], Daniel Jakubczak[1], Akiko Hagiwara[2], Tatjana Paunesku[1], Tetsuya Ono[2], Gayle E. Woloschak[1]. [1]Northwestern University, Chicago, IL, USA, [2]Tohoku University, Sendai, Japan.

We are investigating effects of irradiation on a radiosensitive mouse mutant model - the wasted (wst). Exposure of cells to radiation causes damage of cellular DNA, particularly formation of DNA double-strand breaks (DSBs) and induces formation of gamma-H2AX nuclear foci. In previous experiments we found increased levels of early apoptotic, Annexin V positive cells in irradiated healthy control animals, whereas wasted mice (wst/wst) showed no significant change in Annexin V-staining cells post-irradiation. Also, in non-irradiated and irradiated wasted mice PCNA expression levels were similar to those in irradiated healthy mice. We now decided to estimate expression of PCNA, accumulation of gamma-H2AX and late apoptosis in cells stained with five different fluorophores. Wasted and control mice were sacrificed 3 hours after irradiation. Thymus, spleen and bone marrow were harvested cells were stained first with violet dead-live cell stain and then fixed and stained with anti-PCNA, gammaH2AX and BrDU antibodies (following TUNEL assay BrDU incorporation). PCNA expression pattern was the same as before, with wasted non-irradiated mice showing similar PCNA levels as the irradiated healthy mice. Late apoptosis in wasted and irradiated healthy mice was similar, and similar to the results obtained for early apoptosis as well. Gamma-H2AX staining in wasted mice was increased compared to normal mice: increase in bone marrow was 15 fold following 1 Gy, and 3 fold following 2 Gy dose; in spleen increase of 11 fold at 1 Gy, and 4 fold at 2 Gy; and in thymi 20 fold at 1 Gy and 11 fold at 2 Gy. In conclusion, while irradiated healthy mice show similar extent of apoptosis and level of PCNA expression as wasted mice, DNA double-strand breaks in wasted mice are much more frequent than DNA breaks in healthy mice irradiated with the same gamma-ray doses.

(PS1054) Clustered DNA damage in irradiated human cells: the influence of chromatin organization. Karin Magnander, Ragnar Hultborn, Kristina Claesson, Kecke Elmroth. Department of Oncology, Institute of Clinical Sciences, Göteborg University, Gothenburg, Sweden.

Purpose

Clustered damage is produced when two or more lesions are located within a limited region on the DNA molecule. The aim of the study was to investigate the cellular induction and repair of clustered DNA damage induced by ionizing radiation. The influence of the organization of DNA chromatin on the induction was also examined.

Methods

Human fibroblasts were irradiated with X-rays and the induction of double-strand breaks (DSBs) was quantified using pulsed field gel electrophoresis. To assess the contribution of clustered damage to the total amount of lesions, samples were post-irradiation incubated with a base excision repair endonuclease. The enzyme used in this study was Formamidopyrimidine-DNA N-glycosylase which recognizes oxidized purines and cleaves the strand at the site inducing a strand break. Hence, the additional DSBs detected after enzyme incubation derive from clustered damage. To investigate the influence of chromatin structure on the induction of clustered damage in irradiated cells, different pre-treatments were used that resulted in structures with varying compactness of chromatin.

Results

The number of clustered damages and prompt DSBs increased linearly with dose. For each DSB 0.8 clustered damage was induced with a yield of 0.29 cluster/Gy and Mbp. Analysis of DNA fragment distribution showed that clustered damages and DSBs were randomly allocated after irradiation with X-rays. Clustered damages were rejoined efficiently with a fast and a slow repair component. 20% of initial damage remained after 1 hour but no damages were detected 24 hours after irradiation. When the chromatin was gradually removed the induction of clustered damages and prompt DSBs increased. The yield of clustered damages was 11 times higher when the chromatin was less compact and soluble scavengers were washed away compared with irradiated confluent intact cells. This increase was sufficiently higher than for DSBs (5 times). Irradiation of naked DNA resulted in a yield of cluster damages that was 170 times higher than for intact cells.

Conclusion

Clustered DNA damages contribute significantly to the total amount of complex lesions induced by ionizing radiation. This contribution increases rapidly when the chromatin is gradually stripped-off.

(PS1055) Residual γH2AX foci predict response to cisplatin and fractionated irradiation *in vitro* and *in vivo*. Adriana Banuelos[1], Judit P. Banath[1], Susan H. MacPhail[1], James Byrne[1], Christina Aquino-Parsons[2], Peggy L. Olive[1]. [1]B.C. Cancer

Research Centre, Vancouver, BC, Canada, [2]B.C. Cancer Agency, Vancouver, BC, Canada.

Exposure to ionizing radiation or cisplatin produces DNA double-strand breaks or interstrand crosslinks that result in phosphorylation of histone H2AX. Clusters of γH2AX molecules form over time at sites of double-strand breaks or replication fork collapse and subsequently disappear as repair proceeds. By 24 hours after treatment, the fraction of cells that retain γH2AX foci can be correlated with the fraction of cells that will subsequently die. Since radiation and cisplatin are often combined in the clinic, the ability to use γH2AX foci to predict response to this combination was examined using SiHa and WidR xenografts grown subcutaneously in immunodeficient mice. Mice received a single dose of 5 or 10 mg/kg cisplatin on Monday followed by 3 daily doses of 2 Gy to simulate the clinical schedule. Twenty-four hours after the final radiation dose, tumors were excised and a single cell suspension was prepared and analyzed for clonogenicity and γH2AX foci. The fraction of tumor cells with fewer than 3 foci per cell was correlated with clonogenic fraction for both WiDr and SiHa xenografts, although the 1:1 correlation typically seen for exponentially growing cultured cells was lost. Similar results were obtained when tumor cells close to functional blood vessels or distant from these vessels were analyzed after cell sorting. Based on these results, we have begun to analyze γH2AX foci in clinical samples from cervical cancer patients that have received cisplatin and radiation. Biopsies were obtained before treatment and up to 2 weeks after the start of treatment, and formalin-fixed sections were stained for γH2AX. Preliminary results indicate the feasibility of this approach as a method that may help identify treatment resistant tumors early in the course of therapy.

(PS1056) The effect of heavy-ions on synchronously dividing cell cultures. Takamitsu A. Kato, Yoshihiro Fujii, Akira Fujimori, Ryuichi Okayasu. National Institute of Radiological Sciences, Chiba, Japan.

We investigated the alteration of homologous recombination repair in synchronous CHO cells irradiated with heavy ion particles (290MeV/n carbon ion, LET: 13keV/μm and 70keV/μm) and X-rays. The synchronized cell populations were prepared by mitotic shake-off and sequential harvesting at various time points. Our flow cytometry analysis revealed that the majority of cells enter S-phase at 8 hours, and G2-phase at 12 hours after plating. We scored the number of co-localization sites of gamma-H2AX (DNA double strand breaks) and Rad51 (homologous recombination repair protein) foci after 1Gy irradiation. Carbon irradiated cells showed a significantly higher number of co-localization sites at 30 minutes after irradiation than X-irradiated cells. We also observed delayed disappearance of Rad51 foci in carbon irradiated cells up to 2 hours. Both chromosome- and chromatid-type aberrations were the highest in high LET carbon(70keV/μm), intermediate in low LET carbon (13keV/μm), and the lowest in x-irradiated cells. Furthermore, we observed a significantly increased number of chromatid-type aberrations in cells irradiated at early S-phase by 70 keV/μm carbon ions. The cell survival rates by colony formation seem to reflect the chromosomal data as expected. We did not observe the resistant peak at late-S phase in cells irradiated with high LET (70 keV/μm) carbon ions. These results may indicate the alteration of homologous recombination repair after high LET carbon irradiation lead to various biological effects. Our data are consistent with the idea of complex type DSB induced by high LET heavy ion irradiation.

(PS1057) The mutation of *ric1* induces delayed repair of DNA double strand breaks, cell death inhibition and early checkpoint release. Masayuki Hidaka[1], Shoji Oda[1], Yoshikazu Kuwahara[1], Manabu Fukumoto[2], Hiroshi Mitani[1]. [1]University of Tokyo, Chiba, Japan, [2]Tohoku University, Miyagi, Japan.

RIC1 is the mutant Medaka by ENU-mutagenesis which has the high susceptibility to γ-ray irradiation. It has been shown that RIC1 embryo at the pre-gastrula stage has some deficiencies in the fast repair of DNA double strand breaks (DSBs) by γ-ray irradiation (Aizawa *et al*. 2004). In this study, a cultured cell line was established from a RIC1 embryo and the roles of the *ric1* product in DNA repair, apoptosis and cell cycle checkpoint were investigated. Immortalized cell lines can be established easily from a single Medaka embryo without morphological transformation, and cells can be cultured without a CO_2 incubator under the wide range of temperature. To make clear the roles of *ric1* gene, we performed the neutral comet assay in cultured cells. DSBs by X-ray irradiation were repaired almost completely in CAB cells within 30 mins after irradiation, while DSBs in RIC1 cells were not repaired for 1 hr after irradiation and completely repaired within 2 hrs after irradiation. To investigate the morphological changes and the inhibition of cell proliferation, we performed time-lapse analysis for 24 hrs after γ-ray irradiation (10 Gy). In CAB cells, γ-ray irradiation induced cell fragmentation which are typical of apoptosis. In contrast, γ-ray irradiation did not induce cell fragmentation in RIC1 cells up to 24 hrs after irradiation. The cell proliferation was inhibited immediately after γ-ray irradiation in both CAB and RIC1 cells. CAB cells stopped proliferation for 24 hrs after irradiation. However, RIC1 cells started to proliferate again about 12 hrs after irradiation. Flow cytometry analyses revealed that γ-ray irradiation induced cell cycle arrest at G2 both in CAB and RIC1 cells and that, in RIC1 cells, cell cycle arrest at G1 was also induced. These results suggest that *ric1* gene plays the key roles to induce "repair" or "death"; the mutation of *ric1* impaired function of detection of DNA DSBs. The impair might cause delayed repair of DNA DSBs, cell death inhibition and early checkpoint release. The G1 arrest in RIC1 cells suggests the significant role of *ric1* in the close relationship between DNA repair, cell death inhibition and cell cycle control.

(PS1058) MCT-1 oncogene downregulates p53 and destabilizes genome structure in the response to DNA double-strand damage. Hung-Ju Shih[1], Chik On Choy[1], Ravi Kasiappan[1], Jeffrey R. Sawyer[2], Chung-Li Shu[1], Kang-Lin Chu[1], Yi-Rong Chen[1], Hsin-Fen Hsu[1], Ronald B. Gartenhaus[3], Hsin-Ling Hsu[1]. [1]National Health Research Institutes Division of Molecular and Genomic Medicine, Miaoli County, Taiwan, [2]Department of Pathology, Cytogenetics Laboratory, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [3]University of Maryland Marlene and Stewart Greenebaum Cancer Center, Baltimore, MD, USA.

Tumor suppressor p53 protein mediates checkpoint controls and the apoptotic program that are critical for maintaining genomic integrity and preventing tumorigenesis. Forced induction of MCT-1 decreased p53 expression before and after genomic insults. While inhibiting de nova protein synthesis, the levels of ubiquinated-p53 and the phospho-MDMA2 were significantly increased in ectopic MCT-1 cells. Abrogation of the proteosome degradation process attenuated p53 destabilization and p21 downregulation by MCT-1. Concomitantly, MCT-1 overexpression enhanced the phosphorylation status of MAPK (ERK1/ERK2). While MCT-1 gene knockdown or MEK/ERK pathway inhibition dramatically reduced MAPK phosphorylation, the genotoxin-induced p53 and p21 production were noticeably elevated. Upon Etoposide treatment, ectopic MCT-1 cells relaxed S-phase and G2/M checkpoints followed by G1 phase progressing. Moreover, cells inducing with MCT-1 abridged accumulations of G2/M populations in the response to gamma-irradiation. The polyploidy (DNA content >4N) populations were increased in association with p53 loss in MCT-1 oncogenic cells. Alkaline comet assay validated that ectopic MCT-1 cells were less susceptibility to the genotoxicity. Furthermore, the allocation of nuclear MCT-1 induced by the genotoxic stress was moderately coincided with γ-H2AX appearances. Throughout damage-repairing process, ectopic MCT-1 cells displayed many larger chromosomes and multiple chromosomal fusions compared to the controls that showed increase in chromosomal breaks/gaps and minute chromosomal fragments. Spectral karyotyping analysis precisely identified the acquisition of a single extra copy of chromosome 14 together with a complex genome organizations in ectopic MCT-1 cells, including extra copies of chromosome segments that had been translocated to derivative chromosomes 6 [der(6)] and 9 [der(9)]. In conclusion, MCT-1 deregulates p53-p21 network and impairs the damage

checkpoints those are robustly connected to oncogenic chromosomal abnormalities.

(PS1059) Alteration of mitochondria specific DNA post radiation. Hengshan Zhang, David Maguire, Steven Swarts, Weimin Sun, Shanmin Yang, Wei Wang, Chaomei Liu, Mei Zhang, Peter Keng, Lurong Zhang, Paul Okunieff. Department of Radiation Oncology, Rochester, NY, USA.

The effect of radiation on the mitochondria genome *in vivo* is largely unknown. Since the mitochondria is a vital organelle in cells and has far more less DNA repair machinery compared to the nuclear DNA, the radiation damage of mitochondria genome is likely a cause of some late toxicity in non-proliferating cells. Better understanding of the radiation effects on mitochondrial DNA should lead to new approaches for radiation protection.

In this study, we developed a new system using real-time PCR that sensitively detects the alteration of mitochondria specific DNA. In each sample, the nuclear DNA (coding 18S rRNA) served as a nuclear control run simultaneously with a mitochondria DNA sequence. We found that the alteration of mitochondria specific DNA copy number (MSDCN) differed with **1)** the radiation dose; **2)** the time after radiation exposure; **3)** the organ examined.

The MSDCN of gut tissue collected at 24 hr after 2 Gy or 4 Gy total body radiation (TBI) increased compared to the normal mice. The 4 Gy triggered a higher MSDCN as compared to 2 Gy. Similarly, in the bone marrow collected at 24 hr after 4 Gy or 7 Gy TBI, the 7 Gy triggered a higher MSDCN as compared to 4 Gy. As a function of time, the MSDCN after 7 Gy increased at 48 hr compared to 24 hr.

The damage gut epithelium of BALB/c mice 3.5 days after a 12 Gy exposure had a reduced MSDCN. A subset of animals was give gastrointestinal radioprotectors and these agents improved the MSDCN toward the normal level.

Our preliminary data suggest that the alterations of mitochondria specific DNA induced by radiation are presented within a few days after IR. Further study of the effects of radiation on this understudied category of DNA is indicated.

(PS1060) Dna damage-induced apoptosis in c3h mouse peritoneal resident macrophages. Yoshihisa Kubota, Katsutoshi Suetomi, Akira Fujimori, Sentaro Takahashi. National Institute of Radiological Sciences, Chiba, Japan.

Previously we reported irradiation-induced apoptosis in peritoneal resident macrophages (PRM) of C3H mice but not other strains of mice. Studies with *atm* knockout, *p53* knockout or scid mice suggested that a well-known DNA damage-induced apoptotic pathway involving the activation of ATM, DNA-PK and/or TP53 did not take part in irradiation-induced apoptosis in C3H mouse PRM. Furthermore, superoxide anion and the depletion of Mcl-1, an anti-apoptotic Bcl-2 family protein, through irradiation-induced arrest of global protein synthesis were identified as critical factors for the apoptosis. The present study was planed to reconfirm the involvement of reactive oxygen species but not DNA damage in irradiation-induced apoptosis in C3H mouse PRM. PRM were treated with paraquat (a superoxide inducer), hydrogen peroxide or cadmium at various concentrations to enhance intracellular level of reactive oxygen species or oxidative stress. As a selective indicator of irradiation-induced apoptosis in C3H mouse PRM, Mcl-1 level was examined by western blotting. Hydrogen peroxide and cadmium treatment did not affect the amount of Mcl-1. Paraquat slightly diminished Mcl-1 at extremely high concentrations where oxidative stress was elicited as severely as caspase 3 was completely inactivated. On the other hand, Mcl-1 was markedly depleted by gamma-irradiation or UV irradiation in C3H mouse PRM. PRM were treated with DNA topoisomerase II inhibitor, etoposide or introduced with a restriction endonuclease, Pvu II by means of HVJ envelope vector kit (GenomeONE^{TM}) to generate DNA double-strand breaks. Unexpectedly, these treatments induced marked apoptosis in C3H mouse PRM, but not in radioresistant B6 mouse PRM. Therefore, it is concluded that irradiation-induced apoptosis in C3H mouse PRM is attributable to irradiation-induced DNA damage, possibly DNA double-strand breaks.

(PS1061) Increased chromosome instability and accumulation of DNA double-strand breaks in Werner syndrome cells. Kentaro Ariyoshi[1], Shiraishi Kazunori[1], Keiji Suzuki[2], Makoto Goto[3], Masami Watanabe[4], Seiji Kodama[1]. [1]Radiation Biology Laboratory Radiation Research Center Osaka Prefecture University, Osaka, Japan, [2]Division of Radiation Biology, Department of Radiology and Radiation Biology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan, [3]Division of Anti-Ageing and Longevity Sciences, Faculty of Clinical Engineering, Toin University of Yokohama, Yokohama, Japan, [4]Laboratory of Radiation Biology, Research Reactor Institute, Kyoto University, Osaka, Japan.

Werner syndrome (WS) is a rare human premature aging syndrome caused by mutations in the gene encoding the RecQ helicase WRN. Accumulated evidence indicates that WRN deficiency results in abnormal regulation of telomeres, which possibly correlates with accelerated aging and a high incidence of cancer. Here, we demonstrate that a strain of WS fibroblast cells shows abnormal karyotypes characterized by several complex translocations and 50-fold more frequency of spontaneous abnormal metaphases including dicentric chromosomes without fragments than normal cells when examined at similar culture stages, i.e., almost 30 population doubling numbers (PDN). Further, telomere fluorescence in situ hybridization indicates that the abnormal telomere signals, extra telomere signal and loss of telomere signal, emerge two- to three-fold more frequently in WS cells than in normal cells. Taken together, these results indicate that chromosome instability including dysfunction of telomere maintenance is more prominent in WS cells than in normal cells. In addition, the accumulation of DNA double-strand breaks (DSBs) at the G1 phase, including those co-localized at telomeres, detected by phosphorylated ATM foci is accelerated in WS cells even at a low senescence level, suggesting that lack of WRN contributes to promoting DSB accumulation. The increased accumulation of DSBs in WS cells is substantially reduced in the presence of antioxidant agents, suggesting that enhanced oxidative stress in WS cells is involved in accelerated accumulation of DSBs. Indeed, PDN-dependent increase of intracellular oxidative stress measured by 2′7′-dichlorodihydrofluorescein diacetate (H2DCFDA) is more accelerated in WS cells than in normal cells. These results indicate that WS cells are prone to accumulate DSBs spontaneously due to a defect of WRN, which leads to increased chromosome instability that could activate checkpoints, resulting in accelerated senescence.

(PS1062) Biological studies using human cell lines and the current status of the microbeam irradiation system, SPICE. Teruaki Konishi[1], Takahiro Ishikawa[1], Hiroyuki Iso[1], Nakahiro Yasuda[1], Shunsuke Okuma[2,1], Kumiko Kodama[1], Tsuyoshi Hamano[1], Noriyoshi Suya[1], Hitoshi Imaseki[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Rikkyo University, Tokyo, Japan.

A microbeam irradiation system, SPICE (Single Particle Irradiation system to Cell), is under completion at the National Institute of Radiological Sciences (NIRS). We have improved the beam size, which is now approximately 10 μm diameter, and the cell targeting system, which can irradiated maximum of $1.0x10^5$ cells per hour. The cell dish was specially designed and the cells were cultured on Si_3N_4 plate (3mm×3mm area with 1 μm thickness) consisting of 7.5 mm × 7.5 mm frame of 200 μm thickness. The cell dish is set on the voice coil stage equipped on the cell targeting system, which also consist of a fluorescent microscope and a CCD camera for capturing the cell image. This microscope system captures all the image of dyed cell nuclei in a 3 × 3 mm area and computes the coordinated of the cell position according to the fluorescence and synchronizes this with a single particle irradiation system consisting of a scintillation counter and a beam deflector for irradiation. All the procedures can automatically be performed after setting some parameters, such as a preset number of protons.

SPICE is still under reliability assessment. The first experiment performed was a visualization of phosphorylated histone protein, γ-H2AX, which is known as a marker for DNA double strand breaks using immuno-staining technique, to confirm whether the targeted cell was accurately irradiated. Now we are focusing on low dose effects and how to obtain survival curves to measure hyper-radio sensitivity by irradiating human normal fibroblast and DNA double strand break repair deficient cell lines. We have also performed an experiment to cross-check our survival curves with those obtained using other irradiation techniques, such as broad-beam irradiation. Preliminary results will be presented.

(PS1063) Function of setd4 in dna damage response. Jinjiang Fan, Zhiyuan Shen. Cancer Institute of New Jersey,UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ, USA.

SET Domain is an evolutionarily conserved peptide involved in catalyzing the methylation of lysine residues in proteins. SET domain protein is a family of protein that contains the SET domain, and is essential for the regulation of gene activation and silencing in all eukaryotes. We identified a previously un-characterized human SET domain containing protein, SETD4, which interacts with BCCIP. Bioinformatic analysis had suggested 4 major SETD4 isoforms which are the products of alternative splicing, and we have recently identified a new isoform. BCCIP is a BRCA2 and CDKN1A (p21/Cip1) Interacting Protein, which plays a significant role in DNA repair and cell cycle checkpoint control. Because many previously reported SET-domain proteins function in DNA repair, and SETD4 interact with BCCIP, we investigated the potential role of SETD4 in DNA damage response. We found that exogenous expression of SETD4 increase cell resistance to ionizing radiation by 80–90%. This resistance can be overruled by knockdown of the exogenous SETD4. Furthermore, expression of SETD4 slightly increases the gene targeting efficiency in human cells but had little effect on random gene insertion. These data suggest a role of SETD4 in DNA damage response.

(PS1064) Mitochondrial DNA damage post exposure to simulated sunlight in human skin cells. Luciene Zanchetta[1], James Walsh[2], Fiona Lyng[1], James Murphy[1]. [1]Radiation and Environmental Science Centre, Dublin, Ireland, [2]School of Physics, Dublin, Ireland.

The use of mitochondrial DNA damage as a biomarker of cumulative sunlight exposure in human skin is a relatively new field of research. The aim of this study was to assess mitochondrial damage expressed in human skin cells subjected to different sunlight exposures from a Q-Sun solar spectrum simulating irradiator.

HaCaT, C32 and A375 cells were subjected to a range of simulated sunlight exposures ranging from 30 seconds to 5 minutes with damage being analysed from 4 to 72 hours later. DNA was isolated using DNeasy (Qiagen) and mitochondrial DNA assessed for induction of deletions and for genome frequency per cell. Molecular analysis of the entire 16.5Kbp mitochondrial genome and conserved mitochondrial genomic region were performed by PCR to access the overall mtDNA population of the sample. We had also studied the presence or absence of the so called common deletion (4977bp) as well as the 3895bp deletion found previously in UV radiation exposed skin and skin cells in culture.

Our results demonstrate that exposure of human skin cells *in vitro* to sunlight caused mtDNA damage and altered the number of mitochondrial genomes in exposed cells. The frequency of deletions identified in this study may provide for a potential biomarker for cumulative sunlight exposure in human skin.

(PS1065) Detection of radiation-induced conformational changes in individual DNA molecules using a high-sensitivity flow 'cytometer'. Robert C. Habbersett, James H. Jett, James P. Freyer. Bioscience Division, Los Alamos, NM, USA.

We have developed a specialized high-sensitivity (HS) flow 'cytometer' for analyzing the length of individual DNA fragments (Habbersett & Jett, *Cytometry* 60A: 125, 2004). The HS instrument incorporates relatively slow-flow hydrodynamic focusing and a very small probe volume to dramatically improve fluorescence detection sensitivity (e.g. we can detect a single molecule of B-phycoerythrin). The simple, compact design incorporates several low-cost, low power optical and detection components along with a custom-built digital data acquisition and analysis system. This instrument is capable of resolving DNA fragments as small as 125 bp up to ~500,000 bp, and has been applied in several areas including DNA fingerprinting, bacterial identification and kinetics of DNA-dye interactions. Recently, we have demonstrated that the HS instrument is capable of measuring the conformation of individual DNA molecules in flow, using a more sophisticated waveform analysis of the digitized fluorescence signal as the individual molecules pass through the laser. Through a combination of the photon burst area and the transit time through the laser, we can accurately resolve linear, circular and supercoiled versions of individual DNA plasmid molecules. In a preliminary experiment, we exposed a predominantly supercoiled sample of a 17.4 kbp plasmid to 6 Gy of ^{137}Cs γ-rays. The irradiated sample showed significantly increased fractions of circular and linear molecules, representing the induction of single- and double-strand DNA breaks, respectively. Further experiments are underway to determine if we can use the HS instrument as a DNA damage dosimeter. This flow-based approach to analyzing DNA damage has several advantages over gel-based methods, including: 1) direct quantitation of each conformational type; 2) much less sample required (~2500 individual molecules or 3.6×10^{-20} gr of DNA); and 3) sensitivity to very small DNA fragments that would normally be lost during gel electrophoresis of larger molecules. This work was supported by the National Flow Cytometry Resource (NIH grant RR01315).

(PS1066) Radiation-induced phosphorylated H2a.x foci in human keratinocyte cells expressing histone H2BGFP-tagged protein using the cenbg charged particle microbeam. Hervé SEZNEC, Thomas POUTHIER, Fredrik ANDERSSON, Philippe BARBERET, Sébastien INCERTI, Philippe MORETTO. CENBG, Université Bordeaux 1, CNRS, Gradignan, France.

Charged particle microbeam can be used to generate localized radiation induced-damages within restricted regions of the nuclei in order to study the metabolic responses of single cells after low dose of in vitro radiation exposure. Based upon ion-microbeam techniques developed at the CENBG for targeted irradiation of individual cells, with a counted number of MeV charged particles, we have developed and validated a technical procedure to estimate the DNA damage as a function of dose. In order to avoid external physical and chemical agents as ultraviolet light and Hoechst33342 dye, that could interfere with radiation-induced effects, we have established by transfection a human keratinocyte cell line expressing the histone H2B fused to the Green Fluorescent Protein (named H2BGFP). The ability of our system to routinely irradiate individual cells was checked by estimating the specific cellular damage as DNA DSBs induced after microbeam irradiation. In that purpose, we chose to study the gamma-H2A.X foci formation following exposure of the cell nucleus to a preset number of ions. These data show that the CENBG micro-irradiation setup and the methodology developed, constitute a valuable technique to study the molecular pathways involved in the cellular response after exposure to low doses of radiation.

(PS1067) Abrogation of radiation-induced S-phase checkpoint by oncogenic K-Ras. Moon-Taek Park[1], Min-Jung Kim[1], Joo-Yun Byun[1], Sangwoo Bae[2], Chang-Mo Kang[3], In Chul Park[4], Gyesoon Yoon[5], Sang-Gu Hwang[1], Su-Jae Lee[1]. [1]Laboratory of Radiation Experimental Therapeutics, Korea Institute Radiological & Medical Science, Seoul, Republic of Korea, [2]Laboratory of Radiation Effects, Korea Institute Radiological & Medical Science, Seoul, Republic of Korea, [3]Laboratory of Radiation Cytogenetics and Epidemiology, Korea Institute Radiological & Medical Science,

Seoul, Republic of Korea, [4]Laboratory of Functional Genomics, Korea Institute Radiological & Medical Science, Seoul, Republic of Korea, [5]Department of Biochemistry and Molecular Biology, Ajou University School of Medicine, Suwon, Republic of Korea.

In response to DNA damage, mammalian cells activate a complex network of the checkpoint pathways that inhibit cell cycle progression through the G1 and G2 phases and induce a transient delay in the progression through S phase. In this study we found that oncogenic K-Ras causes abrogation of radiation-induced S-phase checkpoint in MCF-10A, human breast epithelial cells. We show that activations of Raf-1-ERK and PKCδ-p38MAPK pathways are involved in radio-resistant DNA synthesis phenotype in cells overexpressing oncogenic K-Ras. Interference of either pathway clearly restored defects in radiation-induced DNA synthesis inhibition by oncogenic K-Ras. Moreover, we show that Raf-1-ERK pathway is involved in the activation of ubiquitin ligase, Skp1-Cul1-Skp2 complex. Attenuation of the Skp1-Cul1-Skp2 complex formation clearly restored oncogenic K-Ras-induced radio-resistant DNA synthesis. In addition, inhibition of PKCδ-p38MAPK pathway attenuated K-Ras-induced activation of Akt, and enhanced radiation-induced cell death. We conclude that oncogenic K-Ras causes abrogation of radiation-induced S-phase checkpoint through Raf-1-ERK-dependent activation of ubiquitin ligase and PKCδ-p38MAPK-dependent activation of cell survival pathway.

(PS1068) Interactions between IR-induced p53 phosphoforms and ATM/53-BPp1 complexes during DNA-dsb repair. Robert G. Bristow, Shahnaz Al Rashid, Farid Jalali, Shane Harding, Nirmal Bhogal, Richard Hill. Princess Margaret Hospital, Toronto, ON, Canada.

We have recently shown that ionizing radiation (IR)-activated p53 phosphorylated at serine 15 ($p53^{Ser15}$) localizes at sites of IR-induced DNA damage. p53 species can co-localize with DNA-dsb (double-strand break) sensing proteins such as γ-H2AX, 53BP1, ATM, and DNA-PKcs with kinetics similar to that of biochemical DNA-dsb rejoining analyses. In further studies, we have determined that p53 carboxy-terminus is also an important factor in mediating p53′s ability to sites of DNA damage within chromatin. In a human isogenic system, YFP-p53 fusion constructs expressing various carboxy-terminus deletion mutants of p53 have been generated, transiently transfected into $p53^{-/-}$ H1299 cells and analyzed following IR-induced DNA damage. Using fluorescence microscopy and cellular fractionation followed by immunoprecipitation/Western blot analyses, we will present data of differential sub-nuclear and chromatin localization and protein-protein interactions with ATM and 53BP1. We are also determining the role of 53BP-1/p53 interactions in a cell cycle dependent manner in response to endogenous and exogenous DNA damage. Finally, using NHEJ repair-deficient and -proficient mice, we are determining the role of these interactions within whole nulcei in murine skin as a function of dose and time using novel spinning disk confocal microscopy technologies. These studies are supported by CCS-NCIC and NIAID-CMCR/U19 operating grants and RGB is a Canadian Cancer Society Research Scientist.

(PS1069) Tel2 mediates localization of Tel1 to sites of DNA damage. Carol M. Anderson, Dana L. Smith, Svetlana Makovets, Dmitry Korkin, Andrej Sali, Elizabeth H. Blackburn. UCSF, San Francisco, CA, USA.

Telomeres protect chromosome ends from being treated by the cell as DNA damage, yet many proteins involved in the DNA damage response are required for normal telomere function. We are studying one such protein, the product of the yeast *TEL2* gene, which was originally identified in a screen for mutations causing changes in telomere length. Strains carrying the mutant allele *tel2–1* have stably shortened telomeres. *TEL2* encodes a novel 79 kD protein conserved throughout eukaryotes. The *C. elegans* and *S. pombe TEL2* orthologs have been shown to play a role in the checkpoint responses to DNA damage and DNA replication stress. Here, we elucidate the mechanism by which *S. cerevisiae* Tel2 influences the response to DNA damage.

The kinases Tel1 and Mec1 (ATM and ATR in mammals) control the cellular response to DNA damage and replication stress. We report that Tel2 is a critical component of the *TEL1* pathway of DNA damage signaling. The mutant allele *tel2–1* caused defects in DNA damage signaling that mimicked the effects of *TEL1* deletion. By co-immunoprecipitation we found that Tel1 and Tel2 physically interact. Using protein threading, a computational method for structural characterization of proteins, we have predicted that Tel2 is structurally similar to Lcd1 (mammalian ATRIP), a protein that controls the localization of Mec1 to sites of DNA damage. Consistent with this, we found by chromatin IP that wild type *TEL2* was required for localization of Tel1 to an induced double strand break in vivo. We propose that Tel1 and Tel2 form an Lcd1/Mec1-like complex and that Tel2 is responsible for recruiting Tel1 to double-strand breaks.

(PS1070) Intervention of the repair factors of DNA double strand break to micronuclei derivation by radiation. Tomohiro Yoshikawa, Genro Kashino, Koji Ono, Masami Watanabe. Kyoto University Reserch Reactor Institute, Osaka, Japan.

Radiation induces both DNA double strand breaks (DSBs) and micronuclei. DSBs induce the rapid phosphorylation of H2AX that is one of a chromatin protein, and it may act as both DSB sensor and an initial DNA repair factor. However, we have little information about role of phosphorylated H2AX (phospho-H2AX) on micronuclei formation. Therefore, we examined whether micronuclei induced by X-ray have phospho-H2AX foci in it or not.

We irradiated human embryo cells (HE49) and HeLa cells with 6 Gy of X-ray and then cultured them for 6 hours to 5 days. The results showed that 0.3% of nonirradiated HE49 and HeLa cells had micronuclei and 50% of these spontaneous micronuclei had phospho-H2AX foci. On the other hand, cells with micronuclei increased until 3 days after X-irradiation and reached about 20% in both HeLa and HE49.Phospho-H2AX foci were found on 90% and 70% of X-ray-induced micronuclei in HE49 and HeLa, respectively. However, phosphoryated ATM foci were seen in only 20% of X-ray-induced micronuclei at most. These results suggest the possibility that DNA DSBs does not contribute to for the micronuclei formation by radiation.

(PS1071) Distinct roles of xrcc4 and ku80 in non-homologous endjoining of enzyme- and radiation-induced dna double-strand breaks. Jochen Dahm-Daphi[1], Leonie Schulte-Uentrop[1], Raafat A. El-Awady[1], Henning Willers[2]. [1]University of Hamburg, Hamburg, Germany, [2]Harvard Medical School, Charlestown, MA, USA.

Non-homologous endjoining (NHEJ) of DNA double-strand breaks (DSBs) is mediated by two core protein complexes comprising Ku80/Ku70/DNA-PKcs/Artemis and XRCC4/LigaseIV/XLF. Loss of Ku70/80 or XRCC4/LigaseIV function commonly compromises the DSB repair capacity and leads to radiation hypersensitivity while other facetes of the deficient phentypes are less well understood. In this study, we sought to define how XRCC4 and Ku80 affect the rejoining of site-directed chromosomal DSBs in murine fibroblasts. To measure NHEJ, we employed a recently developed reporter system based on the rejoining of I-SceI endonuclease induced DSBs, which leads to transcriptional activation of the bacterial gpt gene. Single enzymatic breaks may resemble'spontaneous' DSBs that arise during normal DNA metabolism. Removal of those breaks likely has different genetic requirements as compared to radiation-induced DSBs. We found that the frequency of NHEJ of the I-SceI-induced DSBs was reduced by 28-fold in XRCC4-/- cells compared to XRCC4+/+ cells while neither Ku80 knock-out nor inhibition of the DNA-PKcs by wortmannin affected the repair efficiency. In contrast, lack of either XRCC4 or Ku80 increased the length of sequence deletions and shifted repair towards the use of longer microhomologies for junction formation. The differential requirement for efficient rejoining of I-SceI breaks may help to explain the lethaliy of

XRCC4 knock-out mice as opposed to Ku80 knock-outs. In contrast, both proteins proved to be essential for the repair of radiation-induced DSBs. Inactivation of Ku80 or XRCC4 increased cellular sensitivity and inhibited DSB repair to a similar extend

(PS1072) Speed of DNA double-strand break processing depends on age. Olga Sedelnikova, Christophe Redon, Izumi Horikawa, Drazen Zimonjic, Nicholas Popescu, William Bonner. National Cancer Institute, Bethesda, MD, USA.

Cellular senescence may have a role in organismal aging. Telomere shortening, oxidative stress and DNA damage are hypothesized to be major factors that negatively affect the process of aging at the cellular level. Although DNA damage has been shown to be involved in both replicative and accelerated cellular senescence, its role in organismal aging is poorly understood. Using immunostaining of a phosphorylated variant of histone H2AX (γ-H2AX), which occurs specifically at sites of DNA double-strand breaks (DSBs), endogenous γ-H2AX foci were visualized in young and senescing fibroblasts, and in human lymphocytes. Additionally, normal individuals and patients with Werner syndrome (WS), a disorder associated with premature aging, genomic instability and increased incidence of cancer, were compared. The incidence of γ-H2AX foci positively correlates with donor age in both normal and WS cells, but the observed values are markedly higher in the latter. These foci co-localized with DSB-repair factors, and the rate of repair protein recruitment to the sites of γ-H2AX foci after irradiation was found to correlate conversely with the age of donors. Slow DSB-repair protein mobilization may be due to slow growth of γ-H2AX foci. Therefore, genomic stability may depend on the rapid formation of γ-H2AX foci and the rapid accumulation of DSB-repair proteins on these foci. These findings establish the physiological importance of DSBs in both normal and pathological aging, thus suggesting that diverse factors affecting the aging process may commonly act through accumulation of DSBs.

(PS1073) Human RAD18 is involved in single-strand break repair independent of PCNA monoubiquitination. Tadahiro Shiomi[1], Naoko Shiomi[1], Masahiko Mori[1], Hideo Tsuji[1], Takashi Imai[1], Hirokazu Inoue[2], Satoshi Tateishi[3], Masaru Yamaizumi[3]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Saitama University, Saitama, Japan, [3]Kumamoto University, Kumamoto, Japan.

Proliferating cell nuclear antigen (PCNA) is monoubiquitinated in a RAD18/RAD6-dependent manner at stalled replication forks caused by DNA lesions. PCNA monoubiquitination mediates a polymerase switch from replicative to translesion polymerase to continue replication. Therefore, *RAD18*-deficient cells become sensitive to various DNA damaging agents. However, PCNA was not monoubiquitinated after X-irradiation in human cell line HCT116, although it's RAD18 deficient mutant was sensitive to X rays and defective in repair of X ray-induced chromosome aberrations. The mutant cells were hypersensitive to topoisomerase I inhibitor camptothecin that generated single strand breaks (SSBs) but not to topoisomerase II inhibitor etoposide that generated double strand breaks. Thus, human RAD18 is required for the repair of chromosomal SSBs and PCNA monoubiquitination is not necessary for the repair process.

(PS1074) NBS1 regulates the induction of apoptosis following radiation damage to DNA. Kenta Iijima[1], Chizuko Muranaka[1], Junya Kobayashi[2], Shuichi Sakamoto[2], Kenshi Komatsu[2], Hiroshi Tauchi[1]. [1]Ibaraki University, Mito, Ibaraki, Japan, [2]RBC, Kyoto University, Kyoto, Japan.

Mutations in the NBS1 protein are responsible for Nijmegen breakage syndrome (NBS), which is characterized by immunodeficiencies and a high incidence of malignances. NBS1 forms a complex with MRE11 and RAD50, and this RAD50/MRE11/NBS1 (R/M/N) complex is known to be an activator of cell cycle checkpoints and homologous recombination repair in response to DNA damage. We report here that NBS1 is required for efficient induction of apoptosis following X-irradiation, and that this function can be independent of R/M/N complex formation.

Apoptosis induction was significantly reduced in Nbs1 deficient chicken DT40 cells after DNA damage induction. Because DT40 cells do not have p53, this Nbs1 regulated apoptosis pathway should be independent of p53. The same observation was also made in lymphoblastoid cells from NBS patients. A series of cells expressing various mutant NBS1 proteins was next examined in order to explore this apoptosis pathway, and in order to locate the critical domain of NBS1 involved in the induction of apoptosis. In parallel with phenotypic observations of apoptosis induction, an insufficient activation of both Bax and caspase-3 was observed in NBS cells. The expression of NBS1 proteins with point mutations in the ATM/ATR phosphorylation sites were not able to complement this behavior, whereas a partial restoration was observed in cells expressing C-terminus deleted NBS1 proteins, such as NBS1 proteins with a deleted MRE11-binding domain or a deleted ATM-interacting domain. These findings suggest that NBS1 is involved in the mitochondria dependent apoptotic pathway, and that the R/M/N complex formation is not essential for this function. Therefore, NBS1 regulates not only the early stages of DNA damage response by forming a complex, but also late stage responses such as apoptosis induction without forming the R/M/N complex. Thus, NBS1 is a key protein for the prevention of carcinogenesis through the regulation of correct repair of damaged DNA, and through the elimination of inappropriately repaired cells.

(PS1075) Phosphorylation and kinase activity of DNA-PK$_{CS}$ regulate its dynamics at DNA double-strand breaks. Eric Weterings. UT Southwestern Medical Center, Dallas, TX, USA.

The process of non-homologous end-joining (NHEJ) is the predominant pathway for repair of DNA double-strand breaks (DSBs) in non-dividing cells. Repair of DSBs via this pathway involves juxtaposition of the two ends of the broken DNA-strand by an enzymatic machinery and subsequent ligation of the tethered DNA-ends. The DNA-dependent protein kinase (DNA-PK), consisting of the Ku70/80 heterodimer and a catalytic subunit with kinase activity (DNA-PK$_{CS}$), most likely plays an important role during this tethering and the subsequent ligation of DNA-ends. Current biochemical literature on DNA-PK suggests that the molecule functions as a 'gate-keeper' which binds to, protects and juxtaposes DNA-ends prior to ligation. In this model, the DNA-PK molecule undergoes a conformational change that liberates the DNA-ends when the repair-complex is ready for correct ligation of the DNA ends. This conformational change is suggested to be mediated by the phosphorylation status and the kinase activity of DNA-PK$_{CS}$. The availability of cells that stably express YFP-labeled DNA-PK$_{CS}$ enabled us to study the influence of specific mutations at the clustered phosphorylation sites and the kinase domain of DNA-PK$_{CS}$ on its recruitment to and behavior at DSB-sites in living cells. We used a laser system to introduce DSBs in a specified region of the cell nucleus. This allowed us to show that (YFP-labeled) DNA-PK$_{CS}$ accumulates at DSB sites in a Ku80-dependent manner and that neither the kinase activity nor the phosphorylation status of DNA-PK$_{CS}$ influences its initial accumulation. However, impairment of both these functions results in deficient DSB-repair and the maintained presence of DNA-PK$_{CS}$ at unrepaired DSBs. In order to understand why the mutated versions of DNA-PK$_{CS}$ did not support effective DSB-repair, we studied the stability of the DNA-PK$_{CS}$ - DNA complexes by the use of photobleaching techniques. This allowed us to determine that disabling the kinase activity and phosphorylation status of DNA-PK$_{CS}$ leads to a more rigid binding of DNA-PK$_{CS}$ to DNA-ends, which may interfere with effective ligation. We suggest a model in which DNA-PK$_{CS}$ autophosphorylation facilitates NHEJ by destabilizing the interaction of DNA-PK$_{CS}$ with the DNA-ends.

(PS1076) A loss of function screening for radiation susceptibility genes. Hitomi Sudo, Atsushi Tsuji, Aya Sgyo, Chizuru Sogawa, Tsuneo Saga, Yoshi-nobu Harada. Diagnostic Imaging Group, Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Japan.

Carcinogenesis is thought to be a multistep process that occurs through the accumulation of mutations in multiple genes required for the maintenance of normal growth control. Genomic instability is considered the earliest cellular event in the process of carcinogenesis. Although genomic instability is induced by ionizing radiation, the molecular mechanisms underlying this process are poorly understood. Cell cycle checkpoints play a key role in cell survival following DNA damage. The failure of DNA repair and cell cycle regulation in response to DNA damage are thought to be important factors in the early stages of genomic instability. Several known genes are reportedly associated not only with DNA damage repair and cell cycle regulation but also with radiation susceptibility. Thus, the identification of novel radiation susceptibility genes will likely help elucidate the molecular mechanisms underlying DNA damage-induced genomic instability.

By the large-scale expression profiling of 15 human cell lines and three mouse strains having varying degrees of susceptibility to ionizing radiation, we identified 200 genes correlated with radiation susceptibility. We constructed the shRNA library of these 200 genes and screened this library using a 96-well format and measuring the cell survivals, as determined by a sulforhodamine B-based cell proliferation assay, after X-irradiation. We identified 12 genes involved in cellular proliferation after irradiation. Three genes, ATM, ATR and CDKN1A, were known to be radiation susceptibility genes and other nine genes have not been reported to be associated with radiation susceptibility. Eight of the 12 genes were reported to directly or potentially regulate cell cycle, and the remaining four genes have not been reported biological functions. We then performed cell cycle analysis of cells transfected with shRNA vectors against these four genes following X-irradiation, and found that knockdown cells of one gene did not accumulate at G2/M phase. This result suggests that one gene is associated with G2/M checkpoint after DNA damage. Further study of these 12 genes would help elucidate the molecular mechanism for genomic instability and carcinogenesis, and develop novel drugs for effective tumor radiotherapy and early diagnosis.

(PS1077) *XRCC1* and *XRCC3* variants and risk of glioma and meningioma. Anne Kiuru[1], Carita Lindholm[1], Sirpa Heinävaara[1], Hannu Haapasalo[2], Tiina Salminen[3], Maria Feychting[4], Christoffer Johansen[5], Beatrice Malmer[6], Anthony Swerdlow[7], Anssi Auvinen[3]. [1]STUK-Radiation and Nuclear Safety Authority, Helsinki, Finland, [2]Tampere University Hospital, Tampere, Finland, [3]University of Tampere, Tampere, Finland, [4]Karolinska Institute, Stockholm, Sweden, [5]Danish Cancer Society, Copenhagen, Denmark, [6]Umeå University Hospital, Umeå, Sweden, [7]Institute of Cancer Research, Sutton, United Kingdom.

Several single nucleotide polymorphisms (SNPs) affecting DNA repair capacity and modifying cancer susceptibility have been described. We evaluated the association of SNPs Arg194Trp, Arg280His, and Arg399Gln in the X-ray cross-complementing group 1 (*XRCC1*) and Thr241Met in the X-ray cross-complementing group 3 (*XRCC3)* genes with the risk of brain tumors. Our Caucasian study population consisted of 701 glioma (including 320 glioblastoma) cases, 524 meningioma cases, and 1560 controls in a prospective population-based case-control study conducted in Denmark, Finland, Sweden, and the UK. In the exploratory analyses of gene-gene interactions, a combination of Gln399Gln in *XRCC1* with Thr241Thr in *XRCC3* was associated with a two-fold increased risk of glioblastoma (OR = 2.22, 95% CI = 1.08–4.57) and meningioma (OR = 1.90, 95% CI = 1.02–3.57). Similarly, a two-fold increased risk of glioblastoma (OR = 2.39, 95% CI = 1.08–5.27) was associated with the combination of Arg399Arg and Arg194Arg in *XRCC1* with Met241Met in *XRCC3*. A combination of Gln399Gln in *XRCC1* with Met241Met in *XRCC3* was associated with a three-fold increased risk of glioma (OR = 3.18, 95% CI = 1.26–8.04) and meningioma (OR = 2.99, 95% CI = 1.16–7.72). In conclusion, our results indicate possible associations between the *XRCC1* and *XRCC3* SNPs and the risk of brain tumors. However, a large number of comparisons were made and p values are modestly significant. Therefore, the results should be interpreted cautiously and need reinvestigation.

(PS1078) Repair of DNA-protein cross-links: Roles of nucleotide excision and recombination repair systems. Toshiaki Nakano[1], Soh Morishita[1], Hiroaki Terato[1], Bennet van Houten[2], Seung Pil Pack[3], Keisuke Makino[3], Hiroshi Ide[1]. [1]Hiroshima Univ., Higashi-Hiroshima, Japan, [2]Lab. Mol. Genet. NIEHS/NIH, Research Triangle Park, NC, USA, [3]Kyoto Univ., Uji City, Japan.

DNA-protein cross-links (DPCs) are produced by ionizing radiation, UV light, aldehydes, and chemotherapeutic platinum compounds. DPCs would inhibit the progression of DNA replication and transcription machineries by the steric hindrance of cross-linked proteins and hence exert adverse effects on cells. However, the repair mechanism of DPCs remains largely elusive. In the present study, to elucidate the repair mechanism of DPCs, we examined the sensitivity of Escherichia coli mutants deficient in nucleotide excision repair (NER), homologous recombination repair (HRR), and translesion synthesis to DPC-inducing agents including formaldehyde (FA) and 5-azacytidine (azaC). FA produces a covalent linkage between the amino group of a DNA base and protein, and azaC is incorporated into DNA and covalently traps the deoxycytidine methyltransferase. When cells were treated with FA, the surviving fractions of uvrA and recA mutants but not the umuDC mutant were significantly lower than that of the wild type cell. In contrast, the recA mutant but not uvrA and umuDC mutants exhibited hypersensitivity to azaC. These results suggest that DPCs formed by FA are repaired by both NER and HRR, whereas those formed by azaC are repaired exclusively by HRR. Further analysis revealed that the HRR of DPCs was dependent on the RecBCD but not RecFOR pathway. We also assessed the damage recognition and excision capacities of the NER system for DPCs using UvrABC nuclease. Oxanine was site-specifically incorporated into oligonucleotides and cross-linked to proteins of various sizes. DPC-DNA was incubated with UvrABC, and incision products were analyzed by denaturing PAGE. The incision activity of UvrABC for DPC-DNA varied markedly depending on the size of cross-linked proteins. DPC-DNA was also incubated with UvrAB, and the formation of DNA-protein complex was analyzed by native PAGE. The efficiency of DNA-UvrB complex formation was dependent on the size of cross-linked proteins. These results indicate that the damage recognition step by UvrA2B is key to the excision of DPCs by UvrABC nuclease. The roles of NER and HRR in the repair of DPCs are discussed.

(PS1079) Missing links in the mechanism of DNA double-strand break repair through the non-homologous end-joining pathway. Yoshihisa Matsumoto[1], Sushma M. Bhosle[2], Masanori Tomita[3], Norio Suzuki[4], Yoshio Hosoi[2], Kiyoshi Miyagawa[2]. [1]Tokyo Institute of Technology, Tokyo, Japan, [2]University of Tokyo, Tokyo, Japan, [3]Central Research Institute of Electric Power Industry, Komae, Japan, [4]National Institute of Radiological Sciences, Chiba, Japan.

In eukaryotic cells, including mammal, DNA double strand breaks (DSBs) are repaired mainly through two pathways: non-homologous end-joining (NHEJ) and homologous recombination (HR). DNA-PK complex, composed of DNA-PKcs, Ku86 and Ku70, and XRCC4/DNA ligase IV complex have been considered key molecules in NHEJ but new molecule, XLF/Cernunnos was found very recently. It is largely unknown how these proteins are recruited to DSB sites and assembled into repair machinery. Another mystery is the role of protein phosphorylating function of DNA-PK. For our understanding of DSB repair mechanism through NHEJ, it is critically important i) to visualize and analyze the recruitment process of NHEJ molecules to DSBs and ii) to elucidate the genuine target of DNA-PKcs and the physiological significance of phosphorylation. Regarding this, we have detected and characterized DNA-PKcs foci induced by ionizing radiation and, this time, we successfully detected the binding of XRCC4 to damaged chromatin DNA. Additionally, we have identified the

phosphorylation sites of XRCC4 by DNA-PKcs, which are phosphorylated in living cells after irradiation and disruption of which resulted in elevated radiosensitivity with partially impaired DNA repair capability. Based on these results, the process of recruitment and assembly of NHEJ machinery and the role of DNA-PK phosphorylation therein will be discussed.

(PS1080) High expression of endogenous DNA repair complexes is associated with reduced DNA double-strand break rejoining but more accurate repair in irradiated murine embryonic stem cells. Judit P. Banath[1], Susan H. MacPhail[1], Adriana Banuelos[1], Dmitry Klokov[2], Peggy L. Olive[1]. [1]B.C. Cancer Research Centre, Vancouver, BC, Canada, [2]DFKZ, Heidelberg, Germany.

Mouse pluripotent embryonic stem cells (mES) give rise to all of the cells of the adult mouse, so DNA repair efficiency should be paramount. However, mES cells display high numbers of endogenous DNA repair foci and a deficiency in rejoining of radiation-induced DNA double-strand breaks. Unlike mouse cell lines that typically show fewer than 3 γH2AX foci per nucleus, mES cells exhibited about 100 endogenous γH2AX repair foci in the absence of measurable DNA double-strand breaks. Furthermore, mES cells failed to rejoin 40% of the radiation-induced double-strand breaks. Two models are proposed to explain these results. First, slow double-strand break rejoining could occur when endogenous DNA repair foci sequester critical repair factors away from sites of radiation-induced breaks. In support of this model, mES cells showed high numbers of endogenous repair foci that contained phospho-ATM, RPA, and RAD51. $H2AX^{-/-}$ cells did not demonstrate these endogenous foci, they activated ATM after irradiation but failed to form ATM foci, and they rejoined DNA double-strand breaks rapidly. Moreover, the rate of rejoining of double-strand breaks was dose-dependent for $H2AX^{+/+}$ cells, consistent with competition between endogenous and radiation-induced foci for repair factors. In the second model, mES cells rely primarily on homologous recombination for repair of double-strand breaks, and $H2AX^{-/-}$ cells are proposed to upregulate the non-homologous end joining (NHEJ) pathway. In support of this model, the amount of DNA-PKcs protein was 4 times higher in $H2AX^{-/-}$ cells than $H2AX^{+/+}$ cells. However, relative radiosensitization by wortmannin and G1 phase radiosensitivity were similar. Therefore, there is some support for both models in explaining the double-strand break rejoining deficiency observed in mES cells.

(PS1081) Induction and persistence of T-cell receptor (TCR) variants in X-irradiated mice depends on p53 status. Toshiyuki Norimura, Hiroyo Kakihara, Kazuyuki Igari, Ryuji Okazaki, Akira Ootsuyama. University of Occupational and Environmental Health, Japan, Kitakyushu, Japan.

The *p53* tumor suppressor gene is integral to the cellular response to genotoxic stress**.** When ionizing radiation cause DNA damage, *p53* activates cell cycle arrest, DNA repair and apoptosis. *p53*-deficient mice are extremely susceptible to radiation-induced carcinogenesis and teratogenesis. Thus the *p53* gene is often referred to as the "guardian of the genome". In order to investigate the role of *p53* gene in surveillance against mutation, and particularly to address the significance of *p53*-dependent apoptosis, the influence of *p53* on the radiation-induced elevation of T-cell receptor (TCR) variant fractions was examined in splenic T lymphocytes of *p53*-proficient and -deficient mice. Wild-type *p53*(+/+), heterozygous *p53*(+/-) and null *p53*(-/-) mice were exposed to 3 Gy of X rays at 8 weeks of age. The fraction of TCR-defective variants was measured at various times after irradiation. Initially, the TCR variant fraction (VF) increased rapidly and reached its maximum level at 9 days after irradiation before decreasing gradually. In *p53*(+/+) and *p53*(+/-) mice, the TCR VF fell to normal background levels at 16 and 20 weeks of age, respectively. In contrast, the TCR VF of *p53*(-/-) mice failed to decrease to background levels during the observation period. Baseline levels were then maintained for approximately 60 weeks in the *p53*(+/+) mice and approximately 40 weeks in the *p53*(+/-) mice. After the long flat period, significant re-increases in the TCR VF were found after 72 weeks of age in the irradiated *p53*(+/+) mice and after 44 weeks of age in the irradiated *p53*(+/-) mice. Measurement of the fraction of apoptotic cells in the spleen and thymus 4 h after X irradiation at these ages in *p53*(+/+) and *p53*(+/-) mice demonstrated a reduction in apoptosis in the irradiated mice compared to the non-irradiated mice. Hence it appears that complete repair of mutagenic DNA damage in irradiated tissues requires the concerted cooperation of two independent functions; proficient DNA repair and competent apoptosis. When p53-dependent apoptotic repair functions efficiently, there is a threshold dose or dose-rate for radiation-induced mutations.

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(PS1082) Roles of DNA repair genes in sustaining cell proliferation under low dose-rate irradiation. Masanori Tomita[1], Yoshihisa Matsumoto[2], Kazuo Sakai[3]. [1]Low Dose Radiation Research Center, Central Institute of Electric Power Industry, Tokyo, Japan, [2]Research Laboratory for Nuclear Reactors, Tokyo Institute of Technology, Tokyo, Japan, [3]Resarch Center for Radiation Protection, National Institute of Radiological Sciences, Chiba, Japan.

Radiation-induced DNA double-strand break (DSB) initiates various kinds of biological effects. There is accumulating evidence indicating that the biological effects of low dose and low dose-rate radiation are different from those of high dose and high dose-rate radiation. To elucidate the molecular mechanisms of cellular response to low dose-rate radiation, it is essential to clarify the role of DSB repair-related genes. In higher vertebrate cells, there are at least two major DSB repair pathways, namely non-homologous end-joining (NHEJ) and homologous recombination (HR). We show that the ratio of the cell growth rate of NHEJ-related $KU70^{-/-}$ chicken DT40 cells irradiated with gamma-rays at 1.0 mGy/hr to unirradiated cells were significantly lower than that of HR-related $RAD51B^{-/-}$, $RAD54^{-/-}$ and $ATM^{-/-}$ cells. NHEJ-related $PRKDC^{-/-/-}$ (catalytic subunit of DNA-dependent protein kinase; DNA-PKcs) cells also indicated higher degree of growth delay than HR-related gene knockout cells. In addition, the extent of growth delay of $ATM^{-/-}KU70^{-/-}$ double knockout cells was also significantly high as $KU70^{-/-}$ cells under low dose-rate irradiation, but that of $RAD54^{-/-}KU70^{-/-}$ cells was similar degree with $RAD54^{-/-}$ cells. On the other hand, both $RAD54^{-/-}KU70^{-/-}$ and $ATM^{-/-}KU70^{-/-}$ cells showed higher degree of growth delay than other knockout cells after irradiation with 0.67 Gy, equivalent to the total dose for 4 weeks irradiation at 1.0 mGy/hr, of X-rays at high dose rate 0.9 Gy/min. The growth delay of $KU70^{-/-}$ and $PRKDC^{-/-/-}$ cells after 0.67 Gy at 0.9 Gy/min was higher than that of $RAD51B^{-/-}$, $RAD54^{-/-}$ and $ATM^{-/-}$ cells. These findings provide that cellular response to low dose-rate radiation is significantly different from that to high dose-rate radiation, even in the initial process of DNA repair and/or cell cycle checkpoint. The growth delay observed in the $KU70^{-/-}$ and $PRKDC^{-/-/-}$ cells irradiated with low dose-rate radiation, suggesting that these NHEJ-related genes may be utilized for the molecular marker to predict the sensitivity to low dose and low dose-rate radiation.

(PS1083) DNA double strand breaks inducing genomic instability in human cells. Masamitsu Honma. National Institute of Health Sciences, Tokyo, Japan.

DNA double strand breaks (DSBs) are particularly dangerous lesions and their repair is important for maintaining the genomic integrity. As a model for studying DSBs repair in mammalian cells, we recently developed a system to trace the fate of a DSB occurring in an endogenous gene in human cells. The lymphoblastoid cell line TSCE5 has a single I-SceI endonuclease sites in an allele of thymidine kinase gene (TK) located on chromosome 17p. The expression of I-SceI enzyme in the cell can certainly generate DSBs at the TK gene. A novel transfection system (Amaxa Nucleofector™) can introduce the I-SceI expression vector into the most of

cell populations and efficiently generate the DSBs. We found mutations in the TK gene associated with the DSB in 3% of randomly isolated clones. Majority of the mutations (80%) were small deletions ranging from 1 to 30-bp, which were presumably generated by non-homologous end-joining (NHEJ). Mutants resulted by interallelic homologous recombination (HR) were also observed infrequently. These results suggested that chromosome integrity against the DSB is appropriately maintained by NHEJ and HR in TSCE5 cells. We next established p53 knock-down TSCE5 cell lines by introducing p53 siRNA expression vector (pSUPERp53/neo). The expression of p53 protein in these cells (TSCE5 p53def) was less than 10-folds in p53-wild type TSCE5 cells. After introducing the DSB, we frequently observed translocations or large chromosome deletions associated with the DSB in the TSCE5-p53def cells, indicating that p53 insufficiency causes chromosome instability through dysfunction of NHEJ and/or HR.

(PS1084) Identification of radiation susceptibility gene of LEC rat by physical map construction and genome sequence comparison. Aya Sugyo[1], Atsushi Tsuji[1], Hitomi Sudo[1], Masashi Sagara[1], Toshiaki Ogiu[2], Chizuru Sogawa[1], Tsuneo Saga[1], Yoshinobu Harada[1]. [1]Diagnostic Imaging Group, Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Japan, [2]Experimental Radiobiology for Children's Health Research Group, Research Center for Radiation Protection, National Institute of Radiological Sciences, Chiba, Japan.

The Long Evans Cinnamon (LEC) rat is highly susceptible to X-irradiation due to defective DNA double-strand break repair and is a model for hepatocellular carcinogenesis. Although radiation susceptibility is controlled by a recessive gene on rat chromosome 4, it has not been identified. We previously determined that the region associated with radiation susceptibility is located in a 1.2-Mb on chromosome 4 using LEC congenic lines and recipient Fischer 344 (F344). Comparison of the coding sequences for seven known genes in the region between F344 and LEC rats showed no changes in deduced amino acid sequences, suggesting that radiation susceptibility gene is not known genes. In the present study, we constructed physical map and compared genome sequences of F344 and LEC rats to identify the radiation susceptibility gene by a positional cloning approach.

We first constructed a bacterial artificial chromosome (BAC) contig of rat chromosome 4 completely covering the region associated with radiation susceptibility. We demonstrated that defective DNA repair in LEC is fully complemented by a 200-kb BAC, 65K18 in transient and stable transfected LEC cells. Further genetic analysis determined that the radiation susceptibility region is located in a 129-kb region of 65K18. We constructed the Fosmid library of the LEC genome and isolated four clones completely covering the region. We compared genome sequences of the region between F344 and LEC and found that an intronless Rpl36a gene is inserted into the LEC genome, but not into F344. The gene expression of Rpl36a of LEC cells was lower than that of F344 cells, suggesting that the intronless Rpl36a in the region is a pseudogene like other intronless genes. It is possible for the insertion of the intronless Rpl36a to disrupt a gene or to change the expression of a gene. We mapped four expressed sequence tags (ESTs) in the region. The expression of three ESTs was not different between F344 and LEC cells, but the remaining one was not expressed in LEC cells. To determine whether the EST is associated with radiation susceptibility, we have isolated a full-length sequence and performed a functional analysis. The identification of the radiation susceptibility gene will potentiate our understanding of the molecular mechanisms of radiation-induced DNA damage repair.

(PS1085) Haplotype effects on chromosomal anomalies in heterozygous BRCA1-deficient primary human fibroblasts exposed to 29 kV mammography X-rays? Marlis Frankenberg-Schwager, Anke Gregus. University of Goettingen, Goettingen, Germany.

Women with an inherited mutation in one allele of the breast cancer suppressor gene BRCA1 show enhanced breast cancer risk and screening by mammography X-rays for early detection of breast cancer is advised. These X-rays efficiently induce of DNA double-strand breaks which can be correctly repaired by homologous recombination requiring Brca1 as partner in a recombination complex. We studied the effect of the degree of truncation of the Brca1 protein on its function in the recombination complex by analyzing chromosomal damage induced by mammography X-rays. BRCA1+/- fibroblasts with three different terminal deletions (C 2860 (1349/1863 aa), C 2899 (699/1863) and C 2852 (434/1863) and controls were derived from biopsies of predisposed women. Confluent cells were irradiated and chromosomal anomalies assayed at first mitosis and at several population doublings after irradiation. The assay included dicentric chromosomes and fragments and anomalies of chromosomes X, 1, 9, 13 and 17 painted by Fish probes.

BRCA1+/- cells C 2860 and its control showed comparable yields of dicentrics and excess acentric fragments at first mitosis post-irradiation. After 1.5 Gy and 11 population doublings higher yields of dicentrics and fragments were observed in C2860 compared to its control. Chromosomal instability of C 2860 was also confirmed for 4 out of 5 Fish-painted chromosomes.

Similar yields of dicentrics and excess acentric fragments were found for the BRCA1+/- cell lines C 2860, C2899 and C 2852 at first mitosis after irradiation. However, several population doublings after exposure to 1.5 Gy a 10-fold increase in dicentrics and fragments was observed for C2860 compared to C 2899, while results for C 2852 and controls were similar. C 2899 revealed an intrinsic chromosome 9 instability, increasing from 11% to 38% during 13 population doublings. C 2899 cells showed a radiation-induced chromosomal instability for chromosome 9 and 17 in contrast to other BRCA1+/- cells.

These data may indicate that the least truncated Brca1 protein (C 2860) can act as a partner in the recombination complex and, thus, lead to chromosomal instability in contrast to the more truncated proteins (C 2899 and C 2852).

(PS1086) Dynamic interactions of non-homologous end-joining proteins with dna ends. Pierre-Olivier Mari, Bogdan I. Florea, Nicole S. Verkaik, Stephan P. Persengiev, Guido Keijzers, Adriaan B. Houtsmuller, Dik C. van Gent. Erasmus Medical Center, Rotterdam, The Netherlands.

Repair of DNA double strand breaks (DSBs) requires assembly of repair complexes at the site of damage. Homologous recombination proteins accumulate in nuclear foci upon DSB induction, but non-homologous end-joining (NHEJ) proteins have not been observed in such structures. Assembly and dynamics of NHEJ complexes is an important factor for radiation sensitivity and formation of chromosomal aberrations. Therefore, we developed techniques to introduce a high concentration of DSBs in a defined subnuclear volume in order to investigate accumulation of these proteins in the damaged region. We used a pulsed near-infrared laser, which induces a large number of DSBs at the focal point of the laser, presumably as a result of multiphoton effects. We showed that NHEJ proteins accumulated in the damaged region.

In order to follow this accumulation in real time in living cells, we tagged NHEJ proteins with green fluorescent protein (GFP). These tagged proteins (which are functional in NHEJ) accumulated in the damaged region within a few minutes. The Ku heterodimer and DNA-PK_{CS} were slightly faster than the XRCC4 protein, which is consistent with a late function of the ligase IV/XRCC4 complex in the NHEJ reaction. The Ku protein disappeared over the course of approximately 2 hours, presumably as a result of diminishing numbers of DSBs because of repair. Within the next 16 hours, several cells went through mitosis, showing that this treatment did not kill the cells. Accumulation of XRCC4 and DNA-PK_{CS} was dependent on Ku protein, showing that protein accumulation was not the result of non-specific aggregation of proteins in the damaged region.

Subsequently, the dynamics of the Ku70/80 heterodimer were studied in more detail. For this purpose, the GFP-tagged protein was allowed to accumulate at the damage and the accumulated protein was bleached. Fluorescence recovery after photobleaching showed that Ku protein could exchange with unbound Ku within approximately 10 minutes. In other words, NHEJ complexes are

very dynamic structures from which proteins can exchange with proteins molecules in solution before completion of a joining event.

(PS1087) Factors forming human individual radiosensitivity. Natalia Ryabchenko, Emilia Dyomina. Institute of Experimental Pathology, Oncology and Radiobiology of NASU, Kyiv, Ukraine.

Genetic features of human organism, its physiological status under ionizing irradiation, specificity of environmental effects are the forming factors in human individual radiosensitivity (IR). Efficiency of repair systems plays the main role in DNA structural maintenance after radiation damage. The purpose of the presented work was to investigate individual variations in chromosomal radiosensitivity of peripheral blood lymphocytes (PBL) of healthy individuals and to estimate contribution of DNA repair system efficiency to their formation.

Materials and methods. Gamma-irradiation of PBL cultures of 103 healthy individuals was carried out in S- and G_2 - stages in 1,5 Gy dose and 1 Gy/min dose rate. Additional hyperthermia (HT) inhibition of cultures was applied after S - stage irradiation (42° C, 1 h).

Results. Analysis of the obtained distributions functions of PBL chromosomal damage in the examined group shows the bimodal character that testify for the existence of two groups of donors among healthy persons. It is supposed that chromosomal G_2-assay makes it possible to indicate persons genetically predisposed to increased IR. In our case 12% of the group demonstrate the elevated levels of chromosomal radiosensitivity.

Comparative analysis of aberrations level after S- stage irradiation and additional HT inhibition of repair enzymes in two groups of healthy donors - with normal and increased IR according to the G_2-assay was carried out. The highest modifying effect of HT was observed in the group with normal chromosomal radiosensitivity. At the same time it was twice less in the group of radiosensitive individuals. The results obtained show the contribution of the efficiency of the DNA damage repair to the formation of human IR on chromosomal level.

(PS1088) Chicken DT40 *PTIP*-null mutants are viable, but defective in proliferation and highly sensitive to ionizing radiation. Fumiko Morohoshi[1], Masanori Tomita[1], Kazutsune Yamagata[2], Mitsumasa Hashimoto[3], Kensuke Otsuka[1], Isamu Hayata[1], Kuniyoshi Iwabuchi[3], Hiroshi Tauchi[4], Kazuo Sakai[5]. [1]Central Res. Inst. of Electric Power Industry, Tokyo, Japan, [2]Natl. Cancer Center Res. Inst., Tokyo, Japan, [3]Kanazawa Med. Univ., Kawakita, Japan, [4]Ibaraki Univ., Mito, Japan, [5]Natl. Inst. of Radiological Science, Chiba, Japan.

The BRCT domain is an evolutionarily conserved domain found in a large number of proteins involved in DNA repair, recombination and damage checkpoints. To elucidate the function of the PTIP protein which carries 6 BRCT domains, we constructed constitutive (*chPTIP*-/-/-) and conditional *PTIP*-null mutants (*tet-off hPTIP, chPTIP*-/-/-) in chicken DT40 cells. In contrast to mouse *PTIP*-null mutant cells, DT40 *PTIP*-null mutants are viable, but appear to have a defective proliferative capacity, since a high proportion of dead cells appear during culture growth, and colony formation ability in methyl cellulose containing medium was less than 1% of that of wild type cells. Conditional *PTIP* mutants express human PTIP (hPTIP) in the absence of doxycycline (Dox) but expression levels decrease to almost undetectable levels within 48 hours after the addition of Dox. Starting from the 7th day after the addition of Dox, the cell cultures begin to produce dead cells and show chromatid-type gaps. Conditional *PTIP* mutants become sensitive to the lethal effects of ionizing radiation (IR) at 1 to 2 days after the addition of Dox [D10(+Dox)/D10(-Dox)=0.8], but not to the effects of UV and MMS, and the cells display low levels of resistance to MMC. The hPTIP polypeptide consisting of the two N-terminal BRCT domains was able to extensively complement cell proliferation defects and the high radiation sensitivity of constitutive *PTIP* mutants.

Since *PTIP* mutants showed a higher sensitivity to IR, we examined whether they were defective in double strand break repair or damage induced checkpoints. Western blot analysis revealed that the phosphorylation kinetics of H2AX after IR irradiation was similar in *PTIP* mutants and in wild type cells. Rad51 foci formation and disappearance after IR irradiation was normal, and homologous recombination operated normally in *PTIP* mutants. These results indicate that the PTIP protein is not involved in double strand break repair. The G2 block after IR irradiation also operated normally in *PTIP* mutants. However IR-induced phosphorylation of S345 in Chk1 was higher in *PTIP* mutants than in wild type cells. This enhanced phosphorylation of Chk1 S345 was not observed after UV irradiation in *PTIP* mutants. These results suggested that repair of IR induced DNA damage or IR induced signaling was defective in *PTIP* mutants.

(PS1089) Lucanthone and hycanthone affect apurinic endonuclease-1(Ape1) by physical interaction. Mamta D. Naidu, Rakhi Agarwal. Brookhaven National Lab, Upton, NY, USA.

Our previous studies in the two glioma cell lines U251 and U87, showed that the differences in their radiation resistance could be correlated to differences in the level of base excision repair (BER) enzyme Ape1, which was specifically inhibited by its known small molecule inhibitors, lucanthone as well as hycanthone, another homologue of lucanthone. Both lucanthone and hycanthone are well known DNA intercalators used in 1950s to treat schistosomiasis. Our Ape1 inhibition studies by these two inhibitors showed that 5 μM of lucanthone pretreatment for 2h resulted in decrease in viability of U251 by 8% and U87 by 15% where as hycanthone at same concentration caused reduction of only 4–5 % in U251 as opposed to significant reduction in viability of U87 by 20%! This indicated that U87 was more sensitive to lucanthone and hycanthone than U251 due to higher Ape1 levels.

5 μM lucanthone was sufficient to inhibit Ape1 in U87 more significantly than in U251 as determined by greater decrease in colony survival in U87 than U251; probably due to possible correlation to higher Ape1 in U87.

Ape1 recombinant protein when treated with 5 μM lucanthone showed significant decrease in Ape1 protein and enzyme activity as compared to N-truncated Ape1which had much lower Ape1 activity. Thus, these *in vitro* studies indicate a possible interaction of Ape1 with lucanthone. Physical interaction studies using circular dichroism(CD) indicate physical interaction of lucanthone and Hycanthone with recombinant Ape1.

Crystal structure of recombinant, full length Ape1 was determined to 2.76Å (PDB id 2ISI). Structural analysis of the active site identified a potential hydrophobic drug target site, which overlaps with the DNA binding site. In order to determine the possible interactions of Ape1 to hycanthone and lucanthone for the inhibition of its catalytic activity, structural studies of their complex is underway.

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(PS1090) 1-Methylxanthine enhances radiosensitivity of tumor cells by abrogating radiation-mediated G_2 checkpoints. Eun Kyung Choi[1], Seong-Yun Jeong[1], Jung Shin Lee[2], Yeon Hee Kook[3], So Lyoung Yi[1], Hyun Jin Ryu[1], Se Hee Son[1], Do Young Song[1], Sung Whan Ha[4], Heon Joo Park[3]. [1]Department of Radiation Oncology, College of Medicine, University of Ulsan, Seoul, Republic of Korea, [2]Department of Internal Medicine, College of Medicine, University of Ulsan, Seoul, Republic of Korea, [3]Department of Microbiology, College of Medicine, Inha University, Inchon, Republic of Korea, [4]Department of Radiation Oncology, Seoul National University College of Medicine, Seoul, Republic of Korea.

Purpose; To study the efficacy of a caffeine metabolite 1-methylxanthine (1-MTX) as a radiosensitizer of cancer and the underlying radiosensitizing mechanisms

Experimental Procedures; Using RKO human colorectal and other cancer cells, we pre-treated cells in culture with 1-MTX, exposed to various doses of IR and then determined the clonogenic cell survival and the cell cycle progression including induction of apoptosis using flow cytometric method. Using Western blot method, we also investigated the effect of 1-MTX on the radiation-induced changes in the expression and activity of various proteins involved in cell cycle progression and apoptosis such as p53, p21, Chk2, Cdc25, Cyclin B1 and Cdc2. The capability of 1-MTX to radiosensitize tumors in vivo was studied using 1-MTX encapsulated in temperature-sensitive liposomes (tsl-MTX). The nude mice bearing RKO xenografts in hind-legs were injected i.p. with tsI-MTX, tumors were heated at mild temperature, irradiated and the tumor growth rate was studied

Results; Pre-treatment of cells with 1-MTX sensitized cells to IR, as determined with clonogenic cell survival assay. Flow cytometric analysis indicated that when cells were treated with 1-MTX and irradiated, the cell population in sub-G1 (apoptotic cells) increased with only a small increase in G2/M cell population, in contrast to a marked increased in G2/M cell population in the cells irradiated without pre-treatment with 1-MTX. These results indicated that 1-MTX radiosensitizes cells by abrogating radiation-induced G2/M arrest, thereby inhibiting repair of radiation damage. 1-MTX treatment was also found to attenuate immensely the IR-mediated activation of Chk2 and p53, which are known to play important role in G_2 arrest. The radiation-induced activation of cyclin B1/cdc2 kinase was slightly suppressed by 1-MTX. The effect of IR to suppress the growth of RKO xenografts in nude mice was markedly enhanced by tsl-MTX

Conclusions; We observed that 1-MTX radiosensitizes tumor cells in vitro as well as in vivo, most likely by abrogating radiation-induced G_2/M arrest and thus by suppressing the repair of radiation damage. Further study is warranted to investigate the feasibility of using 1-MTX to enhance the response of human tumors to radiotherapy

(PS1091) Enhancement of radiation-induced cell killing by inhibiting G2 checkpoint with purvalanol A. Daisuke IIZUKA[1], Osamu INANAMI[2], Mikinori KUWABARA[2]. [1]Experimental Radiobiology for Children's Health Research Group, National Institute of Radiological Sciences, Chiba, Japan, [2]Laboratory of Radiation Biology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan.

[Purpose]

Ionizing radiation is known to cause cell-cycle arrest at G2 phase in various tumor cells. Recently, treatment with drugs such as UCN-01 and caffeine induces cell killing in X-irradiated tumor cells through the abrogation of G2 checkpoint. In this study, we focus on Cdc2 and investigated the mechanism of enhancement of radiation-induced cell killing by the inhibition of Cdc2 kinase activity with cyclin dependent kinase (cdk) inhibitor purvalanol A.

[Materials and Methods]

Human gastric adenocarcinoma MKN45 (wild p53) and MKN28 (mutated p53) cells were exposed to X rays with or without purvalanol A. For measurements of apoptosis, apoptotic morphological changes of nuclei were assessed by fluorescence microscopy. Cell cycle distribution was observed by flow-cytometry. Observation of Cdc2 kinase activity was performed using Cdc2-associated histone H1 kinase assay. Expression of mRNA was assessed by semi-quantitative RT-PCR. Expression of proteins was assessed by Western blotting with corresponding antibodies.

[Results and Discussion]

Cotreatment of cells with X rays and purvalanol A induced the significant increase in the number of apoptosis in MKN45 and MKN28 cells. When cells were exposed to X rays alone, G2/M arrest occurred. Cotreatment of X rays with purvalanol A rendered the decrease in G2/M fraction and the increase in subG1 fraction. Treatment of cells with X rays increased the expression of proteins relating to the G2 checkpoint and Cdc2 kinase activity and resulted in the G2 arrest, and purvalanol A decreased the expression of the G2-related proteins and the Cdc2 kinase activity. On the expression of anti-apoptotic proteins, purvalanol A inhibited the radiation-induced upregulation of Bcl-2 and Bcl-X_L. The expression of survivin and XIAP was slightly increased by X irradiation, but were also inhibited by purvalanol A. On the other hand, pro-apoptotic protein, Bax, was not increased by X irradiation but slightly decreased by purvalanol A. Total RNA synthesis was inhibited by purvalanol A and as a consequence, the expression of mRNA of survivin, Bcl-X_L and Bcl-2 was inhibited. These results indicated that purvalanol A sensitized radiation-induced apoptosis through the abrogation of G2 checkpoint and the downregulation of anti-apoptotic proteins by the inhibition of RNA synthesis.

(PS1092) Hydroxyethyldisulfide as a novel radiation sensitizer of human cancer cells. Kathleen M. Ward, Jie Li, Iraimoudi S. Ayene. Lankenau Institute for Medical Research, Wynnewood, PA, USA.

Using oxidative pentose phosphate cycle (OPPC) deficient Chinese hamster ovary cells (CHO), we previously demonstrated that exposure of OPPC deficient cells to hydroxyethyldisulfide (HEDS), a thiol specific oxidant, caused enhanced radiation sensitivity and an inability to repair DNA double strand breaks (Ayene et al., J. Biol. Chem. 2002; Int. J. Radiat. Biol., 2000). Here we demonstrate that depletion of glucose, a substrate for OPPC, in the culture medium increased the response of human cancer cells to radiation in the presence of HEDS. In glucose containing medium, cancer cells showed HEDS concentration dependent increase in bioreduction i.e. conversion of HEDS to mercaptoethanol (ME). The depletion of intracellular glucose as a result of glucose depletion in the medium resulted in the inability of these cancer cells to convert thiol oxidant HEDS into ME. Comparison of the data in glucose containing- and glucose depleted - medium indicated that glucose deprived cancer cells showed up to a five fold decrease in bioreduction than that measured in glucose containing medium. This suggests that lack of glucose mediated intracellular metabolic activity results in intracellular accumulation of HEDS, which may cause oxidative stress. In the presence of glucose, HEDS did not have any detrimental effects on the growth of human cancer cells with normal pentose cycle activity. However, HEDS inhibited growth of cancer cells in glucose depleted medium. In addition, HEDS specifically sensitized glucose deprived cancer cells to radiation, which is dependent on HEDS concentration and time of incubation. This is consistent with previous reports from our lab, which showed that lack of conversion of HEDS to ME leads to inhibition of DNA repair, loss of DNA repair protein function and enhanced radiation induced cell death in OPPC deficient rodent cells. These results suggest that HEDS in combination with inhibitors of OPPC can be used to sensitize human tumors to radiation. The data also suggest that HEDS alone can be used to sensitize hypoxic tumors to radiation since hypoxic regions of the tumors have low level of glucose.

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(PS1093) Radiosensitization by temozolomide in human glioma cells is independent of MGMT promotor methylation status. Krista van Nifterik[1], Jaap van den Berg[1], Lukas Stalpers[2], Theo Hulsebos[2], Sieger Leenstra[2], Laurine Wedekind[1], Najim Ameziane[1], Ben Slotman[1], Vincent Lafleur[1], Peter Sminia[1]. [1]VU University medical center, Amsterdam, The Netherlands, [2]Academic Medical Center, Amsterdam, The Netherlands.

Purpose

Investigation of the radiosensitizing potential of temozolomide (TMZ) in human glioblastoma multiforme (GBM) cell lines.

Methods and Materials

Experiments were performed on a panel of four established and seven long term primary GBM cell lines. The promotor methylation status of the methylguanine-DNA methyltransferase (MGMT) gene was determined by methylation-specific PCR and methylation specific multiplex ligation-dependent probe amplification assay. MGMT protein expression was studied by Western blotting. TMZ sensitivity of the cells was tested by single exposure to TMZ (0–500 microM) for 24 h. Three GBM cell lines (AMC

3046, VU-109, VU-122), with known sensitivity to TMZ in the clinically feasible dose range (< 50 microM) were selected for combined treatment with irradiation. Cells were irradiated either with single fractions (0–6 Gy) after 96 h pretreatment with TMZ or with five daily 2 Gy fractions with TMZ administered 1 h prior to each irradiation fraction. Treatment response was evaluated by cell growth assay, clonogenic assay and cell cycle analysis.

Results

GBM cell lines showed different sensitivity to TMZ. Three GBM cell lines were found to be TMZ resistant. The other four long-term primary and all three established GBM cell lines were classified as TMZ sensitive, with 24 h exposure to < 50 microM reducing the fraction of surviving clonogenic cells to < 0.5. TMZ sensitivity correlated with MGMT promotor hypermethylation and absent expression of the MGMT protein. TMZ administered concomitant with irradiation further decreased the survival of three methylated GBM cell lines. Significant radiosensitization by TMZ was observed in two of the three cell lines after single dose exposure, but the radiosensitizing effect TMZ was maintained in one cell line only after fractionated exposure. Hence, the radiosensitizing potential of TMZ did not correlate with the MGMT promotor methylation status.

Conclusion

Clinical studies show that radiotherapy combined with TMZ in GBM patients improves survival, particularly in MGMT methylated tumors. This hints to a radiosensitizing potential of TMZ. Our results show that the effect of TMZ is additive to irradiation, but not necessarily synergistic, despite the epigenetically silenced MGMT gene.

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(PS1094) Identification of novel mechanisms of radio-sensitization by histone deacetylase inhibitors in prostate cancer. Seema Gupta[1], Ching-Shih Chen[2], Mansoor M. Ahmed[1]. [1]Weis Center for Rsearch, Geisinger Clinic, Danville, PA, USA, [2]The Ohio State University, Columbus, OH, USA.

Several histone deacetylase inhibitors (HDACI) have shown radiosensitizing effects in various cancer cell lines but the mechanisms have not been studied. In the present study, the effects of novel HDACI, VAD-18, VAD-20 (short chain fatty acids) and (*S*)-**11** [(S)-HDAC-42] (α-branched phenylbutyryl derivative) in prostate cancer cells, PC-3, DU145 and LN-3 with radiation (IR) were investigated. Colony forming assays showed significant radiosensitizing effects with HDACI in all the cell lines. Cell cycle distribution by flow cytometry showed a transient block in G_2/M phase in PC-3 cells. Time dependent changes in p65 localization (Western analysis) and its activity were observed and confirmed by immunofluorescence and gel shift assays implying that HDACI may sensitize the cells to IR by reducing IR-induced p65 translocation to the nucleus. In addition, levels of Bcl_{XL} reduced while Bcl_2 remain unchanged with an increase in Bax following combined treatment. TranSignal protein/DNA array showed that the transactivation function of most of the factors involved in the transcription initiation machinery (C/EBP, p65, CREB and AP-1) or its activators like NFATc were either up-regulated or induced, while most of the STATs and upstream stimulatory factor-1 (USF-1) were down-regulated following treatment with HDACI alone or in combination with IR. Since, the role of USF-1 in IR mediated effects has not been studied, gene expression of USF-1 and its targets involved in cellular proliferation and cell cycle; hTERT, IGF2R, Cyclin B1 and Cdk1 were investigated by real time RT PCR. Although significant changes in USF-1 expression were not observed, its targets were induced following 5 Gy IR while all the other treatment groups showed a reduction indicating the role of post-translational modifications in the regulatory functions of USF-1. These results suggest that IR in combination with HDACI may render effects involving non-epigenetic events such as nucleo-cytoplasmic shuttling. Identification of USF-1 has opened a new avenue for dissecting the signaling pathways induced by HDACI leading to radiosensitization. Further work is in progress to understand the role of USF-1 in the radiosensitizing effects of HDACI by investigating its expression, binding, activity, translocation as well as by intervention. (Funded by DOD-PCRP).

(PS1095) Inhibition of beta1 integrins in three-dimensionally cultured squamous cell carcinoma cells: A potent approach to enhance tumor cell radiation sensitivity. Iris Eke, Yvonne Deuse, Nils Cordes. OncoRay - Radiation Research in Oncology, Dresden, Germany.

Integrin-mediated cell adhesion to extracellular matrix (ECM) increases the resistance to ionizing radiation, a phenomenon termed cell adhesion-mediated radioresistance (CAM-RR). Particularly, beta1 integrins are well-known to be critical for the cellular radiation survival response. To explore integrin beta1 as therapeutic target to enhance the cellular radiosensitivity, different cell lines from human squamous cell carcinomas of the head and neck (HNSCC) were examined for their response to anti-beta1 integrin treatment without or in combination with irradiation. Further, beta1 integrin expression was evaluated in biopsies from human HNSCC. UTSCC-5, −14, −15 or FaDu cells grown in three-dimensional laminin-rich Basement Membrane (lrBM) cell cultures were treated with inhibitory anti-beta1 integrin mAbs (AIIB2; 0–250 μg/ml; vs isotypic, non-specific IgG controls) plus/minus X-rays (0–10 Gy). beta1 integrin cell surface expression (FACS analysis), clonogenic survival, and protein expression and phosphorylation (beta1 integrin, FAK, p130Cas, Paxillin, Akt, GSK3beta, cyclin D1, phospho-tyrosine; Western blotting) were measured in the 3D-lrBM cell cultures. Biopsies from HNSCC exhibited an elevated expression of beta1 integrin in tumor areas as compared to surrounding tumor-associated normal tissue. Besides an AIIB2 concentration-dependent reduction in basal clonogenic survival, all cell lines tested showed a radiosensitization by beta1 integrin inhibition, which could be correlated with beta1 integrin cell surface expression. Blocking of beta1 integrins resulted in strong dephosphorylation of FAK, p130Cas, Paxillin and decreased phospho-tyrosine content. Total protein amounts showed no changes. Intriguingly, Akt, GSK3beta and cyclin D1 remained unaffected upon beta1 integrin blockage. These data strongly underscore a critical role of beta1 integrins in the cellular radiation response. The radiosensitization of human HNSCC cells by beta1 integrin blocking involves FAK, Paxillin and p130Cas but not Akt or GSK3beta, which is distinguishable from the effects mediated by inhibition of growth factor receptors. Future studies are warranted to evaluate the potential of this targeting approach for optimizing radiotherapeutic strategies against HNSCC.

(PS1096) Enhancement of radiation-induced DNA damage and tumor cell cytotoxicity by gold and silver nanoparticles. David G. Hirst[1], Fred Currell[2], Mansukhlal Shah[2], Margaret Brennan Fournet[3], Deirdre Ledwith[4]. [1]School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom, [2]School of Mathematics and Physics, Queen's University Belfast, Belfast, United Kingdom, [3]National Centre for Biomedical Engineering Science Ireland, Galway, Ireland, [4]School of Chemistry, Trinity College Dublin, Dublin, Ireland.

Purpose: We aim to provide a basis for understanding and optimizing the enhancement of radiation damage by high atomic number (Z) metal nanoparticles for application in the treatment of cancer. The enhancement of radiation dose deposition at the interface between materials of high and low (Z) has long been recognized and is attributed to the production of secondary electrons scattered from the surface of the high Z material to create significant local dose enhancement. The concept of using high Z metal nanoparticles as radio-enhancing agents represents a novel therapeutic approach in the treatment of cancer that was first shown to be feasible *in vivo* by Hainfeld et al. (2001) using systemic administration of gold in the form of nanoparticles.

Methods: DNA damage responses were characterised using plasmid DNA irradiated with 160 kVp X-rays. DNA samples were irradiated in the presence and absence of high Z nanoparticle material (5 nm and 20 nm gold; 40 nm silver) and plasmid form analysis conducted by agarose gel electrophoresis. Tumour cell survival curves were determined by clonogenic assay and cellular DNA damage responses investigated using the alkaline comet assay.

Results: The presence of both gold nanoparticles enhanced X-ray induced plasmid DNA damage by 2 fold as measured by an increase in the G value for single strand breaks (G_{SSB}). Silver was less effective. Furthermore, removal of radical scavengers from the

DNA by ultra-filtration had a highly significant effect, increasing the G values for both single and double strand breaks by more than 100 fold and 10 fold respectively. This indicated a high level of radical scavenging by the buffer components. Enhancement of radiation damage by gold nanoparticles was also observed at the level of cell survival *in vitro*, particularly in the clinically relevant radiation dose range (<2Gy).

Conclusions: High Z metal nanoparticles have significant dose enhancing properties, causing increased DNA damage and cell death. This work provides a basis for understanding the biological consequences of enhanced radiation dose deposition at the interfaces between high and low Z materials and represents a novel application high Z nanoparticles in the treatment of cancer.

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(PS1097) Pharmacological approaches for potentiating the radiosensitivity of human breast cancer cell lines. David Murray, Razmik Mirzayans. Cross Cancer Institute, Edmonton, AB, Canada.

There is a growing interest in the use of pharmacological inhibitors of different signal transduction pathways as cancer therapeutic agents. The abilities of such inhibitors to influence the efficacy of conventional radiotherapy and chemotherapy in different cancer cell types are beginning to emerge. Recent studies have shown that drugs such as resveratrol, trichostatin A, or celecoxib, for example, are capable of triggering significant radiosensitizing effects in certain malignant cells (e.g., leukemia, melanoma, cervical carcinoma, colon carcinoma). The aim of our studies is to employ a battery of carefully selected pharmacological modulators of different signaling pathways and an informative panel of human breast cancer cell lines in an attempt to design effective means of improving breast cancer therapy. To this end, we have developed a "High Density Survival" (HDS) assay which offers three advantages over the conventional clonogenic survival and short-term (e.g., the MTT cell proliferation) assays in determining the responses of human cancer cell lines to therapeutic agents: (i) there is no need for the preparation of a single-cell suspension; (ii) cell-to-cell contact (and thus some aspects of intercellular signaling) is maintained during the genotoxic treatment and for an extended time (24 h) thereafter; and (iii) cytotoxicity is evaluated at relatively long times (e.g., 7 days and beyond) post-treatment. Of a panel of sixteen cell lines representing different malignancies that we have evaluated by the HDS assay to date, three exhibited a degree of radiosensitivity comparable to normal human fibroblasts, whereas the remainder (including 10 breast cancer cell lines) were found to be highly radioresistant. The radioresistant phenotype of cancer cell lines correlated inversely with their propensity to undergo accelerated senescence, correlated directly with their propensity to develop genomic instability (e.g., multinucleation), and showed no relationship with their sensitivity to undergo apoptosis/necrosis. We will present the outcome of our studies evaluating the influences of pharmacological modulators of the p53 signaling pathway on these radiation-triggered responses in our panel of human cancer cell lines. (Supported by the Canadian Breast Cancer Foundation-Prairies/NWT Chapter.)

(PS1098) Radiation-induced up-regulation of NQO1 enhances the cytotoxicity of β-lapachone . Chang W. Song[1], Ki-Jung Ahn[1], Jihyung Choi[1], Minoru Suzuki[1], Kaoru Terai[1], Seung-Do Ahn[1], Eun Kyung Choi[2], Robert J. Griffin[1], Heon Joo Park[1,3]. [1]University of Minnesota, Minneapolis, MN, USA, [2]University of Ulsan, Seoul, Republic of Korea, [3]Inha University, Suwon, Republic of Korea.

Purpose: β-Lapachone (β-lap) is a novel anti-cancer drug, which is activated by NAD(P)H:Quinone Oxidoreductase (NQO1) in the cells. The mechanism underlying the synergistic anti-cancer effect of β-lap with ionizing radiation as well as heat-shock and cisplatin was investigated.

Materials and Methods: Various animal and human tumor cell lines were used. The effect of radiation, heating and cisplatin on the cellular level of NQO1 was determined with Western blot and biochemical methods. The cytotoxicity of β-lap alone or in combination with radiation, heat shock or cisplatin was estimated based on clonogenic cell survival and kinetics of apoptosis and their in vivo effect was determined with tumor growth delay assay.

Results: Incubation of cancer cells with β-lap caused clonogenic and apoptotic cell death in vitro in all the cell lines we studied except NQO1-negative cells such as MDA-MB-231-NQ2 cells. Dicoumarol (NQO1 inhibitor) and siRNA-NQO1 significantly inhibited the cell death caused by β-lap, indicating that β-lap plays cardinal role in the β-lap- induced cell death. Irradiation with 2–4 Gy, heating at 41–42oC for 1 h or incubation with 2 μM cisplatin for 1 h up-regulated NQO1 in the cells and increased the sensitivity of the cells to β-lap. A series of experiments indicated that β-lap not only causes cell death but it also inhibits the repair of sub-lethal radiation damage. β-lap treatment transitionally suppressed the expression of p53 and ATM and increased the expression of cytochrome C and NF-kB. The combined effect of β-lap with radiation, hypethermia or cisplatin to suppress the growth of in vivo tumors was greater than additive.

Conclusion: (1) Ionizing radiation, hyperthermia and cisplatin sensitize cells to β-lap by causing a long-lasting up-regulation of NQO1. (2) β-Lap sensitizes cells to radiation by inhibiting the repair of sub-lethal radiation damage.

(PS1099) Acidic microenvironment enhances radiosensitization of human melanoma cells by thermal sensitizers and the Hsp90 inhibitor, 17-AAG. Ronald A. Coss, Dennis B. Leeper, Takahiro Sato, Christopher W. Storck. Thomas Jefferson University, Philadelphia, PA, USA.

Thermal therapy and the HSP90 inhibitor, 17-AAG (17-allylamino-17-demethoxygeldanamycin), are radiation sensitizers. The influence of chronic growth of human melanoma cells at pH 6.7 on radiosensitization by thermal therapy and 17-AAG, and on the ability of lonidamine and quercetin to enhance radiosensitization by thermal therapy and 17-AAG, respectively, was investigated.

Growth of human melanoma cells at pH 6.7 is used as an *in vitro* model of melanoma in the tumor-like acidic microenvironment. DB-1 cells cultured at pH 6.7 and pH 7.3 were exposed to lonidamine for 3h, starting 1h prior to a 42°C (2h) treatment, and to 17-AAG for 24h and quercetin for 48h prior to irradiation. pHi was determined using BCECF and whole spectrum analysis, HSP levels determined by immunoblot analysis and survival determined by colony formation.

DB-1 cells grown at pH 6.7 are sensitized to 42°C by lonidamine, an inhibitor of the H+-linked monocarboxylate transporters. Sensitization is coincident with reduction of intracellular pH and inhibition of the stress response. While 42°C-radiosensitization was comparable in cells grown at pH 7.3 and 6.7, and lonidamine (150 μM) did not enhance cytotoxicity of radiation in cells cultured at pH 6.7, supra-additive sensitization (survival was reduced by more than two additional decades) was observed for the combination of lonidamine, 42°C and 5 Gy in cells grown at pH 6.7.

17-AAG was more cytotoxic to cells grown at pH 6.7 compared to cells cultured at pH 7.3. However, acidification of cells grown at pH 7.3 to pH 6.7 prior to and during exposure to 17-AAG did not modify survival. Quercetin (10 μM), an inhibitor of the stress response induced by 17-AAG, was more cytotoxic to cells grown at low pH, and was even more cytotoxic in combination with 17-AAG. While the cytotoxicity of 5 Gy was slightly enhanced in cells grown at pH 6.7, the cytotoxicity of quercetin and radiation, 17-AAG and radiation, and the combination of quercetin, 17-AAG and radiation all resulted in greater than two additional decades of cell killing.

These results support the concept that cells in a tumor's acidic microenvironment may be uniquely responsive to combinations of radiation sensitizers that reduce the stress response and reduce intracellular pH. Supported by Grant Nos. PO1 CA56690 and R25 CA48010 from NCI, NIH, DHHS.

(PS1100) High-throughput screening for the identification of novel radiosensitizing compounds in a head and neck cancer model. David Katz, Carlo Bastianutto, Fei-Fei Liu. Ontario Cancer Institute, Toronto, ON, Canada.

The purpose of this study is to identify radiosensitizing compounds for the treatment of head and neck cancer via a high-throughput forward chemical genetic screen. Head and neck cancers pose a challenge in their location, as the dose of ionizing radiation (RT) must be limited to avoid damage to nearby critical organs. One strategy to increase RT efficacy is to identify agents, which target cancer cells, and sensitize them to RT. One such compound currently in clinical use is cisplatin. Phase III trials have demonstrated the value of cisplatin combined with RT for head and neck squamous cell carcinomas; however, this strategy achieves a 5-year overall survival rate of only 50%, indicating opportunities for improvement.

The high-throughput screen will utilize a robotic platform to screen chemical libraries using an automated clonogenic assay performed on FaDu cells, a human squamous cancer cell line. This assay assesses whether the specific compound will prevent FaDu cells from reproducing to form daughter cells, which is the ultimate goal in cancer therapy. After treating the cells with the chemical library and subjecting them to ionizing radiation they will be stained and the number of colonies as a proportion of the number of cells seeded will be calculated. The effect of the different chemicals will be compared relative to cisplatin.

Preliminary data, whereby half of the LOPAC library (640 compounds), was screened with RT (4 Gy), identified 7 hits. The method was validated by identification of compounds that have previously been shown to be radiosensitizers, while also identifying compounds with novel radiosensitizing ability.

Novel radiosensitizing compounds will be further evaluated. This will include confirmation of radiosensitization using dose-response (drug and RT) clonogenic assays, in addition to an evaluation on another head & neck cancer, the human nasopharyngeal C666–1 line. The effects of these compounds on the normal human diploid GM05757 fibroblast model will also be assessed, as will cellular and biochemical characterization of their mode of sensitization, including apoptosis, cell cycle, senescence, and other possible mechanisms. *In vivo* validation will be conducted using tumour formation assays, as well as, xenograft models, buttressed by *in vivo* biochemical and histological analyses.

(PS1101) A small molecule high throughput screen for the identification of novel anticancer radiosensitizers. Emma Ito, Fei-Fei Liu. University of Toronto, Toronto, ON, Canada.

Radiation therapy (RT) plays an important role in cancer treatment and remains the primary curative modality for epithelial malignancies such as head and neck carcinoma. Standard radiation treatment, which generally involves administering the maximal tolerable RT dose, achieves a 5-year local control rate of only about 70% in nasopharyngeal carcinoma. Thus, in an effort to improve survival and reduce the long-term morbidity of radiation, novel molecular approaches need to be devised. To that end, we have evaluated the use of a high throughput screening (HTS) approach to discover novel anticancer compounds, preferentially with radiosensitizing activities, for head and neck cancer.

We have already successfully identified two antimicrobials (Benzethonium Chloride and Alexidine Dihydrochloride) with novel anticancer properties through a cell-based, phenotype-driven HTS of two chemical libraries (LOPAC1280 and Prestwick) using the tetrazolium-based MTS assay as the read-out for viability. Further *in vitro* and *in vivo* studies demonstrated that these novel agents ablated the tumorigenicity of FaDu cells, delayed the growth of xenograft tumors, and interacted additively with local RT or chemotherapy (5-fluorouracil and Cisplatin).

In our continued search for novel anticancer agents that could synergize with RT, we have begun to screen another chemical library that contains 2000 natural compounds (The Spectrum Collection). Eighteen compounds were identified to have preferential toxicity against FaDu cells, covering a broad range of functions including antimicrobial, anti-apoptosis, anti-metabolite, and DNA alkylation. The validity of this screen was corroborated by the identification of existing chemotherapeutic agents such as Novantrone, Dactinomycin, and Mechlorethamine, as well as the two novel anticancer agents described in our previous screen. Amongst these 18 "hits", only one compound was identified with novel anticancer properties, and further evaluations regarding its *in vitro* and *in vivo* efficacy, radiosensitizing properties, and mechanism of action will be conducted. In summary, we have designed and performed a rapid, cell-based, and phenotype-driven small molecule screen that can be used for large-scale identification and characterization of novel anticancer agents.

(PS1102) Sodium selenite radiosensitizes prostate cancer xenograft tumors but does not kill intestinal stem cells in vivo. Junqiang Tian, Bryan Husbeck, Donna Peehl, Susan Knox. Stanford University, Stanford, CA, USA.

BACKGROUND: We have previously shown that sodium selenite (SSe) increases radiation-induced cell killing of human prostate carcinoma cells (LAPC-4). In order to determine the potential clinical utility of SSe as a radiosensitizer for the treatment of prostate cancer, the *in vivo* effect of SSe and radiation (XRT) on well-established xenograft tumors (efficacy) and intestinal stem cell survival (toxicity) were determined. METHODS: In a pilot efficacy study, female nude mice with LAPC-4 xenograft tumors (500mm^3) were divided into 4 groups (n=2/group): control (untreated), XRT (7 Gy local tumor irradiation), SSe (IP, 2mg/kg, 3 times/wk for 7 wks), and XRT+SSe. Tumor volume was measured weekly. In the toxicity study, male mice (8 wks of age) were given SSe (IP, 3 times/wk) for 2 or 4 wks in the doses of 0, 2, 3.5, and 6.125 mg/kg (n = 2–3/dose group). Intestinal stem cell survival in duodenum, jejunum, ileum, and colon was determined using a microcolony assay. RESULTS: In the efficacy study, the tumor volume of control mice increased steadily over the study period (to 4.4 fold at 7 wks). XRT and SSe alone each significantly reduced the tumor volume increase to 2.0 and 2.5 fold, respectively, at the 7th wk. The combined treatment of XRT and SSe completely inhibited the tumor growth throughout the study period. In the toxicity study, the number of crypts/cross section in all sections of intestine studied was not significantly decreased by SSe regardless of the treatment dose or duration of the therapy. A similar study is in progress using SSe and XRT. CONCLUSION: Preliminary results from the experiments described above suggest that SSe can significantly enhance the effect of radiation on well established LAPC-4 tumors, and that SSe does not affect normal stem cell survival as a single agent. Ongoing experiments are further testing the efficacy and toxicity of SSe as a radiosensitizer with larger sample sizes, and analyzing intestinal stem cell survival following treatment with both SSe and radiation. Results from these experiments will be presented, and will have important implications regarding the ability of SSe to potentially increase the therapeutic index of XRT for the treatment of local and regional prostate cancer.

(PS1103) Hypoxia-inducible suicide gene therapy approach radiosensitises prostate cancer cells. Laure H. Marignol[1], Foley Ruth[1], Thomas D. Southgate[2], Mary Coffey[3], Donal Hollywood[1], Mark Lawler[1]. [1]Department of Haematology and Academic Unit of Clinical and Molecular Oncology, Institute of Molecular Medicine, St James's Hospital and Trinity College Dublin, Dublin, Ireland, [2]Cancer Research UK Gene Therapy Group, Paterson Institute for Cancer Research, Manchester, UK, Manchester, United Kingdom, [3]TCD School of Radiation Therapy, Trinity College Dublin, Dublin, Ireland.

We have cloned the arrangements of hypoxia response elements (HREs) of the oxygen-responsive genes vascular endothelial growth factor (VEGF) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) upstream of the cytosine deaminase (CD) gene. These constructs will drive the expression of this prodrug activation enzyme, which converts inactive 5-fluorocytosine (5-FC) to active 5-fluorouracil (5-FU), allowing selective killing of vector containing cells.

These constructs were transfected into 2 prostate cancer cell lines (DU145, 22Rv1) and exposed to oxygen concentrations of 0.5% (pO2< 2mmHg) for 48 hours. Western blot analysis of

protein extracts from these cells indicated hypoxic CD induction levels 8.8- and 4-fold with pH5VCD and pH8GCD, respectively. No expression was observed in aerobic cells, confirming the specificity of the approach.

Transfected cells exposed to hypoxia for a 48h period showed a significant decrease in cell number and associated cell death (proliferation assay, p=0.02) following a 4 days aerobic 5-FC treatment at the clinically relevant dose of 1mM, when compared to untransfected as well as transfected aerobic controls. These results correlated with a three-fold increase apoptosis levels on day three of 5-FC treatment (Annexin V assay) in both 22RV1 and DU145 transfectants.

The combination with clinically relevant radiation doses (2Gy) on day one of 5-FC treatment resulted in significant reduction of the surviving fraction of pH5VCD transfected cells (clonogenic assay) compared to either hypoxia or radiation alone, at all 5-FC concentrations tested. There was no significant benefit of the combined protocol in pH8GCD transfectants. The fractionated delivery of the same total dose had a protective effect on the sensitivity of either transfectants to the combined treatment. Finally, HREs of GADPH origin were found to be radiation responsive in 22Rv1 (1.2-, 1.1-fold) and LnCap (1.3-, 2.2-fold) transfectants exposed to radiation doses of 1 and 2Gy.

This data suggests that targeting hypoxia using a gene therapy approach has demonstrated efficacy in selective killing of prostate cancer cells and could be used in combination with ionising radiation. A series of replication deficient adenoviral vectors have been developed and will be tested in this suicide gene therapy approach.

(PS1104) Expression of mIL-3 enhanced a combined course of HSV-sr39tk gene therapy and radiotherapy for prostate cancer. Chi-Shiun Chiang[1], Ching-Fang Yu[1], Sheng-Yung Fu[1], Ji-Hong Hong[2]. [1]National Tsing Hua University, Hsinchu, Taiwan, [2]Chang Gung Memorial Hospital, Tao-Yuan, Taiwan.

This study aimed to test the hypothesis that the expression of mIL-3 and HSV-sr39tk genes within the prostate cancer could enhance its response to GCV prodrug and radiation therapy. To this aim, we employed a murine model of prostate cancer (TRAMP-C1) and a bicistronic vector to express mIL-3 and HSV-sr39tk genes intratumorally. The combining effects of mIL-3 and HSV-sr39tk gene expression with GCV and radiation treatment were evaluated by *in vitro* colony assay and *in vivo* tumor growth delay. The nature of the intratumoral host cell infiltrate and the level of systemic immunity that was generated were measured by flow cytometry and ELISA, respectively. The results show that the expression of mIL-3 and HSV-sr39tk genes within TRAMP-C1 cells does not alter the intrinsic response of TRAMP-C1 cells to GCV and radiation cytotoxicity, but it increases the time of growth delay following a combined course of GCV and radiation therapy. The analysis of flow cytometry shows that the major change of cellular components within mIL-3 and HSV-sr39tk transfected tumors prior to GCV and irradiation is the recruitment of Mac-1 and F4/80 positive cells with enhanced expression of MHC II molecules. Five days after a combined course of GCV and radiation treatment, FACS results showed enhanced expression of Ia signal in Mac-1 positive cells. The TRAMP-C1 specific immune response of spleen lymphocytes was measured by ELSIA. The result showed that mIL-3 and HSV-sr39tk gene expression activates IL-4 producing lymphocytes (Th2 response). However following GCV alone treatment, radiation alone treatment, or combined course of GCV and radiation treatment, the response was shift to Th1 response with more IFN-γ than IL-4 producing cells. The study proves the hypothesis that mIL-3 gene expression can enhance prostate cancer response to a combined course of HSV-sr39tk/GCV therapy and radiotherapy. It also demonstrates that the effect of mIL-3 is mainly through the induction of the infiltration of Mac-1 positive cells into tumors. Moreover, this study shows that the combining mIL-3 gene immunotherapy with HSV-sr39tk/GCV therapy or/and radiotherapy can switch the anti-tumor immune response from Th2 response to Th1 response.

(PS1105) Cooperative effects of armed oncolytic adenovirus with radiotherapy in c3h/hej hepatocarcinoma. Wonwoo KIM. Department of Radiation Oncology, Seoul, Republic of Korea.

Purpose: Armed oncolytic adenovirus (AOA) is currently being developed as novel antitumor therapeutic. To enhance their therapeutic potential, adenovirus is being administered in combination with standard therapy. The objective of this study was to explore whether armed oncolytic adenovirus could potentiate the antitumor efficacy of radiation in vivo, especially on radioresistant murine hepatocarcinoma (HCa-I).

Experimental procedures: C3H/HeJ mice bearing syngeneic hepatocarcinoma were treated with an armed oncolytic adenovirus: Δ B7 or Δ B7/GM-CSF + IL-12 or 25 Gy radiation or both. When tumor size was in the range of 8 mm in diameter, PBS or AOA mixed with lipofectamin and plus solution at a 2:6 ratio were administered intratumorally ($1*10^8$ plaque forming units (PFU) per tumor in 50 ml of PBS) four times once every other day. Tumor response to the treatment was determined by a tumor growth delay (TGD) assay. Possible mechanisms of action were explored by examining the level of apoptosis and regulating molecules. Metastatic potential was evaluated by assessing spontaneous pulmonary metastasis in lung metastasis model. Day 10, 15, 20 after treatment at 8 mm obtained lung and then counted number of nodules. All statistical tests were two-sided.

Summary: In TGD assay, Δ B7/GM-CSF + IL-12 AOA showed a high additive effect of tumor compared with Δ B7 oncolytic adenovirus. In lung metastasis assay, the Δ B7/GM-CSF + IL-12 AOA + radiation (combination) therapy decreased number of nodules. After tumor growth to 8 mm, in 15 days, intratumoral injection of Δ B7/GM-CSF + IL-12 AOA decreased number of lung nodules in mice (Control group = 8.38 ± 0.6 of metastatic tumor per mouse and Combination group = 1 ± 0.3). In 20 days, without drug, it was scored 19.25 ± 2.04 and with combination therapy, number of nodules was scored 1.13 ± 0.3. **Conclusions:** When radiation and Δ B7/GM-CSF + IL-12 AOA were combined, TGD and lung metastasis assay may have potential benefits in cancer treatment. It is suggested that AOA could be useful in combination with radiotherapy by inhibiting tumor growth and metastasis. The mechanism seems to involve the increase of induced apoptosis. Δ B7/GM-CSF + IL-12 AOA, in combination with radiation therapy, may have potential benefits in cancer treatment.

(PS1106) SCC-S2 is a novel androgen-inducible and multifunctional target: implications for radiation and chemosensitization of prostate cancer. Chuanbo Zhang, Isamu Sakabe, Rajshree R. Mewani, Deepak Kumar, Usha N. Kasid. Georgetown University, Washington, DC, USA.

SCC-S2/GG2–1/NDED (approved gene symbol TNFAIP8) is a transcriptional factor NF-kB-inducible, anti-apoptotic, and oncogenic molecule. SCC-S2 gene was discovered by a comparison of the expression profile of a primary human head and neck squamous cell carcinoma (HNSCC) cell line with its matched metastatic and relatively radioresistant HNSCC cell line established from a recurrent lymph node following surgery and radiation therapy. SCC-S2 is a 21 kDa predominantly cytosolic molecule, and a member of the FLIP family of cell death inhibitory proteins. Earlier we have demonstrated a correlation between enhanced expression of SCC-S2 and increased tumor growth, in vitro invasion, and experimental metastasis. Antisense or siRNA inhibition of SCC-S2 expression led to decreased expression of VEGFR-2 in tumor cells and human lung microvascular endothelial cells, and loss of endothelial cell viability. Down regulation of SCC-S2 expression in tumor cells also resulted in decreased expression of known metastasis-related molecules MMP-1 and MMP-9. Consistent with a role of SCC-S2 in oncogenesis, SCC-S2 expression was found to be relatively high in several human renal cell carcinoma and breast carcinoma as compared to their adjacent normal/benign tissues. In "promoter array" studies, differential methylation and regulation of SCC-S2/GG2–1 has been reported in prostate epithelial and cancer cell lines. Here we demonstrate that SCC-S2 mRNA and protein levels are induced by androgen in hormone-responsive LNCaP prostate cancer cells. In athymic mice bearing hormone-refractory PC-3 prostate tumor, intravenous treatment with a liposomal formulation of SCC-S2 antisense oligo caused reduced

expression of SCC-S2 in tumor tissues. A combination of liposomal SCC-S2 antisense oligo and radiation or docetaxel treatments resulted in a significant inhibition of PC-3 tumor growth as compared to radiation or drug alone. These data suggest that SCC-S2 plays a unique role in tumor progression via engagement of multiple signals, and provide a basis for development of SCC-S2-targeted prostate cancer therapy.

(PS1107) Guggulsterone mediated enhancement of radiation response. Rajani Choudhuri, William DeGraff, James B. Mitchell, John A. Cook. NIH/NCI, Bethesda, MD, USA.

Guggulsterone (GS) is a plant sterol used in Indian Ayurvedic medicine to treat inflammatory-related diseases. A recent report indicated that GS inhibits NF-kβ responses in cells. Since NF-kβ is considered a pro-survival pathway, GS was evaluated to determine whether it could influence clonogenic survival after ionizing radiation exposure. PC-Sh cells (pancreatic carcinoma) were exposed to radiation (2–10 Gy) after a 24 exposure to 50 μM GS. GS alone was not toxic; however, GS significantly enhanced the radiation response of these cells (sensitizer enhancement ratio (SER) at 10% survival = 2.5). Full dose survival curves showed that the shoulder of the survival curves was altered suggesting that repair was potentially compromised in the GS treated cells. Additional radiation studies were done using GS incubated for 24 hr with MCF7 and HT29 cells. GS treatment alone (50 μM) was not toxic to HT29 cells; however, survival of MCF7 cells was reduced to 59%. MCF7 cells, but not HT29 cells, were only slightly sensitized (SER = 1.25) after 50 μM GS incubation; however, 100 μM GS did radiosensitize both MCF7 and HT29 cells. Growth inhibition (50%) was achieved in the HT29, PC-Sh, and MCF7 cells after 25, 30–35, and 50 μM GS incubation, respectively. ER alpha protein levels, but not ER beta protein levels, were down regulated after 25 μM GS incubation in MCF7 cells. IGF1R beta also showed a reduction, but only following 100 μM GS treatment in all the cell lines. Potentially associated with the growth inhibition was the up-regulation of p21 (*CDKN1A*) protein levels as a result of GS treatment. Radiosensitization corresponded to changes in the phosphorylation of histone H2AX (γH2AX) as 50 μM GS increased the γH2AX in PC-Sh cells as early as 4 hr post- administration while changes in γH2AX required 100 μM GS incubation in both MCF7 and HT29 cells.

(PS1108) The relation of *p53* status to the radio- or thermo-enhancement effect by adriamycin (ADM) in human lung adenocarcinoma A549 cells and kinetics of apoptosis and hsp72 protein. Sachiko Hayashi[1], Hideki Matsumoto[1], Masanori Hatashita[2]. [1]Division of International Social and Health Science, University of Fukui, Matsuoka-Shimoaizuki, Fukui, Japan, [2]Research and Development Department, The Wakasawan Energy Research Center, Tsuruga, Japan.

Purposes: Previous reports have clarified that human lung adenocarcinoma A549 cells with a mutant *K-ras* and wild-type *p53* gene are low radio- or thermo-sensitivity. Adriamycin (ADM), the anthracycline derivatives, inhibits the activity of topoisomerase II, which was considered to show suppression of DNA synthesis. We have reported that the agent thermo-sensitized Chinese hamster V79 cells in vitro when those were treated with ADM at 42°C simultaneously for short time periods. In the present study, the relation between the *p53* status and the radio- or thermo-enhancement effects of the combination therapy with ADM and X-irradiation or hyperthermia were examined in human lung adenocarcinoma A549 cells.

Procedures: Wild type *p53* (wt*p53*) of A549 cells were transfected with a vector carrying a neomycin-resistant gene (neo) and mutant *p53*-Trp248–19 gene. The radio-or thermo-sensitivity of these cell lines to ADM and X-radiation (or hyperthermia) or the combination therapy were determined by colony formation assay. The induction of apoptosis and the accumulation of hsp72 protein after these treatments were analyzed with double stain methods by Höchst33342/Ethidium bromide and with western blotting methods, respectively.

Results: Surviving fractions from combination therapy with ADM and X-ray (or hyperthermia) sequentially in A549/m*p53*, transfected cells, were higher than that in A549/wt*p53*, original cells. Induced apoptosis after the treatment with ADM and/or hyperthermia at 44°C in these cell lines were examined. While incidence of apoptosis at 24 hr after the combination therapy in A549/m*p53* showed circa 2 times of the single, in A549/wt*p53* it showed circa 3 times. We further analyzed intra-cellular accumulation of p53 and hsp72 protein after the treatments, respectively.

Conclusions: Modification of thermo- or radio-sensitivity with ADM showed significant effects in A549/wt*p53* cells than in transfected A549/m*p53* cells. It was suggested that the mechanisms of thermo- or radio-enhancement effect with ADM were due to *p53*-dependent induction of apoptosis and control of hsp72 protein, because A549 cells with transfected m*p53* gene suppressed the incidence of apoptosis and induced the accumulation of hsp72 protein after combination therapy with ADM and X-radiation (or hyperthermia).

(PS1109) siRNA targeting *NBS1* increases radiation sensitivity of human cancer cells in a *p53*-independent manner. Ken Ohnishi[1], Zorica Scuric[2], Robert H. Schiestl[2], Akihisa Takahashi[1], Takeo Ohnishi[1]. [1]Nara Medical University School of Medicine, Nara, Japan, [2]University of California Los Angeles, Los Angeles, CA, USA.

NBS1 is essential for the repair of radiation induced DNA double-strand breaks (DSBs) in yeast and higher vertebrate cells. In this paper, we examined whether suppressed NBS1 expression by small interference RNA (siRNA) could enhance radiation sensitivity in cancer cells with different *p53* status. We used human non-small cell lung cancer cells, differing in *p53* gene status (H1299/wt*p53* cells bearing wild-type *p53* or H1299/m*p53* cells bearing mutant *p53*). A cassette of DNA expressing siRNA targeted for the *NBS1* gene was transfected into those cell lines and radiation sensitivity was examined with a colony-forming assay. Cellular levels of NBS1 and other proteins were analyzed using Western blot. We found that the radiation sensitivities of H1299/wt*p53* and H1299/m*p53* cells were enhanced by transfection of the DNA cassette. In the *NBS1*-siRNA transfected cells, we observed the decrease of the constitutive expression of NBS1 protein and the decrease of phosphorylated NBS1 induced by radiation. In addition, the radiation-induced expression of the transcription factor NF-κB and XIAP (X-chromosome-linked inhibitor of apoptosis protein) was suppressed by *NBS1*-siRNA. The induced XIAP expression was strongly suppressed by *NBS1*-siRNA in m*p53* cells as compared with wt*p53* cells. Furthermore, we found that NBS1 and phospho-NF-κB were not accumulated after X-ray irradiation in radio-sensitive Gmtert cells (established from a Nijmegen breakage syndrome patient) bearing mutated *NBS1* but in 82–6 normal skin fibroblast cells bearing wild-type *NBS1*. Enhanced X-ray sensitivity, after *NBS1*-siRNA transfection, was achieved in *p53* wild-type and even more pronounced in *p53* mutant cells. The transfection of siRNA targeted for *XIAP* also enhanced X-ray sensitivity even in more pronounced for *p53* mutant cells compared to *p53* wild-type cells. Our data suggest that the sensitization to radiation is a result of *NBS1*-siRNA-mediated suppression of X-ray-induced cell-survival signaling pathways *via* NF-κB and XIAP and that it is a novel radiation therapy-sensitizing agent, particularly in *p53* mutant cancer cells.

(PS1110) Effect of curcumin and ionizing radiation on the activation of wild-type and mutant p53 in prostate cancer cells. Bijaya K. Nayak, Cynthia A. Galindo, Martin L. Meltz, Gregory P. Swanson. University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Mutations in the p53 gene are found with increased frequency in advanced prostate cancers. Radiotherapy failure is more common in prostate cancer patients with abnormal p53. In the present study, the effect of curcumin and ionizing radiation on the p53 response and apoptosis induction was examined in prostate cancer cells. The studies were performed in LNCaP (wild-p53) and PC3 (mutant p53)

prostate cancer cells. The cells were treated with 5 to 50 μm of curcumin and 3 or 5 Gy doses of ionizing radiation. Activation of p53 and the p53 target gene p21 was examined using the RNase protection assay and Western blotting. Induction of apoptosis was measured by examining activation of caspases and annexin-V binding. Activation of p53 and p21 was observed in LNCaP cells after a 3 Gy radiation treatment, while curcumin (7.5 μM) failed to activate wild-type p53 in this cell type. In PC3 cells, there was no activation of the mutant p53 in response to either radiation or curcumin treatment. However, there was activation of p21 in PC3 cells independent of p53 after curcumin treatment. Regardless of p53 status, induction of apoptosis occurred after curcumin treatment in both cell types. Our data thus provide a basis for the combination of curcumin and radiation for the treatment of prostate cancer.

This work is supported by the department of Radiation Oncology, UTHSCSA.

	LNCaP (wild type p53)			PC3 (mutant p53)		
	3 Gy	Curcumin (7.5 μM)	Curcumin (7.5 μM) + 3 Gy	3 Gy	Curcumin (7.5 μM)	Curcumin (7.5 μM) + 3 Gy
Activate p53	yes	no	yes	no	no	no
Activate p21	yes	no	yes	no	yes	yes

(PS1111) Integrative Genitouro-Radiology in Uno-Agenda 21. Eva M. Neu[1], Michael Ch. Michailov[1], Guntram Schulz[1], Ianka Foltinova[2], Walter Seidenbusch[3]. [1]Inst. fuer Umweltmedizin, Muenchen, Germany, [2]Inst. Histology/Embryology, Bratislava, Slovakia, [3]Inst. Experimentalphysik, Univ. Innsbruck, Austria.

INTRODUCTION. Oncological diseases of urogenital system are of first priority:

(Patho-)physiological fundamentals of combined radio-oncotherapy (RT) need up today further investigations to clarify *therapeutic mechanisms* and to prevent large *functional disturbances*. Presently it will be reported about (recent & earlier) results and problems. [Neu, Michailov et al.: see Book of 23rd Int. Radiol (at ISR Montreal): 233, 2004; Faseb J. 19/5, A1355/35 Int.C.Physiol., San Diego 2005; Brit. J. Urol. 305 2004; Urol. 254 2006 (SIU Honolulu & Cape Town); Book of Abstr. (eds Figo,London) B2, 62–69 (18th World Cong. Gyn.): 141 Kuala Lumpur 2006, (eds. R Acoste, Lord N. Patel); Str ther 167, 311–318 (1991), Eur J. Physiol. 443, S334 (2002), Proc ICRR Brisbane 222 (2003) & Würzburg 266 (1995)]

METHOD. Motor and electrical activity of human urogenital-preparations incl. myocytes, urothel are recorded (see intr.).

RESULTS AND PROBLEMS. Action mechanisms of ionizing irradiation and pathogenesis of functional disturbances after RT are till today not clear: e.g. radio-cystitis, ureteral stenosis, etc. Some fundamental results are given.

1. Myometrium generates spontaneous slow *tonic* contractions (STC: 0,22± 0,01/min, n=42): it is radio-insensitive (50–100 Gy).

2. A uterina (presence of spontaneous fast *phasic* contr.: SPC): Hormones (adrenaline, 5-HT) induces STC. **Vv uterina et ovarica** (only SPC)/- hormones augment SPC. After x-rays (0,1–10GY) strong increase of SPC and tonic contr.

3. Vesical detrusor produces SPC (3,2± 0,7/min, n=69), **trigonum**- STC (0,28 ±0,08/min n=56): inhibition of SPC and STC by x-rays (1–10Gy).

4. Vas def. (non active): radio-insensitive (over 100Gy).

5. Membrane potential of **urothel** is decreased after RT.

6. Cytostatics and toxicants have strong effects on SPC/STC.

CONCLUSION: Systematic interdisciplinary investigations in context of **integrative Genitouro-Radiology** are necessary to clarify mechanisms of combined RT and prophylaxis/ therapy of very complex functional disturbances. An int'l cooperation via foundation of int'l institutes/clinics (network of national) could help for higher efficiency in this research and support *UNO-Agenda 21* for better health, ecology, etc. in all countries, i.e. express *high scientific and medical ethics*.(see contrib. in ICRR Michailov et al.).

(PS1112) Integrative Angio-Radiology in UNO-Agenda 21. Michael C. Michailov[1], Ursula E. Welscher[1], Eva M. Neu[1], Viktor Foltin[2], Walter Seidenbusch[3]. [1]Inst. fuer Umweltmedizin, Muenchen, Germany, [2]Inst. Experimentalphysik, Univ. Bratislava, Slovakia, [3]Inst. Experimentalphysik, Univ. Innsbruck, Austria.

INTRODUCTION: Creation of an *integrative oncotherapy* must include not only somatic, but also psycho- /social therapies (psychoneuroimmunomodulation, etc.). The importance of angiology for *radiooncotherapy* is reported by Michailov, Neu, Welscher et al. [Proc ICRR Brisbane 222–223, 276, 2003 & Dublin 186,234, 1999 & Würzburg 234, 400, 434, 1995; Front. Rad. Ther. Oncol. (ed. Vaeth), Karger 22–35,1997 (IORT Conf. San Francisco)] Presently will be given approaches to complex *interaction of therapeutic factors* on vascular effector (recent & earlier data).

METHOD. Recording of vasomotor and electrical activity (rat aorta/ v. portae, human vessels, see Introd.)

RESULTS AND PROBLEMS. *Radiovasocontraction* (**RC**: 0.1–50Gy) *photorelaxation* (**PR**: UV, laser), *thermo-vasodilation* (**TD**: 42–45°C) are fundamental factors for oncotherapy.

1. Sensitization of RC and of motor effects of *cytostatics* (adriblastin, bleomycin) by catecholamines, Ca and PG could be related to *receptor or store-operated Ca-channels*.

2. On the RC, PR, TD are participated probably *pericytes* (electronic signal-transmission of myocytes to endothelium): e.g. x-rays inhibits EDRF, thermo-inhibition (42°C) of RC

3. *Stretch channels of myocytes* (Ca activated K) and *endothelium* (Ca entry by endothelin-1) could be essentially for oncotherapy: e.g. stretch increases RC.

4. *Anaesthetics* potentiated (bupivacain, fentanyl) or inhibited (pro-, tetracain) RC (influence on IORT).

CONCLUSION. Very complex interaction of factors (1–4) leading to vasoconstriction (tissue hypoxia, low drug concentr.) or vasodilation (cooling) is evident. An *adjuvant pharmacotherapy* could counteract negative vasomotor effect *of radio-,photo-,chemo-,thermo-therapy*, i.e. increase of therapeutic efficiency. The future needs a really *integrative angio-radiology* (to radio-oncology) concerning high complex interaction of factors (not only vascular). More effective research could be realised by foundation of int. instit./clinics for *radio oncology* (network of nat. ones) under the auspices of the Intern. Acad. Sci. c/o ICSD, IARR, ISR, IAEA promoting common research/ education progr., personnel, students, whole life work, etc. This could lead to better science, health, etc. in all countries in context of **Agenda 21 of UNO**. (see ICRR contrib. 2007: Neu et al.)

(PS1113) Social Responsibility in Radiation Science for UNO-Agenda 21. ICSD - Scientific Committee Int. Council Sci. Development. ICSD e.V. Muenchen, Inst. für Umweltmedizin, Experimentalphysik, etc., Muenchen, Innsbruck, etc., Germany.

INTRODUCTION: Radiation Sciences (RS) implies a large interdisciplinary spectrum from *atomic physics* (incl. optical, non-/ionizing radiation), *photo-/radiochemistry, photo-/radiobiology, nuclear medicine, radiology, radioecology,* etc. On this way research, education, technology in RS is related to fundamental problems of humanity concerning progress in evolution and misuse of RS in context of peace, medicine, ecology, energy, etc.

This is the reason, why RS, i.e. their scientific representatives (**SR**) -esp. **IARR, IAEA, ISR**, etc.- have high social and scientific responsibility to support UNO-Agenda 21.

PROPOSALS: SR, beginning with ICRR (IARR and American organisations, also directly government) to *support projects* of **International Council for Scientific Development** (**ICSD**) with its central body the International Academy of Science (IAS): Foundation of first (really) int'l interdisciplinary institutions via network of national ones, esp. **International University** (**IU**) / int'l faculties for anthropology, informatics, physics, chemistry, biology, medicine, etc. based on int'l institutes for physics,

chemistry, medicine, philosophy, etc. **Int'l Hospital** to IU, i.e. Int. Clinics for Angiocardiology (ICA), radio-oncology , etc., **Int. Centres for Ecology (ICE)** (incl. inst. for radio-ecology), **Int'l Academy for Solar-energy**, etc. **IU, IH, ICE**, will support the realization of **UNO AGENDA 21** and also the civil use of atomic energy (reactors) as well as spontaneous control of military use by regular presence of scientists from different countries on IU, IH, ICE according to common scientists, (research, post-grad. education) programmes, personnel, possibility for whole life use, etc.

CONCLUSION. Support of ICSD/IAS-projects by IARR, IAEA, ISR could be a new dimension of int. cooperation (incl. developing help) in science and policy, leading to better, peaceful world. A start is possible by organization of extra-ordinary *scientific political session* to ICRR-2007 in SF under participation of high functionaries (Presidents) of responsible organisations (a) some eminent scientists (incl. Noble Laureates) (b) and ministers (science etc.) from America, Africa, Asia, Europe, (c) to start a discussion (e.g. round table) about ICSD proposals. **ICSD-Coord./contact: Guntram Schulz (Guntram@stars.ms)**

(PS1114) Effect of DNA topology on double-strand breaks produced by γ radiation in plasmid DNA. Pichumani Balagurumoorthy, S. James Adelstein, Amin I. Kassis. Harvard Medical School, Boston, MA, USA.

We have studied the effect of DNA topology on the mechanism and magnitude of double-strand breaks (DSB) produced by γ rays in plasmid DNA. Supercoiled (SC), nicked or relaxed-circular (N), and linear (L) forms of ^{3}H-labeled pUC19 DNA were irradiated with ^{60}Co γ rays at a dose rate of 3.75 Gy/min in phosphate-buffered saline, pH 7.4, without and with 0.2 M DMSO. Subsequently, samples were analyzed on 1% agarose gel, and DSB yield per dose unit (gray) per DNA molecule was determined from the fractions of intact SC, N, or L DNA remaining and L DNA produced (Table). The data indicate that (**i**) DSB yield is influenced by DNA topology; and (**ii**) for all three DNA forms, ~90% of DSB are mediated by hydroxyl radicals (•OH), i.e. by indirect mechanisms. DSB production by low-energy, short-range Auger electrons, emitted by the decay of ^{125}I, also depends on DNA topology. However, ~100% of DSB in the SC form are caused by direct mechanisms, whereas those in N and L forms are caused by both direct (~50%) and indirect mechanisms (~50%). For both radiation types, the DSB yield for SC DNA is much lower than that for the torsionally relaxed forms. Our findings show that DNA topology affects the DSB yield produced by γ radiation and ^{125}I decay, seemingly as a result of the extended conformational state of N and L forms compared with highly compact SC DNA.

DSB yield per DNA Molecule per Gray

Topology of plasmid DNA	DSB (−DMSO)	DSB (+DMSO)
SC	**13 ± 0.51 E-4**	**1.57 ± 0.28 E-4**
L	**30.9 ± 2.17 E-4**	**1.99 ± 0.39 E-4**
N	**89.4 ± 7.0 E-4**	**9.10 ± 1.94 E-4**

(PS1115) A comparison of DNA strand break yields in pBR322 plasmid after I-123 and I-125 decay. Ekkehard Pomplun[1], Aude Peudon[2], Michel Terrissol[2], Eberhard Kümmerle[1]. [1]Forschungszentrum Jülich, Jülich, Germany, [2]LAPLACE, Universite Paul Sabatier, Toulouse, France.

The strong radiotoxic effect of DNA incorporated Auger electron emitters is attributed to the numerous short ranging electrons released during the nuclide's decay. Since there are currently no generally accepted radiation weighting factors for these particular nuclides, a detailed understanding of their damaging mechanisms on the molecular and cellular level is necessary. Recently published experimental strand break data after the decay of I-123 and I-125 in plasmid DNA can serve as an appropriate comparison for complex computer simulations.

The computer simulation includes the decay of I-123 and I-125 starting with the electron K capture and the following reorganisation of the excited atomic shells accompanied by the emission of Auger and Coster-Kronig electrons. For 10000 individual decays of each of both nuclides the electron energy emission spectra had been calculated. These data were subsequently used as input for a transport code which simulates the very complete transport of electrons and photons from the physical stage up to primary chemical at 10^{-8} s, inside a complex environment of liquid water, plasmid DNA and scavengers. The decaying nuclides were assumed to be positioned at the methyl groups within the thymine bases of a pBR322 plasmid model with 4362 basepairs. The respective contributions from direct and indirect effects were then evaluated.

The simulation of strand break induction results in 4.7 ssb and 0.8 dsb per I-123 decay and 7.6 ssb and 1.6 dsb per I-125 decay. Adding 2 mol DMSO, the corresponding values are 0.8 ssb and 0.07 dsb for I-123 and 1.4 ssb and 0.13 dsb for I-125. This is in good correlation with the experimental data. Discrepancies may be due to experimental conditions which cannot be simulated adequately. The difference in the efficiency between both nuclides must be attributed to the different distributions of Auger electrons released. To deduce radiation weighting factors additional biological endpoints, in particular those for cellular effects, have to be considered as well as differences in the dose rate due to different half lives of the two iodine isotopes.

(PS1116) The role of hydration in anion electron stimulated desorption from single strands of DNA. Sylwia Ptasinska, Leon Sanche. University of Sherbrooke, Sherbrooke, PQ, Canada.

Energetic electrons can penetrate an organic or biomolecular solid and create secondary species, such as ions and neutral radicals. The formation of anions from surfaces must result from electronic excitation of the molecules of the adsorbate or temporary electron capture by these molecules. Below the dipolar dissociation threshold, anion desorption occurs when an electron with well define energy temporarily occupies one of unfilled molecular orbitals of the target molecule to form a dissociative anionic state. Above a few eV, this dissociative electron attachment (DEA) process usually proceeds via the formation of core-excited resonances since the lifetime of such states is usually longer than the molecular vibrations, thus allowing sufficient time for dissociation of the anion before autoionization.

In order to interpret possible DEA reactions within DNA, we have performed detailed studies on low energy electron interactions with short oligomers [1], double stranded linear DNA and supercoiled plasmid DNA [2].

However, a study of radiation damage to DNA is not complete without taking into account the presence of its native environment which is essentially composed of water molecules. Water provides a medium for many biochemical reactions, it is literally the "matrix of life". Clearly, water is not just an inert bystander in the cell, but a selectively reactive molecule with unique properties that greatly affect biochemical and biological processes.

The present experiments concern electron interactions with a film of short single strands of DNA covered by 3-monolayers of water, which corresponds to 5.25 water molecules per nucleotide. We report on the desorption of H\overline , O\overline , OH\overline from this target induced by 3–20 eV electrons. Below 15 eV, these anions emanate principally from a new type of dissociative core-excited transient anions formed via electron capture by a DNA-H_2O complex. A smaller portion of the H\overline desorption signal arises from weakly bonded H_2O molecules.

According to the present results, DNA damage induced by LEE via DEA is increased by a factor of about 1.6, when an amount of water corresponding to 60% of the first hydration layer is added to vacuum-dried DNA.

[1] S. Ptasinska and L. Sanche *J. Chem. Phys* 125, 144713 (2006)

[2] X. Pan, P. Cloutier, D. Hunting and L. Sanche, *Phys. Rev. Lett*. 90, 208102 (2003)

(PS1117) Chemical yields of DNA strand breaks produced by the direct effect of ionizing radiation: a comparison between samples irradiated at 4 K followed by warming to room temperature with samples irradiated at room temperature. Anita R. Peoples[1], Shubhadeep Purkayastha[1], Jamie R. Milligan[2], William A. Bernhard[1]. [1]University of Rochester, Rochester, NY, USA, [2]University of California at San Diego, La Jolla, CA, USA.

It is our goal to determine the mechanisms underlying the formation of DNA end-products due to the direct effect of ionizing radiation. This entails the study of those free radicals presumed to be precursors to end-products such as strand breaks. Much of our current information on free radical precursors has been obtained by EPR spectroscopy on DNA irradiated at low temperatures, *e.g*., 77 K and 4 K. As part of this effort, we recently measured ssb and dsb in pUC18 DNA films (Purkayastha, S., *et al*., *J. Phys. Chem. B*, **110**, 26286, (2006)). EPR measurements were made on DNA films at 4 K after X-irradiation at 4 K. The same films were then warmed to room temperature (RT) where solutions were prepared for strand break analysis using gel electrophoresis. While these experiments made it possible to correlate strand break events with free radical precursors, they also raised an important question. Do the reactions initiated by direct ionization at 4 K followed by warming to RT result in the same distribution of products as when the samples are irradiated at RT? In this study, we begin to address that question.

Our approach is to use gel electrophoresis to measure the chemical yields of ssb (G(ssb)) and dsb (G(dsb)) in pUC18 DNA films irradiated at RT and compare these yields with films irradiated at 4 K followed by warming to RT. The values of G(ssb) and G(dsb) for plasmid pUC18 samples irradiated at 4 K have been published in the work cited above. Here we report the values for pUC18 films X-irradiated at RT. For DNA hydrated to $\Gamma = 2.5$ mol H_2O/mol nucleotide, we found prompt yields of G(ssb) = 75 ± 9 nmol/J and G(dsb) = 5.2 ± 0.8 nmol/J. These are the same as those obtained previously for pUC18 films X-irradiated at 4 K and warmed to room temperature: G(ssb) = 78 ± 8 nmol/J and G(dsb) = 5.4 ± 1 nmol/J. This raises the possibility, at least with respect to strand breaks, that the chemical yields are not influenced by temperature during sample irradiation; the yields are the same for samples irradiated at 4 K followed by warming to RT as for samples irradiated at RT. These results will increase our ability to link our current knowledge of free radical intermediates observed at low temperatures with DNA end-products formed upon irradiation of DNA in model systems at RT and for DNA under physiological conditions.

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(PS1118) Vibrational excitation of condensed thymidine films by low-energy electron impact. Radmila Panajotovic, Marc Michaud, Leon Sanche. University of Sherbrooke, Sherbrooke, PQ, Canada.

Investigation of the vibrational excitation of nucleosides leading to dissociation through formation of temporary negative ions aims at understanding the correlation of nucleic base-sugar moiety conformational coupling and its consequences on the bond cleavage in DNA. The conformation of the 2'-deoxyribose moiety with respect to the base is expected to influence which species are formed upon exposure of nucleosides to ionizing radiation. Extensive results from measurements on short DNA strands impinged on by 1–30 eV electrons indicate that the damage they induce is due to the chemical nature of the nucleic bases and/or their sequence [1].

Thymidine is one of the most important nucleosides of DNA and an important component of antiviral compounds. In the condensed phase, thymidine's 2'-deoxyribose ring is in the pentose sugar ring form, which is a true conformation of this nucleoside in DNA. As in previous studies on thymine [2], our aim was to investigate a possibility of resonance formation (temporary negative ions) in thymidine for electrons of incident energies below 12 eV. Measurements of the vibrational excitation were performed on a high resolution electron energy-loss (EEL) spectrometer, housed in a cryogenically pumped ultrahigh- vacuum chamber at a base pressure of $\sim 5 \times 10^{-11}$ Torr. EEL measurements were made on a thin film of thymidine deposited on a six-layer spacer of argon condensed on a cold Pt substrate.

The integral cross sections (over the half-space angle) for excitation of the normal vibrational modes of the ground electronic state are calculated from reflectivity EEL spectra following the procedure applied earlier on thymine and pyrimidine [2]. Most of cross sections for vibrational excitation are of the order of 10^{-18} cm^2, except for the stretching of the C=O bond in thymine and the CH2 rocking in sugar (with some contribution of the ring deformation) for which they are of the order of 10^{-17} cm^2. There are two wide resonance features of which the one at 2 eV can be linked to the resonance observed in the study of dissociative ionization of thymidine [3].

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(PS1119) A new Monte Carlo program ETMICRO-CHEM for simulating DNA damage by electrons. Eun-Hee Kim. Seoul National University, Seoul, Republic of Korea.

A new Monte Carlo program ETMICRO-CHEM (Electron Transport code for MICROdosimetry and CHEMical interactions) is now available for simulating the direct and indirect actions of electrons on DNA.

ETMICRO (*Rad Prot Dosim* in press) traces the electrons down to 10 eV in liquid water by considering total 11 (5 ionization, 5 excitation and 1 elastic) scattering modes. The inelastic scattering cross sections adopted in ETMICRO are based on the Drude model parameters that are derived by Emfietzoglou and Nikjoo (*Radiat Res* **163**, 98–111 (2005)) using the optical data set of Hayashi et al. (*Proc Natl Acad* USA **97**, 6264–6266 (2000)). The elastic scattering cross sections originate from various literatures (Danjo and Nishimura, *J Phys Soc Jap* **54**, 1224–1227 (1985); Katase et al., *J Phys B: At Mol Phys* **19**, 2715–2734 (1986); Grosswednt et al., *Nucl Instr Methods* **155**, 145–156 (1975)). ETMICRO informs the spatial distributions of the primary products H_2O^+, H_2O^*, and the sub-excitation electron e^-_{sub} by ionization and excitation of water molecules.

Those primary products in the physical stage are traced further in the CHEM routine, which covers the pre-chemical and the chemical interactions. The radicals H_3O^+, H, OH and the hydrated electrons e^-_{aq} at the start of chemical stage lead to the production of other radicals H_2O_2 and OH^-. In the chemical stage, the time-distributions of the radicals H_3O^+, H, OH, e^-_{aq}, H_2O_2 and OH^- are controlled by diffusion and the reaction radii among reactants. The rate constants and the effective reaction radii are adopted from different sources (Pimblott, *J Phys Chem* **96**, 4485–4491 (1992); Elliot et al., *J Chem Soc Faraday Trans* **86**, 1539–1547 (1990); Buxton et al., *J Phys Chem Ref Data* **17**, 513–885 (1988)).

The ETMICRO-CHEM program has been utilized to estimate the DNA damage by both direct and indirect actions of electrons. The threshold energy of 17.5 eV was assumed for the strand break and base damage by direct action of electrons. Regarding the indirect action, the base damage is assumed to be caused only by OH and e^-_{aq}. The break of sugar backbone is counted only by the reaction with the OH radical. The double strand break was counted with a couple of single strand breaks occurring within 10-base pair distance. This paper compares the estimates of DNA damage by utilizing the ETMICRO-CHEM with those provided in the earlier studies.

(PS1120) Monte Carlo simulations of site-specific radical attack to DNA bases. Bulent Aydogan[1], Wesley E. Bolch[2], Steven G. Swarts[3], David T. Marshall[4]. [1]University of Chicago, Chicago, IL, USA, [2]University of Florida, Ganiesville, FL, USA, [3]University of Rochester, Rochester, NY, USA, [4]The Medical University of South Carolina, Charleston, SC, USA.

An atomistic biophysical model permitting the calculation of initial attacks to a 32-base pair representation of B-DNA base moieties by water radicals is presented. This model is based upon a previous radiation damage model developed by Aydogan *et al*.

(*Radiat. Res.* **157**, 38–44, 2002). Absolute efficiencies for radical attack to the 32-base pair DNA molecule are calculated to be 41%, 0.8% and 15% for hydroxyl radical (•OH), hydrogen radical (H•), and hydrated electron (e^-_{aq}), respectively. Among the nucleobases, guanine is found to have the highest percent •OH attack probability at 36%. Adenine, cytosine, and thymine moieties have initial attack probabilities of 24%, 18%, and 22%, respectively. A systematic study is also performed to investigate •OH attack probabilities at each specified attack site in four DNA structures: free bases, single nucleotides, single base pairs, and a central eight base pairs of the 32-base pair DNA molecule. This work shows that inclusion of steric hindrance and site-specific reactivity at each reactive site are very important when developing a computational model to simulate water radical damage to DNA. Experimental pulse radiolysis data and the calculated initial •OH attack probabilities in this study displayed some differences for each given structure. Cytosine is the base moiety for which the closest agreement is observed between the model prediction and the experimental data. The initial •OH attack probabilities for cytosine as the free base are calculated to be 72% and 28% while experimental data are reported at 87% and 13% for the C5 and C6 positions on the base, respectively. The differences in agreement between calculated and experimentally determined •OH and H• attack probabilities are likely due to the use of atomic charges. These charges are derived from the electrostatic field potentials calculated from density functional theory for the bases, to scale the relative reaction rates of these radicals at the individual atomic positions on the pyrimdine and purine bases. Instead, future updates to the RIDNA model will include the use of electron densities to scale the reaction rates.

(PS1121) Monte-Carlo simulation of liquid water radiolysis: effects of acidity and radiation quality (LET) on the primary yields and application to the Fricke dosimeter. Narongchai Autsavapromporn, Jintana Meesungnoen, Ianik Plante, Jean-Paul Jay-Gerin. University of Sherbrooke, Sherbrooke, PQ, Canada.

The radiolysis of liquid water by ^{60}Co γ-rays, fast electrons, or high-energy protons, where the linear energy transfer (LET) is ~0.3 keV/μm, is generally well understood at neutral pH and 25 °C. However, the situation is not quite as clear when the effects of acid concentration and LET are considered. In this work, Monte-Carlo simulations are used to investigate the effect of acidity (pH) on the primary yields of the various chemical species of the radiolysis of deaerated aqueous sulfuric acid solutions with pH values ranging from 7 to 0.46 (0.4 *M* H_2SO_4). The effects of the quality of radiation have also been studied for LET varying from ~0.3 to 15 keV/μm at 25 °C. Our results show that an increase in acidity ($1 < pH < 4$) leads to an increase in $G_{e\text{-}aq+H^\bullet}$, a slight increase in $G_{^\bullet OH}$ and G_{H2O2}, and a slight decrease in G_{H2}. At $pH < 1$, $^\bullet$OH radicals react with HSO_4^- anions to form $SO_4^{\bullet-}$ radicals, resulting in a steep decrease in $G_{^\bullet OH}$. By contrast, in a pH range from ~4 to 7, the calculated yield values are independent of H_2SO_4 concentration. In both neutral water and 0.4 *M* H_2SO_4 solutions, the primary molecular yields increase upon increasing LET to ~15 keV/μm with a concomitant decrease in those of free radicals. As an exception, $G_{H^\bullet}$ at first increases versus LET, then reaches a maximum near 6.5 keV/μm before decreasing steeply at higher LET. Simulation results are generally in good agreement with experimental results. Finally, as an application, we have simulated the radiation-induced oxidation of ferrous sulfate solutions in aerated aqueous 0.4 *M* H_2SO_4 (Fricke dosimeter) as a function of time up to ~50 s and addressed the effects of LET on the resulting ferric ion yield at 25 °C. The production of Fe^{3+} ions is highly sensitive to free-radical yields, especially $H^\bullet$ atoms (via formation of $HO_2^\bullet$), resulting in a marked decline of $G(Fe^{3+})$ with increasing LET. The general trend of the observed variation of $G(Fe^{3+})$ with radiation quality is well reproduced by our computed Fe^{3+} ion yield values.

(PS1122) Water radiolysis by swift Protons and carbon ions. Benoit Gervais[1], Michael Beuve[2], Anthony Colliaux[2]. [1]Ciril, Caen, France, [2]Liris, Lyon, France.

We present a complete simulation of liquid-water radiolysis by swift carbon ions for Linear Energy Transfer (LET) ranging from 10 to 1000 keV/μm. We consider the water radiolysis as the succession of three temporal stages. The physical stage includes the excitation and ionisation of water molecules by the projectile and the subsequent secondary electron cascade. The physico-chemical stage simulates the fast dissociation and rearrangement processes following immediately the excitation or ionisation of a water molecule in the liquid. Then the chemical stage accounts for diffusion and chemical reactions of these chemical species during the first 10 microseconds following the passage of the ion. Our simulation takes into account explicitly the multiple-ionisation process and the resulting fragmentation, which is specific to high-LET radiations. We will present the time evolution of the radical yields generated by water radiolysis. The comparison of the yields obtained with or without considering multiple ionisation allows identifying this process as the main contribution for HO2 and O2 creation. We will present recent development regarding the scavenging effects of O2 and GSH antioxidant naturally present in cells. We show that, according to the cellular concentrations and the LET of the projectile, these solutes may increase or reduce the yields of oxidative species. A detailed analysis of our high-LET ions simulation shed some new light on the balance between direct and indirect effects for DNA damage.

(PS1123) Ionization by intermediate-energy carbon ions on water vapor. Steven L. McLawhorn, Larry H. Toburen, Robert A. McLawhorn, Edson L. Justiniano, Jefferson L. Shinpaugh. East Carolina University, Greenville, NC, USA.

To enhance understanding of the underlying physical and chemical mechanisms that produce radiation damage it is useful to correlate initial patterns of energy deposition from primary and secondary particles with the patterns of damage observed in DNA and cellular structures. Detailed estimates of these microscopic patterns of energy deposition can be obtained from charged particle Monte Carlo track structure simulations. These simulations rely on comprehensive sets of interaction cross sections to simulate the interaction processes event-by-event along particle tracks. At present, the cross section data needed to extend track structure simulations to heavy ions with energies in the range of those proposed for microbeam studies is exceedingly limited. Accurate track structure simulation for those particles will require both total and differential ionization cross sections as well as cross sections for charge transfer processes for atomic and molecular targets of biological importance. Therefore, we have initiated measurements of doubly differential ionization cross sections, differential in ejected electron energy and emission angle, for carbon ions over a range of initial charge states and energies. Incident ion energies range from 67 keV/u to 350 keV/u with charge states from C^+ to C^{3+}. Ejected electrons are detected over a range of energies from 10 eV to 1500 eV and emission angles from 20 to 120 degrees. In this work we will present new cross section data for electron emission from water vapor owing to collision with carbon ions.

(PS1124) New superparamagnetic polymer nanospheres for the potential separation of radionuclides in nuclear wastes or environmental samples. Yanqin Ji[1], Xianzhang Shao[1], Jinying Li[2], Yueping Guan[3]. [1]National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention, Beijing, China, [2]Department of Radiochemistry, China Institute of Atomic Energy, Beijing, China, [3]School of Materials Science and Engineering, University of Science and Technology, Beijing, China.

The nano- structured magnetic particles have the high-surface-area and high-adsorption -capacity, which exists a high potential to immobilize metal ions, especially radionuclides on their surfaces from the waste and environmental samples. The purpose of this research is to develop a simple, cost-effective and fast separation procedure without production of large secondary waste streams, which combine the simple magnetic separation method with the high selective liquid extraction.

In the present work, the synthesis, characterization and applications of superparamagnetic polymer nanospheres were studied for radionuclides separation.

The magnetite (Fe_3O_4) with a mean diameter of 8nm was prepared by the coprecipitation of ferrous and ferric salts with NH_4OH. The magnetic Fe_3O_4 colloids in hydrophobic monomeric solvent were then introduced into aqueous surfactant solutions to form oil-in-water miniemulsion. By addition of oil-soluble initiator, the polymerization reaction in miniemulsions results in the formation of superparamagnetic polymer nanoparticles with diameter ranging from 100 to 400nm. Data obtained from XRD, TG and TEM indicate that the magnetite contents in particles are about 8–12%, and dispersed homogeneously throughout all particles. Without demolishing the magnetite within the particles have been used for introduction of functional groups, and finally was covalently immobilized on the surface of superparamagnetic polymer nanospheres with extractant agents (crown ether, etc.). The morphology and magnetic properties of the particles were examined by transmission electron microscopy (TEM), field emission scanning electron microscopy (SEM), show that all these particles have similar shape and structure. The VSM magnetometer analysis indicates that these kinds of particles exhibit superparamagnetic behavior with zero coercivity and remanence.

The batch experiments were performed for the radionuclides (strontium, etc) absorption and desorption and the inductivity coupled plasma mass spectrometry (ICP-MS) showed the separation recovery results.

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(PS1125) Oxidative degradation property of sulfonated fep / nafion hybrid proton exchange membranes for pefc. Naohiro Mitani, Yukiko Sato, Kazuki Fujii, Yuji Oshima, Jingye Li, Akihiro Oshima, Masakazu Washio. Advanced Research Institute for Science and Engineering, Waseda University, Tokyo, Japan.

In our resent study on development of polymer electrolyte fuel cells (PEFCs) membranes [1,2], the partial-fluorinated sulfonic acid (part-FSA) membranes based on crosslinked fluorinated-polymers have been fabricated by EB grafting method, and the obtained part-FSA showed high ion exchange capacity and power density.

In our durability study of part-FSA, it was indicated that the oxidative degradation is more suppressive to the sulfonated polytetrafluoroethylene (PTFE) with shorter length of grafted chain, compared with that the longer grafted one [3]. Therefore, it is necessary to control the number of grafting sites and the chain length.

According to our ESR spectroscopy, the number of trapped radicals in fluorinated ethylene-propylene co-polymer (FEP), which are grafting sites have been much higher than that of PTFE. In the case of part-FSA based on FEP, if the grafting yields would be as same as PTFE based one, the chain length of grafted -polymer should be shorter, compared with PTFE based one.

Thus, the part-FSA based on FEP (s-FEP) was fabricated by EB-grafting. The cell performance of MEA based on s-FEP was not so good at high temperatures due to the de-lamination of three-phase interface. In order to get well-laminated MEA, s-FEP/Nafion® hybrid (FN) was fabricated by adding same polymeric binder materials. The power density of obtained FN at 500mA/cm^2 and maximum density were higher than those of Nafion®112 [4].

In this study, in order to evaluate the chemical durability of obtained FN, the accelerated degradation test was carried out in 6vol.% H_2O_2 with 5ppm Fe^{2+} solution at 60°C. The properties of FN before and after degradation tests were measured by means of XPS and TGA, and so on. After oxidative degradation test, the FN became more suppressive to deteriorate than part-FSA based on FEP. This is regarded as a hydrophobic effect of Nafion®.

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(PS1126) A cohort study of thyroid cancer and other thyroid diseases after the Chornobyl accident: Dose-response analysis of thyroid follicular adenomas detected during first screening in Ukraine (1998–2000). Lydia Zablotska. Mailman School of Public Health, New York, NY, USA.

Context: The Chornobyl (Chernobyl) accident in 1986 exposed hundreds of thousands individuals in Ukraine, Belarus and the Russian Federation to radioactive iodine isotopes, primarily ^{131}I. The effects of this exposure in relation to benign thyroid diseases are largely unknown.

Objectives: To estimate the risk of follicular adenoma in relation to individual ^{131}I thyroid radiation dose; to investigate the effects of gender and other effect-modifying factors on the dose-response relationship.

Design: Baseline data from a prospective screening cohort study, 1998–2000.

Setting and Participants: A stratified random sample (N=32,385) was selected from all individuals who had thyroid radioactivity measurements taken within two months after the accident, and at the time of the accident were under the age of 18 years and resided in the three most heavily contaminated areas in Ukraine. Following an extensive tracing and recruitment process, 13,243 (67.5%) of those successfully traced and invited to participate in the study were screened for thyroid diseases. This analysis is based on 12,504 subjects with known history of thyroid diseases.

Main Outcome Measure: Cases with post-surgery pathomorphological diagnosis of follicular adenoma confirmed by an International Pathology Panel.

Results: Twenty-three cases of follicular adenoma were diagnosed. The dose-response relationship was linear with an excess relative risk of 2.07 per gray (95% confidence interval: 0.28, 10.31). The risk was significantly higher in women compared to men, but no clear modifying effects of age at exposure were observed.

Conclusions: Persons exposed to radioactive iodines as children and adolescents have an increased risk of follicular adenoma. Compared to other studies of follicular adenoma and ionizing irradiation, the estimate is smaller, but the confidence intervals overlap suggesting that they are compatible. Risk of follicular adenoma is significantly smaller than the risk of thyroid cancer in the same cohort.

(PS1127) Gastric cancer risk in relation to atomic-bomb radiation and the other risk factors -a nested case-control study. Saeko Fujiwara[1], Gen Suzuki[2], Harry Cullings[1], Nobuo Nishi[1], Midori Soda[3], Eiichi Tahara[4]. [1]Radiation Effects Research Foundation, Hiroshima, Japan, [2]National Institute of Public Health, Wako, Japan, [3]Radiation Effects Research Foundation, Nagasaki, Japan, [4]Hiroshima Cancer Seminar Foundation, Hiroshima, Japan.

Mortality and incidence studies in the Life Span Study at RERF demonstrated increased risk for gastric cancer with atomic-bomb radiation dose. But factors such as *H.pylori* infection and other risk factors were not taken into account in the analyses. Two histological types of non-cardiac gastric cancer, intestinal-type and diffuse-type, have different carcinogenesis pathways. The objective of this study was to determine the atomic-bomb radiation effects on histological type of gastric cancer, after adjusting for potential risk factors, such as *H. pylori* infection using serum collected prior to the development of gastric cancer.

The study was designed as a nested case-control study with 299 gastric cancer cases occurring in a cohort of about 20,000 individuals followed up biennially through health examinations in Hiroshima and Nagasaki since 1958. Three controls per case matched on birth year, gender, city, and counter-matched on radiation dose. There were 152 and 147 cases for intestinal-type and diffuse-type non-cardiac gastric cancer, respectively. Serological diagnosis of atrophic gastritis was based on pepsinogen method, and antibody to *H.pylori* was measured using serum samples obtained shortly before (2.3 years on average) of the development of gastric cancer.

H. pylori infection and atrophic gastritis were independent risk factors of both intestine-type and diffuse-type non-cardiac gastric cancer. Overall gastric cancer risk was marginally associated with

stomach dose, after adjusting for these risk factors. Diffuse-type non-cardiac gastric cancer increased with radiation dose among non-smokers (relative risk [RR] 4.0, 95% confidence interval [CI] 1.0–6.9, p=0.04)), but the intestinal-type was not related to radiation dose (RR 0.8, 95%CI 0.4–1.2, p=0.3).

The nested case-control study among A-bomb survivors suggested that effects of radiation exposure on diffuse-type non-cardiac gastric cancer were observed for non- smokers, but not on intestinal-type. The present results should be validated in a future large scale of pathological review study.

(PS1128) Non-cancer effects in the cohort of workers of the first Russian nuclear facility. Tamara V. Azizova[1], Colin R. Muirhead[2], Maria B. Druzhinina[1], Evgenia S. Grigoryeva[1], Elena V. Vlasenko[1], Margarita V. Sumina[1], Zinaida D. Belyaeva[1], Jackie A. O'Hagan[2], Wei Zhang[2], Richard G.E Haylock[2]. [1]Southern Urals Biophysics Institute, Ozyorsk, Russian Federation, [2]Health Protection Agency, Chilton, United Kingdom.

Cancer risk in humans exposed to ionizing radiation has been widely published in a number of papers. At the same time, there is insufficient information to answer a question about possible non-cancer effects of chronic exposure, especially internal exposure to incorporated plutonium-239.

Cohort of workers of Mayak Production Association, the first Russian nuclear enterprise, as a feasibility study has shown, provide such an opportunity to study morbidity and mortality from non-cancer diseases, taking into account radiation and non-radiation factors. This is because:

- medical follow-up of Mayak workers was carried out on a regular basis according to a standard specially-developed program at the same medical institution;
- vital status is known for 90.1%;
- date of "last medical information" is known for each worker from the cohort, and date of migration from the city, which coincides with the date of "last medical information" in 93%, is known for migrants;
- for about 94% of workers, initial health status before employment at Mayak PA is known from the obligatory pre-employment medical examination;
- high quality information is available on disease morbidity for about 94% of workers owing to the expertise of the medical staff, who observed Mayak workers since Mayak start-up, and to the maintenance of a diagnostic base which was up-to-date for each follow-up period, and this was confirmed by the verification of diagnoses by highly-qualified experts;
- cause of death is known for 99.8% of workers in the study cohort, for whom death was ascertained; autopsy rate is 34% for the whole cohort and 62% for Ozyorsk residents;
- this is one of the few studies of radiation workers to have collected detailed data on potential confounding factors such as smoking, alcohol, blood pressure, body mass index (for more than 90% of workers from the cohort);
- measured individual doses of external gamma and internal alpha exposures are available for 99% and 34% of workers from the study cohort respectively;
- complete information on detailed work history is available for each worker from the study cohort;
- this study has rather high statistical power.This study is funded by the European Commission and RF Federal Medical-Biological Agency within the framework of the project No. FIP6R-516478 "Southern Urals Radiation Risk Research (SOUL)".

(PS1129) Multivariate analysis of effects of radiation and non-radiation risk factors on kidney cancer incidence among Mayak PA nuclear workers. Galina V. Zhuntova, Zoya B. Tokarskaya, Zinaida D. Belyaeva, Evgenia S. Grigoryeva, Viktor A. Syrchikov. Southern Urals Biophysics Institute, Ozyorsk, Russian Federation.

Kidney is considered to be the organ resistant to radiation exposure, however, some studies have shown an increased risk of kidney cancer among patients who underwent gamma-therapy, ^{131}I or ^{224}Ra treatment after Thorotrast injection for diagnostic purposes. To estimate the contributions of radiation (chronic whole-body external gamma exposure, internal alpha exposure due to inhalation of ^{239}Pu aerosols) and non-radiation (smoking, alcohol consumption, chronic inflammatory diseases of urinary system, chemical agents) factors on kidney cancer incidence among Mayak nuclear workers, nested case-control study was performed.

Case group included 83 morphologically verified cases of kidney carcinoma diagnosed among Mayak workers during 1977–2004, and control group included 166 Mayak nuclear workers without malignant neoplasm. Control was matched by sex, age (± 5 years), year of started work at Mayak Production Association (± 2 years), and type of plant. Males made up 80% of the study individuals. Average age at diagnosis was 61.6 ± 1.0 years, duration of period from started occupational radiation exposure till tumor detection was 35.5 ± 1.1 years. Range of whole-body external gamma exposure was 0 to 7.21 Gy, and range of ^{239}Pu body burden was 0 to 16.3 kBq.

Multiple conditional logistic regression was used to estimate adjusted odds ratios and 95% confidence intervals. Attributive risk was also calculated. The study revealed the significant effect of smoking on development of kidney cancer among Mayak nuclear workers: adjusted odds ratio was 2.5 (with 95% confidence interval of 1.5–4.4) for smokers in comparison with non-smokers and those who stopped smoking, attributive risk was 28%. No effect of chronic whole body gamma exposure within the range of 0 to 7.2 Gy, ^{239}Pu body burden within the range of 0 to 16.3 kBq, chronic urinary system diseases, alcohol consumption, and work in the chemical industry before employment at Mayak Production Association on kidney cancer incidence was found.

(PS1130) Minimize offspring radiation exposure following intake of radionuclides by the mother. Hamid Samavat[1], Ali Shabestani Monfared[2]. [1]Hamedan University of Medical science, Hamedan, Iran (Islamic Republic of), [2]Babol University of Medical science, Babol, Iran (Islamic Republic of).

The female patient particularly in childbearing age is of great concern in evaluating possible radiation dose to the embryo and fetus and of course the mother itself. Concern about reducing mother and the embryo dose are addressed through a study of radiation dose in female patients.

It was found that there is a lack of information in medical staff and nurses who provide care for nuclear medicine patients regarding the radiation dose in pregnancy and breast feeding. Many radiopharmaceuticals are administered to women in age of 20–50 years; but with a little knowledge about how much radionuclide crosses the placenta and excretion in breast milk during and after pregnancy. There was no developed model of dose measurement to the offspring following the intake of radionuclides by the mother!

This study suggest that; Medical staff who provide care for female nuclear medicine patients need periodic radiation safety training that is specific for the unique aspects associated with each type of treatment. These training should include the pass way of radionuclide from maternal blood and tissues of mother to the embryo, the physical and biological half-life, formation of decay products and growth of offspring.

(PS1131) Environmental radiation and breast cancer incidence in the Techa River Cohort. Evgenia Ostroumova[1], Dale Preston[2], Elaine Ron[1], Ludmila Krestinina[3], Faith Davis[4], Alexander Akleyev[3]. [1]National Cancer Institute, NIH, Bethesda, MD, USA, [2]Hirosoft International Corporation, Eurika, CA, USA, [3]Urals Reserach Center for Radiation Medicine, Chelyabinsk, Russian Federation, [4]University of Illinois at Chicago, Chicago, IL, USA.

While studies of A-bomb survivors and female patients exposed to medical X-rays have shown that the female breast is especially sensitive to the carcinogenic effects of ionizing radiation, little is known about risks for low-dose chronic environmental exposure. We studied breast cancer risk in women chronically exposed to radioactive waste released into a river from a nuclear weapons facility.

The routine discharge of radioactive waste, a byproduct of production of plutonium at the Mayak Nuclear Facility, into the Techa-Iset river system in the Southern Urals region of Russia resulted in substantial external and internal radiation exposure to residents of the riverside area from 1949 to 1956. External exposures to gamma-radiation were primarily due to Cs-137. Internal exposures were largely determined by consumption of river water for drinking, cooking and other domestic needs. The median, mean and maximum stomach dose from external γ-rays and incorporated ^{137}Cs was 8, 40, and 470 mGy, respectively.

Almost 10,000 women living near the Techa River were followed for cancer incidence from 1956 through 2002 (257,607 person-years of follow-up). Forty-two percent of the cohort were first exposed to radiation before age 20; 68% were Slavs and 32% were Tartars or Bashkirs. At the end of follow-up, 37% of the cohort was alive, 48% had died and 14% were lost to follow-up. A total of 104 primary breast cancer cases fitting the study criteria were diagnosed.

As expected, breast cancer incidence rose with age and years of follow-up. Risk of breast cancer among nulliparous women was 2.7 times higher compared with women who had 3 or more children (95% CI: 1.5 - 5.1). Slightly higher risk (RR=1.12; 95% CI:0.7 - 1.8) was found among women whose age at first full-term pregnancy was 25 and over compared with women under 25 at first full-term pregnancy. Breast cancer incidence among Tartars and Bashkirs was slightly lower then among Slavs. Breast cancer risk increased with dose after adjustment for non-radiation risk factors ($p<0.01$). The attributable risk was 13.8% with approximately 14 breast cancers estimated to be radiation related. Because the number of breast cancers was small, no significant effect modification was observed, but females who were under age 10 at first exposure appeared to have the highest risk.

(PS1132) Considerations in the comparison of cancer risk estimates for a Japanese Thorotrast cohort and the Atomic Bomb Survivors. Harry M. Cullings[1], Takesaburo Mori[2], John B. Cologne[1], Yukiko Shimizu[1]. [1]Radiation Effects Research Foundation, Hiroshima, Japan, [2]Yokohama City University School of Medicine, Yokohama, Japan.

Comparison of radiation-related cancer risk in a Japanese Thorotrast cohort (n =1,912, 284 injected with Thorotrast) to a subset (n = 6,411) of the Life Span Study (LSS) cohort of Atomic Bomb Survivors affords a unique opportunity to compare fundamentally different types of radiation exposure in demographically similar groups: Japanese men aged 20 to 40 in 1945. Thorotrast, a radiological contrast agent containing $^{232}ThO_2$, remained as insoluble deposits in the body. Comparison of mainly low-LET, acute external exposure (atomic bombs) to mainly high-LET, chronic internal exposure (Thorotrast) is a type of comparison of major interest to radiation protection but has not generally been possible. It simultaneously involves two simpler comparisons usually formulated as ratios: radiobiological effectiveness (RBE) and dose and dose rate effectiveness factor (DDREF). If DDREF depends on RBE this involves two equivalent combinations of RBE-specific DDREF and LET-specific RBE whose products must by definition be equal. Thorotrast cases are based exclusively on autopsy whereas LSS data are death certificate mortality and tumor registry incidence. To establish a comparable outcome measure we are attempting to estimate cancers prevalent at death in the LSS subset using an overlapping LSS subset with autopsy results, a previous pathology review of liver cancer, etc. A related concern is paucity of data distinguishing hepatocellular carcinoma from hemangiosarcoma. We revised and extended Thorotrast alpha dosimetry with new nuclear data and data on partitioning into major sites of deposition (liver, spleen, bone marrow) and used Medical Internal Radiation Dose (MIRD) methods to calculate electron and photon dose to those sites and other organs of possible interest such as pancreas. Lagged cumulative alpha dose to liver from Thorotrast is ~40x > electron dose and ~70x > photon dose, and tends to be >> liver dose in the LSS. A possible selective effect of military service on general health of both cohorts prior to exposure is a concern as the Thorotrast cohort were soldiers and the LSS was depleted in young men healthy enough for military service. Possible indirect or non-radiological effects of Thorotrast must also be considered. All-causes mortality in Thorotrast exposed is >> atomic bomb exposed or unexposed in either cohort.

(PS1133) Combination study of indoor radon, gamma activity & inhalation doses in the dwellings of Punjab and Tusham Ring Complex, Haryana. Bikram jit Singh Bajwa, Harmanjit Singh Sandhu, Joga Singh. Guru Nanak Dev University, Amritsar, Amritsar, India.

Since the natural occurring radio-nuclides are responsible for the major contribution to the total effective dose of the ionizing radiations received by the population, so keeping this in view the indoor radon and gamma activities have been carried out in the dwellings of different villages of Punjab and villages situated in and around the Tusham Ring Complex, Bhiwani district, Haryana, known to be composed of acidic volcanics and the associated granites. The LR-115 type-II plastic track detector has been used for measuring the indoor radon levels & inhalation doses in the wide range of dwellings of these villages. *The variations in the indoor radon concentration levels of the two regions have been compared for a data set of two year*. The radon concentration levels have not only been found to be varying with the mode of construction of houses & building materials utilized, but also especially on their location with respect to the nearby high heat producing granite rocks. The indoor radon concentration levels at some places, especially those on or close to granite rock formations have been found to be considerably higher and even at some places it also exceeds the upper limit of the proposed action levels. A good correlation between indoor radon and gamma activities has been found in case of dwellings situated around the exposed high heat producing granitic rock formations.

(PS1134) Lung cancer mortality after exposure to fractionated ionizing radiation in a cohort of Massachusetts tuberculosis patients. Alina V. Brenner, Ethel S. Gilbert, Charles E. Land. Radiation Epidemiology Branch, Bethesda, MD, USA.

We examined lung cancer mortality (through December 31, 2002) in a cohort of 13,572 patients diagnosed with pulmonary tuberculosis (TB) between 1925 and 1954 and discharged alive from 12 Massachusetts hospitals. Forty-seven percent were treated by artificial pneumothorax that required regular fluoroscopic examinations (on average 77 over 32 months resulting in a mean lung dose of 0.84 Gy) and 53% were treated by conventional means. In both groups, 81% of the patients had died and 3% were lost at the end of follow-up. In total, there were 357 deaths from lung cancer. Our dose-response analysis, using a Cox proportional hazard model adjusted for year of birth, gender, smoking status, history of thoracoplasty, TB stage, and age at 1st exposure, found no significant dose response for lung cancer mortality; the relative risk (RR) at 1 Gy was 0.96 with 95% confidence interval (CI) 0.82–1.13. This estimate was essentially unaffected when the first 10 years of follow-up after initial exposure were excluded or when analyses were limited to the exposed group. Radiation-related lung cancer risk did not vary by the adjustment factors or attained age or time since 1st exposure. Our findings are consistent with those obtained in a larger Canadian cohort of TB patients (RR at 1 Gy 1.00, 95% CI: 0.94–1.07) but contrast with a study of atomic bomb survivors (RR at 1 Gy 1.60, 95% CI: 1.27–1.99). In the Canadian study, such differences were interpreted as supporting a substantial fractionation/dose-rate effect for radiation and lung cancer risk, a conclusion that was partially supported by the lack of evidence for dose-related misclassification of lung cancer deaths as deaths from TB. However, in our study TB stage was available for a higher proportion of subjects, and we observed dose responses for TB deaths for which stages were originally classified as minimal (RR at 1 Gy 1.49, 95% CI: 1.16–1.90) and moderate(RR at 1 Gy 1.13, 95% CI: 0.98–1.31). These dose responses, and their relative RR values, are what would be expected if lung cancer deaths were being misdiagnosed as TB. Thus, the lack of lung-cancer dose-response in this study, and perhaps in others, should be interpreted cautiously.

(PS1135) Association of radiation exposure, inflammation, and cancer incidence in atomic bomb survivors - an application of causal pathway model. Kazuo Neriishi, Wan-Ling Hsu, Nobuo Nishi. Radiation Effects Research Foudation, Hiroshima, Japan.

Increasing evidence from the study results of A-bomb survivors has indicated that radiation still causes significant increase in inflammatory markers years after the exposure. With the emerging view that cancer to arise in chronically inflamed tissue(s), it is essential to apply modern statistical models to disentangle the complex association among radiation, inflammation, and cancer. Due to these causal relations (direct and indirect pathways), traditional models, such as logistic or Cox regression models, may underestimate the total radiation effect on cancer incidence. This study aims to apply structural models to fully reflect and capture the health effect of radiation exposure.

There were about 13,000 A-bomb survivors participating in the bi-annual Adult Health Study in 1960. After excluding subjects with existing cancer, not in the cities at bombing, missing covariate information, and diagnosed with cancer within two years of baseline examination, there were 6,444 in the study. They were followed up till 2000 for cancer incidence. Logistic, Cox, and causal pathway models were investigated. In the pathway model, it was hypothesized that radiation, cigarette smoking, and total white blood cell (WBC) count are risk predictors of cancer incidence. The effects of smoking and radiation were also mediated through WBC count. The average of WBC count of the first three exam cycles was used to construct a dichotomous WBC indicator (top quartile is the cut point). Multiple logistic regression functions were estimated in the pathway model.

These models consistently showed significant radiation effect on cancer incidence. However, the pathway model showed significant indirect radiation effect on cancer incidence for subjects exposed under age 15 at 95% confidence level. The attributable proportion of indirect radiation effect on cancer accounted for 1.1% and 1.4% for females and males exposed under age 15, respectively. These attributable proportions declined and became insignificant for older exposed cohorts.

(PS1136) Projected update of the National Institutes of Health radioepidemiological tables and interactive radioepidemiological program (IREP). Charles E. Land[1], Ethel S. Gilbert[1], Deukwoo Kwon[1], F Owen Hoffman[2], Apostoaei Iulian[2], Brian Thomas[2], David C. Kocher[2]. [1]National Cancer Institute, Bethesda, MD, USA, [2]SENES Oak Ridge, Oak Ridge, TN, USA.

The 1985 NIH Radioepidemiological Tables were developed to quantify the actuarial likelihood that a given cancer, diagnosed following a given history of radiation exposure, could be attributed to that exposure history. In 2003 the tables were replaced by IREP, an interactive, web-based computer program, and the underlying statistical information was updated to reflect more recent data. IREP calculates estimates and probabilistic uncertainty distributions for the assigned share (AS, also called "probability of causation", or PC). AS is defined as AS = (RR-1)/RR, where RR is the estimated relative risk in a hypothetical population similar to the cancer case with respect to sex, cancer site, age at cancer diagnosis, and exposure history. IREP, in its original form and as adapted by the National Institute for Occupational Safety and Health (NIOSH-IREP), is used for adjudication of compensation claims against the US government for cancers possibly linked to occupational radiation exposure with the military, the US Dept. of Energy, or its contractors. It is worth emphasizing that IREP does not specify how a claim should be adjudicated but, rather, provides a comprehensive summary of the relevant scientific information in a compact and usable form. Several improvements to IREP are planned for the near future. It will be updated to reflect new epidemiological data including A-bomb survivor tumor registry data through 1998, published by the Radiation Effects Research Foundation, and recommendations by the NAS BEIR VII committee. Given international interest in problems of adjudicating similar compensation claims, we are considering modifications to IREP that would allow it to be used for applications in other countries. A provision for uploading data files containing appropriate population-specific baseline cancer rates would allow the calculations to be tailored to a specific population. Another project is the ongoing development of an IREP-based lifetime risk calculator, which will require input of both site-specific, baseline cancer rates and statistical life tables for applications to different populations.

(PS1137) Longitudinal trends of total white blood cell and differential white blood counts of atomic bomb survivors. Wan-Ling Hsu, Yoshimi Tatsukawa, Michiko Yamada, Kazuo Neriishi. RERF, Hiroshima, Japan.

The total white blood cell (WBC) and differential WBC counts are essential markers for inflammation. Earlier findings of the atomic bomb survivors indicate an increased average WBC and differential WBC counts with radiation dose in the cross-sectional study (Sawada et al, 1986). However, it is unclear whether the radiation-response WBC and differential WBC counts continue to remain elevated over the years. It is also of interest to examine how aging and other risk factors, such as smoking, affect WBC level. The objective of this study aims to depict longitudinal trends of WBC and differential WBC counts in association with different levels of radiation exposure, as well as an overview of the aging process in these inflammatory markers.

This study investigated 7,562 (2,741 male and 4,821 female) A-bomb survivors who participated in the biennial Adult Health Study from 1964 to 2004. A linear mixed model was applied to account for inter- and intra- individual variations, using the repeated leukocyte measurements. Various models were tested for selecting an appropriate age pattern and the best-fitting variance-covariance structure. The comparison of Akaike's Information Criterion (AIC) statistic indicates that an unstructured covariance matrix assumption seems to be the best. A quadratic age function will be modeled for men and women, respectively.

First, we estimated the background model limited to the unexposed or exposed $\leq$ 5 mGy. The total WBC and differential WBC counts declined with age. Smoking results in significantly higher WBC and differential WBC counts over time, for both men and women. Secondly, the full study sample was examined and the effect of radiation exposure on WBC and differential WBC counts was taken into consideration. Though smoking accounted for the major increased proportion, radiation related increase of neutrophil was observed among the subjects who were exposed younger than 10 years old. Furthermore, statistically significant decrease of lymphocyte was observed among older exposed subjects. In conclusion, the radiation effect on longitudinal trend of WBC may be associated with consequent disease development and occurrence.

(PS1138) Gene alterations preferentially occurred in adult-onset papillary thyroid cancer among atomic bomb survivors. Kiyohiro Hamatani[1], Hidetaka Eguchi[1], Masatak Taga[1], Keiko Takahashi[1], John Cologne[1], Midori Soda[2], Kuniko Abe[3], Tomayoshi Hayashi[3], Koji Arihiro[4], Yuzo Hayashi[5], Kei Nakachi[1]. [1]Radiation Effects Research Foundation, Hiroshima, Japan, [2]Radiation Effects Research Foundation, Nagasaki, Japan, [3]Nagasaki University Hospital Medicine and Dentistry, Nagasaki, Japan, [4]Hiroshima University Hospital, Hiroshima, Japan, [5]Geriatric Health Service Facility Hidamari, Hiroshima, Japan.

Thyroid cancer is one of the malignancies most closely associated with ionizing radiation. *RET*/*PTC* rearrangement was frequently observed in child-onset papillary thyroid cancers in post-Chernobyl and also in second-primary papillary thyroid cancers among patients with history of radiation therapy. We have previously reported that *RET*/*PTC* rearrangement was directly induced by X-irradiation in vitro and in vivo. On the other hand, *BRAF* point mutation has been reported to be the most common gene alteration found in non-exposed adult-onset papillary thyroid cancers. However, the mechanisms of how A-bomb exposure influences development of papillary thyroid cancer are not fully clarified. To clarify the mechanisms underlying papillary thyroid carcinogenesis in A-bomb survivors, we have examined *RET*/*PTC* rearrangements and *BRAF* point mutation in 71 papillary thyroid cancer cases (including 21 non-exposed cases) from A-bomb

survivors, in relation to radiation dose as well as years elapsed since A-bomb radiation exposure. We found that *BRAF* point mutation became less frequent in cases exposed to higher radiation dose (P_{trend}= 0.002). Conversely, *RET*/*PTC* rearrangements and other unknown alterations showed increased frequency with increased radiation dose ($P_{trend} < 0.001$ and 0.076, respectively). Papillary thyroid cancer patients harboring *RET*/*PTC* rearrangements contracted cancer sooner after radiation exposure than did patients harboring *BRAF* ^{V600E} point mutation ($P = 0.029$). These results suggest that *RET*/*PTC* rearrangements played an important role in radiation-associated thyroid carcinogenesis.

(PS1139) Effects of inflammation-related gene polymorphisms and atomic-bomb radiation exposure on gastric cancer risk. Tomonori Hayashi[1], Yukari Morishita[1], Hiroko Nagamura[1], Mayumi Maki[1], Misae Sora[1], Kazue Imai[1], Kengo Yoshida[1], Yoichiro Kusunoki[1], Eiichi Tahara[2], Kei Nakachi[1]. [1]Radiation Effects Research Foundation, Hiroshima, Japan, [2]Hiroshima Cancer Seminar Foundation, Hiroshima, Japan.

The purpose of this study is to clarify immune defense mechanisms against cancer, relationship between host immune function and persistent inflammation, relationship between persistent inflammation and cancer, effects of radiation exposure and aging on immune host factors, inter-individual variation in cancer susceptibility, and genetic factors involved in these phenomena. We have found that advancement of persistent inflammatory conditions with age is accelerated among people exposed to atomic-bomb (A-bomb) radiation. We therefore examined the relationship between gastric cancer risk and radiation dose based on inflammation-related gene polymorphisms. In this study, we first identified haplotypes based on polymorphisms of the immunosuppressive/inflammation-related cytokine *IL-10* gene to determine relationship between genotypes/haplotypes and gastric cancer risks and effects of radiation exposure on that relationship. Based on the Adult Health Study cohort study of A-bomb survivors, we studied a gastric-cancer group (238 cases) and a control group (1,757). We identified a single haplotype block composed of four htSNPs (forming two alleles, *IL10-ATTA* and *IL10-GGCG*). Odds ratio of gastric cancer incidence among non-exposed individuals having the *IL10-GGCG*/*IL10-GGCG* haplotype, a homozygote of the *IL10-GGCG* alleles, showed a significantly high value of 2.46 (95% CI: 1.28–4.70). Gastric cancer risk of exposed individuals having *IL10-GGCG*/*IL10-GGCG* was even higher. Association between the *IL-10* haplotype and plasma IL-10 levels was also examined in 615 noncancerous subjects. Levels of IL-10 in the blood of individuals having *IL10-ATTA*/*IL10-GGCG* or *IL10-GGCG*/*IL10-GGCG* among non-exposed controls were significantly higher than those of individuals having *IL10-ATTA*/*IL10-ATTA*. Comparison of plasma levels of IL-10 in non-exposed and exposed individuals having *IL10-ATTA*/*IL10-ATTA* showed higher values for the exposed. This suggests that level of IL-10 in blood is increased not only by genetic effects but also by radiation effects and is closely related to gastric cancer risk. We further analyzed association between the IL-10 haplotype and risk of gastric cancer by histological type of this cancer, since the diffuse type was found to more closely relate to radiation exposure.

(PS1140) Fate of irradiated human fibroblasts: senescence or genetic instability and crisis? Claudia Fournier, Marcus Winter, Sebastian Zahnreich, Sylwester Sommer, Larissa Melnikova, Elena Nasonova, Sylvia Ritter. GSI, Darmstadt, Germany.

The purpose of this study was to follow up the fate of the progeny of human fibroblasts, exposed to different single and fractionated doses of X-rays and subcultured regularly up to the end of their lifespan. During the first weeks after exposure, the expression of cell cycle inhibiting proteins (p21, p16) was elevated and the BrdU labeling index was low. Enhanced activity of β-galactosidase and morphological changes indicated an increased amount of senescent and terminally differentiated cells. However, quantification of proteins in single cells revealed low protein amounts of cell cycle inhibitors and continuous proliferation in a subpopulation of cells, their number depending on the initially delivered dose. Among these cells, the amount of structural and numerical chromosomal aberrations assessed by solid staining (Giemsa) was not different from controls. During a transition period, the control-like cells dominated the populations and the described changes of the markers of senescence and differentiation were no more detectable. However, in the progeny of cells exposed to a high dose of X-rays, cytogenetic analysis by multiplex colour FISH analysis revealed that the frequency of transmissible aberrations was clearly elevated, regardless whether a single or a fractionated dose had been delivered. Among them, abnormal clones of cells (bearing identical aberrations) emerged. In addition, at 4 to 5 months post-irradiation newly formed structural and numerical aberrations were detected. They were accompanied by an enhanced proliferation activity, changes in the expression pattern of cell cycle regulating proteins and a high rate of cell death, indicating genetic instability and crisis. In contrast, the progeny of low dose irradiated cells, cellular and molecular changes associated with senescence appeared again, and now, simultaneously with the controls. Taken together, depending on the dose delivered months before, senescence or genetic instability and crisis were observed in the descendant cells. Based on a more detailed analysis of the detected transmissible and *de novo* formed aberrations the possible impact on the fate of the cells will be discussed. Furthermore, first results on the longterm effects of high LET exposure will be presented.

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(PS1141) Microsatellite instability and related gene alterations in radiation-associated colorectal cancer from atomic-bomb survivors. Hidetaka Eguchi[1], Kiyohiro Hamatani[1], Masataka Taga[1], Hiroaki Katayama[1], Kazunori Kodama[1], Eiichi Tahara[1], Shizue Izumi[2], Shunji Matsumura[3], Naohide Oue[3], Wataru Yasui[3], Kei Nakachi[1]. [1]Radiation Effects Research Foundation, Hiroshima, Japan, [2]Oita University, Oita, Japan, [3]Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan.

Effects of radiation exposure on risk of solid cancers differ by organ in the Life Span Study (LSS) of atomic bomb (A-bomb) survivors. Colon cancer showed a high excess relative risk per Sv of 0.72 (95% confidence interval, 0.29–1.3) for radiation exposure. On the other hand, interestingly, rectal cancer did not show risk elevation for radiation exposure. Among two major molecular events, namely chromosomal instability and microsatellite instability (MSI) in colorectal carcinogenesis, high-MSI (MSI-H) is correlated with a specific subsite of the colon - frequently in the proximal colon. We then hypothesized that MSI phenotype may be associated with radiation exposure among A-bomb survivors. At first, we determined MSI status in 24 colon and 11 rectal cancers from A-bomb survivors with definite radiation dose, in terms of six microsatellite markers (*BAT25*, *BAT26*, *NR-21*, *D2S123*, *D5S346* and *D17S250*). As a result, five MSI-H cases were found in the proximal colon and none in the rectum. The median radiation dose of MSI-H colorectal cancer cases was significantly higher than that of MSS/MSI-L cases (P=0.042). Methylation of the DNA repair gene *MLH1* is a major cause of MSI-H in sporadic colorectal cancer, and therefore, this was studied using combined bisulfite restriction analysis. Methylation of the *MLH1* gene was found in five cases, whose median radiation dose was higher than that of *MLH1*-unmethylated cases (P=0.059). This gene methylation was also associated with MSI status in this study (P=0.017). Furthermore, other MSI-related early molecular events, namely gene alterations relative to Ras-signaling, were studied in relation to radiation dose. On the basis of methylation of the *RASSF2* gene and mutations of the *KRAS* and *BRAF* genes, we found a significant increasing trend of radiation dose with increased number of gene alterations (P=0.016). This Ras-signal alteration status was also found to be closely related to not only MSI but also *MLH1* gene methylation (P=0.045 and P=0.044, respectively). In fact, all MSI-H and *MLH1*-methylated cases were found to carry this alteration. The results thus far obtained imply that radiation exposure may influence MSI status and its related epigenetic and genetic alterations in colorectal carcinogenesis, although further analysis with increased number of cases is required.

(PS1142) Chromosome mutations in iss crew members participating in short duration missions. Wolfgang Goedecke, Alexandra Antonopoulos, Markus Horstmann, Guenter Obe, Christian Johannes. University Duisburg-Essen, Essen, Germany.

Astronauts, cosmonauts and an increasing number of space tourists are currently visiting the International Space Station (ISS) demonstrating the onset of the colonisation of extraterrestrial environments. On board of the ISS, members of the crew are exposed to ionising radiation of cosmic and solar origin, while on the Earth's surface people are well protected by the atmosphere and a deflecting magnetic field. There are now data available for dose and quality of ionising radiation on board of the ISS, but their contribution to mutations of crew members are hard to predict. One reason for this difficulty is the possibility of synergistic effects between radiation and microgravity, which disables the simple transfer of dose response relations from terrestrial environments to those in space. Therefore direct measurements of mutation rates in space are required.

In order to estimate the effect of cosmic and solar radiation on ISS crew members, we present data of chromosome aberration (CA) analyses from lymphocyte metaphase spreads of six ISS crew members. All test persons performed a single one-week shuttle-flight. Therefore exposure to ionising radiation inside the ISS should be comparable to all test persons. Metaphases were scored for CA in preflight and postflight blood samples and the mutation rates were compared. From each sample at least 1000 metaphases were stained with Giemsa and at least 200 metaphases were analysed by m-FISH. Giemsa stained slides were scored for dicentric- and acentric fragments, ring chromosomes and chromatid breaks. Translocations, insertions and complex aberrations were scored from m-FISH karyotypes. Out of the six astronauts analysed in this study, four have performed an extra-vehicular activity (EVA). During an EVA, astronauts were directly exposed to radiation and are not protected by shielding effects of the ISS. Our data suggest that EVAs up to eleven hours do not significantly contribute to CA in ISS crew members.

(PS1143) Interaction among genes influences DNA repair capacity in young lung cancer patients. Sabine Hornhardt[1], Ute Roessler[1], Albert Rosenberger[2], Wiebke Sauter[3], Heike Bickeboeller[2], Thomas Illig[3], Heinz-Erich Wichmann[3], Maria Gomolka[1]. [1]Federal Office for Radiation Protection, Oberschleissheim, Germany, [2]University of Goettingen, Goettingen, Germany, [3]GSF-Research Center, Neuherberg, Germany.

Individuals bearing a radiation sensitive phenotype are often more likely to develop cancer and vice versa. This may be due to defects in genes that are responsible for the integrity of the genome or DNA repair. In contrast to older lung cancer patients, the early onset of the disease in the young, age of 50 years or younger, representing 8% of lung cancer patients, is most likely promoted by genetic components. Goal of the presented project is to identify genes which may contribute to an increased *in vitro* radiation sensitivity as measured by the comet assay. These results are correlated to the lung cancer phenotype.

In an ongoing case control study, initial DNA damage after gamma irradiation with a ^{137}Cs-source (0 Gy, 1 Gy, 2 Gy and 4 Gy) and DNA repair capacity was measured after 10 min, 30 min and 60 min in 100 patients and 100 healthy controls. Single nucleotide polymorphism (SNP) analysis of DNA repair genes *XRCC1-G280A*, *XRCC1-G399A* and *XPD-G312A* was performed by MALDI TOF MS (matrix assisted laser desorption ionisation time of flight mass spectrometer).

No difference between total cases and controls was observed for either induction of DNA damage by irradiation or DNA repair capacity. However, a small group of patients carrying the variant allel A of the XRCC1 gene at position 280 demonstrated reduced DNA repair capacity. When tested in a linear model the genetic impact of this variant was significant ($p = 0.04$) and displayed the highest effect size compared to other factors like smoking, age, sex or radiation therapy. Moreover immortalized lymphoblastoid cell lines from the patients showed decreased survival after irradiation.

The *XRCC1-G280A* genotype influences DNA repair capacity in young lung cancer patients but not in healthy controls. This is possibly due to interaction with genes harboured by lung cancer patients predisposing for cancer.

(PS1144) Radiomodulatory effect of G*rewia asiatica* on liver of Swiss albino mice. K.V. Sharma, Muktika Ahaskar, Smita Singh, Rashmi Sisodia. Department of Zoology, Jaipur, India.

Increasing use of nuclear radiation for human welfare necessitates a new, safe and cost effective radio protector not only for personnel's charged with responsibility of testing or with radiations in laboratories, but also for the general public. Keeping this in view, the study has been undertaken to explore the nutraceutical potential of the *Grewia asiatica* fruit against radiation-induced stress. It has high content of antioxidants like vitamin C, anthocyanin, carotenes and folate that may play a possible role in radioprotection .For experimental study; healthy Swiss Albino mice were selected from an inbred colony and divided into four groups. Group I (normal) did not receive any treatment. Group II was orally supplemented (GAE) at the dose of 700 mg / Kg.b.wt for fifteen consecutive days. Group III (control) received distilled water orally equivalent to GAE for fifteen days than exposed to 5 Gy of gamma radiation. Group IV (experimental) was administered orally (GAE) for 15 consecutive days and exposed to single dose of 5Gy of gamma radiation. Mice were sacrificed at different post irradiation intervals viz. 1, 3, 7, 15 and 30 days. Liver were removed for various biochemical estimations viz. glutathione (GSH), lipid peroxidation (LPO), alkaline phosphatase and acid phosphatase. GAE pre - treatment renders protection against various biochemical changes in mice liver. Radiation induced augmentation in GSH and LPO was significantly ameliorated by GAE. Percentage protection provided by dietary supplementation of GAE prior to irradiation was 49.5% and 9.12% in GSH and LPO respectively, at day 30 post irradiation.It was also observed that GAE treatment after irradiation was highly effective protector against radiation induced damage in liver.

(PS1145) Dietary influence of Grewia asiatica against radiation induced damage on non-cell renewal system of swiss albino mice. Muktika Ahaskar, K.V. Sharma, Smita Singh, Rashmi Sisodia. Department of Zoology, Jaipur, India.

Increasing use of nuclear radiation for human welfare necessitates a new, safe and cost effective radio protector not only for personnel's charged with responsibility of testing or with radiations in laboratories, but also for the general public. Keeping this view, this study has been undertaken to find out the possible radio protective potential of the *Grewia asiatica* fruit extract (GAE). It has a high content of antioxidants like Vitamin C, anthocyanin and folate that may play a possible role in radioprotection. For experimental study, healthy Swiss Albino mice were selected from an inbred colony and divided into four groups. Group I (normal), did not receive any treatment. Group II, was orally supplemented (GAE) once daily at the dose of 700 mg / Kg.b.wt / day for fifteen consecutive days. Group III, (control) received distilled water orally equivalent to GAE for fifteen days than exposed to 5 Gy of gamma radiation. Group IV (experimental) was administered orally (GAE) for 15 consecutive days once daily and exposed to single dose of 5Gy of gamma radiation. Mice were sacrificed at different autopsy intervals viz. 1–30 days. Whole brain and Cerebellum was used for various biochemical estimations. Radiation induced augmentation in LPO and conjugated dienes(CD) was significantly ameliorated by GAE. Whereas deficit produced in GSH, and protein content by radiation could be elevated indicating that GAE pre - treatment renders protection against various biochemical changes in mice cerebellum and whole brain.

(PS1146) Role of *Grewia asiatica* as a potent radioprotector. Smita Singh, K.V. Shrama, Muktika Ahaskar, Rashmi Sisodia. Department of Zoology, Jaipur, India.

The radioprotective effects of *Grewia asiatica* fruit against radiation induced hematological and biochemical alterations in peripheral blood and the survival of Swiss albino mice were studied. *Grewia asiatica* (Phalsa), indigenous plant of Indian subcontinent also found in abundance in western Rajasthan, is known to have ethno medicinal properties. It is rich in antioxidants like vitamin c, anthocyanin and folate that play an important role in radioprotection. A regression analysis of survival data obtained after 5,7, 10 and 12 Gy irradiation to mice yielded $LD_{50/30}$ as 6.21 and 9.53 Gy for the control and experimental group respectively. It and produced a dose reduction factor (DRF) of 1.53.For evaluating haematological parameters, healthy Swiss albino mice from an inbred colony were divided into four groups viz.1. Normal 2. Only Drug treated 3. Control (Irradiated) 4. Experimental (Drug + irradiation). Groups 2 and 4 were orally supplemented with GAE for 15 days, at 700 mg/kg of b. wt./day. After treatment group 3 and 4 were exposed to 5 Gy gamma irradiation. Significant increases in total erythrocyte and leucocytes counts, hemoglobin concentration and hematocrit values were observed in the animals of experimental group in comparison to the hematological values observed in the control group at various post irradiation days viz. 1,3,7,15,30 days. A decrease in reduced glutathione (GSH) content and an increase in lipid peroxidation (LPO) were observed in control animals. However, the animals of the experimental group exhibited a significant ($p<0.001$) increase in GSH and protein content and a significant decrease in LPO level. The findings support the antioxidative properties of GAE against the gamma irradiation. This may be due to scavenging of free radicals by anthocyanin and vitamin c present in *Grewia asiatica* extract.

(PS1147) Radioprotective effects of mentha piperita linn in vivo: studies in swiss albino mice. Ravindra M. Samartha, Meenakshi Panwar, Madhu Kumar, Ashok Kumar. University of Rajasthan, Jaipur, India.

The modifying effects of *Mentha piperita* extract against gamma radiation in Swiss albino mice were explored in several studies. Pretreatment with an aqueous extract of *M. piperita* prior to whole body gamma irradiation at 4, 6, 8 and 10 Gy significantly increased the spleen weight and the number of endogenous spleen colonies. A daily oral dose of 1 g/kg for 3 days prior to irradiation significantly increased hematological parameters, serum alkaline phosphatase and decreased serum acid phosphatase and improved the survival rate compared with irradiated control animals. A regression analysis of the survival data in irradiated mice revealed that mice pretreated with *M. piperita* extract were able to withstand a 1.78-fold higher dose of radiation than untreated mice. The *M. piperita* extract was shown to provide protection against radiation-induced alterations in the intestinal mucosa of mice. *M. piperita* pretreatment protected against the radiation-induced increases in goblet cells/villus section and dead cells/crypt section in the jejunum of mice. Oral administration of *M. piperita* extract prior to radiation exposure was effective against the chromosomal damage in bone marrow. Irradiated animals exhibited chromosomal aberrations in the form of chromatid and chromosome breaks, centric rings, dicentrics exchanges and acentric fragments, while animals pretreated with *M. piperita* extract showed a significantly lesser number of aberrant cells. Animals pretreated with *M. piperita* extract and exposed to 8 Gy gamma radiation showed a significant increases in the activities of reduced glutathione content, glutathione peroxidase, glutathione reductase, glutathione S-transferase, superoxide dismutase, and catalase. Irradiated group pretreated with *M. piperita* extract showed significant decrease in malondialdehyde (MDA) formation in liver. The *M. piperita* extract showed strong radical scavenging activity in both the DPPH and ABTS assays. Thus, the antioxidant and free radical scavenging activities of *M. piperita* extract are the likely mechanism of radiation protection.

(PS1148) Standardized North American ginseng radioprotects human lymphocytes. Tung-Kwang Lee[1], Ron R. Allison[1], Weidong Wang[1], Kevin F. O'Brien[2], Roberta M. Johnke[1]. [1]Dept. Radiation Oncology, Greenville, NC, USA, [2]Dept. Biostatistics, School of Allied Health at ECU, Greenville, NC, USA.

The formation of micronuclei (MN) in cytokinesis-block (CB) peripheral blood lymphocytes (PBL) is one of the most sensitive biomarkers for assessing ionizing radiation damage. To explore the radioprotective potential of standardized North America Ginseng Extract (NAGE, total ginsenoside content: 11.7%), we conducted the CBMN assay *ex vivo* on PBL obtained from 12 healthy volunteers (6M:6F, 46.4±15.7 y) for ^{137}Cs-induced (1 - 2 Gy, 0.6 Gy/min) MN yield. At 24 h before irradiation, 0 h, and at 90 min post irradiation, G_0 PBL from each donor were incubated with different concentrations of NAGE (0, 50, 100, 250, 500, 750, and 1000 μg ml^{-1}). PHA was added to the cultures immediately after irradiation. Cytochalasin B (4 μg ml^{-1}) was applied at 44 h after PHA stimulation, and cultures were terminated following another 24 h. The yield of MN in binucleated (BN) cells was determined by light microscopy. Results from the three different experimental time levels indicated: (1) at 0 Gy and without the presence of NAGE, MN yield (mean ± SEM) ranged from 13.8–15.4 (± 1.4–1.6) per 1000 BN cells; (2) when compared to the 0 Gy response, radiation alone linearly increased MN yield 7.5–8.7 fold and 15–16.7 fold per 1000 BN cells after 1 and 2 Gy exposures (P=0.0001), respectively; (3) MN yields declined linearly as NAGE concentration in irradiated PBL increased (P=0.0001). Compared with radiation alone, NAGE reduced the MN yield in PBL at least 20.4 % (50 μg ml^{-1}) and up to 47.7 % (750 μg ml^{-1}) after 1 Gy, and at least 17.6% (100 μg ml^{-1}) up to 50.1 % (500 μg ml^{-1}) after 2 Gy; and (4) MN yields did not follow a Poisson distribution. Our data unambiguously indicate the ability of standardized NAGE to protect, in a concentration-dependent manner, against ^{137}Cs-induced MN in human G_0 PBL *ex vivo* when applied 24 h before irradiation, at 0 h, and even at 90 min post irradiation. Further, this radioprotection is accomplished without apparent toxicity. NAGE alone at concentrations up to 1000 μg ml^{-1}, demonstrated no adverse effects as evaluated by the MN yield. Thus, we believe that NAGE qualifies as a natural radioprotector with potential benefit for cancer patients undergoing radiotherapy, as well as for situations like nuclear triage, battlefield-related events or nuclear terrorism. This work has been supported by NIH/NCCAM Grant R21AT002639-01A1.

(PS1149) The effect of natural products on hemopoietic function in irradiated mice. Yue Gao. Beijing Institute of Radiation Medicine, Beijing, China.

To elucidate the mechanism of SiWu Decoction , a Traditional Chinese Medicine in irradiated mice, we studied the SiWu and every constituent's effect on hemopoietic function. The results were obtained in irradiated C57 mice exposed to whole body doses of 2.5.5.5 and7.5Gy. and showed that It can enhance hemopoietic function by increasing the number of the granuloid colony forming unit, erythroid colony forming unit, erythroid burst forming unit, monocytic-macrophagic colony forming unit and colony forming unit megakaryocyte. The results also suggested that SiWu has much better effect to reduce the suppressive of mice exposed to dose 5.5Gy. It tell us Traditional Chinese Medicine can modulate the hemopoietic function in limited dosage.

(PS1150) Protection against radiation by *Rosemarinus officinalis* (a medicinal plant) extract. P. K. Goyal. University of Rajasthan, Jaipur, India.

The development of radio protective agents has been the subject of intense research in view of their potential for the use within a radiation environment; however, no ideal, safe radio protectors are available to-date, so the search for alternative sources, including plants, has been on going.

Rosemary *(Rosemarinus officinalis)* plant has been used traditionally for curing various disorders among the people around the world for time immemorial. Its common use, wide acceptability, diverse pharmacological and anti oxidative properties aroused our interest to obtain insight into the radiomodulatory effect of such plant extract against gamma exposure. Oral administration of aqueous extract of Rosemary leaves (1000 mg/kg body weight/day) to Swiss albino mice for 5 consecutive days, half an hour before

whole-body exposure to lethal gamma radiation, enhanced the 30 days survival and also inhibited the radiation induced sickness and life shortening. Dose reduction factor (DRF) for Rosemary leaf extract was calculated as 1.89 from $LD_{50/30}$ values. Rosemary extract also ameliorated anemia, leucopenia, thrombocytopenia, multipotential stem cell death, intestinal and hepatic lesions, induced by radiation at different autopsy intervals between 12 hrs to 30 days, and significantly increased the number of femoral spleen colony forming units (CFU-S) that survived after irradiation. Furthermore, pretreatment with rosemary extract checked depletion of glutathione (GSH) and antioxidant enzymes (SOD, CAT, GST) as well as elevation of lipid per oxidation (LPO) level. The significant reduction in the yield of LPO demonstrates that rosemary protects the membrane against radiation induced oxidative damage.

These findings conclude that such plant extract provides significant radioprotection and it may be potentially valuable in the prevention of injury caused during radiotherapy. It also proves that plant based radioprotectors are cost effective and well within the reach of people living in adverse and hazardous situations and may be of below poverty line.

(PS1151) Radioprotective effects of an Ulmi Cortex Extract and the identification of its effective compounds. Uhee Jung, Hae-Ran Park, Yoon-Ah Lee, Seol-Hee Han, Sung-Kee Jo. Adavanced Radiation Technology Institute, Jeoungeup Campus of KAERI, Jeonbuk, Republic of Korea.

Ulmi Cortex (UC), the bark of the stem and the root of *Ulmus davidiana*, has been used in Oriental Medicine for the treatment of edema, mastitis, gastric cancer and inflammation. Since natural products are gaining more attention as possible modifiers of radiation responses, we investigated the radioprotective effects of an UC extract on the immune and hematopoietic systems and identified the active compounds for reducing radiation-induced oxidative damages. C57BL/6 mice were administered intraperitoneally with the UC extract (100 mg/kg B.W.) prior to and following a whole-body gamma-irradiation (5Gy). The administration of the UC extract reduced the apoptosis of the mouse bone marrow cells ($p<0.01$) and increased the endogenous spleen colony formation ($p<0.001$) implying a protection of the hematopoietic system against a radiation by the UC extract. Also the natural killer (NK) cell activity and Th1/Th2 cytokine (IFN-gamma and IL-4) balance were partially restored when compared with the radiation control group. We performed the column chromatographies to identify active compounds of the UC extract. Two compounds, 3,3',4',5,6,7,8-heptahydroxyflavan (HHF) and 3-O-[β-D-glucopyranosyl]-catechin (GPC), were purified from the UC extract. These two compounds showed higher protective activities than BHA against the radiation-induced DNA damage and they also showed high antioxidative activities. These results suggest that the UC extract has good radioprotective activities for the immune and hematopoietic cells against the gamma-irradiation, and that HHF and GPC are compounds that contribute to these radioprotective effects of the UC extract.

(PS1152) Radioprotective and antimutagenic effects of Oltipraz. Ashok Kumar[1], Ravindra Samarth[1], Madhu kumar[1], Deepali Sharma[1], Patrick Prendergast[2], Hiroshi Kimura[3]. [1]Radiation & Cancer Biology Lab.,Rajasthan University, Jaipur, India, [2]Canopus Corporation, Kenilworth, Byrock 2831, New South Wales, Australia, [3]Department of Biochemistry and Molecular Biology, Shiga University of Medical Science, Otsu, Shiga 520-2192, Japan, Japan.

The radioprotective and antimutagenic activity of *Oltipraz* (5-[2-pyrazinyl]-4-methyl-1, 2–3-thione), formerly a Schistosomal drug, against radiation was studied in Swiss albino mice. Animals were given either double distilled water or *Oltipraz* orally (100 g/kg bwt/day) once a day for two consecutive days. Treatment with *Oltipraz* for 2 consecutive days in mice did not produce any toxic effects. Thirty minutes after the last treatment mice were exposed to 6, 8 and 10 Gy of gamma radiation with or without *Oltipraz*. The animals were observed for 30 days. No radiation sickness was observed in treated animals. In the present study, it was observed that pretreatment of *Oltipraz* enhanced the survival of mice exposed to different doses of gamma radiation. On the basis of $LD_{50/30}$ values dose reduction factor was calculated as 1.34.There was significant increase in the number of spleen colonies formed in the mice and significant decrease in the frequency of aberrant cells in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges and acentric fragments and micronuclei in the animals pretreated with *Oltipraz* (100 mg/kg body weight for two days).Significant decrease in hepatic glutathione, glutathione reductase,glutathione -S-transferase content was observed in control animals (irradiation alone) whereas, experimental animals pretreated with *Oltipraz* before radiation ,showed a significant increase in glutathione, glutathione reductase,glutathione -S-transferase content. Lipid peroxidation (LPO) level in liver and serum was measured in terms of Thiobarbituric Acid Reactive Substance (TBARS). An increase was evident in control animals as compared to normal animals. Maximum decline in LPO level was observed in animals pre-treated with 100mg/kg body weight/day *Oltipraz*.These results suggest that *Oltipraz* pre-treatment provides protection against radiation and chromosomal damage in bone marrow of Swiss albino mice and increases overall survival.

(PS1153) Radioprotection of lactoferrin in sub-lethally x-ray irradiated mice. Yoshikazu Nishimura[1], Shino Homma-Takeda[1], Izuru Kakura[2], Hee Sun Kim[3], Minako Nyui[1], Nobuo Ikota[1]. [1]National Institute of Radiological Sciences, Chiba-shi, Japan, [2]Ishinomaki Senshu University, Ishinomaki-shi, Japan, [3]Korea Hydro & Nuclear Power Co. Ltd., Seoul, Republic of Korea.

Lactoferrin is an iron-binding glycoprotein with a molecular weight of about 80,000. Lactoferrin was originally isolated bovine milk and found in excretions of mammals and is released from neutrophil granules during inflammation. Lactoferrin is considered a multifunctional protein. In this study, we observed the radioprotective effect of lactoferrin in mice following whole-body X-ray irradiation. (1) C3H/He mice were used in this experiment. The mice were separated tow groups One group (25 mice) were fed an AIN-93 diet, which contained 0.1 % w/w lactoferrin. The other group (25 mice) was fed an AIN-93 diet and water *ad libitum*. The mice were kept for 30 days on each diet, and were then exposed 6.8 Gy of whole-body irradiation. The survival rates of these mice were observed within 30 days after irradiation. The survival rate of lactoferrin-diet mice were about 23% higher than those of mice on a standard diet, and the rates dropped sharply to a plateau at day 10 after X-ray irradiation. The scavenging abilities of lactoferrin were evaluated by the ESR spin-trapping method. Lactoferrin is a radical scavenger of hydroxyl radicals and the observations suggested that radical trapping or scavenging might cause the biological response. (2) 52 of C3H/He mice were exposed 6.8 Gy of X-ray irradiation. After the irradiation, LF (4 mg) was administered 26 of mice intraperitonealy and survival rates of these mice were observed within 30 days. The survival rates of lactoferrin-treated mice and non-treated mice were 93 % and 50 %, respectively within 30 days after X-ray irradiation. The radio-protective effects of lactoferrin after X-ray irradiation in mice were investigated by using the micronucleated plychromatic erythrocyte test. Radiation induced apoptosis in mice given a single intraperitoneal injection of lactoferrin after X-ray irradiation was observed by hematoxylin-eosin staining. Lactoferrin inhibited splenocytes apoptosis and damage on a bone marrow cell after X-ray irradiation. These results suggest that lactoferrin can be used as a drug of radiation protective agent.

(PS1154) *Myristica fragrans*: a possible radiomodulator in the testis of swiss albino mice. Madhu Kumar, Mini Sharma. Cell & Molecular Biology Lab., Department of Zoology, University of Rajasthan, Jaipur, India.

Dried seed kernel of the aromatic tree, *Myristica fragrans*, (Family: Myristicaceae), commonly called as nutmeg is widely used in herbal and culinary preparation. Present study evaluates its possible radiomodulatory effects in the testis of Swiss albino mice

Radiomodulatory effect of *Myristica fragrans* (MF), against gamma radiation, was evaluated by 30 day survival assay, histopathological study and biochemical assay. Animals were exposed to different doses of gamma radiation (6, 8 and 10 Gy) in absence or presence of *Myristica fragrans* (10 mg/kg body weight). Regression analysis yielded LD50/30 as 6.83 Gy and 8.89 Gy for irradiated only and MF + radiation groups, respectively. The dose reduction factor was computed as 1.3

Testis is one of the highly radiosensitive organs due to cell renewal system. Irradiated testes showed shrinkage of tubules, germ cell depletion, pyknotic nuclei, broken germinal epithelium and shrinkage of sertoli cell nuclei. However, in the animals pretreated with *Myristica fragrans*, before radiation, all the pathological lesions were reduced. Administration of MF significantly enhanced glutathione (GSH) in the testis and decreased testicular lipid peroxidation (LPO) level whereas acid phosphatase (ACP), alkaline phosphatase (ALP), sialic acid and cholesterol contents did not show any significant alteration. Irradiation resulted in significant elevation in LPO level, ACP activity, and cholesterol level and decreased the GSH content, sialic acid and ALP activity. MF pretreatment effectively protected against radiation induced biochemical alteration as reflected by a decrease in LPO level and ACP activity, and an increase in GSH and ALP activity. A significant decline in cholesterol, sialic acid content and ALP activity was also noticed

The present investigation shows that *Myristica fragrans*, seed extract, is effective in reducing radiation induced testicular damage and animal survival

(PS1155) Radiomodulatory effect of leaf extract of mentha piperita (linn) in Swiss albinio mice. Punar Dutt Meena, Pallavi Kaushik, Anil Soni, Shalini Shukla, Ashok Kumar. University of Rajasthan, Jaipur, India.

Introduction: To investigate the radioprotective activity of 50% ethanolic extract of Mentha piperita leaves (ALM) in Swiss albino mice exposed to different doses of gamma radiation. Methods: To study the radioprotective effect of ALM, mice were administered different doses, 50, 100, 200, 400 mg/kg body weight of ALM orally for 3 consecutive days before exposure to 8 Gy gamma radiation. The optimum dose (100 mg/kg body weight/day) of ALM was administered before exposure to 6,8,10Gy gamma radiation along with their respective controls (radiation alone). Reduced glutathione and lipid peroxidation were estimated in total surviving animals of all groups on day 30 post irradiation. Results: The optimum radioprotective dose was 100 mg/kg body weight/day of ALM where the highest survival (75%) to 8 Gy radiation was observed .The irradiation caused a dose dependent decrease in survival, while treatment of mice with ALM enhanced survival. The dose reduction factor (DRF) was 1.44. Irradiation caused a dose dependent decline in the levels of glutathione accompanied by an elevation in lipid peroxidation, ALM pretreatment arrested glutathione decline and lipid peroxidation elevation significantly. Conclusion: The possible mechanism of radioprotection might be free radical scavenging and arrest of lipid peroxidation accompanied by an elevation of glutathione.

(PS1156) Radioprotective effect of mentha piperita in swiss albino mice. Pallavi Kaushik, Punar Dutt Meena, Shalini Shukla, Ashok Kumar. University of Rajasthan, Jaipur, India.

Introduction-Alcoholic leaf extract of Mentha piperita (ALM) has been found to protect mice against radiation lethality and chromosome damage and to possess significant antioxidant activity. Therefore the present study was conducted to see the radioprotective effect of alcoholic leaf extract of Mentha piperita in Swiss albino mice. Materials & Methods-For radioprotective studies, adult Swiss albino mice were administered different doses of ALM viz. 0, 50, 100, 200, 400 mg/kg body weight/day for three consecutive days before 8Gy gamma radiation. These animals were observed for symptoms of radiation sickness and mortality upto 30 days post-irradiation. Glutathione and lipid peroxidation were estimated in total surviving animals of all groups on day 30 post-irradiation.The radioprotective effect of Mentha piperita was further analyzed on bone marrow chromosomes at 1, 3,7,14,30 day post-irradiation. Results-The optimum radioprotective dose was 100 mg/kg body weight/day for 3 consecutive days where the highest survival to 8Gy gamma radiation was observed.A significant reduction in chromosomal aberrations was observed in ALM treated groups. Irradiation caused a decline in glutathione accompanied by an elevation of lipid peroxidation. The optimum dose of ALM (100 mg/kg body weight/day) arrested glutathione decline and lipid peroxidation elevation significantly. Conclusion-The significant reduction in radiation induced chromosomal aberrations suggests the anti-mutagenic effect of Mentha piperita.ALM treatment reduced the symptoms of radiation induced sickness and increased survival. The radioprotective action of ALM might be due to radical scavenging and arrest of lipid peroxidation accompanied by an elevation of glutathione.

(PS1157) Natural protectors: cytogenetic assessment of radioprotector effect of ethanol extract of propolis. Alegria Montoro. Hospital Universitario La Fe, Valencia, Spain.

A consequence of ionizing radiation interaction at cells is the induction of chromosomal alterations. This relation of causality involves that chromosomal alterations can be considered a good exhibition indicator to the above-mentioned agents.

Some chemical agents can modulate the tissue response to radiation. These compounds are useful when they show certain selectivity, protecting the healthy tissues (radioprotectors) or increasing the sensibility of tissues to radiations (radiosensibilizators).

An evaluation of the propolis substance is performed. Propolis is a product of extraordinary interest for both medicine and pharmaceutical industry, since it is assumed to show diverse beneficial health effects. Among many other attributes of EEP (propolis ethanolic extract), it exhibits the antioxidant and the radical free scavenger properties.

The evaluation of propolis radioprotector effect is aimed at this thesis. With this purpose, propolis ethanolic extract (EEP) has been obtained and samples of peripheral blood have been irradiated under different conditions: to both different radiation doses in EEP absence as well as presence and the same radiation dose to different EEP concentrations. To assess the evaluation, lymphocyte chromosomal alterations have been analysed in the first mitotic division using cytogenetic techniques. The obtained results show a decrease of total alterations when we radiate to a fixed dose and different EEP concentrations, obtaining a protection against radioinduced damage of up to 44 %; as well as when we radiate to different doses in presence and absence of a known EEP concentration. In both cases a significant reduction of the linear and quadratic coefficients for the calibration curve has been obtained.

The proposed concentration for radioprotection would be among 120–500 $\mu g \cdot ml^{-1}$, when a maximum protection against radioinduced damage is obtained and a cytotoxic effect in not irradiated culture of human being lymphocytes is not evidenced.

The cytotoxic effect has been evaluated analysing the EEP effect at the cellular division cycle. Two indexes have been used, the mitotic index and cell proliferation index. For both indexes the cytotoxic effect takes place from 750 $\mu g \cdot ml^{-1}$ concentrations onwards.

(PS1158) In search of new radio-protecting agents. Jaroslaw Dziegielewski, Umut Aypar, Janet E. Baulch, Kurtis E. Bachman, William F. Morgan. University of Maryland, Baltimore, MD, USA.

The goal of this project is to develop novel and effective radio-protecting agents. Free radical scavengers are currently considered superior radioprotectors, however, the inherent reactivity of scavengers renders them highly unstable in biological systems, prone to metabolic deactivation, somewhat toxic, and unsuitable for oral administration. Due to these deficiencies it is important to search for radio-protecting compounds with different mechanism(s) of action. By using a well diversified set of 40,000 small drug-like molecules in high throughput screen (HTS) we should be able to detect compounds capable of modulating radiation effects not only

by direct interaction with free radicals, but also through other mechanisms such as enhancing detoxifying enzymes, DNA repair and other cellular processes. The radio-protective effects of compounds on MCF-10A cells were determined using a 384-well plate HTS Biomek FX workstation (Beckman Coulter). Apparently normal, human mammary epithelial MCF-10A cells were treated with each candidate compound (4 μM), irradiated, incubated 3–4 days and then cell proliferation was determined. Agents enhancing cell proliferation >25% as compared to control were subjected to secondary screening using a clonogenic cell survival assay. In the secondary screen we have found 9 compounds that showed >20% protection. Interestingly, WR-1065, a widely studied active form of the classical radio-protective agent amifostine, requires 10–1000 times higher concentrations to protect MCF-10A cells to a similar extent. As primary and secondary screen results are based on different mechanisms (inhibition of cell growth and clonogenic survival, respectively), we believed that the compounds identified as radioprotectors in our screens are good candidates for further development.

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(PS1159) Development of a primate resource for radiation countermeasures research. Shauna Gray, Esther Arifin, Dan Bourland, Tom Register, Jan Wagner, Mark Cline. Wake Forest University, Winston Salem, NC, USA.

One of the most critical components in our national defense against terrorism is protection from malicious irradiation. As part of the development of a Radiation Countermeasures Center of Research Excellence (RadCCORE), we are developing nonhuman primate models of acute and chronic radiation effects, which will be used for future evaluation of medical countermeasures. Current progress in characterization of the models includes clinical assessments, peripheral blood counts, serum biochemistries, pulse oximetry measurements, and toxicity scoring using a system modified from the Children's Cancer Group. It also includes complete pathologic evaluation of irradiated rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques. Tissue resources from the Primate Core are being used to develop and validate assays for the evaluation of circulating biomarkers, and to assess their changes in response to radiation exposure, as well as for other specific investigator-initiated research studies. As part of our baseline dose-finding studies, we exposed 15 adult female cynomolgus monkeys to whole-body irradiation at low and medium doses (0, 2, and 5 Gy). These doses were not expected to cause life-threatening illness; however, our findings to date indicate that there may be species, sex, and age-related differences in response to low and medium dose exposures to whole-body irradiation. This primate resource will greatly facilitate translational efforts by providing a critical interface between the *in vitro* and rodent studies and their clinical applicability. This work was supported by NIH/NIAID (U19 AI67798–01 [Duke University], subcontract 131714–5).

(PS1160) A soluble peptide (EA230) protects WBI-induced toxicity in a post scenario application. Alan A. Alfieri[1], Laibin Liu[1], Payel Bhanja[1], Zsolt Harsanyi[2], Richard Carlton[2], Chandan Guha[1]. [1]Albert Einstein College of Medicine, Bronx, NY, USA, [2]Exponential Biotherapies, Inc., McLean, VA, USA.

Background: There is a need for non-toxic radioprotectants for military personnel and civilians that may be exposed to a nuclear-radiological scenario or patients therapeutically irradiated within the abdominal area. Current therapeutics are limited and ineffective prophylaxis for gastrointestinal radiation injuries as a post treatment. EA230™, a small soluble peptide (MW 373Da) has already completed Phase 1 GMP safety, pharmacology/pharmacokinetics and formulation development studies as an antisepsis agent that blunts the inflammatory response to LPS in human volunteers and reduces lethal LPS-induced sepsis in mice (EU). The purpose of the present studies was to evaluate whole body (7.4–11Gy at 0.6Gy increments) irradiation (WBI) survival-protection at peptide dose escalations (0.5–12.5mg/mse), route of administration (iv and sc) and treatment intervention (15min to 120hrs post WBI) for optimization. **Methods:** Anesthesized C57Bl6-male mice (NCI) were irradiated (Mark I ^{137}Cs source) a/p and followed for mortality/survival. LD50/30 assay for dose modification assessments (DMF) and Kaplan Meier survival was determined. Single and multiple doses were evaluated for toxicity and efficacy. Immuno-histochemistry, BrdU, TUNEL and H & E morphological evaluations were performed on paraffin and frozen sections 2 −7dys post WBI, along with immuno blot of sera for inflammatory cytokines and jejunal tissue comparisons with and without peptide. **Results and Discussion:** WBI single doses of 8.6 and 9.0Gy (LD80/30 and LD100, respectively) lethality was observed in PBS treated animals whereas fractionated peptide administration produced 100% animal survival at these doses. EA-230 demonstrated an approximate dose modification factor (DMF of >1.3) with single sc dosing. Additional single dose studies (confined to 10.4Gy WBI) demonstrated significant ($p<.01$) animal survival >40% when peptide was administered sc at 0.5, 2.5 and 12.5mg/mse within 15min to 96hrs of the irradiation event. Preliminary indications suggests that EA230′s mechanism(s) of action in protecting against WBI-induced gastrointestinal syndrome is based partly on protecting endothelial components and crypt cell preservation affects. This, in part, had been previously alluded to in the murine lethal sepsis infection protection model.

(PS1161) Adaptation of the yeast DEL assay for rapid identification of radiation protectors and sensitizers. Kurt Hafer, Nikos Hontzeas, Robert Schiestl. Univ of California, Los Angeles, Los Angeles, CA, USA.

We have developed a new method for rapidly qualifying the radioprotective and radiosensitive qualities of compounds in yeast. The yeast deletion (DEL) assay was modified into a liquid-based, colorimetric assay capable of qualifying DNA recombination events in a microwell plate format. The RS112 strain of *Saccharomyces cerevisiae* contains an integrative disruption of the HIS3 gene. Recombination between the two his3 deletion alleles results in reversion to HIS3+ and growth in the absence of histidine. Yeast cells are dispensed into 384-well plate microwells containing complete media and -His media and irradiated with 2000 Gy. Yeast viability is scored using the MTS tetrazolium compound which is reduced by viable cells to a colored formazan product. Growth is quantified by reading 490 nm absorbance 14 hours post radiation exposure.

Irradiated RS112 yeast cells exhibited a greater frequency of recombination events, quantified by the ratio of growth in -His media to growth in complete media, compared to unirradiated yeast. When incubated with the antioxidant N-Acetyl Cysteine (NAC), irradiated yeast cells exhibited both significantly increased growth in complete media and reduced growth in -His media compared to control irradiated cells. Thus two endpoints of radioprotection are able to be qualified for NAC by this method: cytotoxicity (growth in complete media) and genotoxicity (recombination events). Irradiated yeast incubated with ascorbic acid and tempol also exhibited significantly less genotoxicity and cytotoxicity compared to control irradiated yeast cells. Alternatively, irradiated cells incubated with the radiosensitizer bromodeoxyuridine showed greater genotoxicity and cytotoxicity compared to irradiated control cells.

This microplate format of the DEL assay is advantages in that it is capable of scoring both genotoxicity and cytotoxicity simultaneously. This method is well suited for rapidly qualifying the radioprotective and radiosensitive properties of a large number of compounds and is amenable to automation in its current format for high-throughput purposes.

(PS1162) Pulse radiolysis of polystyrene and derivatives. Kazumasa Okamoto, Masafumi Tanaka, Shu Seki, Takahiro Kozawa, Seiichi Tagawa. The Institute of Scientific and Industrial Research, Ibaraki, Osaka, Japan.

Many kinds of polystyrene (PSt) derivatives, such as chloromethylated PSt, are well known as conventional X-ray and electron beam resists. PSt derivatives have also been widely used as backbone polymers for the current standard resists, called chemically amplified resists. As the lithography with ionizing radiation, such as extreme ultraviolet radiation, approaches practical use, better understanding of radiation chemistry in these materials has recently been the focus of much attention. Radiation-induced intermediates (radical cations, excited states and radicals) of PSt and derivatives such as poly (4-hydroxystyrene) (PHS) and poly (4-acetoxystyrene) were studied using pulse radiolysis methods. Characteristic charge resonance (CR) bands were observed in near infrared region. They indicate formations of π-π dimer radical cation in which a positive charge is shared with the two chromophores. Aromatics such as benzene show CR bands, however aromatics with hydroxyl groups have been indicated σ-π or σ-σ dimer radical cation formation exceptionally by the hydrogen bond. It is suggested that polymer structures of PHS restrict the formation of σ-π or σ-σ dimer radical cation. Proton ejection efficiency, which is important in chemically amplified resists, also depends on the substituent.

(PS1163) Selective protection of normal tissues from lethal irradiation by pharmacological imitation of tumor mechanisms suppressing apoptosis. Andrei Gudkov, Elena Komarova, Lyudmila Burdelya, Vadim Krivokrysenk, Joseph DiDonato, Alexander Shakhov, Elena Feinstein. Roswell Park Cancer Institute and Cleveland BioLabs, Inc., Buffalo, NY, USA.

The toxicity of ionizing radiation is associated with induction of massive apoptosis in radiosensitive organs. Tumors frequently acquire apoptosis-suppressing mechanisms through deregulation of major stress response pathways. Our group is exploring pharmacological activation of tumor specific mechanisms of apoptosis resistance as an approach to selective radioprotection of normal tissues. We previously demonstrated isolation of small molecule inhibitors of p53 (PFTα and PFTμ) that effectively protect mice from doses of radiation inducing lethal hematopoietic injury. Even more significant protection from (as well as mitigation of) radiation-induced tissue injury can be achieved by pharmacological imitation of another common property of tumor cells - activation of NF-κB. As NF-kB activators we used natural products, flagellin of *Salmonella* and lipopeptide of *Mycoplasma*, agonists of Toll-like receptors TLR5 and TLR2/6, respectively. A TLR5-activating polypeptide (CBLB502) derived from *Salmonella* flagellin, rescued mice and rhesus monkeys from lethal doses of total body irradiation being injected both before and after irradiation. The mechanism of protection combines direct and indirect effects of CBLB502 on radiosensitive tissues. Direct effect involves the activation of NF-kB-inducible anti-oxidative and anti-apoptotic factors in TLR5-expressing cells in intestine and hematopoietic system. Induction of tissue protecting cytokines (e.g., G-CSF, SCF, KGF, etc.) determines the indirect radioprotective effect of CBLB502 by stimulating regeneration of damaged tissues. At cellular level, CBLB502 protects radiosensitive cells in lamina propria of small intestine and early hematopoietic progenitors of bone marrow thereby being effective against both major components of acute radiation syndromes. An important property of CBLB502 as radioprotectant is high selectivity of its protective effect for normal cells and inability to protect tumor cells - neither *in vitro* nor *in vivo*. Radioprotective properties of TLR2/6 agonists, which are different from those of TLR5 ligands, will be described. Our results demonstrate the potential of TLR agonists such as CBLB502 to serve as biological protectants in radiation emergencies and to improve the therapeutic index of cancer radiotherapy.

(PS1164) Modulation of radiation sensitivity by down-regulation of the PI3K/Akt signalling pathway. Carsten Herskind, Meng Wang, Qi Liu, Christina Ganasinski, Patrick Maier, Frederik Wenz, Frank Lohr. Dept. of Radiation Oncology, Mannheim Medical Center, Mannheim, Germany.

The PI3K/Akt pathway plays an important role in mediating signals for cell growth, metabolism and survival. Thus signalling via PI3K/Akt/BAD protects against apoptosis. However, downstream pathways modulating radiation-induced cell inactivation are less well characterized. The aim was to study the modulation of radiation sensitivity by down-regulation of Akt signalling in cells that do not undergo apoptosis via BAD. Hamster (V79) and human cell lines (U87, U251, U343 glioblastoma; TK6 lymphoblasts) were used with clonogenic cell survival, activation of signalling, cell cycle distribution and apoptosis (subG1) as end points. Inhibition of Akt signalling activation by graded concentrations of the PI3K inhibitor Wortmannin showed no effect or even minor radioprotection with low, specific concentrations (5–500 nM). These concentrations were able to perturb the cell cycle of V79 cells. In TK6 cells, which undergo apoptosis via BAX, low concentrations of Wortmannin had no effect on apoptosis. In all cell types, much higher concentrations (5–20 μM) were required for radiosensitization. Similarly, radiosensitization of V79 cells with Ly294002 was achieved only at high concentration (25 μM) which was partly toxic. Sensitization of U251 cells by 20 μM Wortmannin occurred, albeit slightly attenuated, even when the drug was added 3–7 hours after irradiation but was nearly absent when added 24h after irradiation, thus showing a time kinetics similar to that of cell recovery. Downstream inhibition of mTORC1 by 20–100 nM Rapamycin had no effect on the radiosensitivity of glioblastoma cells in vitro. In summary, down-regulation of PI3K/Akt signalling in these cells was not sufficient for radiosensitization which was seen only at high concentrations where other PI3K family kinases (e.g. DNA-PK) may be inhibited. We propose that specific down-regulation of PI3K/Akt affects the cell cycle and that radiosensitization by high concentrations of PI3K inhibitors may involve inhibition of DNA repair. Further experiments, including knock-down of Akt1 expression, are in progress to test this hypothesis.

(PS1165) An essential role of integrin-linked kinase on the cellular radiosensitivity during the process of cell adhesion and spreading. Stephanie Hehlgans, Iris Eke, Nils Cordes. OncoRay - Center for Radiation Research in Oncology, Dresden, Germany.

In addition to focal adhesion kinase (FAK), Paxillin and p130Cas, integrin-linked kinase (ILK) mediates signals from beta integrins for controlling e.g. survival, proliferation, adhesion and spreading. An overexpression of ILK causes enhanced radiosensitivity in tumor cells. To evaluate the role of ILK in the cellular radiosensitivity of ILKfl/fl and ILK-/- mouse fibroblasts at different stages of cell adhesion and spreading, cells were irradiated (0–6 Gy, X-rays) a) in suspension, b) at varying time periods after Fn adhesion (5 min - 6 h), c) after 24 h on different matrix proteins (2D) or d) after 24 h in a three-dimensional laminin-rich basement membrane (3D lrBM) culture model. These conditions were combined with 30 min treatment using the phosphatidylinositol-3 kinase (PI3K) inhibitor Ly294002. Cell adhesion, clonogenic survival and protein expression and phosphorylation (Western blotting; FAK, Paxillin, p130Cas, Akt) were examined. Ilk knockout status resulted in a strong adhesion defect. In contrast to suspension and short term adhesion, radiation survival was independent from ILK after 24 h growth in 2D and 3D. In suspension and during the first 6 hours after plating on Fn, ILKfl/fl cells showed significantly increased radiation survival compared to ILK-/- cells. Since PI3K inhibition diminished the survival advantage of ILKfl/fl cells up to survival rates detected in ILK-/- cells, this process seems strongly dependent on PI3K/Akt signaling. In parallel experiments for protein expression and phosphorylation kinetics after plating on Fn, Akt phosphorylation was completely abrogated while FAK, Paxillin and p130Cas were hyperphosphorylated. Dependent on ilk gene status, substratum and culture model, Akt phosphorylation was induced after 2 Gy whereas phosphorylation of FAK, Paxillin and p130Cas declined. These findings indicate a critical function of ILK in the cellular radiosensitivity during cell adhesion and spreading. Associated with this are converse modulations between PI3K/Akt and FAK/Paxillin/p130Cas cascades. The understanding of the underlying mechanisms for adhesion- and spreading-related changes in the cellular radiosensitivity might be important for the behavior of irradiated cells located at the invasive edge of malignant tumors.

(PS1166) Modulation of irradiation-induced microglial inflammation by PPARα activation. Sriram Ramanan[1], Mitra Kooshki[2], Weiling Zhao[2], Michael E. Robbins[2]. [1]Dept. of Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC, USA, [2]Dept. of Radiation Oncology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.

Brain irradiation can lead to devastating functional impairment several months to years after irradiation. Recent studies suggest that progressive dementia occurs in up to 50% of brain tumor patients who are long-term survivors after treatment with partial or whole brain irradiation (WBI). Currently, there are no successful long-term treatments for radiation-induced brain injury, nor are there any known effective preventive strategies. Microglia are the principal immune cells of the brain. Studies on rodents have shown a rapid increase in activated microglia following brain irradiation, contributing to a chronic inflammatory response and a corresponding decrease in hippocampal neurogenesis. Thus, alleviating microglial activation following radiation is a key strategy to minimize radiation-induced morbidity. Peroxisomal proliferator-activated receptors (PPARs) are ligand-activated transcription factors that possess anti-inflammatory properties and upregulate antioxidant enzyme expression. We hypothesize that pre-incubation with PPAR agonists will ameliorate the radiation-induced pro-inflammatory responses in the microglia. The presence of the PPARα gene was confirmed in the immortalized murine microglial cell line, BV-2. Irradiating BV-2 cells with single doses (2–10 Gy) of ^{137}Cs γ-rays (dose rate 4 Gy/min) led to dose-dependent increases in the gene expression of the pro-inflammatory cytokines Interleukin-1beta (IL-1β), Tumor necrosis factor alpha (TNFα) as well as increases in intracellular ROS measured one hour postirradiation; these were 5- and 7- fold at 5 Gy and 10 Gy, respectively. An increase in DNA-binding of the pro-inflammatory transcription factors AP-1 and NF-κB was observed as early as 30 min post-irradiation. Pre-treating BV-2 cells with the specific and potent PPARα agonist GW7647 (1 μM) inhibited the radiation-induced increase in IL-1β and TNFα mRNA by $>$ 50% postirradiation. Our data suggest that this modulation is via anti-inflammatory mechanisms of PPARα; pre-treatment of the BV-2 cells with GW7647 did not appear to inhibit the radiation-induced ROS generation. These data support the hypothesis that radiation-induced microglial inflammation can be modulated by activation of PPARα (Supported by grant number CA112593).

(PS1167) The involvement of protein kinase C alpha in radioadaptive response. Akira Tachibana[1], Katsuyuki Ito[1], Hiroshi Tauchi[1], Masao S. Sasaki[2]. [1]Faculty of Science, Ibaraki Univ., Mito, Japan, [2]National Institute of Biomedical Innovation, Ibaraki, Japan.

Radioadaptive response is a biological defense mechanism that is induced by low-dose ionizing irradiation for cellular resistance to the genotoxic effects of subsequent irradiation. We have reported the effect of pre-irradiation with 2 cGy X-rays prior to the challenging irradiation with 3 Gy on the induction of chromosome aberrations in quiescent mouse m5S fibroblasts. Similar effects have been reported by many others groups using other biological end-points, such as cell killing, mutation induction, the induction of micronuclei (MN), and single-cell gel electrophoresis (comet assay). We also showed that the pre-treatment with low concentration of TPA or hydrogen peroxide mimics the pre-irradiation with low dose of X-rays. We have suggested that the radioadaptive response is mediated through the pathways involving protein kinase C (PKC) alpha and p38 mitogen-activated protein kinase. However, the molecular role of PKC is still largely elusive. Here, we examined the involvement of PKC alpha in radioadaptive response by the use of RNAi.

We introduced three kinds of siRNA into m5S cells, each of which targets different site of the mouse PKC alpha gene, Prkca. One of them greatly reduced the amount of Prkca mRNA, indicationg that RNAi is effective for suppressing the expression of the Prkca gene.

We examined the radioadaptive response in the m5S cells in which the Prkca mRNA was suppressed. Cells were pre-treated with low concentration of hydrogen peroxide followed by irradiation with 5 Gy X-rays, and analyzed the induction of MN. The radioadaptive response was suppressed in the cells with low amount of the Prkca mRNA. This results indicate that PKC alpha is one of the critical factor of the signal transduction pathway in the radioadaptive response.

(PS1168) Low-dose of ionizing radiation enhances cell proliferation through Ikaros phosphorylation in IM-9 B lymphoblast cells. Min Young Kim[1], Kwang Hee Yang[1], Sung-Ryul Lee[2], Seon Young Nam[1], Meeseon Jeong[1], Cha Soon Kim[1], Hee Sun Kim[1], Young-Woo Jin[1], Suhkneung Pyo[2], Chong Soon Kim[1]. [1]Radiation Health Research Institute, Seoul, Republic of Korea, [2]College of Pharmacy, Sungkyunkwan University, Suwon, Kyunggi-do, Republic of Korea.

Exposure of cells in vitro or in vivo to low-dose irradiation (LDIR) has been reported to increase the immune activation such as the significant prevention or inhibition of carcinogenesis and tumor growth. In this study, we examined the effects of LDIR on the immune system in IM-9 B lymphoblast cells in comparison with the effects of high-dose irradiation (HDIR). Cell proliferation was enhanced significantly by LDIR at 0.01, 0.05 Gy but not by HDIR above 1 Gy. We found that the effect of LDIR-induced B lymphoblast cell proliferation could be modulated by the phosphorylation of Ikaros, a key regulator of lymphocyte proliferative responses. LDIR induced Ikaros phosphorylation in its serine/threonine region during the G1-S transition and then decreased DNA binding activity. In contrast, the Ikaros-DNA binding activity decreased by HDIR did not affect Ikaros phosphorylation. We also found that protein kinase casein kinase II (CKII) was one of the key molecules in relation to Ikaros phosphorylation. These results indicate that Ikaros phosphorylation is at least partially responsible for the enhanced cell proliferation after LDIR at 0.05 Gy in IM-9 B lymphoblast cells.

(PS1169) Altered phosphorylation of p53 in Nijmegen breakage syndrome cells. Luitpold V. Distel, Matthias Uhl, Anne Hofmann, Ulrike Keller, Leonhard Kuehn, Rolf Sauer, Gerhard G. Grabenbauer. Radiation Oncology, University of Erlangen, Germany.

The Nijmegen breakage syndrome (NBS) is a rare disease leading to a dramatic radiosensitivity phenotype. The Nbs protein is involved in DNA double-strand break (dsb) repair and is a signal modifier. Phosphorylation of Ser 15, 20 and 37 on p53 are directly associated with dsb repair. Our question was whether NBS$^{-/-}$ cells showed alterations in p53 phosphorylation only at these DNA-repair signalling sites. We were especially interested to analyse later time points of p53 phosphorylation since DNA-repair in NBS$^{-/-}$ cells is ongoing even several days after insult. We studied both the frequency of phosphorylation and site-specific phosphorylation of Ser 6, 9, 15, 20, 33, 37, 46 and 392 on p53 in NBS and control cell lines.

Lymphoblastoid and primary fibroblast cell lines from a healthy individual and a NBS$^{-/-}$ patient were used. The amount of phosphorylation sites on p53 was quantified using the isoelectric point-shift by the phosphate molecules in a 2D-electrophoresis combined with immunoblotting. Phosphorylation sites in single cells were analysed by immunostaining.

Unirradiated cells have in average of 4.8 phosphorylation sites on each p53 molecule. A dose of 2Gy (5Gy) increased the number of phosphorylation sites to 5.3 (6.6) for control cells and to 5.8 (6.9) sites in NBS$^{-/-}$ cells three hours after irradiation. Ser 33, 37 and 46 were not spontaneously phosphorylated in both fibroblast cell lines, and Ser 15 was found to be phosphorylated only in the NBS$^{-/-}$ cell line. Ser 6, Ser 9, Ser 20 and Ser 392 showed spontaneous phosphorylation in both cell lines. Phosphorylation at various sites was monitored after a dose of 2 Gy up to 6 days. Practically no difference between the control and NBS$^{-/-}$ cell line was observed at the Ser 33 site initially, however, after 6 hours, differences between the two cell lines were observed. The most striking difference was that phosphorylation at Ser 20 and Ser 46 increased in the control cell line from 3 hours to a maximum at about 24 hours, while in the NBS$^{-/-}$ cells the increase didn't begin until about 24 hours.

Although the Nbs protein is only known to be involved in phosphorylation of p53 at a few sites, nearly all phosphorylation patterns are different among control and $NBS^{-/-}$ cell lines. Our results show that nearly all functions of p53 like cell cycle arrest, apoptosis and differentiation are altered in NBS cells.

(PS1170) The effect of radiation and repeated sub-culturing on TGF-β1 signaling in FRTL-5 cells. Cheryl G. Burrell[1], Leticia Ortloff[1], Lora Green[1,2]. [1]Loma Linda University, Loma Linda, CA, USA, [2]JL Pettis Memorial Veterans Medical Center, Loma Linda, CA, USA.

From our ongoing *in vitro* studies using the Fisher Rat Thyroid cell line-5 (FRTL-5) we recorded accelerated growth, reduced follicularization and reduction in thyroxin release that occurred as the cells were repeatedly sub-cultured. We also recorded that these changes occurred earlier and more rapidly following radiation exposure. We determined that TGF-β1 production increases under both conditions. We hypothesize that alteration in the TGF-β1 signaling pathway contributed to the changes observed in the cellular properties in FRTL-5 cells. Our objective was to examine some of the players in the TGF-β1 signaling pathway to determine whether radiation and/or repeated sub-culturing promoted changes in their levels and activity.

We quantified changes in cellular growth rate using the MTS cell proliferation assay. TGF-β1 ligand and receptor levels were quantified via ELISA, immuno-cytochemistry and western analysis respectively. The levels and activity of Smad 2, 3, 4 and 7, downstream effectors in the TGF-β1 signaling pathway were also measured via western blotting. Lastly, we examined whether TGF-β1 was correctly regulating the expression of its response genes; cyclin A and plasminogen activator inhibitor-1 (PAI-1) under our experimental conditions. To determine this we used luciferase reporter constructs containing promoters for cyclin A and PAI introduced to the cultures by transfection. PAI-1 production in response to exogenously added TGF-β was also tested using a PAI-1 specific ELISA.

We observed an acceleration of growth that occurred earlier in irradiated cells than it did in cells subjected to repeat sub-culturing. The TGF-β1 receptor levels remained unchanged as result of radiation and continual passage. We, however, observed differences in TGF-β1 induced Smad 2 and 3 phosphorylation levels but not in steady-state levels of these and the other Smads. Alterations were also observed in TGF-β1 ability to control the expression of cyclin A and PAI-1.

Collectively, these results support that alterations in the TGF-β1 signaling pathway were contributing to the changes in cellular properties that we measured in our cell line, FRTL-5′s. These alterations were evident at the level of Smad signaling and transcription initiation.

(PS1171) p38MAPK plays a cytoprotective role in response to radiation through Akt activation in human cervical cancer cells. Min-Jung Kim[1], In-Chul Park[2], Chang-Mo Kang[3], Sangwoo Bae[4], Yun-Sil Lee[4], Sang-Gu Hwang[1], Su-Jae Lee[1]. [1]Laboratory of Radiation Experimental Therapeutics, Korea Insutitute Radiological & Medical Science, Seoul, Republic of Korea, [2]Radiation Function Genomics, Korea Insutitute Radiological & Medical Science, Seoul, Republic of Korea, [3]Radiation Cytogenetics and Epidemiology, Korea Insutitute Radiological & Medical Science, Seoul, Republic of Korea, [4]Radiation Effect, Korea Insutitute Radiological & Medical Science, Seoul, Republic of Korea.

The treatment of cells to ionizing radiation results in the simultaneous activation or down regulation of multiple signaling pathways, which play critical role in controlling cell death or cell survival after irradiation in a cell type specific manner. In this study, we investigated the cell survival signaling in response to radiation in human cervical cancer cells. Ionizing radiation caused suppression of ERK and activations of JNK and p38 MAPK in cervical cancer cells. Inhibition of JNK suppressed radiation-induced Bax and Bak activations, down-regulation of Bcl-2, and apoptotic cell death, while inhibition of p38 MAPK slightly enhanced radiation-induced apoptotic cell death. Moreover, p38 MAPK inhibition completely attenuated radiation-induced Akt phosphorylation at Ser473. Conversely, overexpression of p38 MAPK enhanced Ser473 phosphorylation of Akt after irradiation. In addition, ectopic expression of RacN17, a dominant negative form of Rac1, markedly inhibited p38 MAPK activation and Akt phosphorylation at Ser473, but did not affect JNK activation. Upon stimulation of cells with radiation, Src was activated. Moreover, pretreatment of PP2 or siRNA targeting of c-Src attenuated Rac1 and p38 MAPK activations, and Ser473 phosphorylation of Akt. These results suggest that Src-Rac1-p38 MAPK pathway plays cytoprotective role against radiation-induced apoptosis through Ser473 phosphorylation of Akt.

(PS1172) Knock down of the TGFβ type III receptor results in modulation of NFkB signaling and radio-resistance of normal mouse mammary epithelial cells. Tracy Criswell, Carlos L. Arteaga. Vanderbilt University, Nashville, TN, USA.

Radiation therapy is part of the standard of care for women with breast cancer, although it is estimated that up to 50% of these tumors will be unresponsive to this therapy. Understanding the mechanisms of radiation resistance will allow clinicians to identify patients that may best benefit from this type of therapy as well as develop new treatments that can target therapy-resistant tumors and prevent tumor metastasis.

Transforming growth factor beta (TGF-B) is a pleitropic growth factor known to regulate cell growth and motility. TGFB1 and TGFB3 can bind with high affinity to the TGFB type II receptor (TBRII). In contrast, TGFB2 binds with low affinity to the type II receptor. The type III receptor (TBRIII) binds with high affinity to all three TGFB isoforms, and is required for presenting TGFB2 to TBRII.

TGF-B is growth inhibitory in normal cells, but becomes growth promoting during tumorigenesis. The role of the type I and II receptors in tumorigenesis has been extensively studied. We hypothesized that TBRIII is involved in tumorigenesis of breast cancer. In order to investigate this hypothesis, we used the normal mouse mammary epithelial NMuMG cell line. In these cells, we stably expressed short hairpins RNAs (shRNAs) specific to TBRIII. The NMuMG cells that have decreased RIII (NM-kd) expression show an increased growth rate and increased motility and invasion into matrigel, as compared to the control cells (NM-con). In addition, the NM-kd cells are also radio-resistant as compared to the NM-con cells as determined by colony forming assay, and radiation sensitivity is restored when TBRIII expression is reconstituted.

The family of NFκB transcription factors are known to regulate cell growth and survival, so we decided to investigate whether NFκB signaling is modulated in the more aggressive radio-resistant NM-kd cells. We found that NFκB activity is higher in the NM-kd cells in comparison to control cells using an NFκB responsive luciferase reporter. Transient infection of the NM-kd cells with a dominant negative IκBα containing adenovirus abrogated NFκB activity and the ability of these cells to invade matrigel. In conclusion, TBRIII differentially regulates cell motility, invasion and radiation sensitivity in normal mammary epithelial cells through the modulation of NFκB activity.

(PS1173) Src tyrosine kinase inhibitor PP2 suppresses activation of ERK1/2 and epidermal growth factor receptor induced by X-irradiation. Yoshio Hosoi, Kiyoshi Miyagawa. Section of Radiation Biology, Center for Disease Biology and Integrative Medicine, Tokyo, Japan.

Ionizing radiation (IR) has been shown to activate epidermal growth factor receptor (EGFR) and extracellular signal-regulated kinase (ERK). EGFR activation by IR initiates the Ras/Raf/ERK signaling cascade, stimulates cell proliferation, and leads cells to be resistant to IR. The molecular mechanisms underlying IR-induced activation of EGFR are not clear. Treatment with hydrogen peroxide activates Src, and specific EGFR inhibitor AG1478 and specific inhibitor PP1 inhibit the activation of EGFR and ERK1/2 induced by hydrogen peroxide. Because IR induces production of hydrogen

peroxide, we considered the possibility that IR-induced intracellular or extracellular hydrogen peroxide caused activation of EGFR and ERK1/2 through Src activation just as hydrogen peroxide. To investigate this, we studied the effects of IR on activities of ERK1/2, EGFR, SHP-2, and Src as indicated by their tyrosine phosphorylation and effects of AG1478 and PP2 on them using human breast cancer cell line MDA-MB-468. Exposure of MDA-MB-468 cells to ionizing radiation (IR) caused biphasic activation of ERK as indicated by its phosphorylation at Thr202/Tyr204. AG1478 and PP2 inhibited IR-induced ERK1/2 activation but phosphatidylinositol-3 kinase inhibitor wortmannin did not. IR caused EGFR tyrosine phosphorylation, whereas it did not induced EGFR autophosphorylation at Tyr992, Tyr1045 and Tyr1068 or Src-dependent EGFR phosphorylation at Tyr845. SHP-2, which positively regulates EGFR/Ras/ERK signaling cascade, became activated by IR as indicated by its phosphorylation at Tyr542. This activation was inhibited by PP2 not by AG1478, which suggests Src-dependent activation of SHP-2. Src became activated as indicated by phosphorylation at Tyr416. These data suggest that IR-induced ERK1/2 activation involves EGFR through a Src-dependent pathway that is distinct from EGFR ligand activation.

(PS1174) Developing novel imaging techniques to study radiation induced signaling in 3D model systems. Marianne B. Sowa, Lee Opresko, Derek F. Hopkins, H. Steven Wiley. PNNL, Richland, WA, USA.

Using a three dimensional (3D) model of nonmalignant human breast epithelial cells, we have demonstrated that low dose low- linear energy transfer (LET) radiation exposure leads to increased cellular invasiveness into the surrounding matrix and we propose this is due to activated proteolytic cascades. The 3D model employed organizes into tissue-like structures and recapitulates intact human mammary gland morphology enabling molecular level study of how normal tissues respond following exposure to low doses of ionizing radiation. To study radiation induced signaling in such complex systems, we have developed and assembled a fully automated high speed confocal microscope designed to simultaneously capture the output from two intensified CCD cameras.

Three unique capabilities have recently been added to this system: real-time image registration, active montage, and remote control of hardware and software. Real time two-channel image registration corrects for differences in the optical path and camera alignment as images are acquired. Every pixel from the two cameras is matched before the image appears on the screen. This calculation is so efficient we can obtain two-channel image registration at the full camera frame rate (30 FPS). An extension of this method provides the ability to monitor flouresence resonance energy transfer (FRET) in living cells in real-time. Another advance, the active montage capability, stitches multiple images in real time with associate stage coordinate information. The resulting single image is comprised of hundreds of fields of view but can be processed in a single step. This novel technology allows for the images of the entire culture dish (300–400 individual images) to be treated as a single image during the analysis. All aspects of the microscope are capable of being controlled remotely over a computer network, including the streaming of live video and control the microscope over vast distances (thousands of miles) allowing for easy collaboration with distant users. We conclude that advanced imaging techniques can be applied to the study of radiation induced signaling in 3D culture systems.

(PS1175) Tumor hypoxia, necrosis and targeting: it's the gradient, dummy. John P. Kirkpatrick, Thies Schroeder, Mark Oldham, Mark W. Dewhirst. Duke University Medical Center, Durham, NC, USA.

Introduction: Tumor hypoxia is associated with increased metastatic potential, radioresistance and worse clinical outcome. It has been proposed that hypoxic regions be targeted to maximize cell kill. Emerging techniques offer the promise of non-invasively imaging hypoxia, while current technology, such as IMRT, appears capable of delivering radiation to specific subvolumes. To determine if hypoxic fraction alone defines optimum volumes for irradiation, we employ some simple models of tumor mass transport and dose distribution.

Model & Results: The tumor model consists of four compartments - severely, moderately and mildly hypoxic and normoxic - with median pO_2 of 0.5, 2, 5 and 25 mm Hg, respectively. We assume these to have relative volumes of 0.42, 0.21, 0.20 and 0.17, corresponding to a severely hypoxic core of relative radius 0.75 surrounded by successively better-oxygenated "shells" of thickness 0.11, 0.08 and 0.06. Using a power-law approximation for the oxygen-enhancement ratio, the algorithm for optimal dose redistribution across compartments of varying radiosensitivity (Søvik, 2007) and the assumption that compartments have equal clonogen density, then the compartments would ideally receive 2.47, 1.95, 1.65 and 1.30 Gy, (most to least hypoxic) based on a mean tumor dose (MTD) of 2 Gy. Assuming linear exponential cell kill with $\alpha = 0.3\ Gy^{-1}$, initial clonogen density of 10^7 clonogens/ml, initial tumor volume of 100 ml and 21 2-Gy fractions, the tumor control probability (TCP) is 0.83. In fact, severely hypoxic regions are often necrotic and irradiating a "dead" core should not be beneficial. In this case, assuming the core contains 0 clonogens, clonogens are redistributed equally across the shells, total clonogen number (10^9) is preserved and a TCP of 0.83 is desired, the compartments would receive 0, 1.95, 1.65 and 1.30 Gy (MTD 1.63 Gy). Alternatively, a MTD of 2 Gy yields a TCP of 0.999 for this dose distribution. Mass transport modeling shows that substrate gradient reflects substrate utilization and zero gradient is characteristic of necrotic tissue.

Conclusions: While areas of necrosis are hypoxic when adjacent to tissues that deplete oxygen, high-gradient areas contain the highest density of clonogens and should be targeted to maximize TCP. Avoiding irradiation of necrotic volumes optimizes treatment.

(PS1176) LSDCAS studies of cells undergoing reductive division following radiation-induced mitotic catastrophe. Fiorenza Ianzini, Elizabeth A. Kosmacek, Jennifer M. Symonds, Paul J. Davis, Michael A. Mackey. University of Iowa, Iowa City, IA, USA.

Mitotic catastrophe (MC) has been observed following alterations in specific cellular proteins, or as a consequence of treatment of cells with chemicals, heat and/or ionizing radiation. MC is a common mechanism underlying mitotic-linked cell death and it is the result of premature entry into mitosis occurring during delays late in the cell cycle. MC is characterized by an aberrant nuclear morphology and often results in the generation of aneuploid and polyploid cell progeny. Although generally lethal, some cells can survive MC through mechanisms which are incompletely understood. The mechanism underlying the restoration of clonogenicity in populations which have undergone MC is an active research area and cytological and molecular biology studies conducted by us and other groups are pointing to the presence of features associated with meiotic cell division in the polyploid cell populations produced *via* MC. This finding might be particularly relevant in the understanding of tumor progression, and might provide a novel mechanism for the generation of quasi-diploid progeny from MC-induced polyploid cell populations. From these studies, however, an important piece of information is missing as it is not possible to know what the fate of these "reverting" cells might be. In the present report we will discuss the fate of the MC cells by means of live cell imaging. By using the Large Scale Digital Cell Analysis System (LSDCAS) and the developed in-house "Cell Event Analysis Software" we are able to measure a myriad of different cell parameters and events. Among other parameters, the event data is queried to generate cell population statistics for cell death, cell proliferation, and cell division including unique events such as failed divisions, multipolar divisions, sister cell fusions, non-sister cells fusions. Through application of the LSDCAS analytical tools, we show that cells that have undergone MC can re-initiate normal cell division after many rounds of abnormal cell division and can thus generate cell populations that are still alive at day 15 post-irradiation. These cells are good candidates to be those responsible for cancer resistance to treatment and cancer progression. *(Support: NIH CA74899, CA58648, CA/GM94801; NASA NRA NNJ06HH68G; Whitaker Foundation Special Opportunity Award).*

(PS1177) Imaging of the redox state and of hypoxia in human head and neck tumor xenografts. Ala Yaromina[1], Ulrike Sattler[2], Verena Quennet[2], Christian Hoerner[2], Daniel Zips[1], Stefan Walenta[2], Michael Baumann[1], Wolfgang Mueller-Klieser[2]. [1]Department of Radiotherapy and Oncology, Medical Faculty, University of Technology, Dresden, Germany, [2]Institute of Physiology and Pathophysiology, Johannes Gutenberg-University, Mainz, Germany.

Lactate accumulation quantified with imaging bioluminescence in solid malignant tumors is extremely variable even when different individual lesions at the same clinical stage and with the same pathohistological grading are compared. In several independent studies on three different tumor entities high lactate levels in the primary lesions predicted for a high incidence of distant metastases, local recurrence, and poor survival of patients (Walenta et al., Curr Med Chem 2004;11:2195).

In the current experimental study, metabolic imaging was extended to structure-associated mapping of pyruvate and the lactate-to-pyruvate (L/P) ratio in solid tumors (Sattler et al., Lab Invest 2007, 87:84–92). The novel approach was combined with Pimonidazole labelling in eight established head and neck squamous cell carcinoma lines that were xenografted in nude mice and characterized with regard to their radiosensitivity. ATP, glucose, lactate, pyruvate, and L/P ratios were measured before radiotherapy via bioluminescence including data acquisition exclusively in vital tumor regions.

Mean tissue concentrations (μmol/g) of metabolites in Cal33, FaDu, SAS, UTSCC-5, UTSCC-14, UTSCC-15, UTSCC-45, and XF354 tumors ranged from 7.3–25.9 for lactate, 0.9–1.5 for pyruvate, 0.8–2.5 for ATP, and 1.1–3.1 for glucose, respectively. The L/P ratio ranged from 7–27 and was positively and closely correlated with the lactate concentration across the tumor lines. The hypoxic tissue fractions determined by Pimonidazole ranged from 6.2–35.2%. A spearman rank order correlation revealed a trend towards pHF and L/P ratio being positively correlated ($r = 0.6190$), but this was not statistically significant ($p = 0.1017$). On the other hand, previous data analysis demonstrated a positive and statistically significant correlation between TCD_{50} and the L/P ratio.

In conclusion, neither lactate nor L/P ratio were strongly correlated with the Pimonidazole hypoxic fraction. The L/P ratio as a pretherapeutic predictive parameter of radiosensitivity has to be evaluated in a larger multiparametric study.

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(PS1178) Temporal changes in tumor blood supply measured in A-07 melanoma xenografts. Kjetil G. Brurberg, Camilla Mollatt, Jon-Vidar Gaustad, Einar K. Rofstad. The Norwegian Radium Hospital, Oslo, Norway.

The purpose of the work was to image the temporal heterogeneity in tumor blood supply time (BST).

A plastic chamber was implanted over a circular incision in the dorsal skin fold of BALB/c-nu/nu mice, and a spheroid of A-07 cells (ø ~ 0.1 mm) was implanted into the opposing skin fold. A glass window was placed over the incision site. Tumors were subjected to imaging 9–12 days after implantation. Prior to imaging, all mice were anesthetized and fixed on a custom-made and heated microscope stage. Then, 4 mg of TRITC-labeled 155kDa dextran was administered intravenously while epifluorescense images were acquired subsequently at a frame rate of 9 per second. This imaging procedure was performed thrice for each tumor, with 20 min between each imaging series. Custom-made software was used to calculate the time needed by the blood to flow from an artery in the normal tissue to the respective vessels. BST was defined as the difference in the time to maximal contrast enhancement between the reference vessel and the respective tumor vessel. BST was calculated pixel-by-pixel for all tumor vessels.

Five A-07 tumors with different vascular morphology were included in the study. Typically, the BST was higher in the tumor periphery than in the center. It was seen that most tumors included vessels in which BST changed significantly within 40 min. The magnitude of the changes in BST ranged from 0 to 1 second. Moreover, the fraction of vessels undergoing changes in BST differed among the tumors. In some tumors BST changed only in single vessels, whereas other tumors experienced a general up or down regulation of the BST.

The BST to particular tumor regions may change significantly within 40 min, and may reflect changes in the oxygen delivery efficiency to the tumor.

(PS1179) Fraction of radiobiologically hypoxic cells and fluctuations in tumor blood perfusion assessed by dynamic contrast-enhanced MRI. Jon-Vidar Gaustad, Kjetil G. Brurberg, Tormod A. M. Egeland, Ilana C. Benjaminsen, Einar K. Rofstad. Group of Radiation Biology and Tumor Physiology, Department of Radiation Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway.

The purpose of this study was to investigate the potential usefulness of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) for detection of fluctuations in tumor blood perfusion, and to evaluate whether parameters derived from DCE-MRI data can predict the fraction of hypoxic cells.

To investigate the occurrence of blood flow fluctuations, A-07 melanoma xenografts were subjected to two DCE-MRI acquisitions separated by one hour. Images of $E{\cdot}F$ (E is the initial extraction fraction of Gd-DTPA and F is perfusion) and λ (the partition coefficient of Gd-DTPA, which is proportional to extracellular volume fraction) were obtained by Kety-analysis of DCE-MRI data. $E{\cdot}F$ images were used to determine changes in blood perfusion arising between the two imaging sessions. λ images were used to control the reproducibility of the experimental procedure. To evaluate whether parameters derived from DCE-MRI data can predict the fraction of hypoxic cells, A-07 melanoma xenografts were first subjected to DCE-MRI with subsequent Kety analysis, and then to measurement of fraction of hypoxic cells using a radiobiological assay.

Fraction of voxels indicating significant fluctuations in blood perfusion ranged from 6 % to 51%. Some tumors were characterized by a general increase or a general decrease in $E{\cdot}F$, whereas others showed similar fractions of voxels with increased or decreased $E{\cdot}F$ values. All tumors showed voxel clusters (# voxels > 10) where $E{\cdot}F$ changed in the same direction. Such clusters were usually observed in the tumor periphery. The hypoxic fraction of the tumors differed from 1.2% to 48%. Median $E{\cdot}F$ and median λ differed among the tumors by factors of ~5.8 and ~1.8 respectively. $E{\cdot}F$ was found to be strongly correlated to fraction of hypoxic cells ($P<0.000001$), whereas significant correlation between λ and fraction of hypoxic cells could not be detected.

The study showed that DCE-MRI with subsequent Kety analysis is a useful method for detection of blood flow fluctuations in tumors, and strongly suggested that the peripheral regions of A-07 tumors are more exposed to temporal changes in blood perfusion than are the central parts. The study also suggested that $E{\cdot}F$ may be a useful parameter for the extent of hypoxia in experimental and human tumors with properties similar to those of A-07 tumors.

(PS1180) Spatiotemporal imaging of hif-1 mediated tumor cell-stromal adaptation to hypoxia following radiation. David L. Schwartz, Jung Hwan Oh, Ryan Williams, Yi He, Robert Lemos, Norihito Kuno, Sunil Krishnan, William Bornmann, Edward Jackson, Garth Powis, Juri Gelovani. UT M.D. Anderson Cancer Center, Houston, TX, USA.

Background: XRT induces HIF-1 signaling in tumors, although the exact mechanisms and dynamics of this remain undefined. We hypothesized that XRT disrupts stromal vasculature, leading to acute hypoxia responsible for HIF-1 signaling. We also reasoned that HIF-1 inhibitor PX-478 combined with XRT would prevent stromal adaptation and XRT resistance

Methods: We used a C6#4 glioma cell line stably transfected with a hypoxia response element-driven HSVtkGFP reporter to examine HIF-1 signaling before and after *in vitro* irradiation of monolayer and 3D spheroid cultures. For *in vivo* experiments, immunohistochemistry (IHC), ^{18}FEAU-PET imaging, and dynamic contrast enhanced (DCE)-MRI were performed on murine xeno-

grafts after single dose 8 Gy. Mice received PX-478 (120 mg/kg) for 4 days, starting the day of XRT

Results: Exposure of C6#4 monolayers to 1% 0_2 prior to 8 Gy increases HIF-1 signaling, and augments post-radiation expression of VEGF mRNA at 2 hr post-XRT and secreted VEGF at 48 hr post-XRT. In spheroids, radiation inhibits HIF-1 signaling within central core regions. Control xenografts develop diffuse centralized FEAU uptake on PET over time. 8 Gy stimulates intense, localized HIF-1 signaling by 48 hours. IHC demonstrates a reduction in Ki-67 and pimonidazole staining at 24 hr, followed by focal development of pimonidazole staining co-localizing with HIF-1, VEGF and decreased CD105 vascular staining. Xenograft tissue 8 days post-XRT reveals continued overlap of hypoxia and HIF-1 signaling outside of areas of dense CD105 signal. DCE-MRI revealed localized areas of decreased vascular integrity following XRT. Although PX-478 alone temporarily inhibits HIF-1 signaling, combined PX-478 and 8 Gy yields sustained HIF-1 inhibition and blunted xenograft growth

Conclusions: XRT induces downregulation of HIF-1 in spheroids, but promotes delayed HIF-1 signaling in vascularized tumors, implicating XRT-induced stromal vessel collapse and ischemic injury. HIF-1 signaling leads to downstream VEGF-dependent stromal recovery, reperfusion, and tumor XRT resistance. Interruption of tumor cell-stromal interactions critical to tumor adaptation to XRT via inhibition of HIF-1 is a promising strategy for radiosensitization

(PS1181) Use of optical spectroscopy for characterization of tumor oxygenation and metabolic redox ratio. Gregory M. Palmer, Ronald J. Viola, Thies Schroeder, Pavel S. Yarmolenko, Lauren E. Tochacek, Nimmi Ramanujam, Mark W. Dewhirst. Duke University, Durham, NC, USA.

This study investigated the use of non-invasive optical spectroscopy to monitor tumor physiology, including tissue oxygenation and metabolic redox ratio, in response to a perturbation by carbogen breathing. Measurements were performed on 4T1 mammary tumors grown in the flank of nude mice (n=14), by placing a fiber optic probe in contact with the surface of the tumor. Two Oxylite pO_2 sensors inserted into the tumor were used for comparative purposes. Two sets of optical measurements were made, (1) the diffuse reflectance spectra (350–600 nm), and (2) the fluorescence emission spectra obtained at 350 and 460 nm excitation, which excite NADH and FAD, respectively.

A Monte Carlo model of light transport was used to model the reflectance and fluorescence spectra to extract the concentrations of oxy and deoxy-hemoglobin (and thus the hemoglobin saturation), as well as the intrinsic fluorescence spectra (which is proportional to the concentrations of the fluorophores NADH and FAD). These physiologic parameters are shown in Table 1, for the case before and during carbogen breathing.

For the set of 14 animals, a significant increase was seen in pO_2 ($p<0.05$) and Hb saturation ($p<0.001$), while a significant decrease was seen in the redox ratio ($p<0.05$) using a paired t-test. As seen in the standard errors, remarkable consistency was seen in the hemoglobin saturation, suggesting this technique could be useful in monitoring response to anti-angiogenic and other therapies.

	Before Carbogen Breathing	During Carbogen Breathing
Hemoglobin Saturation (%)	57±2	72±3
Redox Ratio	9.9±0.8	9.0±0.7
Baseline-Normalized Redox Ratio	1±0	0.92±0.02
pO_2 (mmHg)	3.8±1.2	28±9

Table 1: Physiologic parameters recorded before and after administration of carbogen breathing.

(PS1182) Does [18F]FDG-uptake predict therapy response to single dose irradiation in FaDu tumors in nude mice? Bettina Beuthien-Baumann[1], Christina Schuetze[2], Ralf Bergmann[3], Franziska Hessel[4], Michael Baumann[4]. [1]Universitaetsklinikum Carl Gustav Carus, Klinik für Nuklearmedizin, Dresden, Germany, [2]Universitaetsklinikum Carl Gustav Carus, Klinik für Strahlentherapie und Radioonkologie, Dresden, Germany, [3]PET-Zentrum, Forschungszentrum Dresden-Rossendorf, Dresden, Germany, [4]Universitätsklinikum Carl Gustav Carus, Klinik für Strahlentherapie und Radioonkologie, Dresden, Germany.

Objectives: Not only tumors of different patients but also the tumor of an individual patient may show heterogeneous [18F]FDG uptake. It is currently unknown whether this intratumoral heterogeneity correlates with tumour response in subvolumes of the tumor and may be used as a biological marker for heterogeneous dose prescription. We used a preclinical model situation to investigate the heterogeneity of [18F]FDG uptake and dose response in a single human tumor line transplanted in nude mice.

Material and Methods: The human squamous cell carcinoma FaDu was transplanted subcutaneously into the hind leg of NMRI nude mice. Animals entered the study at a tumor diameter of 7 mm. [18F]FDG-PET scanning was performed on a dedicated animal PET scanner. After [18F]FDG-PET the tumors were irradiated with either 25 Gy or 35 Gy single dose (ambient) using 200 kV x-rays. Sixty-two animals are included in this study. The maximal Standard Uptake Value (SUVmax) was evaluated by 3D regions of interest delineating the tumor. Mice were observed for 120 days, experimental endpoint was local tumor control.

Results The SUVmax values ranged from 0.72 to 3.47, the median value was 1.59. In all 62 animals local tumor control rates were 28 % after irradiation with 25 Gy and 57 % after 35 Gy (Kaplan-Meier analysis, Logrank-Test p=0.007) When tumors were stratified above or below median SUVmax , no significant difference in local tumor control after 25 Gy (p=0.89) but a significant higher local control for tumors with SUVmax above median SUVmax after 35 Gy (p=0.002) was found. The COX model with dose and SUVmax as continues variables shows a significant decreasing hazard of recurrence with increasing dose (p=0,007) or SUVmax (p=0.05).

Conclusions: Our data show that radiation dose had a greater effect on local control in tumors with high FDG uptake than tumors of the same line with low FDG uptake. This supports the hypothesis that pretreatment FDG-PET may provide useful information for heterogeneous radiation dose prescription. As only one tumour model was studied and single doses were applied, confirmatory investigations using further tumour models and fractionated radiotherapy are warranted.

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(PS1183) Molecular imaging of a murine monoclonal antibody that binds to the β3 subunit of αvβ3 integrin in the experimental model of the Lewis lung cancer in mice. Marek Bilski[1], Ireneusz P. Grudzinski[1], Urszula Karczmarczyk[2], Robert Zdanowski[1], Jacek Pietrzykowski[3], Renata Mikolajczak[4], Piotr Garnuszek[2], Eugeniusz Dziuk[3], Marek P. Dabrowski[1], Marek K. Janiak[1]. [1]Military Institute of Hygiene and Epidemiology, Warsaw, Poland, [2]National Institute of Drugs, Warsaw, Poland, [3]Military Medical Institute, Warsaw, Poland, [4]Radioisotope Centre Polatom, Otwock, Poland.

Targeting integrin αvβ3 in both the tumor and endothelial cells that line tumor infiltrating blood vessels is a new diagnostic strategy that has gained widespread support from preclinical studies. Here we developed the noninvasive diagnostic method to image and quantify the subunit β3 of integrin αvβ3 in a murine model of the Lewis lung cancer (LLC).

Monoclonal antibody anti-CD 61 (2C9.G2) against the subunit β3 of the integrin αvβ3 was labeled with iodine ^{131}I using a standard iodogen method. The radiochemical purity of the resulting mAb-^{131}I of 99% was achieved in a reaction time just after the iodination, and 95.6 and 93.7 % after 2 and 24 hr. The diagnostic value of the mAb-^{131}I radiotracer was elucidated by scintigraphic imaging followed by quantification of the tumor integrin subunit β3 level in male C57BL/6 mice 3 wk after s.c. inoculation of the syngeneic LLC. The in vivo pharmacokinetic (PK) of the mAb-^{131}I radiotracer was studied 1.5–72 hr after a

single i.v. injection of 1.5 to 50 μg mAb-^{131}I in tumor (LLC)-bearing mice.

After injection of mAb-^{131}I, the radioactivity cleared rapidly from the blood ranging from 21 to 3.7 %ID/ml between 1.5 and 6 hr post-dosing followed by slow decreases in next hours (0.86 %ID/ml after 72 hr). There was also considerable uptake of mAb-^{131}I in various normal tissues such as liver, lung, spleen, and kidney. Muscle, brain and skin tissues had relatively low activity uptake. In the course of the applied dose, the tumor-to-non-tumor tissue (T/NT) ratio rose up to 20 μg dosage. Uptake of the radiolabeled mAb-^{131}I in the tumor was rapid and high (4.9%ID/g at 1.5 hr). On the scintigraphic images acquired at 24 hr after the injection of mAb-^{131}I, the liver and kidneys and the bladder were the organs with the highest activity levels, illuminating the renal excretion pattern of the radiotracer. The transplanted tumors were visualized on the images of the mice that received mAb-^{131}I. Regions of interest (ROIs) analysis revealed the T/NT count ratio of 0.8 and 12.1 for liver and muscle, respectively. The binding potential extrapolated from scintigraphic data correlated well with the in vivo PK studies.

The present study showed that the radiolabeled monoclonal antibody, mAb-^{131}I with high integrin subunit β3 specificity and favorable pharmacokinetic, is able to target and image tumor tissue in a mouse LLC model.

(PS1184) Molecular probe for the detection of hydroxyl radicals (•OH) within DNA. Amarjit Singh, Yongliang Yang, Pichumani Balagurumoorthy, S. James Adelstein, Amin I. Kassis. Harvard Medical School, Boston, MA, USA.

We have been developing DNA-binding coumarin (**C**) analogs that can report the formation of hydroxyl radicals (•OH) within DNA. In this approach, a nonfluorescent coumarin analog is converted to a fluorescent derivative after interaction with •OH in aqueous solution. Using computer-generated molecular modeling, two coumarin analogs, coumarin-polyamine (**C-polyA**) and coumarin-polyamine-coumarin (**C-polyA-C**), with favorable binding energies (**Δ*G***) to DNA were identified. The compounds (and their hydroxylated fluorescent analogs) were synthesized and characterized. The fluorescence yield of **HO••C-polyA** is ~3.3 times that of **HO••C** and **HO••C-polyA-C••OH**. When **C**, **polyA-C**, and **C-polyA-C** are dissolved in PBS (pH 7.0) and irradiated with γ rays (0–10 Gy) or ^{125}I (0–7E8 decays), (**i**) the samples become fluorescent, (**ii**) the emission spectrum of each analog is similar to that of its corresponding hydroxylated derivative, (**iii**) the relationship between induction of fluorescence and dose is linear, and (**iv**) the fluorescence yield of irradiated **C-polyA** is ~3 times that of **C** and **C-polyA-C**. Since this increase in yield is similar to that observed when the spectrum of each hydroxylated analog is measured, the three derivatives are apparently hydroxylated with the same efficiency when irradiated. Gel electrophoresis shows that the addition of these polyamine derivatives to plasmid DNA (≤50:1) does not lead to its aggregation, and the fluorescence yield of the hydroxylated analogs is not quenched in the presence of DNA. We believe that the highly sensitive **C-polyA** will enable the quantitative detection of •OH present within the immediate vicinity of the DNA double helix in solution (~7-nm-radius cylinder) and advance our comprehension of the molecular basis of •OH-mediated DNA breaks.

(PS1185) Optical analyses of radiobiological effects in irradiated cellular systems. Aidan D. Meade[1], Hugh J. Byrne[1], Fiona M. Lyng[2]. [1]Dublin Institute of Technology, Dublin, Ireland, [2]RESC, Dublin Institute of Technology, Dublin, Ireland.

Exposure of in-vitro cultures to ionizing radiation produces complex responses that are dependent on dose, dose-rate and relevant biological effectiveness of the radiation. In order to completely elucidate the response of the system to ionizing radiation exposure, the assay of many relevant endpoints and the replication of experiments for genetically independent cultures, is required. This can often result in the use of a vast array of biological samples and assay material, which can be prohibitive. Recently, biospectroscopy has emerged as a potential non-invasive tool that may provide multiplexed assays of radiobiological responses in these systems. The present work investigates this potential further in the case of human epithelial keratinocytes (HaCaT) and bronchial epithelial cells (BEAS2B) exposed to 1 MeV g-radiation from a Co-60 therapy source.

HaCaT and BEAS2B cells were cultured on quartz for Raman spectroscopy and on MirrIR slides (Kevley Technologies Inc.) for FTIR spectroscopy. Cells were irradiated with dose points from 0 to 5Gy and were assayed from 6 hours to 5 days post-irradiation. The spectroscopic measurements were referenced to assays of proliferative capacity, cellular viability and cellular reproductive capacity. Investigations of the effects of inhibitors of free-radicals (Catalase, and L-NAME) on spectroscopic measurements were also conducted.

Results of fluorescence assays demonstrate a time and dose-dependance in proliferative effects, with Raman and FTIR measurements exhibiting variations in band intensity in regions (amide I, II and DNA PO2- stretching vibrations) that may be associated with apoptotic, necrotic and protective responses in the cell. Furthermore, Principal Components Analysis discriminates the progression of these responses in terms of the molecular content of the cell providing insights into the changes in molecular content associated with different radiobiological responses.

These results demonstrate that complex biochemical responses in cellular systems may be non-invasively assayed using vibrational spectroscopy and are comparable with those measured with standard radiobiological assays. It also demonstrates that insights into radiobiological processes at the molecular level can be elucidated directly with minimum sample preparation.

(PS1186) Targeted Therapeutic Evaluation on Inhibition of Fatty Acid Synthase in a Human Prostate Carcinoma LNCaP/*tk-luc* bearing animal model with Molecular Imaging. Wen-Tien Tai[1], Ya-Fang Chang[1], Jyh-Der Leu[2], Jeng-Jong Hwang[1]. [1]Department of Biomedical Imaging and Radiological Sciences (BIRS),National Yang-Ming University, Taipei, Taiwan, [2]2. Division Radiation Oncology, Taipei City Hospital, Renai Branch, Taipei, Taiwan.

Fatty acid synthase (FAS), with ability of *de novo* fatty acid synthesis, is highly expressed in most human cancers, including prostate carcinoma. FAS is overexpressed at both mRNA and protein levels in prostate carcinoma associated with a 4-fold risk of disease recurrence and higher stage. The high-level FAS expression in patients with prostate cancers were also found with high risk in bone metastases. As a novel potential therapeutic target, inhibition of FAS could arrest cell cycle in S phase and trigger apoptosis rapidly, implying the reliance of cancer cell survival on FAS activity. In this study, we used the FAS inhibitor, C75, and small interfering RNA (siRNA) to manifest the inhibition effect of endogenous fatty acid metabolism in a human prostate carcinoma LNCaP/*tk-luc* cells both in vitro and in vivo. Multimodalities of molecular imaging were used to demonstrate the inhibition effects of FAS in a LNCaP-*tk*/*luc* bearing mouse model, which constitutively expresses herpes simplex virus type-1 thymidine kinase (HSV1-tk) and luciferase (luc) genes. Bioluminescent imaging (BLI) and nuclear imaging (gamma scintigraphy, PET and autoradiography) were used to monitor tumor progression and metastaic spreading.

LNCaP-*tk*/*luc* cells were implanted subcutaneously into NOD/SCID mice. Animals were i.p. injected with high-dose C75 (total 120 mg/kg, i.e. 30 mg/kg once a wk for 4 weeks) or locally administered with FAS sequence-specific siRNA. The results showed that the intensity levels of BLI from in vivo and ex vivo post treatments were well correlated to the tumor growth inhibition, and were further confirmed by histopathology. [I-131]FIAU, [C-11]acetate, [I-124]FIAU, and [F-18]FHBG were also used to evaluate the *tk* expression in the tumor. In conclusion, the targeted therapy using enzyme inhibitor, such as C75, or siRNA for specific protein could be explored using reporter genes combined with multimodalities of molecular imaging.

(PS1187) Primary tumor volume measurements of nasopharyngeal carcinoma determined with computed tomography: study of variability. Cheng-Chaun Chang, Mu-Kuan Chen, Hwa-Koon Wu. Changhua Christian Hospital, Changhua City, Taiwan.

Objective: To investigate the intraobserver and interobserver variability of computed tomography-based volume measurements of nasopharyngeal carcinoma. Desgin: Prospective study. Setting: Tertiary care centre. Methods: The primary tumour volume of 13 nasopharyngeal carcinomas was repeatedly measured by two trained observers independently in two different sessions, using the summation of area technique. Main Outcome Measures: Mean tumour volume and its standard deviation were calculated for each tumour. Statistical analysis was done with multivariate analysis, linear regression, and a two-way analysis of variance (ANOVA) random effects model. Results: The coefficient of variation was less than 20% in 11 volume measurements, but a large discrepancy between observers was noted in two tumours with involvement of the paranasal sinuses. A good linear correlation was found between mean tumour volume and its standard deviation: standard deviation = 0.26 volume - 2.48 ($r = .80$). When the two tumours with a large coefficient of variation were excluded, the two-way ANOVA random effects model revealed that both the interobserver ($p = .83$) and the intraobserver ($p = .90$) effect are not statistically significant; interobserver variability was the major component of total variability (71.0%). Conclusions: Total variability in the computed tomography-based measurement of nasopharyngeal carcinoma volume is small by having the measurements done by a trained observer, except in tumours with involvement of the paranasal sinuses.

(PS1188) Application of a high-brightness electrodeless z-pinch soft x-ray source to water window imaging and microbeam research. Stephen F. Horne, Matthew M. Besen, Robert D'Agostino, Donald K. Smith, Paul Blackborow. Energetiq Technology, Inc., Woburn, MA, USA.

Soft X-Ray light, in the so-called "water window" between the carbon and oxygen absorption edges at 2.3nm and 4.4nm, is a candidate for microbeam studies into the effects of ionizing radiation on biological cells. At these wavelengths, due to the relatively high soft x-ray transmission of water, radiation can penetrate depths of 10 micrometers or more in typical samples. Soft x-ray light can be readily focused into a small spot using nano-fabricated Fresnel zone plate lenses. This approach has enabled soft x-ray microscopy and tomography of biological samples with resolution in the 20 nanometer range. Soft x-ray light for microbeam studies and microscopy can readily be found at the synchrotron light sources of the national labs. However, this limits the availability of soft x-rays to those researchers who are able to obtain beam time and are able to bring their experiments to the synchrotrons.

Energetiq Technology has developed a compact source of soft x-ray light based on a novel plasma approach. The device is a modification of our commercially successful 13.5 nm (92 eV), 10 Watt source, and is being optimized primarily as a source of 2.88 nm radiation to enable a compact water window microscope. The technical requirements for an interesting microbeam device are somewhat less demanding than for an imaging light source, and we expect that a device delivering >1 Gy/sec in a sub-micron beam is achievable.

We will present source performance data, and describe the current state of our programs in X-ray microscopy and microbeams.

(PS1189) Curcumin disrupts radiation induced positive feed back (NFκB-TNFα-NFκB) cycle and inhibits NFκB mediated radio-adaptation in neuroblastoma cells. Natarajan Aravindan, Rakhesh Madhusoodhanan, Salahuddin Ahmad, Daniel Johnson, Terence Herman. Oklahoma University Health Science Center, Oklahoma City, OK, USA.

The induced radio-resistance in surviving cancer cells after radiotherapy could be associated with clonal selection leading to tumor regrowth at treatment site. We recently demonstrated the mechanism of induced radio-resistance, at-least in part, is mediated by the persistent activation of transcription factor NFκB through a positive feed back (NFκB-TNFα-NFκB) cycle. Curcumin, a dietary polyphenol, is a pharmacologically safe and effective agent that has been demonstrated to have anti-inflammatory, antiproliferative, and antitumor effects by modulating many potential molecular targets including NFκB. Accordingly we investigated the effect of curcumin on the radiation induced-persistent activation of NFκB, feed back cycle, survival advantage and initiation of clonal expansion. Human neuroblastoma (SK-N-MC) cells pretreated with 100nM/L curcumin for 1 h and exposed to radiation (2Gy) were examined for inhibition of persistent activation of NFκB and the disruption of the feed back cycle. NFκB DNA-binding activity was measured using electrophoretic mobility shift assay, TNFα mRNA expression using real-time quantitative-PCR (Q-PCR). Intracellular expression of TNFα protein was quantified using ELISA. Effect of curcumin on radiation induced survival advantage is measured after exposing the curcumin pretreated cells to either single (2Gy) dose or after repeated radiation (2Gy x 5). mRNA and protein expression of pro-survival molecules (IAP1, IAP2, survivin, XIAP) were examined by Q-PCR and immunoblotting. Telomerase activity was determined by TRAP assay. The results clearly indicated that curcumin significantly inhibited the radiation-induced NFκB DNA-binding activity and this inhibition is persistent (even after 72 h). The disruption of radiation-induced positive feed back cycle is indicated by the suppressed expression of intracellular TNFα. Curcumin significantly suppressed the radiation induced pro-survival molecules (both mRNA and protein). Similarly, pretreating the cells with curcumin markedly inhibited the radiation-induced telomerase activity. These results suggest that curcumin reverted radiation-induced radio-adaptation and survival advantage in human neuroblastoma cells by inhibiting NFκB-TNFα-NFκB feed back cycle mediated persistent activation of NFκB.

(PS2001) Robotic blood handling in a cytogenetic biodosimetry laboratory for dose assessment in radiological and nuclear mass casualties. Pataje G. Prasanna, Patrick R. Martin, Uma Subramanian, Maria Moroni, Roman E. Berdychevski. Armed Forces Radiobiology Research Institute, Bethesda, MD, USA.

Purpose: The chromosome aberration-based dicentric assay may be used for individual dose assessment in radiation mass casualties, confirm clinical triage, and aid medical treatment. This will necessitate processing of a large number of blood samples in a cytogenetic biodosimetry laboratory. We developed a high throughput, flexible, modular, and scalable robotic blood handling system, which represents a "beta" version.

System Description: Development of robotic blood handling system involved integration and customization of commercially available hardware including (a) a robotic liquid handler (Tecan Freedom EVO, Tecan, NC), (b) a robotic swinging bucket centrifuge (ROTANTA 46RSC, Helmer, IN), and (c) an automated cell viability analyzer (Cedex Innovatis, Germany). This integrated system is enclosed in a bio-safety level II cabinet (BIOPROtect II, JT Baker, ME). Customized software interfaces with CytoTrack, a sample tracking and data management laboratory information management system.

Summary of Data. Customization to Tecan's large work deck include (a) a barcode reader, (b) a Liquid Handling arm (LiHa) with clot detection, liquid sensing, and vacutainer septa piercing capabilities for all liquid handling operations, (c) a Pick and Place robotic arm for moving tubes around the work deck, (d) a Robotic Manipulator with a customized extended Z-axis for transporting centrifuge buckets to and from the integrated robotic centrifuge, (e) troughs for reagents, (f) racks for blood vacutainers, and (g) wash station for pipette tips. Two scalable and flexible automated sample processing scripts are developed for short-term blood/lymphocyte cultures for harvesting metaphase spreads for dose assessment: (a) a micro-culture script for processing whole blood for triage and (b) a mini-culture script for a cell density corrected isolated lymphocyte cultures for more precise dose estimation. A throughput of up to 96 samples per run (in less than 1 hour) and 72 samples per run (in less than 4 hours) can be achieved using the micro- and mini-culture scripts respectively.

Conclusion: Automated blood handling can increase throughput, assure quality and ensure occupational safety.

Acknowledgement. AFRRI and National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, supported this research.

(PS2002) An improvement of the metaphase finder system for the biological dosimetry by chromosome aberration. Akira Furukawa, Masako Minamihisamatsu, Isamu Hayata. National Institute of Radiological Sciences, Chiba, Japan.

For biological dosimetry by counting chromosomes aberrations, automation technique has been required to process large number of sample preparations at low dose radiation exposure, or at large number of people who wants the exposure test.

The metaphase finder is an automated optical microscope system, which automatically scans and finds metaphase cells on the slide glass, and relocates metaphase cells to the center of the field of view of the microscope to observe chromosomes in high magnification.

We have constructed a Metaphase Finder system by assembling commercially available components, such as microscope, motorized sample stage, personal computer and general purpose image analysis software package, instead of purchasing one dedicated system.

The new points of recent system were camera's view field became larger than before, and morphology-based improved image processing method was used. Our system has begun to practically use in several domestic laboratories.

The purpose of this paper is to test the performance of this new Metaphase Finder system. The results were: The scanning speed was 22 min/cm^2. The false positive rate was 2 %.

The speed was slower than several commercial metaphase finders but enough for practical use.

(PS2003) Rapid and accurate *in vivo* tooth dosimetry: experimental procedure for positioning the resonator. Ruhong Dong, Artur Sucheta, Eugene Demidenko, Benjamin B. Williams, Maciej Kmiec, Gregory Burke, Piotr Lesniewski, Firdus F. Gubaydullin, Andres Ruuge, Harold M. Swartz. Dartmouth College, Hanover, NH, USA.

EPR dosimetry was recognized almost 40 years ago as a method that could be used to measure doses of ionizing radiation received by humans through measurements on teeth, bones, and fingernails. This approach, as applied earlier, required removal of the teeth or bone samples for the assay. EPR dosimetry with teeth has been usually performed at the more sensitive high frequency X-band (9~10 GHz) in vitro. The availability of low-frequency L-band EPR spectroscopy provides the potential for making accurate and sensitive measurements of absorbed radiation dose in teeth without extracting the teeth form the mouth. *In vivo* EPR tooth dosimetry is more challenging as compared to measurements *in vitro* because there are several potential additional sources of variation when the measurements are made in the mouth of a person. Moreover, the use of EPR spectroscopy at the lower ~1.2 GHz frequency inherently lowers the attainable sensitivity. Placing the resonator in the mouth results in several challenges to overcome, e.g., the effect of saliva, swallowing and other motions of the subject, the nonresonant attenuation of microwave energy by the tongue and adjacent tissues, and the requirement of exact positioning the resonator loop on the tooth surface _ all these sources can reduce sensitivity and accuracy.

We have developed a procedure to reduce the effects of motion near the resonator that greatly improves the signal quality and reduces the amount of time needed to complete the measurements for measurements *in vivo in situ*. We have made measurements on irradiated teeth placed in appropriate gaps of volunteers dentition, and for dosimetry *in vivo* in natural teeth of patients who have undergone radiation therapy that resulted in a dose to the teeth. This has made it feasible to obtain initial results of absorbed dose in teeth *in vivo* with a dose response relationship with about ±0.5 Gy based on measurements of a set of six teeth with doses from 0.5 to 30 Gy. The time required for acquisition was about 10 min per tooth. These data support the expectation that *in vivo* EPR dosimetry can provide the data needed for an effective triage of a population exposed to radiation in the range that can cause acute clinical syndromes.

Acknowledgement

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(PS2004) The automatic dicentric detection system efficiency in a real case population triage. Laurence Roy, Aurélie Vaurijoux, Eric Grégoire, Cécile Martin, Pascale Voisin, Sandrine Roch-Lefevre, Partick Gourmelon. Institut de Radioprotection et de Sûreté Nucléaire, Fontenay-aux roses, France.

In 2006 sixty five workers were accidentally exposed to an Iridium source. The source was used for gammagraphy controls and remained blocked in the pipe during two months. Due to the complexity of the exposure it was not possible to reconstruct individual doses using physical dosimetry. Therefore the only way to estimate the dose was to perform biological dosimetry. Such technique is based on the scoring of dicentrics in lymphocytes from blood. When only few individuals are accidentally overexposed, at least 500 lymphocyte cells have to be scored to have a good estimation of the dose. But such a practice is too time consuming when many people are exposed. In order to reduce the time required to estimate a dose, successive specific strategies have been developed in the laboratory and were tested on this real case. The first strategy is based on the scoring of 50 cells (50C) instead of 500. Then the 95% confidence interval of the dose is 1 Gy. The second one is based on the automatic dicentrics scoring (ADS) by an image analysis system (METASYSTEMS). The system proposes to the operator some candidate dicentrics which have to be verified manually. Fifty percent of the dicentrics are correctly detected and the 95% confidence limit of the dose is 0.4 Gy.

In the first step both strategies were used to identify as fast as possible highly exposed workers. Results were classified as exposure not detected (class 0), exposure detected but a lower range of the confident limit of 0 (class 1); positive exposure (class 2). In a second step doses were precisely measured by scoring several hundred of cells per person and the classification was reevaluated. This second step was used as the reference to validate the results of both triage strategies.

Compared to the reference, 69 % of the persons were correctly classified using 50C strategy against 78 % for ADS. In 0 % for 50C and 17 % for ADS an over classification was done and in 31 % for 50C vs 5 % for ADS a under classification was found.

When doses are compared the correlation is better between ADS and the reference than between 50 cells and the reference.

The ADS is efficient to classify persons according to their level of exposure. A tendency of overexposure is observed but without major health consequences. This technique would be a good alternative to the manual scoring which is still the technique of choice.

(PS2005) Development of an ultrahigh-throughput robotically-based biodosimetry workstation using in-situ assays. Guy Garty[1], Gerhard Randers-Pehrson[1], Oleksandra V. Lyulko[1], Aparajita Dutta[2], Jing Nie[2], Giuseppe Schettino[1,3], Anubha Bhatla[4], Jian Zhang[4], Alessio Salerno[4], Nabil Simaan[4], Y. Lawrence Yao[4], David J. Brenner[1]. [1]Columbia University - Radiological Research Accelerator Facility, Irvington, NY, USA, [2]Columbia University - Center for Radiological Research, New York, NY, USA, [3]Currently at: Gray Cancer Institute - Mount Vernon Hospital, Northwood, Middlesex, United Kingdom, [4]Columbia University - Department of Mechanical Engineering, New York, NY, USA.

Following a mass radiological event, there will be a significant requirement to assess, within a few days, the radiation doses received by tens or hundreds of thousands of individuals. We present the design of a fully automated high-throughput robotic biodosimetry workstation, based on in-situ analysis in multiwell plates, capable of assessing radiation exposure in up to 30,000 blood samples per day.

The inputs to the system are 50 µl blood samples in plastic hematocrit capillaries, collected in the field using a standard lancet. After the capillary is input into the system, no further operator handling is involved. The samples are centrifuged to separate the lymphocytes, and a preliminary lymphocyte count indicates if emergency treatment is required. The capillary is then laser cut below the lymphocyte band, and the lymphocytes and plasma are deposited into a filter bottomed multiwell plate.

Depending on the time since exposure, either the micronucleus or the γ-H2AX assay is then performed in an automated liquid handling system with custom made robotic incubators. The use of filter bottomed plates allows rapid washes without the need to pellet and resuspend after each step, simplifying the liquid handling system and increasing throughput. Finally the filter bottoms are transferred to a rigid substrate for automated imaging and final archiving. Transfer between the various stations uses a selective compliant articulated robot arm with custom designed grippers.

Both assays require initial separation of lymphocytes from whole blood. 50 µl of whole blood and 50 µl of lymphocyte separating medium is used, centrifuged at 40g for 20 minutes. This yields good separation of the lymphocytes as a well defined white band, for guiding the cutting laser. The lymphocyte separating medium with a density of 1.114 g/ml yielded better lymphocyte counts and sharper bands, as compared with lower-density (1.077 g/ml) separation medium.

Both assays have been optimized for processing in filter bottomed multiwell plates and are being optimized both for sensitivity and speed. We will present the layout of the system as well as preliminary results of the various components and optimized assays.

Work supported by NIAID grant 5 U19 AI067773

(PS2006) Achieving requirements for dosimetry for management of potential radiation exposures to a large population with EPR technqiues. Harold M. Swartz, Artur Sucheta, Ruhong Dong, Eugene Demidenko, Ben Williams, Piotr Lesniewski, Maciej Kmiec, Yasuko Sakata. Dartmouth Medical School, Hanover, NH, USA.

The exposure of large numbers of people to levels of ionizing radiation that could lead to the acute radiation syndrome (ARS) is now a significant possibility due to terrorism or nuclear war, and also could occur from accidents. There is a pressing need to determine the exposure dose to individuals immediately after such an event, in order to provide effective guidance under what is likely to be very chaotic conditions. There currently is no plausible procedure for doing this in spite of published guidelines. Under such chaotic conditions these procedures cannot provide prompt and accurate classification of many individuals into the needed categories: 1) unlikely to experience the ARS; 2) likely to have ARS that potentially could respond to medical intervention; 3) has an exposure that is unlikely to respond to treatment.

This presentation describes how electron paramagnetic resonance (EPR) dosimetry can meet these needs. The technique utilizes stable radiation-induced unpaired electrons in teeth and fingernails, whose magnitude is proportional to the absorbed dose. The principal method uses a specially built low frequency (1200 MHz) *in vivo* EPR dosimeter with a resonator that fits comfortably over the teeth within the mouth. It also is feasible to use a conventional EPR spectrometer and measure the radiation induced signals in clippings from fingernails or toenails. Transportable versions of the EPR spectrometers are fully feasible. In studies with normal volunteers who have had irradiated teeth placed within their mouths and in patients whose radiation fields for head and neck tumors have resulted in significant doses to the teeth, measurements have been made successfully. The current capabilities have a SEM of about 50 cGy for *in vivo* calibrations in normal volunteers for doses from 100 to 3000 cGy. In patients who received significant radiation doses to their teeth from therapy, the EPR measurements were within 10% of the calculated doses. The time required for the individual measurements was about 10 minutes. The measurements can be made any time after the irradiation occurs.

We conclude that EPR dosimetry can meet the need for appropriately rapid and accurate triage in a population that potentially has been exposed to doses that could cause the ARS.

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(PS2007) Fully-automated rapid in-situ cellular imaging for a high-throughput biodosimetry workstation. Oleksandra V. Lyulko, Guy Garty, Gerhard Randers-Pehrson, David J. Brenner. Columbia University - Radiological Research Accelerator Facility, Irvington, NY, USA.

In the context of response to a large-scale radiological event, a high-throughput in-situ cell imaging system is being developed as part of a fully-automated mass screening radiation biodosimetry workstation. The system is exclusively dedicated to quantifying the micronucleus and γ-H2AX assays in lymphocytes cultured in multi-well plates. This specificity allows for rapid imaging solutions specific to these assays. Along with rapid sample manipulation and automated focusing, the resulting estimated throughput is 20–30,000 samples per day.

To move between adjacent samples in a multi-well plate (9 mm well-to-well separation), a high-speed stage is used with a transit time of 50 msec. Moving between the fields of view within a single well is performed by steering light with ultrafast galvanometric mirrors located in a 2-D scan head just above the objective lens; typical transit times are 1–2 msec.

To overcome the time-consuming iterative nature of conventional focusing, a cylindrical lens is placed in the optics path, which enables one-step focusing: this is based on imaging a circular object, which appears circular when in focus and elliptical when out of focus. The elongation of the elliptical image is proportional to the distance from the focal plane, and so a fast piezo stage can make a one-step focusing correction.

CMOS cameras are used in the system, which have much faster readout times than CCD cameras; the increased noise associated with CMOS cameras does not limit system performance. To decrease the volume of data transferred to the controlling computer, which would otherwise be a throughput bottleneck, analysis of the image is split between the camera and the frame grabber board. By using a dichroic mirror and two cameras attached to the same frame grabber board, it is possible to visualize nuclei and cytoplasm simultaneously, and to rapidly analyze their overlap, yielding the number and the size of nuclei in each cell.

This rapid automated imaging system, which is tightly integrated into the overall design of the high-throughput radiation biodosimetry workstation, will be described. The light steering and the automated focusing mechanisms are among the novelties of this system; preliminary results will be presented, along with comparison to ray tracing simulations.

Work supported by NIAID grant 5 U19 AI067773

(PS2008) Validation of high throughput micronucleus analysis in peripheral reticulocytes for radiation biodosimetry. Yuh-chyau Chen[1], Ollivier Hyrien[1], Irena Nowak[1], Ying Tsai[1], Nancy Wang[1], Ruth Wilkins[2], Catherine Ferrarotto[2], Stephen Dertinger[3]. [1]University of Rochester, Rochester, NY, USA, [2]Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, ON, Canada, [3]Litron Laboratories, Rochester, NY, USA.

Purpose: Micronucleated reticulocyte (MN-RET) analysis is an established, rodent-based in vivo genotoxicity test for drugs. We have previously reported increased frequencies of peripheral blood MN-RETs in cancer patients upon exposure to radiation (RT) or chemotherapy [Mutat Res 626 (2007) 111–119]. Given the high-throughput capacity of this technology, it offers the potential in radiation biodosimetry to screen large populations when physical dosimetry is not available. We conducted investigations to validate flow-cytometry based MN-RET analysis for radiation biodosimetry by benchmarking against traditional microscopy assays of lymphocytes for RT biodosimetry. **Methods:** We applied animal total body irradiation (TBI) model using C57Bl/6 mice at 8–9 week-old, exposed to Cs-137 source to radiation from 0 Gy to 4 Gy. Blood from tail vein was collected at 43 hrs post-TBI, fixed in methanol, stained, and analyzed by flow-cytometry, for CD71+ MN-RET frequency and RET frequency. Mice were then anesthetized for

rapid cardiac puncture according to protocol of institutional animal facility. Whole blood samples were prepared for cytogenetic analysis of chromosomal aberrations (dicentrics, centric rings and accentrics) and of micronucleus in lymphocytes by cytokinesis-block micronucleus assay (CBMN). **Results**: MN-RET frequency increases linearly from 0 Gy to 1 Gy after TBI, followed by a leveling off and down trend. RET frequency continues to decline with increasing RT doses, reflecting direct bone marrow toxicity by TBI. In contrast, the frequency of dicentrics and micronuclei in lymphocytes continues to increase exponentially up to 4 Gy. A bivariate regression model was built to describe jointly the RET and MN-RET frequencies as a function of RT doses. From this model, the dose of RT can be reconstructed using both endpoints by the method of maximum likelihood. **Conclusions**: High throughput, automated analysis of MN-RET by flow-cytometry is a sensitive and reliable method for detecting recent radiation exposure, ideal for rapid triage after radiation incidences. The technology offers great potential for low-dose radiation biodosimetry. MN-RET analysis can provide reliable estimates of the dose of irradiation when using both the RET and MN-RET frequencies simultaneously, even for doses greater than 1 Gy.

(PS2009) Oxidative effects related to proton irradiation in Hepatoma cell line at the Lund Nuclear Probe. Natalia Arteaga-Marrero[1], Magnus G. Olsson[2], Jan Pallon[1], Bo Åkerström[2], Mikael Elfman[1], Per Kristiansson[1], Charlotta Nilsson[1], Christer Nilsson[1], Marie Wegdén[1]. [1]LTH, Lund University, Lund, Sweden, [2]BMC, Lund, Sweden.

The Lund Nuclear Probe has been modified lately in order to create a Single Ion Hit Facility for biological applications. Up to now, the Facility is operative regarding non-targeted cell irradiation thus, a collaboration between BMC (Bio Medical Centre) and LTH (Lund Institute of Technology) at the Lund University has been established.

The purpose of the cooperation is to perform non-targeted irradiation of the human hepatoma cell line HepG2 using single 2.5 MeV protons. Irradiation effects on the cells will be investigated checking the oxidation status of the surrounding cells. Furthermore, the antioxidative effects of alpha-1-microglobulin (α_1m) on this process are also studied.

The horizontal arrangement of the Lund Facility in addition to the post-cell detection system force us to use special irradiation surfaces. The cells can be grown, cultivated and irradiated in the same epoxy-based surface which facilitates the achievement of the technical requirements.

2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) is an oxidation sensitive probe which is loaded into the cell culture. This probe passes freely through cell membranes, is cleaved intracellularly by esterases and consequently unable to cross the membranes. Oxidative products formed and released from cells exposed to irradiation have the ability to oxidise the intracellularly loaded probe. The oxidative effects of the irradiation are then detected using fluorescence microscopy.

The relation between the number of targeted protons and the oxidative effects produced by the irradiation will be presented.

(PS2010) Mechanisms underlying α-particle induced bystander effects in normal human fibroblasts. Zhi Yang[1], Perumal Venkatachalam[2], Sonia M. de Toledo[1], John B. Little[3], Edouard Azzam[1]. [1]University of Medicine & Dentistry of New Jersey, Newark, NJ, USA, [2]Indira Gandhi Center for Atomic Research, Kalpakkam, India, [3]Harvard School of Public Health, Boston, MA, USA.

Evidence accumulated over the past two decades has indicated that exposure of cell populations to ionizing radiation results in significant biological effects occurring in both irradiated and non-irradiated cells in the population. This phenomenon, termed the 'bystander response', occurs *in vitro* and *in vivo* and has been postulated to impact the estimation of risks of exposure to low dose/low fluence ionizing radiation. Mechanisms involving secreted soluble factors, oxidative metabolism and gap-junction intercellular communication have been shown to regulate the propagation of radiation-induced bystander effects in a variety of cell types. However, the exact molecular steps by which bystander effects are elicited have not been entirely defined. Elucidation of these steps should increase our understanding of cellular processes that determine risks to low level radiation exposure.

Using density-inhibited normal human skin fibroblast cultures exposed to low fluences of α-particles, we show that partial oxygen tension (Po_2) in cells, and signaling pathways involving *ATM* and *P53* are critical in expression of bystander effects in normal cells. Attenuation of Po_2 or inhibition of either ATM or p53 activity reduces the propagation of stressful effects from irradiated to non-irradiated cells. Whereas micronucleus formation and stress-responsive proteins were induced in bystander cells in cultures exposed to low mean doses of α-particles (1–5% of cells' nuclei traversed by a particle track), such effects were significantly attenuated when the oxygen concentration in the culture atmosphere was gradually decreased from ambient 21% to 0.5% as found in skin, and the cell cultures were maintained at the low Po_2 for 48 h prior to irradiation and 3 h post irradiation. Attenuated bystander effects were also observed in α-particle exposed confluent cultures of cells homozygous for *ATM* or normal human cell cultures incubated with the ATM kinase inhibitor Wortmannin. Bystander effects were also decreased in normal human cell cultures incubated with α-pifithrin, an inhibitor of p53. Collectively, these data indicate that cellular Po_2 and the ATM/p53 signaling pathway play a significant role in regulating signaling of α-particle induced bystander effects in normal human cells. *Supported by grant CA92262-01A1 from the NIH*

(PS2011) Caspases are involved in the induction of apoptosis in irradiated and bystander cells. Martin Purschke, Zhixiang Zhou, Kathryn D. Held. Massachusetts General Hospital, Charlestown, MA, USA.

Ionizing radiation (IR) causes apoptosis in many cell types. Bystander cells, cells which are not traversed by irradiation, can also undergo apoptosis. While the apoptosis pathway in irradiated cells is already well studied, little is known about the apoptotic process in bystander cells. This study contributes to the understanding of apoptosis in bystander cells.

In this study we used c-myc transfected rat embryonic fibroblasts (REC:Myc) in the transwell insert system where directly irradiated cells and bystander cells share the same medium, but do not have cell to cell contact. Apoptosis was determined morphologically after DAPI or Hoechst 33342 staining. At 48 h irradiated cells (up to 6 Gy x-rays) showed a dose dependent increase in apoptosis up to 15x compared to control. Bystander cells also generated apoptosis which was about 3x higher than control and dose independent. In addition we used caspase 8 and 9 inhibitors to assess the involvement of those caspases in our observed radiation induced apoptosis. These proteins are known as initiator caspases for the extrinsic or the intrinsic apoptosis pathway, respectively. Inhibition of caspase 8 reduced the level of apoptosis in both irradiated and bystander cells by about 50 %, whereas caspase 9 inhibition showed differences. In irradiated cells we observed a greater reduction of 75 % compared to 50 % in the bystander cells. We are now using computerized time lapse microscopy (CTLM) in combination with caspase 8 and 9 substrates to determine the apoptosis pathways in directly irradiated and bystander cells.

Our data indicate that both the directly irradiated and bystander cells undergo apoptosis after irradiation. However, the induction of apoptosis might be triggered by different pathways and time courses, since we found differences in the reduction of apoptosis in directly irradiated and bystander cells by using caspase inhibitors. CTLM seems to be a useful tool to study possible differences in apoptosis pathways and their time courses.

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(PS2012) Functional genomics of the radiation bystander response in normal human fibroblasts and epithelial cells.

Shanaz A. Ghandhi, Jaeyoung Ahn, Sally Amundson. Columbia University Medical Center, New York, NY, USA.

The goal of this study is to use a functional genomics approach to identify mechanisms mediating indirect effects of ionizing radiation (IR), called bystander effects. The ultimate aim is to integrate the knowledge of gene expression patterns that are significant to the bystander effect into the physiological response of organisms to radiation. Specifically, we are using the Agilent whole human genome oligonucleotide array platform to develop gene expression profiles of cultured primary human lung fibroblasts and small airway epithelial cells that have been exposed to alpha particles and comparing them with those of nearby in-contact bystander cells. As the Agilent microarrays have 44,000 unique gene sequences that can be analyzed in each experiment, this represents coverage of essentially the entire human genome.

We have also monitored micronucleus formation frequency as an indicator of irradiation damage, and individual gene expression changes such as up-regulation of $p21^{WAF1}$ mRNA, which is known to be an early response to radiation. Our results show that both human fibroblast and epithelial cells respond with increased levels of micronucleus formation as an index of DNA damage in both directly irradiated and bystander cells, though the levels in directly irradiated cells are consistently higher than in bystander cells. Our functional genomics approach will allow us to identify candidate signaling pathways that can be useful for further studies to understand the mechanisms regulating the bystander effect. We are currently in the process of data collection and analysis and our observations thus far indicate that this approach will provide us with strong candidate genes and signaling networks for further studies.

Supported by NIH grant CA049062

(PS2013) Radiation induced bystander effect in plants is generated by radicals formed in irradiated media. Igor Kovalchuk, Franz Zemp, Trang Bui. University of Lethbridge, Lethbridge, AB, Canada.

Radiation induced bystander effect (BSE) is described by the destabilization of the genome of non-targeted cells that have been in physical or spatial contact with radiation exposed cells. This phenomenon has been commonly observed *in vitro* in irradiated cell cultures and even *in vivo* on the level of whole organism in mammals. Very little information is available about plants. Recently we reported bystander like effect in plants by showing that local UV exposure resulted in global genome instability.

In current work we attempted to identify whether the BSE can be generated by exposing plants grown on liquid media and measuring the genome stability in "standing by" non-irradiated plants. We germinated *Arabidopsis* plants on plates with liquid MS media. We covered half of the plates with either aluminum foil or lead shield and irradiated the plants with either UVC or X-ray. In this way we obtained two groups of plants, irradiated and bystander. Analysis of the homologous recombination frequency (HRF) revealed an over 2-fold increase in both, UVC and X-ray irradiated plants and bystander plants. Next we hypothesized that there is a residual radiation dose under the aluminum and lead cover. We measured the HRF in plants that were completely covered during irradiation and did not find any increase. We thus hypothesized that BSE was generated either via liquid or via air transmission. We carried on two experiments. In one experiment, we grew plants on plates with plastic separator and found no BSE generated upon irradiation of half covered plates. In another experiment, we germinated seeds of *Arabidopsis* plants on one half of the plate, whereas another half was empty. Both parts of the plate were either connected by liquid media or were separated by plastic separator. As a control we had plates with plants on both sides. We found that irradiation of empty half of the plate resulted in comparable increase in HRF in covered plants to the one observed by irradiation of plates with plants. Plants grown on plates with separator showed very little difference to the control non-irradiated plants.

These experiments suggested that the BSE in our experiments was most probably triggered by radicals formed in the liquid media rather than by irradiated plants.

(PS2014) Role of epigenetic effectors in the radiation-induced bystander effects in vivo. Olga Kovalchuk[1], Igor Koturbash[1], Yaroslav Ilnystky[1], Kristy R. Kutanzi[1], Igor Pogribny[2]. [1]University of Lethbridge, Lethbridge, AB, Canada, [2]National Center for Toxicological Research, Jefferson, AR, USA.

Bystander effect occurs when irradiated cells communicate damage to nearby, non-irradiated 'bystander' cells, ultimately contributing to genome destabilization in the non-exposed cells. While bystander effects are well studied using cell cultures, data on somatic bystander effects *in vivo* are scarce. The long-term persistence of the distant bystander lesions in somatic organs upon irradiation has never been addressed. Also, no exact mechanistic data were proposed to explain the molecular etiology of bystander effects.

Recent evidence suggests that bystander effect may be epigenetic in nature; however, characterization of epigenetic mechanisms (DNA methylation, histone modifications and RNA-mediated silencing) involved in bystander effect generation and its long-term persistence has yet to be defined.

To analyze *in vivo* bystander effects we developed a rodent model whereby the animal head was exposed to radiation while lead shielding protected the rest of the body. To investigate the possibility that localized X-ray-irradiation induces persistent bystander effects in the distant tissue, we monitored the induction of epigenetic changes (i.e. DNA methylation, histone methylation and microRNA expression) as well as DNA damage in spleen tissue 6 hours, 4 days, 14 days, 56 days and 7 months after localized cranial irradiation.

We noted a significant accumulation of DNA damage, and alterations in the levels of cellular proliferation and apoptosis in the bystander spleen. We found that localized cranial radiation exposure leads to decreased levels of global DNA methylation, alters the levels of key proteins known to modulate methylation patterns and silencing (e.i. *de novo* methyltransferase DNMT3a and methyl-binding protein MeCP2) and contributes to reactivation of LINE1 retrotransposon in the bystander spleen. We also noted pronounced alterations in the bystander spleen microRNAome. We also found that cranial irradiation resulted in the altered levels of microRNA in plasma of animals. Thus, microRNAs may serve as important mediators of the bystander effects in the distant organs. Importantly, the observed epigenetic dysregulation was a cross-species phenomenon and was not caused by radiation scattering.

(PS2015) The role of miRNAs in the epigenetic regulation of bystander responses in 3D human tissue models. Franz J. Zemp[1], Jennifer Dickey[2], Gloria Jenkins-Baker[3], Stephen A. Marino[3], David J. Brenner[3], William M. Bonner[2], Olga A. Sedelnikova[2], Olga Kovalchuk[1]. [1]University of Lethbridge, Lethbridge, AB, Canada, [2]National Cancer Institute, Bethesda, MD, USA, [3]Columbia University, New York, NY, USA.

Small RNA species in animals are beginning to establish themselves as important regulators of cellular homeostasis and control. In fact, the emergence of 'oncomirs' has given them a notable role in the future of oncology. Thus, as the genomic stability and epigenetic mechanisms underlying bystander effects have the potential to be regulated by small RNAs, it is the goal of this research to investigate the role of small RNAs in radiation-induced bystander effects.

Artificial 3D human tissues are important tools in researching bystander effects, as these tissue models preserve the 3D geometric arrangement and communication of cells present in tissues *in vivo*. It has been shown that bystander effects exist in human *'in vivo'* models using 3D artificial tissue. These experiments found increased levels of apoptosis, micronuclei formation, and strand breaks in bystander tissue, as well as a global loss of DNA methylation.

Here, EpiAirway (Air-100) tissues were microbeam-irradiated, and exposed and bystander tissues harvested at 8 hours and 7 days post-irradiation. MicroRNA microarray analysis was performed, and inductions confirmed by RT-PCR. Surprisingly, only a single miRNA, miR-26, was regulated 8 hours after exposure in irradiated tissue. Seven days after exposure, 18 and 10 miRNAs were up- and down-regulated, respectively, in the directly exposed tissue, while 31 and 21 miRNAs were up- and down-regulated in bystander

tissue, respectively. Interestingly, 16 miRNAs (12 up- and 4 down-regulated) were common to both irradiated and bystander tissue. The most predominantly regulated miRNA found in the array was miRNA-206, which was up-regulated >6-fold in the bystander tissue 7 days after exposure. The most strongly regulated miRNA among the shared group was miRNA-20b. Further, in correlation with the results found from the previous bystander studies in 3D-tissue models, both of these miRNAs putatively target DNMT1, a methyltransferase critical to maintaining methylation patterns. Additionally, a number of regulated miRNAs were found to target apoptotic factors and factors involved in the modulation of DNA repair and cellular senescence, possibly regulating the aforementioned processes in bystander tissue. The model of the putative roles of miRNAs in bystander effects will be presented.

(PS2016) Proteome analysis of proliferative response of bystander cells adjacent to cells exposed to ionizing radiation. Bogdan I. Gerashchenko[1], Akira Yamagata[2], Ken Offusa[2], Katsutoshi Yoshizato[3], Sonia M. de Toledo[4], Roger W. Howell[5]. [1]Indiana University School of Medicine, Indianapolis, IN, USA, [2]Japan Science and Technology Corporation, Higashi-Hiroshima, Japan, [3]Hiroshima University, Higashi-Hiroshima, Japan, [4]University of Medicine and Dentistry of New Jersey, Newark, NJ, USA, [5]University of Medicine and Dentistry of New Jersey,, Newark, NJ, USA.

Recently (Cytometry 2003, 56A, 71–80) we reported that direct cell-to-cell contact is required for stimulating proliferation of bystander rat liver cells (WB-F344) co-cultured with irradiated cells, and neither functional gap junction intercellular communication nor long-range extracellular factors appear to be involved in this proliferative bystander response. The molecular basis for this response is unknown. Confluent monolayers of WB-F344 cells were exposed to 5-Gy of γ-rays. Irradiated cells were mixed with unirradiated cells and co-cultured for 24 h. Cells were harvested and protein expression was examined using 2-D electrophoresis. Protein expression was also determined in cultures of unirradiated and 5-Gy irradiated cells. Proteins were identified by mass spectrometry. Nucleophosmin-1, a multifunctional nucleolar protein, was more highly expressed in bystander cells than in either unirradiated or 5-Gy irradiated cells. Enolase-α, a glycolytic enzyme, was present in acidic and basic variants in unirradiated cells. In bystander and 5-Gy irradiated cells, the basic variant was weakly expressed whereas the acidic variant was overwhelmingly present. These data indicate that the presence of irradiated cells can affect nucleophosmin-1 and enolase-α in adjacent bystander cells. These proteins appear to participate in molecular events related to the proliferative bystander response and suggest that this response may involve cellular defense, proliferation, and metabolism.

(PS2017) Molecular mechanisms of the sex differences in the radiation induced bystander effect in vivo. Igor Koturbash, Kristy Robin Kutanzi, Karl Hendrickson, Dmitriy Kogosov, Olga Kovalchuk. University of Lethbridge, Lethbridge, AB, Canada.

It has been shown that irradiated cells may 'forward' ionizing radiation (IR)-induced genome instability to non-irradiated neighbor cells, a phenomenon known as the 'bystander effect'. The rare evidence on somatic bystander effect *in vivo* points that this phenomenon may be distinct in males and females. The exact mechanisms of IR-induced bystander effects and the underlying mechanisms responsible for the sex differences have yet to be established.

We developed an *in vivo* model, whereby bystander effects are studied in the spleen of animals subjected to head exposure, when the rest of the body is protected by a medical grade lead shield.

Using this model, we analyzed the sex differences in the IR-induced bystander effects. We examined changes in spleen tissue of male and female mice following *in vivo* cranial IR exposure. The 50 day-old male and female mice were randomly assigned to different treatment groups. The first cohort received 1 Gy of X rays delivered to the entire body. The second cohort received 1 Gy of X rays exposure to scull only, while the rest of animal body protected by a lead shield. Control mice were sham treated. A fourth cohort of animals was exposed to the calculated scatter dose. The animals were sacrificed either 6 hours or 96 hours (4 days) after exposure to analyze initial and persistent responses.

We analyzed the changes in the levels of DNA damage, cellular proliferation and apoptosis as well as the three the main epigenetic phenomena - global DNA methylation, histone modifications and microRNA expression. We noted that whole body X-ray exposure led to a significant increase in the levels of DNA strand breaks in spleen of both male and female mice 6 hours upon whole body and head exposure, however, 4 days following irradiation the DSBs were repaired.

Interestingly, significant sex differences were found in the levels of DNA methylation and expression of DNA methyltransferases and methyl-binding proteins in the exposed and bystander spleen 6 hours and 4 days after exposure. Similarly, IR exposure induced sex-specific changes in the levels of microRNAs in the exposed and bystander spleen. The sex-specific microRNAome changes were correlated with the sex-specific expression of microRNA target proteins. These are some of the first data showing on the sex differences in the radiation-induced bystander effects.

(PS2018) Imaging of chemical and structural aspects of low-LET bystander effects in living mammary epithelial cells. Hoi-Ying N. Holman, Kathy A. Bjornstad, Al C. Thompson, Chris J. Rosen, Eleanor A. Blakely. Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

Our aim is to apply the high-resolution Fourier transform infrared (FTIR) spectroscopy imaging technique for directly characterizing the molecular chemistry within individual targeted and non-targeted cells *in vivo*. Existing approaches often involve measuring "marked" cellular events using methods that require labeling, fixing, or staining of targeted biomolecules. Our results can add a vital extra dimension to the knowledge of bystander effects (BSE).

In this investigation, we compare chemical and structural aspects of the early and delayed responses of low-LET radiation in targeted and non-targeted cells in two phenotypically normal and non-malignant human mammary epithelial cell (HMEC) models: the HMEC HMT-3522 (S1) with an infinite lifespan, and a subtype of HMEC 184 (184V) with a finite lifespan. Mono-layer cells are grown until nearly confluent. They are irradiated with precise stripes of dose ranging from 10 cGy to 50 cGy from a 12.5 keV X-ray microbeam. After irradiation, cells are incubated in fresh medium for a range of time points prior to the FTIR spectroscopy imaging and analysis.

The spatial range of effectiveness of BSE, measured at single-cell resolutions, is quantified by analyzing infrared absorption spectral character (e.g., absorption band shape, intensity, and peak position). The infrared spectra of single cells are sensitive to chemical and conformational changes in biomolecules. Ongoing assessment of spectral endpoints of damages and responses in targeted and non-targeted cells include global analysis of spectral characters of lipid peroxidation, protein phosphorylation, protein oxidation, and DNA and nucleic acid modifications inside individual cells. Preliminary spectral data after the x-ray irradiation demonstrate (1) the presence of oxidative damages in targeted and non-targeted cells, immediately and 6 minutes post- irradiation, which decreases at 30 minutes post-irradiation; (2) the spatial distance for the range of the bystander effect is greater for the lower dose at 30 minutes post-irradiation; and (3) a higher degree of phosphorylation occurs in bystander cells at 24-hours post-irradiation. Comparisons of differences in bystander responses between the two cell models will be discussed.

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(PS2019) DNA double-strand break formation in normal human fibroblasts can be triggered by exposure to human cancerous cultured cells in a bystander-like effect. Mykyta

Sokolov, Olga Sedelnikova, William Bonner. NIH, Bethesda, MD, USA.

Radiation-induced bystander effect (RIBE) is a non-targeted effect seen in cells that were not directly irradiated but were either in contact with or received conditioned medium from irradiated cells. These non-hit bystander cells can exhibit damage typically associated with direct radiation exposure. There is a growing body of evidence showing that the phenotype of normal human cultured cells can be significantly altered if these cells are exposed to cancerous cells. This phenomenon in some respects is reminiscent of RIBE. Having shown that the gamma-H2AX focus formation assay can be used for sensitive detection of DNA double-stranded breaks (DSBs) in bystander cells, in the present study we set out to determine whether cancerous cells can induce a DNA damage response (DDR) in normal human fibroblast cells. To this end, we employed a cell co-culture protocol using a panel of human cancerous cell lines (glioma T406, fibrosarcoma HT1080 and transformed HEK 293). We also explored whether media transfered from human cancerous cell lines (HeLa, M059J, T406 and SW756) and DNA repair deficient cell lines (ATM, ATR, TP53 and BLM) could elicit the bystander DDR in normal human breast fibroblasts. Surprisingly, we found that the incidence of DNA DSB formation in normal cells conditioned with media from irradiated tumor cell cultures does not change compared to the incidence of DNA DSB formation in normal cells receiving media from unirradiated cancerous cells. Oxidative stress, calcium signaling and cell cycle progression may be involved in the manifestation of bystander DDR. Taken together, our results indicate that human cancerous cells can create a stressful environment for normal human cells similar to radiation which elicits a DDR in the latter.

(PS2020) Effect of low dose of X rays and/or microwaves on human blood mononuclear cells *in vitro*. Wanda Stankiewicz[1], Aneta Cheda[2], Ewa M. Nowosielska[2], Jolanta Wrembel-Wargocka[2], Marek P. Dabrowski[1], Roman Kubacki[1], Marek K. Janiak[2], Stanisław Szmigielski[1]. [1]Military Institute of Hygiene and Epidemiology, Dept. of Microwave Safety, Warsaw, Poland, [2]Military Institute of Hygiene and Epidemiology, Dept. of Radiobiology and Radiation Protection, Warsaw, Poland.

Nowadays, humans are often exposed to combined exposure to ionizing radiation (IR) and microwaves (MF), which may affect many functions of living organisms. Recent development and common use of different low energy and high frequency MF emitters as well as widespread exposures to low doses of ionising radiation increased the interest on the risk of their possible harmful effects, and, on the other hand, on the potential of their therapeutic applications.

In view of this, the aim of the present study was to determine the possible effect of low-dose IR and/or MF, on the selected parameters of monocytes and lymphocytes collected from human blood.

Peripheral blood mononuclear cells (PBMC) from healthy donors (N = 20) were exposed to 0.1 Gy of X rays or pulse modulated MF (2850 MHz, SAR 0,1 W/kg) on the day of culture initiating or exposed to X rays (on the day of culture initiating) and 48 hours later to MF. Colorimetric assay with the Griess reagent was used to measure production of nitric oxide (NO). LM index was estimated as the effect of monokine (IL-1ra/IL-1β) on lymphocyte proliferative response. IL-1ra and IL-1β were assessed using the respective ELISA assays.

Irradiation of PBMC with high immunogenic activity (control LM index values >8,0) with 0.1 Gy of X rays or combined exposure of these cells to IR and MF, but not exposure to MF alone, decreased this activity. In turn, PBMC representing control, normal values of their immunogenic activity remained insensitive to exposures to IR, MF or combined exposures to IR and MF. Irradiation of PBMC with IR, MF or combined exposures to IR and MF resulted in the significantly enhanced production of NO when the control values were low, whereas high control values were not affected by each of the applied exposures. Analysis of IL-1β production by PBMC revealed that normal values of this cytokine level did not change after each of the applied exposures, but higher than normal IL-1β concentrations decreased only after exposure to IR. The low initial production of IL-1ra remained unchanged after each exposure, whereas its high initial synthesis decreased after the exposure to IR.

Overall, the obtained results suggest that in vitro exposures of monocytes and T lymphocytes in PBMC to 0.1 Gy of X rays and/or low energy microwaves (2850 MHz) modulate the activity of these immune system effector cells.

(PS2021) The noninvasive modulation of the blood stem cell's pool in human: circulating lymphocytes and somatic effects. Marat A. Karamullin[1], Alexey V. Babak[2], Ludmila P. Ekimova[1], Vladimir V. Salukhov[2], Vjacheslav A. Phedorov[1], Elena B. Kireeva[1], Anatoly E. Sosukin[2], Alexey N. Shoutko[1]. [1]Central Research Institute of Roentgenology and Radiology, St.-Petersburg, Russian Federation, [2]Military Medical Academy, St.-Petersburg, Russian Federation.

We've reported earlier about spontaneous regular fluctuations of concentration of the $CD34^+$-cells recorded in a blood of Chernobyl's clean-up workers (CUW) and about the opportunity of artificial growth of the previously mentioned parameter by transcutaneous vibrational impact on the awake medullary hemopoiesis zones (Karamullin M. et al., 2004; Shoutko A. et al., 2005). Methods: The exercise stress tolerance in the bicycle ergometry test was studied in the group of CUW (n=40, age 49,8±0,7) before and after a course of a vibratory activation of a hemopoiesis in the remote period after Chernobyl accident. Various mononuclear cell subpopulations in peripheral blood were estimated by laser cross flow-cytometry method (FACScan, Beckton-Dickinson) with monoclones (DAKO, BD). Results: Improving of parameters describing a state of aerobic work capacity in CUW's group after an activation of a hemopoiesis authentically correlated with the contents of CD34 of cells and lymphocytes of an intermediate stage of maturity in peripheral blood. So, the augmentation of the maximal oxygen consumption had strong direct dependence on the contents of early CD34 cells with phenotype CD811b (R=0,77±0,1, p<0,001). The level of the increase of Robinson index at the peak of an exercise stress directly correlate with contents of $CD34^+$ (R=0,74±0,11, p=0,001), $CD4^+Leu8^+$ (R=0,67±0,13, p<0,001), $CD2^+,35^+$ (R=0,67±0,13, p=0,001); CD19 (R=0,56±0,16, p=0,003). As a whole, augmentation in observation group of persons that became capable to execute full exercise stress test, has found dependence on the circulating contents of CD4Leu8 lymphocytes in a range of 2–24% of a mononuclear fraction (R=0,76±0,11, p<0,001); of $CD34^+$ cells in a range of 0–0,46% (R=0,55±0,18, p=0,009); of CD2,35 lymphocytes in a range of 0–14% (R=0,46±0,20, p=0,04).

Conclusion: As time of the transition in a peripheral blood of the cell with phenotype CD34 in phenotypes TdT, CD8,11b and CD4Leu8 usually lasts from 1 to 4 weeks (Shoutko A. et al, 2005), the received data specify probable participation in realization of some positive somatic phenomena of so-called therapy by founder cells of more differentiated forms of lymphocytes which are not reaching however stages of a differentiation, considered as a level for functionally mature, immunocompetent forms.

(PS2022) Metabolomics as a tool for understanding the cellular stress response of TK6 cells following ionizing radiation exposure. Andrew D. Patterson[1], Henghong Li[2], Kristopher W. Krausz[1], Albert J. Fornace, Jr.[2], Frank J. Gonzalez[1], Jeffrey R. Idle[3]. [1]Lab of Metabolism, National Cancer Institute, Bethesda, MD, USA, [2]Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC, USA, [3]Institute of Clinical Pharmacology, University of Bern, Bern, Switzerland.

The purpose of the study was to define changes in the intracellular metabolome of the lymphoid TK6 cell line after ionizing radiation (IR) exposure. The cellular response to IR is well documented in terms of its direct and indirect effects on macromolecules such as nucleic acids and proteins. The use of global profiling technologies has contributed substantially to the understanding of the IR cellular stress response and has elucidated many of the complex biological networks associated with gene expression and signal transduction. On a similar level, however,

global understanding of how IR affects small molecule concentrations (such as metabolites) in the cell is incomplete. Metabolomics is a rapidly advancing field that aims to identify and quantify the concentration changes of all metabolites (i.e., the metabolome) in a given biofluid. In order to assess the metabolic changes associated with IR, we employed a metabolomics approach using the high-resolution of ultra-performance liquid chromatography coupled with the accurate mass determination of electrospray time-of-flight mass spectrometry (operating in both positive and negative ion mode) to profile TK6 cells exposed to increasing doses of IR. We performed principal components analysis and supervised orthogonal projection to latent structures analysis using SIMCA-P+ in order to identify perturbed metabolic pathways. This approach revealed several biomarkers of IR exposure in these lymphoid cells including products of oxidative stress, energy pathway intermediates, and nucleotide and amino acid pool alterations. The application of metabolomic analysis to the field of radiobiology, or radiation metabolomics, promises to increase our understanding of how cells respond to IR by profiling the dynamic changes of cellular metabolites and may provide a basis for developing biomarkers of IR exposure.

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(PS2023) Inhibition of p38 MAPK reduces ionizing radiation (IR)-induced hematopoietic suppression by preventing hematopoietic stem/progenitor cell senescence. Yong Wang, Lingbo Liu, Daohong Zhou. Medical University of South Carolina, Charleston, SC, USA.

Bone marrow (BM) injury is one of the major concerns for cancer patients undergoing chemotherapy and/or radiotherapy. It has been demonstrated that p38 mitogen-activated protein kinase (p38 MAPK) plays a critical role in the induction of apoptosis and senescence in response to genotoxic stress caused by these therapies. To elucidate the role of p38 MAPK in radiation-induced BM injury, we monitored the activation of p38 MAPK in BM hematopoietic cells at different time after exposure to IR using our well-established long-term BM cell culture (LTBMC) system. The activation of p38 MAPK was detected within 24 hours after BM hematopoietic cells were exposed to 4 Gy IR and this activation sustained up to 5 weeks after radiation. Inhibition of p38 MAPK activity with a p38 MAPK specific inhibitor (SB202190) attenuated IR-induced suppression of the hematopoietic function of BM hematopoietic cells in an *in vitro* colony forming cell (CFC) assay. Moreover, the number of hematopoietic progenitor cells produced by SB202190-treated BM cells was significantly greater than that by the cells without SB202190 treatment after exposure to IR and followed by a five-week LTBMC. Interestingly, p38 MAPK inhibition showed no effect on IR-induced apoptosis in both hematopoietic stem cells (HSCs) and progenitor cells (HPCs), whereas the radioprotection effect of SB202190 was associated with a significantly reduction of p16INK4a expression and senescence-associated β-galactosidase (SA-β-Gal) activity in irradiated BM cells after five weeks of LTBMC. These findings suggest that activation of p38 MAPK may mediate IR-induced hematopoietic suppression by induction of hematopoietic cell senescence and pharmacological inhibition of the p38 MAPK pathway may have the potential to be developed as an innovatively therapeutic strategy to ameliorate IR- and chemotherapy-induced BM toxicity.

(PS2024) Glutamate transporter response after exposure to low-dose gamma and proton radiation. Martha C. Sanchez, Abigail Benitez, Leticia S. Ortloff, Lora M. Green. Loma Linda University, Loma Linda, CA, USA.

Exposure to low-dose irradiation can result in cognitive and motor deficits soon after irradiation. However, some effects, usually progressive and fatal, do not become apparent for months or even years after exposure. Fundamental to understanding how radiation can produce neurodegenerative effects, is characterizing the cellular and molecular mechanisms that promote radiation-induced central nervous system damage. Glutamate-triggered excitotoxicity is believed to be a major factor in neurodegeneration. Glutamate metabolism and transport are tightly coupled processes in neurons and astrocytes, and failure of glutamate transporters to clear extracellular glutamate can result in excitotoxicity. We hypothesize that ionizing radiation results in glutamate transport dysfunction which would impair neuron-astrocyte coupling, and ultimately result in neurotoxicity.

Glutamate transporter expression and function were assessed using cultures of fully differentiated neurons and astrocytes derived from the NTera2/D1 cell line after retinoic acid treatment. Pure cultures of each cell type were irradiated with 10, 50, and 200 cGy gamma or 250MeV protons. Measurements were then taken at 3 hours, 2 days, and 7 days following irradiation to assess transporter expression and function. Glutamate transporters 1–4 (EAATs 1–4) were quantified using western blot analysis, whereas transporter function was assessed by ^{3}H-glutamate uptake.

At gamma doses of 50 and 200 cGy, there was a reciprocal response of neurons and astrocytes to gamma exposure, observed as early as 3 hours post-irradiation. Neuronal glutamate transporter activity increased, whereas astrocytic glutamate transporter activity decreased. However, in astrocytes transporter activity returned to baseline 2 days after exposure, while transporter activity remained elevated in neurons as late as 7 days post-gamma irradiation. EAAT3 and EAAT4 showed the most dramatic changes in protein expression, at the slightly higher doses of 50 and 200 cGy. Changes in transporter expression level and activity could represent a reparative response to a stressor such a gamma irradiation. The results, thus far, revealed that proton exposure does not produce any significant change in transporter activity in either cell type after exposure at these low doses.

(PS2025) Local radiotherapy induces recruitment of hematopoietic stem cells to the irradiated bone marrow. Carlo Bastianutto, Asim Mian, Julie Symes, Joseph Mocanu, Nehad Alajez, Will Shi, Jeff medin, armand keating, michael crump, mark minden, mary gospodarowicz, Fei-Fei Liu. Princess Margaret Hospital, Toronto, ON, Canada.

Secondary Acute Myeloid Leukemia (sAML) is the most common therapy induced malignancy affecting breast cancer (BC) patients. Local breast radiation therapy (RT) is associated with a 3-fold increased risk in this iatrogenic complication; the mechanism of which remains unknown. Based on the assumption that AML is a stem cell-derived malignancy, we hypothesized that breast (and/or lymph node) RT actively recruits hematopoietic stem cells (HSC) to the site of RT, thereby increasing the risk of generating a leukemic stem cell. Hence, the objective of this study is to demonstrate this recruitment, and to identify the mediators involved in this process.

Using bioluminescence imaging (BLI) in a murine model, we show that local RT delivered to the left leg causes preferential accumulation of bone marrow cells (BMC) to the irradiated site, with maximum signal intensity observed at 6 days post-RT. Using flow cytometry, this is associated with a 4-fold higher number of donor-derived HSC present in the left leg on day 6, confirming preferential recruitment of HSC to the site of RT. The chemokines mediating this recruitment include SDF1, MMP2 and MMP9, wherein at 16 hrs post-RT, there is a 2.4, 14.5 and 2.9 fold respective increase in these transcript levels in the irradiated, compared to the un-irradiated leg. In turn, inhibitors of MMP2 and 9 reduced HSC recruitment significantly by 60%; inhibiting CXCR reduced HSC recruitment by 50%.

Our data demonstrate for the first time, that local RT has significant systemic effects, by recruiting HSC to the irradiated BM site; a process mediated by SDF1, MMP2 and MMP9. These results provide important potential opportunities by which sAML might be prevented in BC patients undergoing RT.

(PS2026) Evidence for genomic instability in human haemopoietic stem cells containing radiation-induced chromosome

aberrations. Natalia D. Sumption[1], Dudley T. Goodhead[2], Rhona M. Anderson[3]. [1]GlaxoSmithKline, Ware, United Kingdom, [2]MRC Radiation and Genome Stability Unit, Harwell, United Kingdom, [3]Brunel University, West London, United Kingdom.

To assess the long-term stability of chromosome aberrations initially induced in the haemopoietic compartment, we have optimised a cell culture system that supports the differentiation of irradiated stem cells into mature T-cells *in vitro*. *De novo* T-cells are sampled and subsequently analysed using multiplex fluorescence *in situ* hybridisation (m-FISH) for chromosome and chromatid type aberrations. The stem cell differentiation method, termed the extra-thymic T-cell differentiation method, hinges on the principle that if you deplete all of the mature T-cells from a fresh whole bone marrow mononuclear cell (BM MNC) sample, then there will be a homeostatic response to replete the T-cells by differentiation of stem cells within the BM-MNC fraction. Depletion and repletion of all intermediate and mature T-cells was assessed by fluorescence assorted cell analysis to identify T-cell specific cell surface markers. M-FISH data will be presented that demonstrates the persistence of radiation-induced chromosome clonal aberrations in *de novo* T-cells. Further, we will show evidence for the occurrence of genomic instability in *de novo* T-cells derived from irradiated stem cells. Specifically, non-clonal aberrations such as translocations, chromatid breaks and aneuploidy were observed in cells carrying clonal radiation-induced chromosome aberrations.

(PS2027) Possible influence of multiple SNP markers to urological morbidity induced by radiotherapy with carbon-ions among 133 prostate cancer patients. Tomo Suga[1], Mayumi Iwakawa[1], Shuhei Noda[1], Hiroshi Tsuji[1], Eisei Oda[2], Yoshimi Otsuka[1], Atsuko Ishikawa[1], Hirohiko Tsujii[1], Takashi Imai[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Medical Toukei Corporation, Tokyo, Japan.

Purpose: Based on the assumption that clinical radiosensitivity should be caused by multi-genetic factors dependent on the cumulative effect of a number of genetic alterations, we evaluated multiple single nucleotide polymorphism (SNP) markers of candidate genes whether to correlate with radiation morbidity in prostate cancer patients treated with carbon ion radiotherapy (C-ion RT), and investigated the possibility to construct the marker set, which maximizes the discriminating ability of risk for adverse effect.

Patients and Methods: The 133 prostate cancer patients included in this study were underwent C-ion RT at a total dose of 65.7 +/- 1.7 GyE and subsequently evaluated with regard to urinary morbidity, polyuria, which was claimed by individuals as subjective scoring according to the Late Effects of Normal Tissue/ Subjective, Objective, Management, and Analytic scoring system. One thousand and one SNPs in 138 candidate genes were genotyped by a matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. Patients were divided into a training set ($n = 73$) and a test set ($n = 60$). We dichotomized into grade 0 and grade 1+. Case-control study was designed in each set. In the training set, candidate SNP markers were explored using the Fisher's exact test ($P < 0.05$). The multiple marker sets were constructed using candidate markers, and the receiver operating characteristic (ROC) curve and area under the curve (AUC) were used to compare their diagnostic value. In the test set, the reproducibility for diagnostic ability of the most appropriate marker set was evaluated by AUC-ROC method.

Results: No grade 3 or higher toxicities were observed in this study and the incidence of grade 2 and grade 1 were 1% and 24%, respectively. In thirty-three patients, grade 1+ morbidity was observed. The AUC of SNP marker set of *LIG1, CAP1, RAD9, REV3L,* and *SERP1* in the training set and in the test set were 0.914 and 0.641, respectively.Conclusions: Although larger numbers of patients are needed to validate the results, this study provides new insights to support the assumption that radiosensitivity is caused by multi-genetic factors and the possibility that the number of high-risk genotype on SNPs might predict radiosensitivity.

(PS2028) Relative biological effectiveness (RBE) of carbon ions in the rat spinal cord. Peter Peschke[1], Christian P. Karger[1], Michael Scholz[2], Jürgen Debus[3], Peter Huber[1]. [1]German Cancer Research Center, Heidelberg, Germany, [2]Gesellschaft für Schwerionenforschung (GSI), Darmstadt, Germany, [3]Universität Heidelberg, Heidelberg, Germany.

Purpose: Particle beams have achieved growing interest for tumor therapy. To exploit the role of carbon ions (^{12}C) for late complications in the normal central nervous system (CNS), rat spinal cord was used as a late effect model to determine the radiotolerance of photon and carbon ion irradiations.

Methods: The cranial part of the cervical spinal cords were irradiated with 1, 2, 6 and 18 fractions of ^{12}C or photons. The target volume was positioned either in the plateau-region of a 270 MeV/u ^{12}C-beam or in the middle of an extended Bragg-peak of a 140 MeV/u ^{12}C-beam. Photons were delivered by a linear accelerator (Siemens MPX, 15 MeV). Treatment fields were defined by a multileaf collimator with an aperture of 10×15mm. Animals were kept under daily observation with a special focus on radiation-induced acute reactions, latency and incidence of neurological disorders. Dose-response curves for the endpoint symptomatic myelopathy (paresis II) were established and the resulting values for ED_{50} (dose at 50% complication probability) for radiation myelopathy were used to determine RBE-values.

Results: Based on the biological endpoint D_{50} (dose at 50% complication probability) we determined rather constant RBE-values for the plateau-region ranging from 1.43 ± 0.08, 1.37 ± 0.12, 1.33 ± 0.02, and 1.42 ± 0.02 for 1, 2, 6 and 18 fractions of ^{12}C-ions, respectively. In contrast, in an extended Bragg-peak RBE increases from 1.76 ± 0.05 for single doses of ^{12}C-ions to 5.04 ± 0.08, when the total dose is devided into 18 fractions.

Conclusion: Carbon ion irradiations of the spinal cord are significantly more effective in the peak than in the plateau region. The higher efficacy is related to a clear fractionation effect for plateau irradiations, while no clinically relevant effect of fractionation was obtained, when carbon ion irradiation was performed in the Bragg maximum. The acquired data contribute significantly to the validation of the RBE-model presently applied for ^{12}C treatment planing in the clinical situation.

(PS2029) Effect of radiation therapy of carbon -ion to Mesothelioma. Kumie Nojima, Masao Suzuki, Yoshinobu Harada. NIRS, Chiba, Japan.

Malignant pleural mesothelioma (MPM) is an asbestos-related malignancy that is highly resistant to current therapeutic modalities. NIRS started the research that detected a MPM at the early stage by using the molecular imaging technology in 2005. We examined whether the radiation therapy by the carbon-ion beams showed the effectiveness to MPM. We measured the radiation sensitivity of the MPM cells by X rays and the carbon-ion beam, used five kinds of MPM cell lines.

The dose response curves for cell death in two different MEM cell lines by X ray as well as low-LET (13keV/micrometer) and high-LET (70keV/micrometer) carbon-ion beams. The relative biological effectiveness (RBE) values calculated by the D10 which is determined as the dose (Gy) required to reduce the surviving fraction to 10%, relative to X-rays. The results were that the MPM cells were resistant for X ray and low-LET carbon-ion beams, but the high-LET carbon-ion beams was effective for the MPM cells. The RBE values were 1.1 to 1.29 for the low-LET beams and 2.5 to 3.1 for the high-LET carbon-ion beams.

This result suggested that, it was possibility that the radiation therapy by the carbon-ion beam was effective for the MPM.

(PS2030) Research of boron neutron capture therapy (bnct) for cancer at kyoto iniversity reactor (kur). Koji Ono[1], Shin-ichiro Masunaga[1], Minoru Suzuki[1], Kenji Nagata[1], Yuko Kinashi[1], Akira Maruhashi[1], Yoshinori Sakurai[2], Shin-ichi Miyatake[3], Junich Hiratsuka[4], Junich Hiratsuka[4], Itsuro Kato[5]. [1]Kyoto University, Kumatori-cho, Osaka, Japan, [2]Sapporo Medical University, Sapporo, Japan, [3]Osaka Medical College, Takatsuki-shi, Osaka, Japan,

[4]Kawasaki Medical School, Kurashiki-shi, Okayama, Japan, [5]Osaka University, Suita-shi, Osaka, Japan.

Introduction : BNCT is a treatment in which selective irradiation to cancer cell is possible in principle. Here, we report the present status of BNCT at KUR.

Materials and Methods : Borocaptate and Boronophenylalanine (BPA) were used, and 75–100 mg/kg or 250–900 mg/kg were respectively prescribed for the patient. The epithermal neutron and a thermal neutron were properly used by a tumor. The physical dose was calculated from the B-10 concentrations in the blood concentration, neutron fluence and γ-ray dose, and then biological dose which multiplied the physical dose by Compound Biological Effectiveness factor, which is apparent RBE, was displayed. An object was selected by based on F-18 BPA PET. In case of a brain tumor, the tumor dose of >=40 Gy-Eq and a brain dose of <=18 Gy-Eq (case without an irradiation history), <=15 Gy-Eq (case with an irradiation history), and the skin dose at <=10 Gy-Eq were required. Moreover, in head and neck cancer, it was considered as tumor dose >=30 Gy-Eq and skin-dose <=10 Gy-Eq.

Results : We performed the BNCT of 173 affairs in and after 2001 when BNCT by the epithermal neutron was started. The five year survival rates of AA and GB, the malignant gliomas, are 53% and 15% respectively, and, BNCT surpasses in both histopathologies compared with the standard treatment reported by the Japan Neurosurgical Society. Even if analyzed according to 6 group classifications of RTOG, the BNCT group was superior to the standard treatment group. It being characteristic of BNCT is that reduction of a tumor shadow or an edema region is observed at a post-irradiation early stage. Such a phenomenon is not observed in the ordinary X-ray irradiation to glioma.

According to the recurrent head and neck cancer after standard treatment including X-ray therapy, almost all cases show regression of a tumor, and the rate of effectiveness of treatment is >90%. There are also several cases in which perfect elimination and control of tumors were obtained.

Although there is little number of cases, we also carries out treatment of the liver cancers which have multiple lesions, the lung tumor including malignant mesothelioma, etc., and the effect was approved.

Conclusion : To X-ray resistance cancer, BNCT is effective, and it needs to develop an accelerator neutron source and to promote the further research.

(PS2031) Gene expression profiling in normal human fibroblasts following the irradiation with heavy ion particles by HIMAC. Akira Fujimori, Katsutoshi Suetomi, Ryuichi Okayasu, Sentaro Takahashi. National Institute of Radiological Sciences, Chiba, Japan.

HiCEP (High-coverage expression profiling) is a novel comprehensive analysis method which is based on DNA finger printing and PCR amplification. It enables to detect any altered gene expression among 60–70% of all the actually transcribed genes in any eukaryotic cells and tissues. We previously applied HiCEP to a primary culture of normal human fibroblasts and observed gene expression responding to X-ray at the very low dose (10 mGy). As the result of screening approximately 23.000 transcripts, we have identified a set of CXC chemokines (CXCL1, CXCL2, CXCL6 and CXCL8) up-regulated by the 10 mGy X-rays in the normal human fibroblasts (HFL III) (Cancer Res 2005; 65: 10159–10163). Those genes have hardly been expected from the previous studies using the higher (>100 mGy) doses of radiation. Our observation indicated that different molecular mechanisms are involved in the response to ionizing radiation with different doses /dose rates, suggesting that different cellular responses could be induced by ionizing radiation with different LETs. Accelerated heavy ion particles (at high LET) provide promising effects for radiotherapy of certain types of malignancy, however, the molecular basis of its advantage to gamma rays is not fully understood. This time, we applied HiCEP to compare the gene expression profiles in normal human fibroblasts (HFL III) irradiated with radiation with different LET's. More than 40 genes were found to be up-regulated in the irradiated cells by >3 folds within 4 hrs post-irradiation of carbon ion at 2Gy (~70 keV/μm). Those included the DNA damage-inducible genes and also some unexpected genes. We are challenging to identify some characteristic cellular responses to low (~13 keV/μm) and high (~70 keV/μm) LET's in order to understand the molecular bases for the heavy ion particle therapy.

(PS2032) 17-allylamino-17-demethoxygeldanamycin enhances the cytotoxicity of tumor cells irradiated with carbon ions. Miho Noguchi, Dong Yu, Ryoichi Hirayama, Emiko Sekine, Koichi Ando, Ryuichi Okayasu. National Institute of Radiological Sciences, Chiba-shi, Japan.

We investigated radiosensitization effect and its mechanism of Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) in human tumor cell lines irradiated with high LET carbon ions. Two human tumor cell lines, DU145 and SQ-5 derived from prostate carcinoma and lung carcinoma, respectively, and normal human fibroblasts HFL III were incubated for 24 h in the presence of 17-AAG at concentration of 100nM. The cells were then irradiated with carbon ions (290MeV/nucleon, LET70keV/μm) and several biological endpoints were compared. Cellular radiation sensitivity was determined by clonogenic assay and DNA double strand break (DSB) repair kinetics were examined by constant field gel electrophoresis. Two tumor cell lines showed an increase in carbon ions-induced cell death when pre-treated with 17-AAG. The radiosensitivity enhancement ratios measured at a survival rate of 10% were 2.13 and 1.40 for DU145 and SQ-5 cells, respectively. In contrast to the tumor cell lines, normal human fibroblasts with carbon irradiation showed no radiosensitization with 17-AAG pretreatment. Our constant field gel electrophoresis studies indicated that 17-AAG had almost no effect on carbon ion-induced DSB repair in DU145 and SQ-5 cells. On the other hand, radiation induced Rad51 foci formation showed different kinetics between the carbon ion alone and the combined treatment with 17-AAG and carbon ions in DU145 cells. Our findings suggest that mechanisms other than inhibition of DSB repair could be involved with the radiosensitization by 17-AAG. in tumor cells irradiated with carbon ions. However, limited inhibition of HRR by this agent may still be a possibility.

(PS2033) Characterization of cell death induced by high LET irradiation in head and neck squamous cell carcinomas : implications for future clinical application in hadrontherapy. Mira Maalouf[1], Gersende Alphonse[1], Michael Beuve[2], Priscilla Battiston-Montagne[1], Claudia Fournier[3], Gisela Taucher-Scholtz[3], Claire Rodriguez-Lafrasse[1]. [1]Department of Cellular and Molecular Radiobiology, EA3738, Oullins, France, [2]IPNL-LIRIS, Villeurbanne, France, [3]GSI, Darmstadt, Germany.

Increased interest in the biological effects of high-LET carbon irradiation lies in the emerging development of hadrontherapy. However a number of molecular issues regarding the mechanisms of action of carbon ions remain to be clarified. We initiated studies on the mechanisms of cell death in two p53-mutated head and neck squamous carcinoma cell lines (HNSCC) with different radiosensitivity, since recent clinical trials (Mizoe J *et al.,* 2004) had shown that the local treatment of the HNSCC by carbon hadrontherapy is much less efficient than in other radioresistant cancer.

We first demonstrated that carbon irradiation (9.8MeV/u) induces a higher level of clonogenic cell death than carbon (75MeV/u) and x-irradiation, for SCC61 (radiosensitive to x-rays), and SQ20B cells (radioresistant to x-rays) which are systematically less sensitive. In SCC61 cells, a dose- and time-dependent induction of apoptosis was observed in response to photons which was even more pronounced in response to both carbon beams. The percentage of cells in sub-G1 phase reached 49 % at 72 h following a 10 Gy photon irradiation, 58 % for C75MeV/n and 78 % for C9.8MeV/n. This increase was directly related to that of ceramide production.

In the radioresistant SQ20B cell line, the lack of apoptotic induction during the 120h following low or high LET irradiation correlated with a deficiency in ceramide production. The number of cells remained virtually unchanged from 2 to 10 days following carbon irradiation confirming a very slow proliferation. A transient arrest in G2 phase was measured at 24h in response to x-rays. This was even more pronounced and maintained 5 days after carbon

irradiations. From 5 days post-irradiation, a significant percentage of cells appeared as positively stained for β-galactosidase activity and showed a senescence-like phenotype.

We conclude that in both cell lines, carbon ion irradiation does not modify the type of death involved, but amplifies it. In the radiosensitive SCC61 cell line, the ceramide p53-independent apoptotic pathway is involved following photon and carbon ions exposure. The high efficiency of carbon irradiation in radioresistant SQ20B cells could not be explained by the induction of apoptosis but by a persistence of the cell cycle arrest and further studies, also including p53 wild type cell lines, are in progress.

(PS2034) Hydrogen peroxide enhances radiation-induced apoptosis in the PC-3 prostate cancer cell line. Shinji Kariya, Ken Sawada, Toshihiro Kobayashi, Akihito Nishioka, Yasuhiro Ogawa. Kochi Medical School, Nankoku, Japan.

Purpose: It is well known that the PC-3 prostate cancer cell line is resistant to X-ray-induced apoptosis. Hydrogen peroxide induces apoptosis in a variety of cell lines at a certain concentration; cells are brought to necrosis at high concentrations. We studied the effect of hydrogen peroxide on radiation-induced apoptosis in the PC-3 prostate cancer cell line.

Materials and Methods: PC-3 cells were exposed to NH_4Cl concentrations of 0 or 10 mM for 4 hours before irradiation. The cells were exposed to hydrogen peroxide concentrations of 0 or 0.1 mM just before irradiation. Irradiation was administered with 10 MV X-rays (3.5 Gy/min) at doses of 0 or 10 Gy. The percentage of apoptotic cells was determined by flow cytometry. Detection of apoptotic cells, ROS production, and morphemic change of lysosomes and mitochondria were examined using a CCD camera-equipped fluorescence microscope. Analysis of cytochrome c was examined using ELISA.

Results: The percentage of apoptotic cells at 48 h after X-irradiation alone, administration of hydrogen peroxide alone, and combined X-irradiation and hydrogen peroxide were 1.85, 4.85, and 28.4%, respectively. In the case of combined X-irradiation and hydrogen peroxide administration, 1) ROS (reactive oxygen species) production occurred at both 1 and 4 h after X-irradiation, 2) lysosomes were ruptured at 4 h after X-irradiation, 3) mitochondria were fragmented at 4 h after X-irradiation, and 4) the release of cytochrome c occurred from the mitochondria to the cytoplasm. In contrast, in the case where the cells were exposed to NH_4Cl before X-irradiation and hydrogen peroxide administration, 1) apoptosis was almost completely suppressed, 2) ROS production did not occur, 3) lysosomal rupture was blocked, 4) fragmentation of mitochondria was blocked, and 5) cytochrome c was not released.

Conclusions: These results indicate that hydrogen peroxide strongly enhances radiation-induced apoptosis in the PC-3 prostate cancer cell line, that this apoptosis is lysosome-dependent, and that mitochondrial fragmentation exists downstream from lysosome rupture in the apoptotic pathway. These results also suggest that ROS production may be followed by injury of cytoplasmic organelles such as mitochondria by X-ray and hydrogen peroxide, and that this ROS production may itself induce apoptosis.

(PS2035) Elevated expression of Prx1 and Nrf2 in stage I non-small cell lung cancer: Prx1, but not Nrf2, is an independent prognostic factor for disease recurrence and reduced survival after surgery. Joo-Heon Kim[1], Paul N. Bogner[2], Nithya Ramnath[3], Yoorim Park[1], Xiaofei Yu[1], Jihnhee Yu[4], and Young-Mee Park[1]. Department of [1]Cell Stress Biology, [2]Pathology, and [3]Medicine, Roswell Park Cancer Institute, Buffalo, NY 14263, USA; [4]Department of Statistics, State University of NY at Buffalo, Buffalo, NY 14215, USA.

Abstract

Purpose: Lung cancer is the leading cause of cancer death with chance of survival restricted to a subset of NSCLC patients able to undergo surgical resection. However, the recurrence rate of NSCLC after surgery remains high with few prognostic indicators of clinical outcome. Prx1 is shown to be elevated in various cancers and confers an aggressive survival phenotype. We recently cloned the *prx1* promoter and found that Nrf2 is a key transcription factor for *prx1* up-regulation. Previous studies suggest that Nrf2 may be constitutively activated in NSCLC. Based on the above information, we investigated whether Prx1 and/or Nrf2 levels have prognostic significance in stage I NSCLC.

Methods and Results: Immunohistochemical expression of Prx1 and Nrf2 was evaluated in paraffin embedded tissues from 90 patients who underwent a curative surgical resection. Increased expression of cytosolic Prx1 (66.7%) and nuclear Nrf2 (61.8%) was observed in this series. Prx1 elevation, but not Nrf2, correlated with reduced recurrence free survival and overall survival on univariate ($P = 0.01$ and $P = 0.03$) and multivariate ($P = 0.003$ and $P = 0.005$) analyses.

Conclusion: This is the first study to test the prognostic significance of Prx1 and Nrf2 in human cancers. Our results demonstrate that Prx1 expression status predicts for recurrence and shorter survival in stage I NSCLC after surgery. Considering the possible role of Prx1 and Nrf2 in radio-/chemo-resistance, it warrants future investigation to evaluate whether elevated Prx1 and/or Nrf2 levels are predictive of treatment response in advanced lung cancer and other malignancies.

(PS2036) Hypofractionation results in reduced tumor cell kill compared to conventional fractionation for tumors with regions of hypoxia. David J. Carlson, Paul J. Keall, J. Martin Brown. Stanford University, Stanford, CA, USA.

Tumor hypoxia has been observed in many human cancers and has been shown to correlate with treatment failure in radiation therapy. The purpose of this study was to quantify the effect of dose per fraction on tumor cell killing assuming a realistic distribution of tumor oxygenation and full reoxygenation between dose fractions. We assessed the sensitivity of the results to variations in the radiobiologically hypoxic fraction, the dose per fraction, oxygen diffusion model parameters, and intrinsic radiosensitivity parameters. We calculated a probability density function for the partial pressure of oxygen in a tumor cell population as a function of radial distance from the capillary wall. Estimates of the oxygen partial pressures for subpopulations of tumor cells were used to determine the corresponding oxygen enhancement ratios (*OER*s) for cell killing. The overall surviving fraction of a tumor cell population consisting of maximally resistant cells, cells at intermediate levels of hypoxia, and well-oxygenated cells was calculated as a function of dose per fraction for an equivalent tumor biological effective dose (BED). Our model predicts that increasing hypoxia as a function of distance from blood vessels causes a decrease in tumor cell killing by a factor of 10^5 over a radial distance of 130 μm (assuming a partial oxygen pressure of 60 mmHg at the capillary wall). For tumor cells with $\alpha/\beta = 10$ Gy (typical of head and neck cancers), the overall surviving fraction for the tumor over a full treatment course increases by a factor of 10^3 as the dose per fraction is increased from 2–24 Gy. For tumors cells with $\alpha/\beta = 3.0$ Gy (typical of prostate cancers), the surviving fraction over a full treatment course increases by a factor of 10^3 as the dose per fraction is increased from 2–18 Gy. The total dose delivered for each dose per fraction has been calculated to achieve equivalent tumor BED values of 80.5 Gy and 130.0 Gy for reference head and neck (30 fractions of 2.2 Gy) and prostate (39 fractions of 2.0 Gy) treatments, respectively. The modeling studies presented in this work suggest that hypofractionation of a radiotherapy regimen results in a significant decrease in tumor cell killing compared to standard fractionation as a result of tumor hypoxia.

(PS2037) The correlation of intrinsic radiosensitivity with bystander response in individual colorectal carcinoma patients undergoing radiotherapy treatment. Orla L. Howe[1], Jacintha O Sullivan[2], Blathnaid Nolan[3], Brenden McClean[4], Fiona M. Lyng[1]. [1]Dublin Institute of technology, Dublin, Ireland, [2]University College Dublin, Dublin, Ireland, [3]St. Vincents Hospital, Dublin, Ireland, [4]St. Lukes Hospital, Dublin, Ireland.

Intrinsic radiosensitivity is an important factor that influences the individual response of tumours to radiotherapy treatment. This

factor also influences the levels of cell death in bystander cells and thus may enhance or reduce bystander responses. The main objective of this work is to assess intrinsic radiosensitivity and the corresponding bystander responses 'in vitro' in individual colorectal carcinoma patient's cells throughout a course of radiotherapy treatment. These radiobiological endpoints will be compared and contrasted with patient clinical prognosis post radiotherapy treatment.

Whole blood was taken from colorectal carcinoma patients attending St. Vincents hospital at three phases of their radiotherapy treatment; Phase 1: At diagnosis, Phase 2: During radiotherapy, Phase 3: 1 year after radiotherapy. The blood samples were used to generate whole blood lymphocyte cultures for both the G2 chromosomal radiosensitivity assay (to assess intrinsic radiosensitivity) and for the bystander assay. The cultures were exposed to gamma radiation (+ and - 0.5Gy) from a cobalt teletherapy unit). The intrinsic radiosensitivity and radiation induced bystander data for each individual colorectal carcinoma patient blood sample was compared and contrasted.

To date, a number of blood samples from colorectal carcinoma patients at phase 1 and some at phase 2 of their radiotherapy treatment have been collected and analysed. The preliminary data shows variation in individual intrinsic radiosensitivity and radiation-induced bystander responses. No correlation pattern was observed between the two radiobiological endpoints at phase 1 to date.

It is proposed that the data generated from this research will give us a further insight into the influence of intrinsic radiosensitivity on bystander response in individual colorectal carcinoma 'in vitro' cultures, and further more demonstrate the influence these two endpoints have on patient response to radiotherapy treatment plans. As a result of this, it is possible that radiotherapy treatment plans could be modulated to improve efficacy for each individual colorectal carcinoma patient in the future.

(PS2038) Relative effectiveness equation of proliferative tumour cells: concept of hyper-extended fractionation: application to lung radiotherapy. Rakesh M. Chandola. Pt.J.N.M.Medical College Raipur 492001 Chhattisgarh INDIA, Raipur, India.

In this work, a new iso-effect dose equation is developed for immediate proliferative tumour cells. This equation is based on the linear - quadratic model of radiotherapy. To account the proliferation of tumour cells, a factor exp(γT) is induced here in the previously developed iso - survival equation, were, "γ" is the prolifration rate of tumour cells, and "T" is the total irradiation time in days. To compensate the entire time span of proliferation effect, a new concept of hyper - extended fractionation (HEF) radiotherapy is induced here for estimation of new iso - effect doses. In this new technique, the total irradiation time is taken upto the proliferation period of tumour and the dose per day with Multiple Fractions a Day (MFD) is taken equal to the dose per fraction of conventional equivalent dose with irradiation upto the proliferation period of tumour. This Relative Effectiveness (RE) equation along with this hyper - extended fractionated concept is applied here to radiation induced acute lung damage (radiation pneumonitis). For this, to account the proliferation period of 60 days of lung after start of it's irradiation, the Extrapolated Response Dose (ERD) of HEF for lung irradiation upto 60 days is taken equal to the ERD of it's equivalent conventional irradiation of 52.48 Gy in 43 fractions in 60 days with 1.22 Gy/fractions with 5 days a week treatment. In result, new iso-effect dose chart is presented here. It is concluded here that the hyper - extended fractionation with 2 or 3 or 4 fractions per day up to 60 days of irradiation may provide the better therapeutic window of lung radiotherapy.

Key words : Proliferation, MFD, L-Q model, HEF.

(PS2039) Tumor carbonic anhydrase 9 expression is associated with the presence of lymph node metastases in uterine cervical cancer. Hye-Jin Shin, Sun Lee, Jooyoung Kim. National Cancer Center, Korea, Goyang-si, Gyeonggi-do, Republic of Korea.

Purpose: Tumor hypoxia has a pronounced effect on malignant progression and metastatic spread of human tumors. As carbonic anhydrases (CA) 9 and 12 are induced by the low-oxygen environment within tumors, we investigated the relationship between the expression of these two CA and the presence of metastatic lymph nodes (LN) in uterine cervical cancer.

Materials and methods: CA9/CA12 expression was evaluated histochemically in primary cervical cancer tissues of 73 patients who underwent laparoscopic LN staging and two patients with clinical staging before definitive radiotherapy at the National Cancer Center, Korea. We also evaluated CA9 expression in 33 patients with pathologically confirmed metastatic LN.

Result: CA9 expression in the primary tumors was significantly associated with LN metastasis ($P = 0.03$) and poorer disease-free survival (relative risk, 6.1;95% confidence interval, 1.3.28.3, $P = 0.02$, multivariate analysis), whereas CA12 expression did not show such a relationship. In addition, 21 of 24 metastatic LN revealed similar CA9 expression ($P = 0.001$), suggesting that CA9-expressing tumor cells had a higher metastatic potential. CA9 was expressed in 45 of 75 (60%) primary tumors, with positive tumor cells observed predominantly in the area away from the blood vessels. In contrast, CA12 expression was observed in only 29 of 74 primary tumors (39%), without a specific pattern.

Conclusions: These findings indicate that expression of CA9, but not CA12, in tumors is associated with the presence of LN metastases and poorer prognosis. Selective application of new treatment modalities based on CA9 expression to prevent LN metastases may improve overall treatment outcome in patients with uterine cervical cancer. Currently, further studies are underway to unravel the cellular characteristics of CA9 overexpression by microarray analysis using CA9-overexpressing cancer cells as well as a series of human cervical cancer tissues. (Cancer Science 2007)

(PS2040) Cluster effects within the Local Effect Model - Application to in vitro and in vivo experiments. Thilo Elsässer, Michael Scholz. Gesellschaft für Schwerionenforschung, Darmstadt, Germany.

The treatment of tumors with heavy ions requires a biophysical model in order to predict the relative biological effectiveness (RBE) at each position in the irradiation field. Generally, the RBE depends on the energy and LET of the primary beam and all its fragments, the tissue under consideration and the dose level.

The Local Effect Model (LEM) developed at GSI is currently the only model, which is implemented in treatment planning and takes these dependences into account. It calculates the RBE for different ions and cell lines starting from the corresponding experimental photon data and an amorphous track structure model. Here, we present an extension of the model which takes cluster effects of single strand breaks at the nanometer scale into account. In line with the main idea of the LEM, we take the yield of single (SSB) and double strand breaks (DSB) from experimental photon data and use a Monte-Carlo method to distribute them onto the DNA. We score clusters of SSBs where individual SSBs are separated by less than 25 bp as additional DSBs. Assuming that the number of DSBs is a measure for cell lethality, we derive a modified cell survival curve for photons which takes these cluster effects into account. Additionally we improved the representation of the radial dose distribution by explicitly considering radical diffusion.

We compare this extended version of the LEM to various experimental *in vitro* data and find a better agreement of the RBE than for the original model. The key feature of the extension is an increased ratio of RBE values for high-LET particles of several hundred keV/μm with respect to low-LET particles.

Furthermore, we combined the extended version with the treatment planning software TRiP98 facilitating the calculation of the biologically equivalent dose in complex three dimensional geometries. We simulate the RBE values for the irradiation of CHO cells in a two-dimensional therapy-like setup mimicking an extended tumor volume with an organ at risk. Finally, the cluster extension is applied to compare the RBE predictions to experimental data of the radiation tolerance of the rat spinal cord following fractionated irradiations with carbon ions. For both comparisons we also find a better agreement with the measurements.

(PS2041) IGF-IR gene expression as a predictor to radiation response in patients with advanced cervical carcinoma. Pablo Moreno Acosta[1], Myriam Sánchez de Gómez[2], Alejandro García Carrancá[3], Ricardo Cendales[1], Jaime Triana[1], German Dario Díaz[4], Zoila Conrado[5], Antonio Huertas[1], Monica Molano[1], Maria Mercedes Bravo[1], Rosalba Ospino[6], Maria Cristina Plazas[2]. [1]Instituto Nacional de Cancerología, Bogotá, Colombia, [2]Universidad Nacional de Colombia, Bogotá, Colombia, [3]Instituto Nacional de Cancerologia de México/Universidad Nacional Autonoma de México, México D.F, Mexico, [4]Universidad Javeriana, Bogotá, Colombia, [5]Fundación SantaFe, Bogotá, Colombia, [6]Instituto Nacional de Cancerologia, Bogota, Colombia.

Cervical cancer is the leading cause of cancer related deaths in Colombian women. Most of cases are diagnosed in advanced stages and its treatment is based in the application of radiotherapy. However, several studies have shown different risk factors associated with lack of treatment response. Available evidences suggest that insulin-like growth factor I receptor (IGF-IR) expression leads to increased cellular radioresistance. Nevertheless the IGF-IR expression and other clinical parameters have not been evaluated in relation to the clinical radioresponse in patients with advanced cervical carcinoma. The aim of this investigation was to evaluate the effect of IGF-I, IGF-II, IGF-IR and GAPDH expression on clinical radioresponse.

A sequential, non probabilistic sample was designed. The treatment response was evaluated three months after radiotherapy treatment completion. The complete response was considered as a 100% reduction of the initial tumoral volume; partial response with a partial reduction (greater than 50%) of the initial tumoral volume. The gene expression of IGF-IR, IGF-I, IGF-II and GAPDH was determined by Real Time PCR and IGF-IR was detected using Western Blot. The hemoglobin level was evaluated as parameter of oxigenation before treatment (hb> 10g/dl; hb< 10g/dl). The gene expression levels were compared between complete responders and partial or non responders using Anova or Kruskall-Wallis Anova. A multivariate analysis was developed using a binomial logistic inconditional regression.

In this study 37 patients with cervical cancers (Stage IIB: n=6; Stage IIIA: n=1; Stage IIIB: n=27; stage IVB: n=1; HPV16 positives) were recruited. IGF-IR expression (p= 0.04) and incomplete treatment (p= 0.019) were associated with the lack of treatment response. Patients expressing IGF-IR had 3.6 times more risk of non treatment response. GAPDH expression was directly correlated with the IGF-IR expression, IGF-IR and IGF-II co-expression under hypoxic conditions (hb< 10g/dl), demonstrated a possible activation of a glicolytic pathway as an answer to the high metabolic rate of tumoral cells. This is the first *in vivo* report relating the IGF-IR expression with the lack of treatment response. Further studies will be completed in order to confirm this finding.

(PS2042) Radio-sensitization by 17-AAG in insulin growth factor 1 receptor over-expressed tumor cells. Dong Yu[1], Miho Noguchi[1], Emiko Sekine[1], Akira Fujimori[1], Masahiko Miura[2], Ryuichi Okayasu[1]. [1]Heavy-Ion Radiobiology Research, Chiba, Japan, [2]Oral Radiation Oncology Tokyo Medical and Dental University, Tokyo, Japan.

Heat-shock protein 90 (Hsp90) is a ubiquitous molecular chaperone protein, which is related to the stabilization and activation of various cell cycle checkpoints and signal transduction proteins. An effective Hsp90 inhibitor 17-AAG has now entered clinical trials. Insulin-like growth factor I receptor (IGF-IR) over-expressed HeLa cells (HeLa-IGF-IR) exhibit a radioresistant phenotype and are difficult to be treated with radio-therapy. We studied the radio-sensitization effects of 17-AAG in HeLa-IGF-IR cells. After pretreatment with 17-AAG for 24 hours, characteristics of irradiated cells were examined by colony-forming assay, MTT assay, western blotting, senescence like growth arrest (SLGA), and immunostaining. 150nM of 17-AAG pretreatment significantly radio-sensitized HeLa-IGF-IR cells, but this sensitization was not observed with non-transfected HeLa cells. The protein expression of PARP after irradiation was reduced with 17-AAG pretreatment, indicating an increase in apoptosis formation. X-irradiation with 17-AAG pretreatment in HeLa-IGF-IR cells led to reduction in cell growth kinetics, spheroid formation prevention, and hyper-sensitivity to gamma-H2AX and p-ATM foci formation when compared to HeLa cells. The downstream pathways of IGF-IR (PI3-K/Akt and Raf/MEK/ERK pathways, proliferation signal pathways) were inhibited by 17-AAG treatment, and more cells were led to apoptosis and SLGA. We also have used the tumor xenografts model using SQ5 lung carcinoma cells to examine the antitumor effect of 17-AAG in vivo. Treatment with 17-AAG (80mg/kg, 1–3 times/week, i.p.) alone did not inhibit the tumor growth, but the combined treatment with 8 Gy gamma-rays and 17-AAG significantly inhibited tumor growth. 17AAG treatment would be a useful method for certain radio-resistant tumor cells.

(PS2043) Loratadine-mediated enhancement of radiation response. Benjamin P. Soule, Nicole L. Simone, William DeGraff, John A. Cook, James B. Mitchell. National Cancer Institute, Bethesda, MD, USA.

The histamine receptor-1 antagonist, loratadine (ethyl4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1- piperidinecarboxylate) has been shown to inhibit growth of human colon cancer xenografts. Mechanistic studies to date indicate that inhibition of tumor growth is due in part to cell cycle arrest in G2/M and caspase 9-mediated apoptosis. Since the anti-tumor activity of loratadine is related to cell cycle blocks in G2/M, which are radiation sensitive phases of the cell cycle, we have initiated preliminary studies to determine if loratadine treatment modifies radiosensitivity. A variety of human tumor cell lines are being used with emphasis on human colon carcinoma (HT-29 cells). HT-29 cells were treated with a range of loratadine concentrations (0–150 μM) for 24 hr followed by exposure to radiation and survival was assessed by the clonogenic assay. Based on full radiation dose response curves, pre-treatment of exponentially growing cells with loratadine (75 μM) yielded a radiation dose modification factor (DMF) of 1.95. The survival of cells treated with loratadine alone was 45%. Single dose radiation studies conducted at various times (4–24 hr) after loratadine addition showed that maximal radiation modification occurred at 24 hr. Likewise, pre-treating cells for 24 hr with various concentrations of loratadine (10–75 μM) showed that only 75 μM was effective. Consistent with published studies, loratadine treatment (75 μM, 24 hr) imposed a G2/M block in HT-29 cells (47% compared to 17% for control cells). Interestingly, the radiation response of non-cycling plateau phase cultures of HT-29 cells yielded a DMF of 1.4, suggesting that loratadine-mediated radiation modification involves more than cell cycle arrest. Loratadine (75 μM, 24 hr pre-treatment) was also found to enhance radiosensitivity of DU145 (prostate), SF295 (glioblastoma), and HeLa (cervical) cell lines. Ongoing studies include evaluation of other histamine receptor antagonist analogues, effects of loratadine on radiation-induced DNA damage repair, and potential loratadine-mediated radiation enhancement in vivo. Loratadine is a potentially promising drug that may warrant evaluation in chemo-radiation clinical trials, since loratadine is already FDA approved for oral administration.

(PS2044) Potential role for 13-cis-retinoic acid and alpha interferon to increase radiation effectiveness via abrogation of bcl-2 over expression in prostate tumor cells while sparing normal human colonic cells. Colin K. Hill, Grant Dagliyan, Parvesh Kumar. USC Keck School of Medicine, Los Angeles, CA, USA.

Uncontrolled or over expression of anti-apoptotic proteins such as bcl-2 is considered a major cause of resistance to radiation therapy (RT) and recurrence in prostate cancer patients treated with RT. To determine if Cis-retinoic acid /Alpha interferon (CRA/IFN) could down regulate bcl-2 expression, induce apoptosis and hence increase cell death and reduce resistance to radiation, we conducted experiments in LNCAP tumor, and normal human Co-18 and IMR-91 cell lines. We chose Co-18 because the rectum is the radiation dose limiting structure in prostate cancer patients treated with RT. Methods: LNCAP, Co-18 and IMR-91 cells were treated with CRA (200 nmolar) and IFN (100U/mL) for 48 hours. Bcl-2, Bcl-X, and Bax expression and percent apoptosis were determined at time

points after removal of the drugs (0,12,24,48, and 96 hours). For gene expression analysis, cells were washed then scraped from the flasks in a small amount of RIPA buffer. They were then sonicated in the RIPA buffer and centrifuged. The target protein was determined by Western blot analysis of the supernatant. To determine apoptosis, cells in multi-well dishes were washed and fixed in para-formaldehyde, and then digested and stained using a Roche Chemicals fluorescent apoptosis kit. The number of fluorescing cells versus the total number of cells was counted in at least 9 microscope fields to determine percent apopotosis. Cells were also treated with gamma rays in one or two fractions (200 –400cgy) after CRA/IFN exposure. Results. In LNCAP cells, CRA/IFN reduced bcl-2 over-expression and increased apoptosis (to 70%) by 48 hours after drugs were removed. In co-18 and IMR-91 cells, CRA/IFN marginally decreased bcl-2 expression with no induction of apoptosis. After CRA/IFN exposure and treatment with radiation, the level of apoptosis in Co-18 and IMR-91 was 22% and 0% respectively at 48 hours. We conclude that CRA/IFN selectively reduces bcl-2 expression and induces apoptosis in LNCAP tumor cells as compared to normal Co-18 and IMR-91 cells and the induction of apoptosis is significantly less in Co-18 and IMR-91 cells treated with CRA/IFN followed by radiation than in LNCAP cells treated with CRA/IFN alone. These results suggest CRA/IFN treatment prior to radiation will increase radio-sensitivity in tumor cells but not significantly in critical normal cells.

(PS2045) TP53 and TP53-related genes associated with protection from apoptosis in the radioadaptive response. Ryuji Okazaki, Akira Ootsuyama, Toshiyuki Norimura. University of Occupational and Environmental Health, Kitakyushu, Japan.

To clarify the characteristics of the radioadaptive response in mice, we compared the incidence of radiation-induced malformations and investigated the effect of administering priming low-dose irradiation prior to high-dose irradiation on the level of apoptosis and on the expression of TP53 and TP53-related genes in mouse splenocytes

Pregnant ICR mice were exposed to a priming dose of 2 cGy on day 9.5 of gestation and to a challenging dose four hours later of 2 Gy, and were sacrificed on day 18.5 of gestation. The incidence of malformations, prenatal death and fetal body weights were studied. The incidence of external malformations was significantly lower (by approximately 10%) in the primed (2 cGy + 2 Gy) mice compared to the unprimed (2 Gy alone) mice. However, there were no differences in the incidence of prenatal death, the skeletal malformations or the body weights between primed and unprimed mice. These results suggest that primary conditioning with low doses of irradiation suppresses radiation-induced teratogenesis

The percentage of apoptotic cells was significantly lower in $TP53^{+/+}$ mice receiving priming irradiation 2 to 168 h before the high-dose irradiation, compared to $TP53^{+/+}$ mice exposed to 2 Gy alone. In contrast, $TP53^{+/-}$ mice only exhibited a reduced level of apoptosis when priming was performed only for the 2 h or 4 h prior to the high-dose irradiation. In $TP53^{+/+}$ mice, primed mice had higher TP53 expressions than 2 Gy exposed mice. Phospho-TP53 (ser15/18) expression was the highest in 2 Gy exposed mice, and intermediate in primed mice groups. Expression of p21 was higher in primed mice groups compared with 2 Gy exposed mice. MDM2 expression remained at a high level in all mice receiving 2 Gy. Elevated phospho-ATM expression was observed only in 2 Gy exposed mice. We conclude that TP53 plays a critical role in the radioadaptive response and that TP53 and TP53-related genes might protect cells from apoptosis through activation of the intracellular repair system

(PS2046) A mechanism for the radiosensitizing effect of gold nanoparticles in radiotherapy: the increased generation of secondary electrons around DNA. Yi Zheng, Darel Hunting, Leon Sanche, Patrick Ayotte. University of Sherbrooke, Sherbrooke, PQ, Canada.

It has recently been shown that the preferential accumulation of gold nanoparticles (GNP) in cancer cells followed by X-ray irradiation of these cells could considerably increase the survival rate of tumor bearing mice [1]. To study the basic mechanism behind this finding, we performed experiments on thin films of pGEM-3Zf(-) plasmid DNA directly bombarded by **60 keV electrons** with and without gold GNP, and measured the damage to DNA by agarose gel electrophoresis. Transmission electron microscopy (TEM) revealed that GNP could closely link to DNA scaffolds by electrostatic binding. The exposure curves were recorded for the formation of crosslink, single-strand break (SSB), double-strand break (DSB) and loss of supercoiled DNA. The quantum yield of SSB and DSB for GNP/DNA mixtures with molecular ratio of 1:1 were 1.8 and 6.3 folds increased, respectively, compared to that for the pure DNA sample. The previously observed radiosensitizing effect of GNP may therefore be due to their localization close to cellular DNA. Owing to the high molecular weight of gold, the absorption of ionizing radiation is increased close to DNA and leads to a considerably increased production of short range secondary electrons that have a high probability to damage DNA.

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(PS2047) Effect of thorium and rare earth mixed dust on cytokines and genetics in occupational exposed workers. Huimin Lu[1], Kejun Jia[2], Cuilan Zhang[1], Chunyan Wang[1], Wei Zhang[1], Hui Zheng[1], Yufei Liu[2], Xumin Tu[1], Shuxia Hao[1], Rong Zhen[1], Xu Su[1]. [1]National Institute for Radiologcal Protection, Chinese Center for Disease Control and Prevention, Beijing, China, [2]Institute of labour hygiene, Baotou Iron and Steel Company, Baotou, China.

In order to evaluate the effects of thorium and rare earth mixed dust on cytokines and genetics, we examined the peripheral bloods of workers who occupationally exposed to low dose radiation due to thorium and rare earth mixed dust. Serum IL-2, IL-6 and TNF were analysed by radioimmunoassay. DNA damage was evaluated by the DNA comet length of lymphocyte using gelatin electrophoresis method. Micronucleus (MN) rate of lymphocyte was examined by direct method in the present study. Activities of serum IL-2 were 2.267 ± 1.032 and 4.608 ± 1.411 pg/ml for exposed and non-exposed group respectively. Activities of serum IL-6 were 66.55 ± 27.22 and 124.3 ± 56.32 pg/ml for exposed and non-exposed group respectively. Levels of IL-2 and IL-6 for exposed group were significantly lower than that of non-exposed group ($p<0.01$). Activities of TNF were 0.96 ± 0.33 and 0.84 ± 0.35ng/ml respectively. TNT level of exposed group was significantly higher than that of non-exposed group ($p<0.01$). The average values of DNA comet length for exposed and non-exposed group were 40.2 ± 4.14μm and 39.5 ± 2.54μm respectively and no significant difference was found between these two groups ($p>0.05$). MN rates for two groups were 2.71 ± 2.04‰ and 0.79 ± 0.91‰ respectively and a tendency difference was found between these two groups. It was concluded that thorium and rare earth mixed dust might affect some immunological functions and genetic indexes of occupational workers chronically exposed to low dose radiation.

(PS2048) The maximal protection by DMSO in mammalian cells exposed to very high LET radiation. Ryoichi Hirayama[1], Atsushi Ito[2], Yoshiya Furusawa[1], Koichi Ando[1], Masanori Tomita[3], Teruyo Tsukada[4], Masako Izumi[4], Fumio Yatagai[4], Ryuichi Okayasu[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Tokai University, Hiratsuka, Japan, [3]Central Research Institute of Electric Power Industry, Komae, Japan, [4]The Institute of Physical and Chemical Research, Wako, Japan.

To understand indirect action for high linear energy transfer (LET), we examined the radioprotection effects of dimethylsulfoxide (DMSO) on the cell killing for cells irradiated with very high LET heavy ions. Exponentially growing Chinese hamster V79 cells were exposed to low- and high-LET radiations. We estimated that a maximal protectable fraction from a regression lines in the reciprocal plots degree of radical scavenger against the concentra-

tion from the cell survival curves. The contribution of indirect action decreased with increasing LET, but remained at the very high LET region. The contribution of indirect action in the cell killing were 52, 39 and 32% at LET of 797, 1298 and 2106 keV/μm, respectively. When cell survival curves were analyzed by the linear-quadratic equation, dependence of the DMSO concentration on the survival curve parameters were found. The quadratic (β) term depends on DMSO concentration in X-rays, and the linear (α) term depends on that in the very high LET radiation. The results suggest that an indirect action to cell killing results from a double-event mechanism in X-rays, and from a single-event mechanism in the very high LET radiation

(PS2049) American ginseng reduces micronuclei yield in human lymphocytes after low dose radiation exposure. Wei-Dong Wang[1], T.K. Lee[1], H. Mota[1], R.R. Allison[1], R.M. Johnke[1], C. Sibata[1], A.L. Wiley[2]. [1]Brody School of Medicine at ECU, Greenville, NC, USA, [2]Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA.

Background: Since the clinical significance of low dose radiation (LDR, ≤ 0.2 Gy) exposure is unclear, we applied in this study the cytokinesis-block micronuclei (MN) assay in human peripheral blood lymphocytes (PBL), to explore whether LDR induces cytogenetic damage in PBL, and to assess the radioprotective potential of standardized North American ginseng extract (NAGE) in PBL following LDR exposure. **Methods:** PBL were obtained from four healthy donors. At 24 h before irradiation, PBL from each individual were incubated with different concentrations of NAGE up to 750 μg ml^{-1}. IR absorbed doses (0, 0.05, 0.1, 0.5, & 1 Gy) were delivered with 6 MV photon beam by a linear accelerator (Siemens Primus) at a dose rate of 0.9 Gy/min in a water phantom. The yield of MN in PBL was determined microscopically on Hema-3 stained slides. **Results:** At 0 Gy and without the presence of NAGE, MN yield was 15.8 ± 0.8 per 1000 BN cells, and this baseline declined with the application of increasing concentrations of NAGE ($P<0.04$). IR increased MN levels to 27.5 ± 3.2, 30.5 ± 4.2, 51.7 ± 6.2, 89.1 ± 10.0 per 1000 BN cells for 0.05, 0.1, 0.5, & 1 Gy, respectively ($P<0.01$). However, the pretreatment with NAGE significantly decreased these levels, with optimal responses occurring at concentrations of 500 μg ml^{-1} for doses of 0.05 - 0.10 Gy ($P \leq 0.001$) and 250 μg ml^{-1} for doses of 0.5 - 1.0 Gy ($P \leq 0.03$). **Conclusion:** our findings suggest that (1) LDR induces cytogenetic damage in human PBL as evaluated by MN yield; and (2) the standardized NAGE appears to have the potential to protect against this damage and exerting no apparent cytotoxic effects. Supported by NIH/NCCAM grant R21AT002639-01A1.

(PS2050) Histone H2AX phosphorylation (γ-H2AX) and cell survival following ionizing radiation with thiol containing drugs in human microvascular endothelial cells (HMEC). Yasushi Kataoka, Jeffrey S. Murley, David J. Grdina. The University of Chicago, Chicago, IL, USA.

The purpose of this study is to investigate the relationship(s) between the frequency of radiation-induced histone H2AX phosphorylation at serine 139 (γ-H2AX) in cells and subsequent survival as assessed by colony forming ability in the presence or absence of thiol containing compounds during irradiation. Human microvascular endothelial cells (HMEC) in confluent conditions were exposed to X-rays in the presence of active thiol forms of amifostine (WR-1065), phosphonol (WR-255591), N-acetyl-l-cysteine (NAC), captopril or mesna, all of which are known to exhibit anti-oxidant properties. Each of these thiols was administered to HMEC at a 4 mM concentration 30 min prior to irradiation. γ-H2AX formation in irradiated cells was quantified by bivariant flow cytometric analysis with FITC-conjugated γ-H2AX antibody and nuclear DAPI staining. The frequency of γ-H2AX positive cells increased with increasing dose of radiation followed by a dose and time-dependent decay. The most robust response for γ-H2AX formation occurred 1 h following radiation. To assess the effects of the various thiols on γ-H2AX formation, all measurements were made 1 h following irradiation. WR-1065, free thiol form of amifostine, was not only effective in decreasing γ-H2AX formation across the entire dose range of radiation exposures used, but also it was significantly more cyto-protective than either its prodrug (WR-2721) or disulfide (WR-33278) analogues. WR-1065alone, however, did not show a significant effect on γ-H2AX formation if administered immediately or up to 30 min following radiation exposure. NAC, captopril and mesna each reduced the frequency of γ-H2AX formation. While all five thiols were effective in reducing γ-H2AX formation, NAC, captopril and mesna afforded no cyto-protection as determined using a colony forming survival assay. Only WR-1065 and WR-255591 reduced the frequencies of radiation-induced γ-H2AX positive cells and against radiation-induced cell death. These results suggest caution in the use of γ-H2AX as a biomarker for screening effective radioprotective drugs. This work was supported in part by NIH/NCI RO1 Grant CA99005 (DJG), DOE Grant DE-FG02-05ER64086 (DJG).

(PS2051) Modulation of radiosensitivity in lung cancer cell line by a selenium-metabolizing enzyme. Ji Hyun Lee, Su-Jae Lee, Yun-Sil Lee, Sangwoo Bae. KIRAMS, Seoul, Republic of Korea.

Selenium is an essential trace element that has fundamental importance in maintaining health. Selenium deficiency can have adverse consequences for disease susceptibility and the maintenance of optimal health. Selenium has gained much interest due to its cancer preventive effect. Human epidemiological data revealed inverse relationship between selenium intake and risk of cancer.

Despite vast knowledge of selenium effect on various health conditions, functional characterization of selenium metabolic enzymes on cellular physiology has been limited. In particular, information about the relationship between selenium metabolism and radiation sensitivity has been absent.

Therefore to gain insight into the mechanisms underlying cancer prevention by selenium and its role in radiosensitivity, we investigated *sps1*, one of the two human selenophosphate synthetase genes for its role in cancer cell's response to ionizing radiation.

We constructed a stable cell line that over-express sps1 in a lung cancer cell line, NCI-H460 which has wild type p53.

Although stable expression of Sps1 protein per se had little effect on cell proliferation, concurrent irradiation decreased viability of the sps1 cell line as assessed by MTT and clonogenic assay respectively. The increased sensitivity of the cell lines to ionizing radiation was correlated with increased p53 activity as well as with simultaneous up- and down-regulation of Bax and Bcl2 respectively.

Knockdown of *sps1* and p53 by small interfering RNA method revealed that the level of p53 was proportional to that of Sps1 and that the increased radiosensitivity was dependent upon p53.

Interestingly, Sps1 cell lines displayed decreased level of reactive oxygen species with concomitant increase of certain redox enzymes. Furthermore, p53 activity was regulated by cellular redox via Ref1 in sps1 cell lines.

Collectively our results demonstrated that *sps1* was able to affect cell viability upon ionizing radiation via modulation of p53 activity. They further suggest that Sps1 and its reaction product selenophosphate might be involved in cancer prevention in a p53-dependent manner and could be applied to development of a novel cancer therapy.

(PS2052) Ionizing radiation and free radical induced DNA strand breaks: Effects of ion chelator and free radical scavenger. Meriyani M. Odyuo, Rajeshwar N. Sharan. North-Eastern Hill University, Shillong, India.

Ionizing radiation induces direct effect by ionization of DNA molecule and an indirect effect by generation of free radicals. It is generally believed that approximately 70% of radiation induced DNA damage is caused by the secondary species of free radicals generated by primary chemical events, essentially radiolysis of water in the vicinity of the DNA molecule. Some chemicals, aptly

called 'radio-mimetic chemicals' also generate free radicals and, hence, mimic the indirect action of ionizing radiation. Therefore, use of such chemicals in understanding interaction of radiation with DNA is quite popular. Depending on the dose and quality of intervention, both ionizing radiation and radio-mimetic chemicals are known to induce single strand breaks (SSB) and double strand breaks (DSB).

The plasmid is a convenient tool for studies of radiation induced DNA damage since the SSB and DSB can be easily identified, quantified and analyzed. In this investigation, plasmid DNA construct, pMTa4, has been used *in vitro* to identify and quantify strand break type of damage such as SSB and DSB induced by γ-radiation (5–60 Gy) and two free radical generating systems, namely, Fenton reagent and Haber-Weiss reagent. Two interventions were used to modify the radiation and/or free radical induced strand breakage. In the first, an ion chelator, ethylenediaminetetraacetic acid (EDTA) (10 mM), was used. Secondly, a free radical scavenger, alcohol (400 mM), was used. Presence of EDTA or alcohol completely abolished induction of DSB by both Fenton and Haber-Weiss reagents. EDTA was unable to abolish the dose dependent induction of SSB by Fenton as well as Haber-Weiss reagents unlike alcohol, which completely prevented further induction of SSB. Dose dependent reduction in total plasmid DNA was observed in all cases. Results suggest that EDTA and alcohol mediated protections to induction of strand breakage in plasmid DNA were qualitatively and quantitatively different for free radical as well as γ-radiation. The presentation shall focus on the mechanistic aspect of these effects.

(PS2053) Hypersensitive γH2AX dose response and infrequent apoptosis in normal epidermal skin from radiotherapy patients. Martin Simonsson[1], Fredrik Qvarnström[1], Jan Nyman[2], Karl-Axel Johansson[3], Ingela Turesson[1]. [1]Uppsala University, Department of Oncology, Radiology and Clinical Immunology, Section of Oncology, Uppsala, Sweden, [2]Göteborg University, Sahlgrenska University Hospital, Department of Oncology, Gothenburg, Sweden, [3]Göteborg University, Sahlgrenska University Hospital, Department of Radiophysics, Gothenburg, Sweden.

The histone H2AX is phosphorylated in response to severe DNA damage, such as double strand breaks (DSBs). The phosphorylated form, γH2AX, can be visualised by immunohistochemistry as distinct foci in cells exposed to DNA damaging agents.

To evaluate DSB dose response and repair in normal tissue, skin biopsies were taken from five prostate cancer patients undergoing the first week of radiotherapy. Areas of the skin exposed to doses between 0.05 to 1.2Gy were sampled for biopsies in which γH2AX foci were quantified by immunohistochemistry and digital image analysis.

Biopsies taken 30 minutes after delivered fractions revealed a low-dose hypersensitivity effect for doses below 0.2Gy, suggesting that early DSB repair is dose dependent with a reduced repair rate for low doses. This interpretation also implies that following exposure to doses between 0.4 and 1.2Gy, a portion of the DSBs are repaired within just 30 minutes. Besides the initial fast repair, the presence of a much slower repair component was inferred since no decrease in numbers of γH2AX foci could be detected when analysing biopsies taken 2 hours after administered fractions.

Furthermore, no differences in repair kinetics or dose response could be detected when comparing biopsies taken at the start of the treatment week, with those taken at the end of the week.

γH2AX is also associated with DNA fragmentation during apoptosis. A subset of intense and evenly stained cells expressing several apoptotic morphological features was observed within the immunostainings. Co-localisation with the apoptosis marker cPARP-1 was confirmed in a double staining. However, the γH2AX staining proved superior in both contrast and intensity, which enabled positive identification of 43 apoptotic cells out of 168 000 epidermal cells analysed. Despite infrequent apoptosis, a dose dependency could still be detected at the end of the first week of radiotherapy ($p<0.05$).

In conclusion, γH2AX dose response in normal epidermal skin reveals a low-dose hypersenstitivity effect for doses below 0.2Gy and suggests the involvement of both fast and slow repair mechanisms. γH2AX proved to be a potent marker for apoptosis in paraffin embedded biopsies and its quantification revealed infrequent yet dose dependent apoptosis after one week of fractionated radiotherapy.

(PS2054) γ-H2AX in blood as a biomarker for low dose irradiation exposure. Christophe E. Redon, Olga A. Sedelnikova, William M. Bonner. NIH/NCI/CCR/LMP, Bethesda, MD, USA.

We are living in a world where ionizing radiation exposure is inevitable. Adding to exposure from cosmic rays, sun and radioactive substances, modern society has created new sources of radiation exposure such as space and high altitude journeys, X-rays diagnostics and radiological treatments. Adding to these is the increasing threat of radiobiological terrorism

Histone H2AX is unique because upon exposure of cells to DNA damaging agents, its COOH terminus gets rapidly phosphorylated. The phosphorylation of several thousand molecules of H2AX to form γH2AX in the chromatin flanking the double-strand break (DSB) site can be detected *in situ* using the anti-γH2AX antibody. Immunocytochemical analysis reveals the number and position of each nuclear DSB as a γ-H2AX focus. We used γ-irradiation to produce DSBs in blood cells. After a period of recovery, the blood was fixed and the lymphocytes were isolated. The number of γ-H2AX foci peaks 30 min after irradiation and then declines at a relatively steady pace as the cell repairs the DNA damage. Several doses of irradiation, ranging from 0 to 150 Rads, were applied. The higher the dose of irradiation, the higher the numbers of γ-H2AX foci seen in the nuclei. In fact, the amount of DNA damage is linear (0.13 γH2AX focus per rad) and does not depend on the age of the blood donor

The high sensitivity of the assay permits detection of DSBs formed after as little as 2 rad of gamma irradiation. Blood fixation eliminates the decay of the biological response to preserve information. Fixed blood samples and/or lymphocytes can be stored up to six months at minus 20°C and as little as 15 µl and 500 µl blood can be used for analysis of white blood cells and lymphocytes respectively. This method can also be applied to study other nuclear and cytoplasmic proteins and used with small animal models to overcome volume limits of blood samples. By detecting DNA damage in lymphocytes and estimating the amount of damage, this non-invasive blood test could be useful to analyze or adjust clinical treatments, drug development and to monitor persons exposed to voluntary or accidental sources of radiation

(PS2055) Evaluation of radioprotection of a new chemical entity ON01210 (Ex-Rad™) using alkaline Comet assay. K. S. Kumar[1], M. W. Perkins[1], S Ghosh[1], T-C. Kao[1], K Hieber[1], S. Cosenza[2], MVR Reddy[2], E. P. Reddy[2], M. Maniar[3], A.A. Alfieri[3], T. M. Seed[4]. [1]AFRRI, Bethesda, MD, USA, [2]Temple University, Philadelphia, PA, USA, [3]Onconova Therapeutics Inc, Lawrenceville, NJ, USA, [4]Radiation Effects Research Foundation, Hiroshima, Japan.

Several derivatives of benzyl sulphonyl chloride were screened for their anticancer and radioprotective activities by Onconova Therapeutics Inc, NJ. One of these derivatives ON01210 (Ex-Rad), was found to be a good radioprotectant in mice. Subcutaneous injection of a suspension of ExRad at 24 hrs and 15 min before irradiation of mice at 7.5 Gy (dose rate 0.6 Gy/min), provided a dose dependent protection. ExRad was effective also when it was given only 15 minutes before irradiation at 7.5 Gy. When the drug was given at 500 mg/kg body weight, the protection obtained was in the range of 75–100%. The mechanism of this radioprotective effect was evaluated using alkaline comet assay that measures DNA damage within irradiated cells. In these studies, 3 cell lines with differing radiosensitivities were used. These were human umbilical vein epithelial cells (HUVEC), Chinese hamster ovary cells (CHO), and ataxia telangiectasia (AT) cells. The cells were grown to confluence, treated with 20 µM ExRad and irradiated after 24 hrs at radiation doses ranging from 1–9 Gy. Control cells received the vehicle in which ExRad was prepared. Irradiated cells were mixed with low melting agarose, subjected to electrophoresis, and processed for Comet tail length assessment. HUVEC and CHO cells treated with ExRad showed a statistically significant ($p <$

0.05) decrease in Comet length, indicating the ExRad treatments had reduced DNA damage associated with irradiation at 9 Gy, but not at the other radiation doses tested. By contrast, with DNA-repair deficient AT cells there was no difference in comet length between control and drug treated cells at any radiation doses tested. These data suggest that one possible mechanism by which ExRad radioprotects involves the prevention of radiation-induced DNA damage.

(PS2056) Hemolytic anemia and iron accumulation in CuZn-superoxide dismutase knockout mice. Micael Granström[1], Stefan L. Marklund[2], Göran Roos[1]. [1]FOI NBC-Defence, Umeå, Sweden, [2]Umeå University, Umeå, Sweden.

One of the major reasons for damage to DNA from ionizing radiation is the formation of free radicals. Free radicals are also produced in normal cellular processes in the oxygen metabolism. In mammals there are a number of enzymes that protect the organism against these radicals. Among these are the glutathione peroxidases, catalase and the superoxide dismutases.

In the present study we compared CuZn superoxide dismutase (SOD-1) knockout mice and wildtype mice. The SOD-1 knockout mice develop normally but show reduced fertility, reduced ability of repairing neuronal damage and cells derived from the mice are difficult to keep in long-term cultures. They also show a shortened survival, probably due to an overfrequency of hepatocellular carcinomas in old mice. Studies have also showed lowered aconitase activity in the liver in the knockout mice.

This study included micronuclei frequency in erythrocytes in vivo, a number of hematological parameters and the basal aconitase activity in different organs. We also studied the iron content in different tissues. Our results show elevated background frequencies of micronuclei in erythrocytes in SOD-1 knockout mice as compared to wild type mice. A number of hematological parameters were altered hemoglobin levels were lower in the knockout mice than in wildtype mice. In liver and kidney of the knockout mice the cytosolic aconitase activity was almost abolished.

The total iron content in the liver and the kidney of the CuZn-SOD knockout mice was higher than in the wildtype control mice.

The conclusions that can be drawn from this study are that the absence of CuZn-SOD in the mice leads to oxidative stress and elevated levels of DNA damage, which in turn leads to hemolytic anemia and iron accumulation.

(PS2057) Protection against direct type DNA damage by ligands containing tyrosine and tryptophan. Sam Bullick, Jamie Milligan. UC San Diego, La Jolla, CA, USA.

We have condensed plasmid DNA using cationic tetralysine ligands containing an additional single amino acid located either between the two central lysines or at the C-terminus. This additional amino acid was either tyrosine or tryptophan. These condensates were gamma irradiated in the presence of thiocyanate ions to produce direct type damage to guanine bases. After irradiation, the plasmid was decondensed, incubated with the base excision repair endonuclease FPG, and the yield of the resulting SSBs were quantified. The presence of tyrosine or of tryptophan was found to decrease the SSB yield. Tryptophan was more effective than tyrosine, which is consistent with the behavior of the monomeric amino acids. The central location was more effective than the C-terminus. Based upon fluorescence quenching and structural considerations, we interpret this to the larger distance between the C terminal amino acid and the guanine base. Thus the rate of the electron transfer from the amino acid donor to the guanine radical acceptor appears to exhibit the distance dependence found with other electron transfer reactions.

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(PS2058) Hot spot occurrence in a nickel resistance gene (*nrp*) of *Enterobacter* sp. Ni15 after a gamma ray irradiation. Young-Keun Lee. Korea Atomic Energy Research Institute, Jeongeup-si, Jeonbuk-do, Republic of Korea.

Enterobacter sp. Ni15 has a nickel resistance determinant, the *nrp* gene in pNi15, and an increased nickel resistance to *Escherichia coli* DH5α *in trans*. To understand the effect of a gamma radiation on a nickel resistance determinant, *nrp* gene, *Enterobcter* sp. pNi15 was irradiated by a Co-60 irradiator at a dose of 1.0 kGy. Thirty five mutants of the irradiated *Enterobacter* sp. Ni15 showed a wide range of a nickel resistance (from 7.5 mM up to 12 mM) in Luria-Bertani media, but the wild type showed a medium-level (up to 10 mM). Among the 35 mutants, 27 mutants had two simultaneous mutated sites (hot spots) which were found in the NrpA coding region (one base deletion) and the promoter region (one base (T→C) transition). All the mutants with a hot spot in the NrpA coding region (nt-145 deletion) caused a frame shift mutation and produced a truncated NrpAM1 product. As a result, a gamma radiation induced two hot spots in 27 of the 35 tested mutants (77.1%) but the truncated NrpAM1 product of the base deletion also had a similar nickel resistance to the NrpA.

(PS2059) Comparative studies on spontaneous and ionizing radiation induced mutagenesis between somatic and male germ cells. Naoko Shiomi[1], Katsuko Noshiro[1], Seiji Kito[1], Kenichi Masumura[2], Takehiko Nohmi[2], Tadahiro Shiomi[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]National Institute of Health Sciences, Tokyo, Japan.

We have carried out mutation assays in germ cells from the transgenic mice after irradiation of various doses of ionizing radiation at the post-meiotic spermatid stage, to learn the difference in sensitivity to ionizing radiation in mutation induction among somatic cells and male germ cells (pre-meiotic spermatogonial stem cell and post-meiotic spermatid stage). The transgenic mice used for the assay are the gpt-delta mouse strain, which carries about 80 copies of the bacterial gpt gene per cell as targets for mutagenesis. To measure the induced mutation frequencies in male germ cells (spermatid stage), sperms were extracted at the 14th day after irradiation of 2.5 or 5 Gy of X rays, corresponding to the spermatid stage at the time of treatment. The spontaneous gpt gene mutation frequency in male germ cells was 0.36×10^{-5}. The mutation frequencies in male germ cells irradiated with 2.5 or 5 Gy of X rays at the spermatid stage were 0.60 or 1.03×10^{-5}, respectively. The induced mutation frequencies in male germ cells irradiated at the spermatid stage were nearly the same as those irradiated at the spermatogonial stem cell stage (0.53 or 1.05×10^{-5} for 2.5 or 5 Gy of X-ray-treatment, respectively), and about three to four times lower than those in somatic cells (2.43 or 3.46×10^{-5} for 2.5 or 5 Gy of X-ray-treatment, respectively). This difference between somatic and male germ cells in the mutation frequency would be mainly due to the high base excision repair activity in male germ cells.

(PS2060) Estimation of mutation induction rates in AT-rich sequences using a genome scanning approach following X-irradiation of mouse spermatogonia. Jun-ichi Asakawa, Nori Nakamura, Hiroaki Katayama, Harry M. Cullings. Radiation effects research foundation, Hiroshima, Japan.

We have previously used *Not*I as the landmark enzyme (recognizes GCGGCCGC) in a genome scanning approach for detection of mutations induced in mouse spermatogonia and estimated the mutation induction rate as about 0.7×10^{-5}/locus Gy^{-1}. To see if different parts of the genome have different sensitivities for mutation induction, we used *Afl*II enzyme (recognizes CTTAAG) in the present study as the landmark enzyme. Following the screening of 1,120 spots in each mouse offspring, five mutations were found among 92,655 spots from unirradiated paternal genome, five mutations among 218,411 spots from unirradiated maternal genome, and 13 mutations among 92,789 spots from 5 Gy-exposed paternal genome. Among the 23 mutations, 11 involved mouse satellite DNA sequences (AT-rich), and the remaining 12 mutations also involved AT-rich but non-

satellite sequences. Both types of sequences were found as multiple-, similar-sequence blocks in the genome and hence molecular characterization of most mutations was not possible. Counting each member of cluster mutations separately and excluding results on one hypermutable spot, the spontaneous mutation rates were estimated as 3.2 ($\pm$ 1.9)$\times 10^{-5}$ and 2.3 ($\pm$ 1.0)$\times 10^{-5}$ per locus/generation in the male and female genomes, respectively, and the mutation induction rate as 1.1 ($\pm$ 1.2)$\times 10^{-5}$/locus Gy^{-1}. The induction rate would be reduced to 0.9×10^{-5}/locus Gy^{-1} if satellite sequence mutations were excluded from this analysis. The results indicate that mutation induction rates do not largely differ between GC-rich and AT-rich regions; 1×10^{-5}/locus Gy^{-1} or less, which is close to 1.08×10^{-5}/locus Gy^{-1}, the current estimate for the mean mutation induction rate in mice.

(PS2061) Complex mutations in rpoB gene produced from irradiated Bacillus subtilis spores. Nobuo Munakata, Toshiyuki Natsume, Atsushi Kamata, Kotaro Hieda. Biophysics Laboratory, Rikkyo University, Tokyo, Japan.

In previous works, we analyzed the spectra of radiation-induced mutations in the gyrA gene, and demonstrated several types of tandem base changes were produced from irradiated B. subtilis spores (N. Takahashi et al. J. Radiat. Res. vol. 40, 115–124, 1999). In this work, we extended the analysis to rifampicin-resistant mutations that exhibited wider varieties of sequence changes including insertions and deletions.

All of >400 rifampicin-resistant mutations from strains HA101 and TKJ6412 (recF7) have been identified as carrying sequence changes in two N-terminal regions of rpoB gene. In both strains, the majority of spontaneous mutations were single base substitutions with predominant hot spots, exceptions being insertions of three bases. Among the mutations produced upon the irradiation with heavy ions (Fe, C, Ar), X or gamma rays, about 2.7% were tandem-double substitutions in the HA101. Also, several double substitutions within several base pairs and small deletions were observed in the HA101. In contrast, a few double substitutions but a large number (>10%) of deletions of 3, 6, 9 and more bases were obtained from the TKJ6412.

Results indicate clustered or complex damage covering two to several base pairs might be produced by the exposure to radiations, and these types of damage induce multiple base changes in the repair-proficient HA101. Daughter-strand gaps generated from such damage might be repaired by the RecF-dependent pathway of recombinational repair. If this pathway is defective, they result in the deletions possibly due to the non-homologous end joining type of mechanism.

(PS2062) Effects of particle LET and dose fractionation on tissue-specific mutations in vivo. Polly Y. Chang, James Bakke, Angela Puey, Sylvia Lin. SRI International, Menlo Park, CA, USA.

As NASA's space exploration program extends towards the moon and Mars, there is an increasing need to understand the potential risks of human exposure to protracted radiations of diverse ionization quality in long-term manned space missions. The goal of our research is to advance our understanding of the molecular basis of mutations in tissues after exposure to space radiation components. Our approach is to evaluate mutations in a genomically-integrated reporter transgene after particle irradiations with beams of different linear energy transfer (LET) values and dosing regimes.

Plasmid-based transgenic mice were either sham- or whole-body irradiated with a range of doses of high energy protons (LET = 0.24 keV/μm), Oxygen (LET = 14 keV/μm), Titanium (LET= 108 keV/μm) or Iron ions (LET = 150 keV/μm). In separate experiments, animals were exposed to either a single or fractionated doses of the iron ions or protons. Brain and spleen tissues were collected at different times up to 8 weeks after irradiation. Transgene *lacZ* mutant frequencies (MF) were determined according to methods previously established and mutant spectrum was characterized by Restriction Fragment Length Polymorphism (RFLP).

We noted that *lacZ* MF in both brain and spleen tissues are dependent on the LET of the particle beam. Point mutations dominate the mutant spectrum at 8 weeks after irradiation for protons, oxygen and titanium beams. As the LET of the beam increased, we noted a progressive increase in the percentage of size-change mutants. Size- change mutants include deletion mutants, as well as mutants containing inserted mouse genome sequences. The MF after fractionated protons or iron ions were lower than if the radiation was given in a single exposure. Despite the lowered frequencies, the characteristics of the mutant spectrum remained similar, independent of the dosing regime. Our results demonstrate for the first time that particle radiation-induced genomic damage is retained in tissues up to 8 weeks post irradiation, and that recombinational events are involved in the damage-repair process.

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(PS2063) Kinetics of CHO A_L mutant expression after treatment with radiation, EMS, MNNG and asbestos. Stephen B. Keysar, MIchael H. Fox. Cell and Molecular Biology Program, Colorado State University, Fort Collins, CO, USA.

We have previously described a flow cytometry mutation assay (FCMA) using the hybrid CHO A_L cell line that measures mutant yield in less than two weeks. Mutations in the *cd59* gene located on human chromosome 11 are scored by the absence of fluorochrome-conjugated antibody binding to the CD59 antigen on the cell surface. After treatment with various genotoxic agents, the mutant fraction increases to a maximum in 6 to 12 days and quickly drops to a plateau similar to clonogenic assays. We hypothesized that peak mutant expression occurs only after normal cell cycling resumes and the reduction in the mutant fraction to a plateau is due to cells that have lost CD59 expression but are either nonviable or have a reduced growth rate compared to wild type cells.

Clonogenic survival was measured at various times after treatment with EMS, MNNG or asbestos. The surviving fraction was approximately 50% the day after treatment but returned to control values just prior to or at the day of peak mutant expression. Potential doubling time (Tpot) and cell cycle analysis was measured by uptake of BrdU and PI staining. Only irradiated cells had significant G2 blocks but cells treated with EMS or asbestos had Tpot values much longer than those of controls. Normal proliferation and Tpot values resumed prior to the peak day of mutant expression. Mutant and non-mutant cells were sorted based on CD59 expression and survival was measured. Survival of isolated CD59 negative cells on the day of peak expression after treatment with EMS or γ-radiation was about 50% of cells sorted from the CD59 positive peak of the same sample and about 40% of cells from an untreated control. Isolated mutant populations were cloned and subsequently mixed with untreated CHO A_L cells, then measured at various times later. The mutants were lost from the population exponentially from 50% initially to 10% after 10 days.

The range in time after treatment before the peak expression of mutants occurs when using the FCMA is likely due to the varying degrees of toxicity between agents and only arises when normal cell proliferation and survival return and shuttling of mutated antigen can occur. The drop from peak mutant fraction to plateau is most likely a result of more slowly growing or nonviable CD59 negative cells.

(PS2064) Germline minisatellite mutation rate in the offspring of radiation workers from Sellafield Nuclear Facility, UK. Gwen S. Rees[1], Laura Guyatt[1], Patricia A. Jonas[1], E Janet Tawn[1], David H. Macgregor[2]. [1]Westlakes Research Institute, Cumbria, United Kingdom, [2]British Nuclear Group, Cumbria, United Kingdom.

Radiation-induced germline mutation has been demonstrated in experimental studies, but to date, there has been no consistent epidemiological evidence that this effect occurs in humans. Accordingly, calculation of the risk of radiation-induced genetic disease in humans must rely on extrapolation from the animal data. It has recently been suggested that germline mutations at hypervariable minisatellite loci may provide a new approach to

the study of radiation-induced genetic effects in man. The purpose of this study is to investigate the germline mutation rate in relation to paternal pre-conceptional exposure in families of occupationally exposed radiation workers.

Germline mutation rate at eight hypervariable minisatellite loci was investigated by Southern hybridisation in 79 families where the father was employed at the Sellafield Nuclear Facility, UK. Offspring from these families were divided into two groups according to their pre-conceptional exposure, offspring in the control group had a pre-conceptional dose of <50mSv (mean 7.5mSv, range 0 - 40.7mSv) and offspring in the exposed group had a pre-conceptional dose of ≥50mSv (mean 192.6mSv, range 50.8 - 739.1mSv). Twenty paternal germline mutations were observed for 352 informative alleles in 48 control offspring (total mutation rate 5.7%), compared to 26 mutations for 513 informative alleles in 70 exposed offspring (total mutation rate 5.1%). No statistically significant difference between the two study groups was observed for the total paternal mutation rate or the mutation rate at any single locus.

The data from this study demonstrate no statistically significant increase in germline minisatellite mutation rate associated with paternal occupational exposure to radiation. The study is ongoing and will be expanded to include 150 families.

(PS2065) Role of DNA polymerase β in response to ionizing radiation: studies with a dominant negative. Sari Neijenhuis[1], Manon Verwijs-Janssen[1], Gaby Rumping[1], Kerstin Borgmann[2], Ulla Kasten-Pisula[2], Ekkehard Dikomey[2], Conchita Vens[1], Adrian Begg[1]. [1]Netherlands Cancer Institute, Amsterdam, The Netherlands, [2]University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Several types of DNA lesion are induced after ionizing irradiation (IR) of which double strand breaks (DSB) are expected to be the most lethal, although single strand breaks (SSB) and base damages are quantitatively in the majority. These numerous lesions are predominantly repaired by the base excision repair (BER) and SSBR pathway, in which DNA polymerase β (polβ) has been shown to play a key role.

We previously showed that inhibition of these repair processes by expressing a dominant negative to DNA polymerase β (polβDN) resulted in radiosensitization, confirming polβ's proposed critical role in repair of IR induced DNA damage.

Chinese hamster ovary cells deficient in XRCC1, critical in BER and SSBR, are radiosensitive, but were not further sensitized by polβDN. This implies that polβDN acts via XRCC1-dependent pathways, consistent with interference in BER/SSBR. Unrepaired BER/SSBR intermediates can result in additional residual DSBs, leading to aberrations and death. Indeed, we observed an increased induction of both chromosomal aberrations and γH2AX foci after IR in polβDN expressing cells, indicating the occurrence of secondary DSBs. These aberrations did not result from changes in induction or repair of the majority of DSBs, as determined by constant field gel electrophoresis. Residual DSBs appeared to occur in a replication-independent manner, since the polβDN also radiosensitized cells in confluence. In addition, we observed an increase of typical G1-phase aberrations after IR. We further found that the polβDN does not sensitize cells to H_2O_2, an agent producing oxidative base damage and SSBs. This indicates that polβDN is a specific sensitizer for IR, and suggests involvement in repair of clustered damage, which occurs after ionizing radiation but not after H_2O_2.

These data confirm a critical role of efficient and successful BER/SSBR after IR for cell survival. They also have potential clinical implications for radiotherapy and other modalities, since a significant proportion of tumors contain polβ mutations and/or have altered expression that, due to their structure, probably act as a dominant negative.

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(PS2066) Accelerated formation of colour junctions directly after irradiation by manipulation of homologous recombination with mild hyperthermia. Nicolaas A. Franken[1], Judith W. Bergs[1], Przemek Krwawczyk[2], Tony Cisouw[2], Jan Stap[2], Chris van Bree[1], Jaap Haveman[1], Jan Paul Medema[1], Jacob A. Aten[2]. [1]LEXOR, Laboratory for Experimental Oncology and Radiobiology, Dept . Radiotherapy, Academic Medical Center, Amsterdam, The Netherlands, [2]Center for Microscopic Research, Academic Medical Center, Amsterdam, The Netherlands.

Hyperthermic treatments of mammalian cells at temperatures in excess of 42.5 °C are known to enhance effects of ionizing radiation. We have observed radiosensitization after 1 h at 43°C in both SW1573 lung and RKO colorectal tumor cells. However after 1 h at 41 °C we only observed significant radiosensitization in RKO cells. The mechanism of radiosensitization at this mild hyperthermic treatment is investigated with respect to induction of chromosome aberrations in relation to DNA repair.

We studied the induction of chromosomal aberrations and kinetics of the γ-H2AX, MRE-11, DNA-PK and Rad51 foci in two human tumor cell lines, SW-1573 and RKO, after combined mild hyperthermia (HT: 41 °C for one hour) and ionizing radiation (4 Gy) treatment.

Color junctions and fragments of chromosome 2 and 18 of SW-1573 cells and chromosomes 18 and 19 of RKO cells were analyzed by FISH in PCCs both immediately and after a delay of 24 hours after treatment. Kinetics of γ-H2AX, MRE11, DNA-PK and Rad51 foci was studied in interphase cells growing on glass slides in the first hour after treatment.

Our results show that

1: Immediately after the combined treatment in all studied chromosomes of both cell lines more color junctions were found as compared to radiation alone. 24 hour after treatment no difference in the number of color junctions was found after 4 Gy alone and the combined treatment and was similar to the number found directly after the combined treatment. The number of fragments directly after combined treatment or 4 Gy only was similar and was decreased after twenty-four hours.

2: A transient but significant decrease in the number of cells with co-localized γ-H2AX and Rad51 foci within 60 min after the combined treatment was observed. The number of positive cells with co-localized foci of γ-H2AX with MRE11 or DNA-PK was not influenced.

Based on these results we suppose that the decrease of the co-localization of γ-H2AX with Rad51 foci is caused by a temporarily impairment of homologous recombination repair due to the treatment of 1 hour at a temperature of 41 °C. This transient impairment of homologous recombination might enhance NHEJ repair causing an increase in the number of color junctions directly after the combined treatment. However, these results do not explain the difference in radiosenstization of the two cell lines.

(PS2067) Development of a non-mammalian vertebrate model system to investigate mechanisms and pathways of genomic instability in vivo. Wendy W. Kuhne, Lingling Ding, William S. Dynan. Medical College of Georgia, Augusta, GA, USA.

Genomic instability is a phenomenon whereby radiation-related genetic damage manifests itself one or more cell generations following the generation in which the damage was inflicted. Although not fully understood at a molecular level, radiation-induced genomic instability may reflect the presence of unstable chromosomes formed by misrepair of DNA double-strand breaks. It may also reflect the non-DNA targeted effects of radiation, including persistent oxidative stress and bystander signaling. One approach for detection of genomic instability is based on the use of locus-specific genetic tests that detect non-conservative homologous recombination events. Tests have been devised in the mouse based on a naturally-occurring mutation, the pink-eyed unstable allele, and on an artificial transgene, unstable fluorescent green direct repeat (FDGR) locus. The latter contains two tandemly repeated, but incomplete, copies of the enhanced green fluorescent protein that must undergo recombination to be active (Jonnalagadda et al., DNA Repair (4) 594–605 (2005)). Here we describe the adaptation of the FGDR technology to a non-mammalian model system, the Japanese medaka fish (Oryzias latipes). As vertebrates, medaka share with humans a large complement of vertebrate-specific organs of radiobiological interest (brain, spinal cord, vasculature, digestive,

excretory, and hematopoietic systems). The short life cycle of the medaka and the transparent chorion make this vertebrate model an excellent candidate for transgenerational studies and determination of tissue-specific responses to ionizing radiation. Chimeric medaka containing the FGDR transgene will be established by microinjecting embryos at the one or two-cell stage with the test cassette. A homologous recombination event can restore the full function of the test cassette through the subpathways of either single strand annealing or a gene conversion with or without crossing over. Mature chimeric medaka will be bred with wildtype medaka of the same strain to produce an F1 generation. Fluorescent green direct repeat (FGDR) positive F1 embryos will be determined by PCR-genotyping to determine the frequency of germ-line transmission. Preliminary results of the development and characterization of the transgenic fish will be reported.

(PS2068) Imaging the direct interaction of Artemis with DNA in irradiated cells. Geoffrey E. Brand, Kai Rothkamm, Kevin Prise. Gray Cancer Institute, Northwood, United Kingdom.

Complex DNA double-strand breaks (DSBs) induced by ionising radiation contain additional base lesions close to the break ends which may require specific processing steps involving the Artemis nuclease before repair by non-homologous end joining can occur. Artemis-deficient cells are radiosensitive and cannot repair a small subset of DSBs. A greater fraction of complex breaks can be generated using densely ionising radiation such as α-particles. The aim of this work is to study the recruitment of Artemis to sites of radiation-induced DNA damage and to image its interaction with DNA in irradiated cells using fluorescence resonance energy transfer (FRET). This approach allows interactions in the nanometer range to be examined, providing a valuable and novel method of imaging protein-protein interactions as well as protein-DNA interactions in situ.

N- and C-terminal fusion constructs of Artemis and enhanced green fluorescent protein (EGFP) were generated and expressed in HeLa cells. Recruitment of Artemis to sites of alpha-particle and X-ray-induced DSBs was investigated by co-immunofluorescence microscopy for EGFP-Artemis and the DSB marker 53BP1. For FRET analysis of the interaction of Artemis with DNA, Hela cells expressing EGFP-Artemis as FRET donor were stained with the membrane permeable DNA dye Syto81 as FRET acceptor. The spatial proximity between donor and acceptor was determined by time correlated single photon counting fluorescence lifetime imaging microscopy. FRET between EGFP and Syto81 reduces the fluorescence lifetime of the donor and thus allows the proximity of donor and acceptor to be calculated.

Cells irradiated with α-particles produced 53BP1 foci that were large and intense indicating that multiple DSBs were induced along each particle track. The fluorescence lifetime of EGFP-Artemis decreased in unirradiated cells after staining with Syto81, indicating that Artemis interacts with intact DNA. Further changes in the donor lifetime after irradiation suggest a modified association of Artemis with damaged DNA.

We conclude that Artemis is recruited to sites of multiple DSBs induced by alpha-particles. Furthermore, we have established an *in situ* FRET assay for measuring Artemis' binding to DNA in response to radiation.

(PS2069) Interactions of the c-terminal domain of human ku70 with dna substrate: a molecular dynamics study. Shaowen Hu[1], Claudio Carra[1], Janice L. Huff[1], Janice M. Pluth[2], Francis A. Cucinotta[3]. [1]USRA, Space Life Sciences Division, Houston, TX, USA, [2]Lawrence Berkeley National Laboratory, Life Sciences Division, Berkeley, CA, USA, [3]NASA, Lyndon B. Johnson Space Center, Houston, TX, USA.

NASA is developing a systems biology approach to improve the assessment of health risks associated with space radiation. The primary toxic and mutagenic lesion following radiation exposure is the DNA double strand break (DSB), thus a model incorporating proteins and pathways important in response and repair of this lesion is critical. One key protein heterodimer for systems models of radiation effects is the Ku70/80 complex. The Ku70/80 complex is important in the initial binding of DSB ends following DNA damage, and is a component of nonhomologous end joining repair, the primary pathway for DSB repair in mammalian cells. The C-terminal domain of Ku70 (Ku70c, residues 559–609), contains an α helix-extended strand-helix motif and similar motifs have been found in other nucleic acid-binding proteins critical for DNA repair. However, the exact mechanism of damage recognition and substrate specificity for the Ku heterodimer remains unclear in part due to the absence of a high-resolution structure of the Ku70c/DNA complex.

We performed a series of molecular dynamics (MD) simulations on a system with the subunit Ku70c and a 14 base pairs DNA duplex, whose starting structures are designed to be variable so as to mimic their different binding modes. By analyzing conformational changes and energetic properties of the complex during MD simulations, we found that interactions are preferred at DNA ends, and within the major groove, which is consistent with previous experimental investigations. In addition, the results indicate that cooperation of Ku70c with other subunits of Ku70/80 is necessary to explain the high affinity of binding as observed in experiments.

(PS2070) DNA double strand breaks are not sufficient to initiate recruitment of TRF2. Eli S. Williams[1], Jan Stap[2], Jeroen Essers[3], Brian Ponnaiya[4], Martijn S. Luijsterburg[5], Przemek M. Krawczyk[2], Robert L. Ullrich[1], Jacob A. Aten[2], Susan M. Bailey[1]. [1]Colorado State University, Fort Collins, CO, USA, [2]Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, [3]Erasmus MC, Rotterdam, The Netherlands, [4]RARAF, Center for Radiological Research, Columbia University, Irvington, NY, USA, [5]Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands.

The human telomere binding factor TRF2 is rapidly recruited to sites of damage produced by high intensity laser microbeams, prompting the conclusion that it plays an early role in the repair of DNA double strand breaks (DSBs). To characterize the types of damage initiating this recruitment, we generated well-defined, localized nuclear damage utilizing numerous sources of ultra-violet (UV) and ionizing radiation (IR), and then quantified accumulation of TRF2 at these sites. Whereas TRF2 was recruited to sites damaged by a high intensity multiphoton laser beam, no evidence for such recruitment was observed following exposure to lower intensity sources of UV. Furthermore, exposures to various types of IR - agents notorious for their ability to produce copious quantities of DSBs - generated easily recognizable damage foci but failed to elicit a TRF2 response. Our results cast serious doubt on TRF2 playing a biologically relevant role in response to exogenous DNA damage, particularly DSBs. Moreover, they serve to illustrate meaningful differences in the cellular response to high-intensity laser systems versus other types of radiation damage.

(PS2071) Studies on response to the challenging dose of x-rays in lymphocytes of prostate cancer patients and healthy donors. Mateusz Krzysiek[1], Antonina Cebulska-Wasilewska[2], Zygmunt Dobrowolski[3], Agnieszka Panek[1], Wacław Lipczyński[3], Barbara Dobrowolska[3]. [1]Department of Radiation and Environmental Biology INP PAN, Kraków, Poland, [2]Department of Radiation and Environmental Biology INP PAN, Chair of Epidemiology and Preventive Medicine Collegium Medicum Jagiellonian University, Kraków, Poland, [3]Department and Clinic of Urology CM UJ, Kraków, Poland.

Variability was investigated among patients with cancer or benign prostate stage (BPS) diseases in cellular susceptibility to the challenging dose of X-rays and competence to repair the induced DNA damage. DNA damage was detected, with the alkaline version

of single cell gel electrophoresis assay, before and immediately after irradiation and after incubation for a period allowing cells to proceed with the DNA repair (40min). All results were standardized on the base of responses of internal standard cells applied for all procedures.

The difference between groups of prostate cancer and BPS patients in susceptibility to the dose of X-ray was insignificant, although, a strong variation between patients was observed. Percentage of unrepaired and remaining as a residual DNA damage (RD), was significantly higher in lymphocytes of prostate cancer patients ($RD_{TDNA} = 64{,}72 \pm 12{,}60$) than that in cells of BPS or healthy donors ($RD_{TDNA} = 55{,}11 \pm 16{,}97$; $p < .05$). Neither in prostate cancer nor in BPS patients group was observed a serious difference in RD between smokers and non-smokers. However, in a subgroup of recent or former smokers, prostate cancer patients have shown significantly higher amount of residual DNA damage ($RD_{TDNA} = 65{,}11 \pm 13{,}09$), that means lower efficiency of the DNA repair, comparing that of BPS patients ($RD_{TDNA} = 50{,}18 \pm 15{,}97$; $p < .005$). That difference was statistically insignificant for non-smokers. In both, prostate cancer or BPS, no significant differences were observed in DNA repair between groups of those who reported or not, the occurrence of the cancer in instant family. Essentially lower efficiency of DNA repair was observed in lymphocytes of prostate cancer patients ($RD_{TDNA} = 67{,}84 \pm 10{,}55$) comparing that of BPS ($RD_{TDNA} = 53{,}81 \pm 17{,}71$; $p < .05$) from subgroup of patients with no reported cancers in their families, although, that difference was insignificant in the other subgroup.

Our results have shown a significant variability among investigated groups of patients in their cellular susceptibility to the challenging dose of X-rays and in the DNA damage repair competence. That can be valuable tool in radiotherapy planning. Furthermore, results displayed that tobacco smoking and genetic factor can influence DNA damage repair competence that might be important in a both, cancer prevention and treatment.

(PS2072) A structure:function analysis of ku86. Junghun Kweon, Eric A. Hendrickson. University of minnesota, twin cities, Minneapolis, MN, USA.

DNA-PK (the DNA-dependent protein kinase complex) is known to play a crucial role in four distinct, but mechanistically overlapping, processes: V(D)J and 2) switch recombination in the immune system, 3) the repair of DNA DSBs (double-strand breaks) via NHEJ (non-homologous end joining) and 4) telomere length maintenance and function. DNA-PK consists of a catalytic subunit ($DNA\text{-}PK_{cs}$) and the Ku proteins, Ku70 and Ku86. Ku70 and Ku86 from a tight heterodimer, which acts as the regulatory (DNA binding) subunit for the complex. As the focus of my research, I would like to determine whether there are different domains of Ku86 required for each of the 4 separate functions of DNA-PK. Precedent for this hypothesis comes from work in yeast, where four independent laboratories have described separation-of-function (for DNA repair, DNA recombination and telomere maintenance) mutations for yKu70 and yKu86. These mutations presumably define domains in the Ku proteins that interact with repair-, recombination-, or telomere-specific proteins, respectively. To try and extend these studies to mammals, I have introduced a series of site-directed (based on the yeast studies) point mutations into a hamster Ku86 cDNA. These constructs were then separately introduced into hamster *sxi-3* (*sensitive to X-irradiation-3*; $Ku86^{-/-}$) cells at the same chromosomal locus using an integrated FRT (FLP recombinase target) site and the Flp (flippase) recombinase. EMSAs (electrophoretic mobility shift assays) demonstrated that Hammut #2 (V57H) & Hammut #6 (K343P) had significantly decreased DEB (DNA end binding) activity, a result that was not expected based upon the yeast studies. Even more unexpected, Hammut #2 and Hammut #6 appeared to be very resistant to etoposide _ a DNA double strand break inducing reagent _ and were indistinguishable from wild-type using PFGE (pulsed field gel electrophoresis) to directly measure the repair of DNA double-strand breaks. Additional characterization of these and other mutants is currently underway. Future experiments include introducing identical mutations into a human Ku86 cDNA to measure their effects on telomeric end structure and overall telomere maintenance.

(PS2073) Telomeric sister chromatid exchange, dna repair and aging. Robert Hagelstrom[1], Sandy Chang[2], Laura Niedernhofer[3], Susan M. Bailey[1]. [1]Colorado State University, Fort Collins, CO, USA, [2]MD Anderson Cancer Center, Houston, TX, USA, [3]University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA.

Sister chromatid exchange (SCE) is a chromosomal event associated with genomic instability. The mechanism of SCE formation remains unclear, but homologous recombination (HR) is strongly implicated. We have identified repair genes involved in regulating SCE frequency dependent on genomic location, i.e., genomic (G-SCE) vs. telomeric DNA (T-SCE). Werner Syndrome (WS) and Bloom Syndrome (BS) result from defects in the HR proteins Werner (WRN) and Bloom (BLM) respectively, and display premature aging symptoms including graying of hair, atherosclerosis, diabetes, and increased cancer incidence. Both proteins belong to the RecQ helicase family, both possess 5′ to 3′ helicase activity, but only WRN has 5′ to 3′ exonuclease activity. Using Chromosome-Orientation Fluorescence In Situ Hybridization (CO-FISH) and fluorescence plus giemsa (FPG) staining to determine SCE frequencies in a mouse model null for WRN and Terc (telomerase RNA component), we have demonstrated significantly elevated T-SCE levels while G-SCE levels remain near background [Laud et al., 2005]. To extend these studies, we are analyzing SCE frequencies in human immortalized lymphoblastoid lines deficient in either WRN or BLM; we are also utilizing siRNA to knockdown expression in primary human fibroblasts (telomerase negative). This strategy will facilitate determination of whether BLM also demonstrates differential roles in regulating SCE. Finally, we are examining SCE frequencies in a mouse model deficient in the endonuclease Ercc1-XPF that is required for nucleotide excision repair (NER) of helix distorting DNA adducts and interstrand crosslinks. Ercc1-XPF has recently been shown to have an accelerated aging phenotype [Niedernhofer et al., 2006]. Our preliminary results suggest that Ercc1-XPF does not influence G-SCE levels. These studies will serve to further enlighten links between telomeres, DNA repair and aging.

(PS2074) Oxidative stress-induced intestinal tumorigenesis in mice with a targeted disruption of the Mutyh gene. Teruhisa Tsuzuki, Takuro Isoda, Kazumi Yamauchi, Yusaku Nakabeppu, Yoshimichi Nakatsu. Kyushu University, Fukuoka, Japan.

Oxygen radicals are produced through normal cellular metabolism, and the formation of such radicals is further enhanced by ionizing radiation and by various chemicals. The oxygen radicals attack DNA and its precursor nucleotides, and consequently induce various oxidized forms of bases in DNA within normally growing cells. Among such modified bases, 8-oxo-7, 8-dihydroguanine (8-oxoG) and 2-hydroxyadenine (2-OH-A) are highly mutagenic lesions, if not repaired. MUTYH, a mammalian ortholog of *E. coli* MutY, is a DNA glycosylase that excises adenine and 2-OH-A incorporated opposite 8-oxoG or guanine, thus considered to prevent G:C to T:A transversions in mammalian cells. Recently, biallelic germ-line mutations of *MUTYH* gene have been found in patients predisposed to a recessive form of hereditary multiple colorectal adenoma and carcinoma. Tumor analyses from the patients revealed a significant excess of G:C to T:A somatic mutations in the *APC* gene. To investigate the role of the *MUTYH* gene in mutagenesis and tumorigenesis, we have established *Mutyh* gene-knockout mice by gene targeting. The *Mutyh*-deficient mice appeared normal in their development and growth but showed a marked predisposition to spontaneous tumorigenesis in various tissues when examined at 18 months of age. The incidence of adenoma/carcinoma in the intestine was significantly increased in *Mutyh*-deficient mice, as compared with wild-type mice. This high susceptibility of *Mutyh*-deficient mice to intestinal tumor-development was well correlated with the observation in MAP patients (MUTYH associated polyposis). We performed mutation analysis of the tumor-associated genes (*Apc*, *β-catenin*, *k-ras*, *p53*), using the amplified genomic DNA recovered from 62 samples of intestinal tumors of four *Mutyh*-deficient mice that had been treated with $KBrO_3$. Altogether, fifty-four out of 62 tumors (87.1%) had a mutation in either *Apc* or *Ctnnb1*, however, no tumor had simultaneous mutations in the both genes. In total, 59 out of 60 mutations (98.3%) identified were G:C to T:A transversions. No

mutations were found in either *k-ras* (exon 2) or *p53* (exon 5–8). Our findings suggest that the abnormality in Wnt signal transduction pathway is causatively associated with oxidative stress induced tumor-development in the small intestines of *Mutyh*-deficient mice.

(PS2075) Characterization of a human DNA helicase, *PIF1*, which is responsible for chromosomal integrity. Yongqing Gu, Yuji Masuda, Kenji Kamiya. Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan.

Introduction: DNA helicase has a vital role for DNA metabolism. Some genetic diseases are linked to defects in specific DNA helicases. It has been demonstrated that such DNA helicase has essential function to protect cells from UV or ionizing radiation, since cells defective in such DNA helicases exhibited severe sensitivity to UV or ionizing radiation

The *S. cerevisiae PIF1*, which is encoding a SF I superfamily of 5′ to 3′ DNA helicase, was identified by yeast genetics as a gene required for prevention of replication fork stalling. This gene has been well conserved from yeast to mammals, suggesting crucial function for chromosome maintenance

Purpose: The purpose of our research is to elucidate the function of hPif1 in prevention of replication fork stalling

Experimental Procedure: At first, we identified a human *PIF1* homologue in database, and cloned it from HeLa cDNA library. *hPIF1* expression in human tissues was monitored by RT-PCR using commercially available muti-tissue cDNA panels. For further analysis of the gene product, we purified the recombinant proteins from over produced *E. coli* cells. Then we characterized the ATPase and helicase activity under different conditions and with different substrates

Results: The *PIF1* cDNA encoded a 69 kDa protein consisting of 641 amino acid residues. The *PIF1* gene consisted of 13 exons located on chromosome 15q22. *hPIF1* was ubiquitously expressed in all the tested 16 tissues, higher in the thymus, spleen , testis and very low in the pancreas, prostate, peripheral leukocytes. We demonstrated that the gene has an ATPase activity with different preference for different substrates, and also has unwinding activity, which is much stimulated by substrates of fork structure

Conclusions: Our report is the first report of purification of the full-length hPif1 helicase. And the characterization of its ATPase activity and unwinding activity would help us to understand its function in prevention of replication fork stalling

(PS2076) Deoxycytidyl transferase activity of human REV1 and its substrate specificity. Jinlian Piao, Yuji Masuda, kenji Kamiya. Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan.

Introduction: Translesion DNA synthesis (TLS) is essential for the maintenance of chromosomal integrity as well as the DNA repair function. It has been suggested that functions of the *REV* genes are required for error-prone post-replication repair, essential for induction of mutations and prevention of cell death caused by ionizing radiation. REV1 is the deoxycytidyl transferase and a member of the Y-family DNA polymerase. The activity is capable of extending a primer terminus by insertion of dCMP opposite a variety of damaged bases. *REV3* and *REV7* encode an error-prone DNA polymerase zeta, pol ξ. Genetic data suggest that those proteins form specialized machinery for translesion DNA synthesis. We have demonstrated that human REV1 forms a stable heterodimer with REV7. Recently, it has been found that REV1 interacts with all of the Y-family DNA polymerases, pol η, and κ. These results suggested the central role of REV1 in the translesion DNA synthesis.

Purpose: In this study, we examined roles of amino acid residues for substrate discrimination using purified mutant REV1 proteins.

Experimental procedures: Structure analysis of human REV1 protein revealed several amino acid residues conserved in eukaryotes. We produced the mutant REV1 proteins by base substitutions using PCR. His-tagged mutant REV1 proteins were purified from over-expressing *E. coli* cells. By Nickel-chelating column and gel filtration chromatography. The deoxycytidyl transferase activites of REV1 proteins were analyzed by primer extension assays.

Result: we identified amino acid residues, which are responsible for substrate discrimination of REV1 protein and TLS activity.

(PS2077) Loss of FANCD2 sensitizes cells to topoisomerase II poisons but does not disrupt non-homologous end-joining. Henning Willers[1], Li Li[1], Chen-Mei Luo[1], Jochen Dahm-Daphi[2], Lisa A. Kachnic[1]. [1]Massachusetts General Hospital, Boston, MA, USA, [2]University of Hamburg, Hamburg, Germany.

Cells derived from individuals with Fanconi Anemia (FA) display a profound hypersensitivity to DNA interstrand crosslinks. Since defects in the FA pathway have been detected in a variety of cancers, it is important to define additional types of DNA damage that FA cells may be sensitive to. DNA topoisomerase (topo) poisons are commonly used as chemotherapeutic drugs, with or without concurrent radiation. In this study, we sought to investigate whether the FA pathway mediates cellular resistance to the topo II poison VP16. We utilized well-characterized isogenic pairs of fibroblasts derived from patients with FA group D2 (PD20) or group A (PD220) and their retrovirally complemented counterparts expressing the respective wild-type protein. We found that FANCA-mutant PD220 cells displayed the same clonogenic survival as the wild-type complemented cells in response to VP16. In contrast, PD220 cells were markedly hypersensitive to the cross linker mitomycin C. Surprisingly, FANCD2-mutant PD20 cells were about 10-fold more sensitive to VP16 than cells with wild-type FANCD2, suggesting that FANCA and FANCD2 do not function exclusively in a linear pathway. The ability of cells to survive topo II poisoning is dependent upon non-homologous end-joining (NHEJ) proteins such as DNA ligase IV/XRCC4. The FA proteins also have been implicated in NHEJ, although the line of evidence is not consistent. To clarify the role of FANCD2 in NHEJ as a possible mechanism for VP16 resistance, we stably transfected FANCD2-proficient MCF-7 cells with a plasmid substrate in which the rejoining of I-SceI breaks results in reconstitution of a reporter gene. We then used siRNA to disrupt FANCD2 function, but we did not detect a reduction in the frequency of NHEJ ($\sim 1 \times 10^{-2}$). In addition, PD20 cells showed normal levels of NHEJ, and preliminary sequencing analysis of the I-SceI break junctions in these cells revealed an intact fidelity of end-joining. In contrast, we showed that loss of XRCC4 significantly impairs NHEJ of I-SceI breaks. We conclude that FANCD2-deficient cells are hypersensitive to topo II poisoning, but this phenotype is not the result of a primary NHEJ defect. Importantly, our data also suggest that FANCD2 and FANCA have distinct roles in the cellular response to cytotoxic agents. Supported by a grant from Susan G. Komen for the Cure (to HW).

(PS2078) A novel role of DNA repair factor NBS1 in centrosome maintenance. Mikio Shimada, Junya Kobayashi, Kenshi Komatsu. Radiation Biology Center, Kyoto University, Kyoto, Japan.

Nijmegen syndrome is the genetic disorder, which is characterized by high sensitivity to radiation, chromosome instability and aberrant cell cycle checkpoint. NBS1, the gene responsible for Nijmegen syndrome, forms a protein complex with hMRE11 nuclease and hRAD50, and functions in homologous recombination (HR) repair from DNA double strand breaks, which are elicited by ionizing radiation or other stresses. NBS1 also binds to ATM at the C-terminus and disruption of the interaction fails to recruit ATM to damage sites. Both ATM and NBS1 could have pivotal roles in regulations of cell cycle checkpoints. Therefore, the chromosome instability in NBS cells is considered to be due to defects in both DNA repair and cell cycle checkpoints.

Thus, DNA repair and cell cycle checkpoint genes are responsible for genome instability. However, it is suggested that genome instability in tumor is related to centrosome aberration.

Centrosome is the complex organelles comprising two microtubule-based centrioles surrounded by a protein matrix (pericentriolar material, PCM) and other structural elements, a key regulator for chromosome separation in mitosis. Proper centorosome duplication and spindle formation are crucial for prevention of chromosomal instability. Therefore, the normal function of centrosome is essential for maintenance of genome stability. Recent studies suggest that DNA repair factors are involved in centrosome function. BRCA1 is HR repair protein, which interacts with NBS1 and localizes to the centrosome in mitosis. Therefore, BRCA1-defective cells showed the abnormal duplication of centrosome, leading to aberrant chromosome. On the other hands, DNA damage checkpoint factor p53 is also important for centrosome dynamics. The p53-defective cells showed centrosome-mediated G1/S arrest. Since NBS cells show a significant chromosome instability, NBS1 is considered to have a possible role in centrosome dynamics.

When we examined the localization of NBS1 and ATM by using both protein antibodies, they showed to be accumulated in centrosomes. Moreover, NBS cells showed defect in centrosome amplification, suggesting an indispensable role of NBS1 in centrosome maintenance. We further discuss this novel role of NBS1 in centrosome maintenance.

(PS2079) The meiosis-specific synaptonemal complex protein SCP3 is expressed in cancer and induces aneuploidy in somatic cells. Noriko Hosoya, Sho Hangai, Kiyoshi Miyagawa. Section of Radiation Biology, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, the University of Tokyo, Tokyo, Japan.

The synaptonemal complex proteins, SCP1, SCP2, and SCP3, are components of the synaptonemal complex (SC), a meiosis-specific protein structure essential for synapsis of homologous chromosomes, and play a crucial role in the process of homologous recombination in meiotic cell division. Among these, SCP1 has previously been reported to be ectopically expressed in some tumors, suggesting that it might belong to the cancer/testis antigens group. However, the biological functions of the SC proteins in somatic cells and in tumorigenesis are still unknown. In this study, we investigated expression and biological function of another SC protein SCP3. Expression of SCP3 was examined by Western blotting analysis using human cell lines of various tissue origins, including, breast, bladder, colorectal and hematopoietic tumors. Ectopic expression of SCP3 was detected in some cancer cell lines derived from different tissues. In order to clarify the role of SCP3 in somatic cells, we forcedly expressed the cDNA encoding *SCP3* in retinal pigmented epithelial (RPE) cells and analyzed the cell growth and chromosome aberrations of two independent RPE clones stably expressing SCP3. The growth rates of RPE cells expressing SCP3 were significantly lower than those of the mock cells transfected with an empty expression vector. Fluorescence in situ hybridization analysis using chromosome-specific centromeric probes showed a statistically significant increase in the frequency of the total of one, three, four, or five signal(s) in both of the two SCP3-expressing RPE clones in comparison to the mock cells, suggesting that aneuploidy is caused by forced expression of SCP3 in RPE cells. In conclusion, the meiosis-specific protein SCP3 was shown to be expressed in cancer and induce aneuploidy when ectopically expressed in somatic cells. This report provides the first evidence that the abnormal regulation of proteins involved in SC formation could lead to genetic instability in somatic cells and initiate tumor development. Further investigation to clarify the molecular mechanisms underlying chromosome instability induced by SCP3 would be of great interest.

(PS2080) Low dose-rate effects and non-homologous end-joining repair pathway of double strand breaks. Hiroshi Utsumi[1], Kuniyoshi IWABUCHI[2], Akihisa TAKAHASHI[3], Akira TACHIBANA[4]. [1]Health Research Foundation, Kyoto, Japan, [2]Dept. Biochem., Kanazawa Medical Univ., Kanazawa, Japan, [3]Nara Med. Univ., Nara, Japan, [4]Fac.Science Ibaraki Univ., Mito, Japan.

Introduction :In general, the dose rate of sparsely ionizing radiation is reduced over a period of time the biological effect of a given total dose is reduced. This low dose-rate effects (LDRE) has been generally accepted to result from the sub-lethal damage (SLD) recovery. To study the molecular mechanism of LDRE, we analyzed the knock-out mutants KU70$^{-/-}$, RAD54$^{-/-}$, and KU70$^{-/-}$/RAD54$^{-/-}$ of the chicken B-cell line, DT40 (*Cell* (1997) **89**: 185–193, *EMBO J* (1998) **17**: 5497–5508), since our recent studies on SLD recovery, using these DT40 cells showed that the recovery of SLD is due to DSB repair mediated by homologous recombination (HR) (*Radiat. Res* (2001) **155**, 680–686).

Materials and Methods :Our results were obtained with these knockout mutant clones of DT40. The length of cell cycle is almost the same, ~8 h, between the four types of cells. LDR radiation was delivered at under 1Gy/day using a ^{137}Cs γ-ray source (Sangyo Kagaku Co., Ltd., Tokyo) at the RBC, Kyoto University. Cells were irradiated in suspension continuously at 39.5°C in an incubator set in front of the ^{137}Cs source. Subsequently, serially diluted cells were inoculated into several individual culture dishes (Iwaki, Japan) containing 6 ml of 1.5% (w/v) methylcellulose (Aldrich, Milwaukee, WI) containing D-MEM/F-12 (Gibco-BRL), 15% FCS, 1.5% chicken serum and 10mM β-mercaptoethanol for each radiation dose. The cells were allowed to grow for 7 days.

Results and Discussions :Survival enhancement by LDR irradiation was observed in parent DT40 and RAD54$^{-/-}$ cells but not in non-homologous end-joining (NHEJ) deficient KU70$^{-/-}$ and KU70$^{-/-}$/RAD54$^{-/-}$ cells. Under continuous LDR irradiation, NHEJ-deficient cells will be irradiated in G1 phase and killed since there is no repair system of DSBs in G1 phase. In LDRE, NHEJ pathway shall be more important than HR pathway because NHEJ pathway is working in whole cell cycle. We studied further LDRE using the deficient KU70$^{-/-}$ and KU70$^{-/-}$/53BP1$^{-/-}$ cells since 53BP1 is reported to play a role in new NHEJ repair (*Genes to Cells* (2006) **11**: 935–948). KU70$^{-/-}$/53BP1$^{-/-}$ cells were sensitive than KU70$^{-/-}$ cells under 1 Gy/day dose rate irradiation. These results suggest that 53BP1 play a role in a pathway distinct form Ku-dependent NHEJ pathways, and that both NHEJ pathway is important for LDRE.

(PS2081) DNA Double-strand break repair *in vivo* assessed by gamma-H2AX in blood lymphocytes and normal tissues of repair-proficient and -deficient mouse strains. Martin Kühne[1], Nicole Rief[2], Xiaorong Dong[1], Saskia Grudzenski[2], Christian Rübe[1], Markus Löbrich[2], Claudia E. Rübe[1]. [1]Dept. of Radio-oncology, Homburg/Saar, Germany, [2]Dept. of Biophysics, Homburg/Saar, Germany.

Double-strand breaks (DSBs) are the most deleterious form of DNA damage after ionising radiation, and deficiencies in repairing DSBs lead to pronounced radiosensitivity. Organs and tissues show substantially varying levels of sensitivity during radiotherapy but only little is known about how DSBs are repaired differentially in various normal tissues. By enumerating γH2AX foci in blood lymphocytes and normal tissues, we performed a quantitative analysis of the induction and repair of DSBs *in vivo*, in repair-proficient (C57BL/6) and various repair-deficient mouse strains (SCID, A-T, BALB/c) after whole body irradiation with clinically relevant doses. We observed identical γH2AX foci levels with a clear linear dose correlation and very low backgrounds in the brain, lung, heart, and small intestine. Scoring the loss of γH2AX foci allowed us to verify the different, genetically determined DSB repair deficiencies, including the minor impairment of BALB/c mice. γH2AX foci kinetics measured in the brain, lung, heart, and small intestine were similar to kinetics in peripheral blood lymphocytes demonstrating that data obtained in blood samples can be utilized to screen for DSB repair deficiencies as predictor for clinical radiosensitivity. Strikingly, the various analysed tissues exhibited similar kinetics for γH2AX foci loss despite their clearly different clinical radiation responses. Hence, the varying radiosensitivities of different parenchymal cells cannot readily be explained by tissue-specific differences in DSB repair.

(PS2082) Measurements of DNA-double strand breaks after tumour therapy-related carbon irradiation and comparison with γH2AX analysis after induction of highly complex DNA-lesions. Yvonne Eva Schweinfurth, Jana Topsch, Philippe Barberet, Burkhard Jakob, Gisela Taucher-Scholz. Gesellschaft fuer Schwerionenforschung, Darmstadt, Germany.

Radiotherapy with carbon beams is characterised by an inverse dose profile, extreme precision of irradiation and enhanced relative biological effectiveness (RBE) in the tumour. The increased RBE is assumed to be due to the clustered DNA double strand breaks (DSBs).

To study DSBs under conditions mimicking therapeutical ion irradiation, cells were exposed to high energy carbon ions in a water phantom. Human fibroblasts (AG 1522), hamster cells (CHO-K1) or prostate tumour cells (R-AT1) were placed in the entrance channel (healthy tissue) and in the extended Bragg-Peak (tumour) region. The γH2AX signals were analysed semi-automatically from confocal images. This foci counting was complemented by flow cytometry to account for overlapping foci and multiple DSBs produced along the ion tracks. Both methods indicate a dose-dependent induction of DSBs in the Bragg peak as well as in the entrance channel shortly after irradiation of AG and CHO cells. A clear reduction of γH2AX (both foci and mean fluorescence) was observed with time, reflecting an efficient repair even of ion-induced DSBs. However, the level of residual DSBs was slightly but consistently increased after exposure to Bragg peak ions. Prostate cancer cells showed no differences between the entrance channel and Bragg peak region in the amount of γH2AX foci but clearly in the induction of micronuclei in Bragg peak.

Additionally, we used heavy ions to observe γH2AX during processing of complex DNA-lesions. Irradiation with heavy ions under standard and microbeam conditions induced distinct γH2AX foci. For the later time points (> 2 h) after irradiation we observed splitting of the primary γH2AX foci in sub-compartments or small foci. The generated secondary foci were localized in different layers of the nucleus and persisted for several days (for very heavy ions). Colocalization with 53BP1, Mre11, PML and p53 was analysed. The dispersion and splitting of ion induced foci was also detected in live cell imaging experiments with HeLa 53BP1-GFP cells. Furthermore, the localisation of the residual γH2AX signals within the nuclear chromatin structure in HeLa-GFP cells was strongly influenced by chromatin structure.

The data obtained contribute to a better understanding of the spatial "reorganisation" of nuclear lesion processing after induction of DSBs.

(PS2083) Functional interaction between histone H2AX and NBS1 on ATM-dependent DNA damage response. Junya Kobayashi[1], Hiroshi Tauchi[2], Shinya Matsuura[3], David Chen[4], Kenshi Komatsu[1]. [1]Radiation Biology Center, Kyoto University, Kyoto, Japan, [2]Ibaraki University, Mito, Japan, [3]Hiroshima University, Hiroshima, Japan, [4]University of Texas Southwestern Medical Center, Dallas, TX, USA.

Histone H2AX, one of histone H2A family, is phosphorylated within a few minutes in response to generation of double-strand breaks (DSBs) and the phosphorylated H2AX (gamma-H2AX) forms foci at the region of DSBs. Histone H2AX is essential for the IR-induced focus formation of DNA repair-related proteins such as BRCA1, NBS1, MDC1 and 53BP1 and also important for DSB repair such as homologous recombination (HR). On the other hand, the role of H2AX in ATM-dependent DNA damage response such as cell cycle checkpoints was unclear. Although we previously reported that NBS1 binds to gamma-H2AX in response to DSBs, NBS1 also interacts with ATM through the C-terminus of NBS1 and this interaction is indispensable for recruitment of ATM to DSB sites and ATM activity. Hence, we investigated whether gamma-H2AX functions in ATM-dependent DNA damage response through interaction with NBS1. We showed here that gamma-H2AX forms the complex with ATM and NBS1 in irradiated cells. When the expression of H2AX was repressed by H2AX siRNA, chromatin-bound NBS1 and ATM in irradiated cells was significantly decreased. Focus formation of NBS1 and phosphorylated ATM was also attenuated. H2AX siRNA also reduced IR-induced phosphorylation of ATM substrates in normal cells, but did not in NBS cells. Moreover, the immuno-complex by anti-gamma-H2AX antibody from normal cells showed the similar kinase activity to ATM and recombinant phospho-mimic H2AX increased ATM activity in vitro. Moreover, H2AX-defective cells showed the deficiency of S and G2 checkpoints as AT and NBS cells do. These results suggest that histone H2AX could cooperate with NBS1 in ATM-dependent cell cycle checkpoints. However, DR-GFP assay (HR assay) showed that H2AX and NBS1 functioned for HR repair, but ATM did not. Furthermore, in vivo NHEJ assay show that ATM is indispensable for NHEJ pathway. Taken together, there might be distinct functional interaction of ATM with NBS1 and H2AX in DSB-induced cellular damage response.

(PS2084) Homologous recombination repair is regulated by domains at the N- and C-terminus of NBS1 and is dissociated with ATM functions. Kyosuke Nakamura[1], Syuichi Sakamoto[1], Kenta Iijima[2], Daisuke Mochizuki[2], Keisuke Teshigawara[3], Junya Kobayashi[1], Shinya Matsuura[4], Hiroshi Tauchi[2], Kenshi Komatsu[1]. [1]Radiation Biology Center, Kyoto University, Kyoto, Japan, [2]Ibaraki University, Mito, Japan, [3]Lymphocyte Bank Co., Ltd., Kyoto, Japan, [4]Hiroshima University, Hiroshima, Japan.

DNA double strand breaks (DSBs), which can be induced by ionizing radiation (IR) and which also occur during normal DNA processing, pose a considerable threat to cell viability. In order to maintain genomic integrity, eukaryotic cells respond to DSBs through a variety of pathways such as cell-cycle checkpoints, induction of apoptosis, and direct DNA repair reactions. NBS1 is a component of the RAD50/MRE11/NBS1 (RMN) complex and this complex functions for homologous recombination (HR) repair. The cell lines derived from NBS patients show elevated sensitivity to IR, chromosome instability and defects in cell cycle checkpoints after DNA damage. It has been demonstrated that NBS1 is phosphorylated by ATM on Ser278 and Ser343 when cells are irradiated, and that these phosphorylation events in NBS1 are critical for intra-S phase checkpoint activation as a downstream event of ATM. Recent reports showed that NBS1 physically interacts with ATM at the extreme C-terminus of NBS1 and this interaction is critical for recruitment of ATM to damaged sites and the subsequent activation of ATM-dependent cell-cycle checkpoint. However, the relationship between NBS1 and ATM in DBS repair is unclear. Therefore, we investigated the role of ATM in NBS1-dependent HR repair.

We measured the ability of several NBS1 mutant clones and A-T cells to regulate HR repair using the DR-GFP or SCneo systems. ATM deficiency did not reduce the HR repair frequency of an induced DSB and it was confirmed by findings that HR frequencies are only slightly affected by deletion of ATM binding site at the extreme C-terminus of NBS1. In contrast, The HR-regulating ability is dramatically reduced by deletion of the MRE11-binding domain at the C-terminus of NBS1, and markedly inhibited by mutations in the FHA/BRCT domains at the N-terminus. This impaired capability in HR is consistent with a failure to observe MRE11 foci formation. Furthermore, normal HR using sister chromatid was completely inhibited by the absence of FHA/BRCT domains. These results suggested that the N- and C-terminal domains of NBS1 are the major regulatory domains for HR pathways, very likely through the recruitment and retention of the MRE11 nuclease to DSB sites in an ATM-independent fashion.

(PS2085) Inhibition of ATM may induce high frequency of misrepair in normal and AT heterozygous fibroblast cells. Tetsuya Kawata[1], Francis Cucinotta[2], Kerry George[3], Naoyuki Shigematsu[4], Masayoshi Saito[1], Kouhei Inoue[1], Cuihua Liu[1], Hisao Ito[1]. [1]Department of Radiology, Chiba shi, Japan, [2]NASA Johnson Space Center, Houston, TX, USA, [3]Wyle Laboratory, Houston, TX, USA, [4]Department of Radiology, Keio University, Japan.

Caffeine sensitizes cells to ionizing radiation and this effect is believed to be associated with the disruption of DNA damage-responsive cell cycle checkpoints controlled by ATM (Ataxia Telangiectasia mutated). In this work, we investigated the effects of caffeine and radiation on non-growing G-0 phase human fibroblast cells (normal and AT heterozygous) by determining cell survival

and scoring chromosome aberrations. Results from the cell survival study indicate that after X-ray exposure cells were sensitized by treatment with caffeine. Analysis of chromosome aberrations using FISH (fluorescence in situ hybridization) revealed a high frequency of aberrant cells and color-junctions in the caffeine treated cells. Enhancement seems to be marked in AT heterozygous cells compared to normal fibroblast cells. Since the majority of DNA repair in non-growing G-0 cells is believed to undergo non homologous end joining (NHEJ), caffeine may influence the fidelity of NHEJ pathway in irradiated G-0 phase cells.

(PS2086) The Complexity of Phosphorylated H2AX foci and DNA repair proteins at ionizing radiation induced DNA double-strand breaks in mammalian cells. Asako Nakamura, William M. Bonner. NIH/NCI/LMP, Bethesda, MD, USA.

Histone H2AX, which is a key protein in DNA repair pathways, is rapidly phosphorylated at the site of DNA double-strand breaks (DSBs). Phosphorylated H2AX (γ-H2AX) may induce chromatin remodeling following exposure of cells to ionizing radiation in order to allow DNA repair proteins and/or checkpoint proteins (such as 53BP1 or Mre11/Rad50/NBS1 complex) to accumulate at the DSB sites. The accumulation of thousands molecule of various proteins including γ-H2AX makes a large focus which is known as Ionizing Radiation Induced Foci (IRIF). IRIFs are dynamic, microscopically assembled structures. The kinetics of IRIF formation is a critical factor for efficient DNA repair and the maintenance of genome stability. In fact, mutations of DNA repair proteins involved in IRIFs result in well characterized diseases, such as Nijmegen Breakage syndrome (NBS1) or Ataxia Telangiectasia (ATM), diseases characterized by high radiation sensitivity, high incidence of cancer, and genomic instability. Studying the mechanism of IRIF formation is a necessary step to better understand cellular DNA repair process.

In yeast, chromatin immunoprecipitation assay revealed that H2AX is phosphorylated in large regions flanking DNA DSB site. However DNA repair proteins such as Mre11 and Rad51 show a different distribution, accumulating at tight regions along DSB site. These data suggested that DNA repair proteins have their own territory at the DSB site according to their function. However temporal and spatial distribution of DNA repair proteins including γ-H2AX in mammalian cells is still not clear. To better understand mechanism of IRIF in mammalian cells, we analyzed γ-H2AX and other DNA repair proteins distribution at DSB site using DNA swelling procedure, chromatin fiber and immunofluorescence on metaphase chromosomes.

Our data show that some but not all DNA repair proteins formed IRIF on metaphase chromosomes. In addition, while γ-H2AX is found in large domains at DSB sites, other repair factors are more localized. These findings demonstrate the complex nature of γ-H2AX foci, and provide insights into the structure and function of focal subcomplexes.

(PS2087) Late phase activation of ATM and DNA-PKcs kinases upon UV-induced replication stress. Hirohiko Yajima, Kyung-Jong Lee, Benjamin P.C. Chen. U. Texas Southwestern Medical Center, Dallas, TX, USA.

The phosphatidylinositol 3-kinase-like protein kinases (PIKK) including ATM, ATR, and DNA-PKcs are the major kinases activated in respond to various DNA assaults for damage signaling and DNA repair. It is generally believed that DNA-PKcs is participating only in double strand break (DSB) repair whereas ATM and ATR are required for signal transduction upon DSB and replication stress respectively. However, a growing body of evidence suggests that there may be overlapping role of these PIKK kinases. For example, ATM is shown to play a role in replication stress response whereas ATR is activated upon DSB in an ATM-dependent manner. In addition, we have reported that DNA-PKcs is associated with ATR and is rapidly phosphorylated by ATR upon UV irradiation. The involvement of ATM and DNA-PKcs in replication stress response however is still not clear. Using UV-induced replication stress as a model, our results demonstrate that there are two distinct phases of response. Furthermore, both ATM and DNA-PKcs are activated at late phase after UV irradiation. In early phase response, UV-induced S/TQ phosphorylations are predominantly mediated by ATR kinase including many of the known ATR substrates as well as phosphorylation of DNA-PKcs and ATM. Several of the ATR-dependent phosphorylations, however, are defective in A-T cells at later stage, suggesting that ATM kinase activity may be required to sustain these phosphorylations. The late phase activation of ATM upon UV irradiation is also supported by the late increase of ATM-dependent Chk2 and KAP-1 phosphorylations. Furthermore, we observed a sharp increase of H2AX phosphorylation and DNA-PKcs autophosphorylation at late phase after UV. These results consistently point out that the late phase activation of ATM and DNA-PKcs kinases upon UV irradiation are likely dependent on DSB formation, possibly, resulting from the collapse of replication forks.

(PS2088) The mechanism of DNA-PKcs regulating the phosphorylation of H2AX. Jing An, Qin-Zhi Xu, Jian-Li Sui, Bei Bai, Ping-Kun Zhou. Department of Radiation Toxicology and Oncology, Beijing Institute of Radiation Medicine, BeiJing, China.

Abstract: DNA Dependent Protein Kinase Catalytic Subunit (DNA-PKcs), a sub member of Phosphatidylinositol-3 kinase related protein kinase (PIKK) family, is involved in repair of DNA double sequence breaks (DSBs). Many researches reported that a number of PIKK family members were activated to phosphorylate H2AX when DSBs were induced by different damage agents, but the specific mechanism is still not well understand. In our study, we used the DNA-PKcs knock-down cells (HeLa-H1) with siRNA strategy and control cells (HeLa-NC) to investigate the mechanism of H2AX phosphorylating. Results showed that the radiation sensitivity of HeLa-H1 cells strikingly increased and the ability to repair the DSBs decreased. After 4Gy γ ray irradiation, the lever of γH2AX in the HeLa-NC rised rapidly with a peak level at 1h; while the level in the HeLa-H1 was markedly lower, indicating the γH2AX induced by irradiation was closely correlated with expression status of DNA-PKcs. HeLa-NC and HeLa-H1 cells were synchronized at G1 phase and then released. The γH2AX level of HeLa-NC rised after cell getting into G2 phase; while the level of HeLa-H1 had no marked alteration with changing of phase, suggesting the γH2AX was inhibited after DNA-PKcs silencing. But the status of DNA-PKcs did not affect the cell cycle progression. Using ATM knock-out cells (ATS4) and control cells (A549 and HEL), we investigated the effect of ATM on γH2AX expression after radiation. The results showed that deletion of ATM can also inhibit the γH2AX lever. Furthermore, inhibition of PDK, a regulatory molecule of PIKK signal pathway, by siRNA could also down regulate the expression of γH2AX, and PIKK inhibitor wortmannin had the same effect. GSK3 is a critical downstream target of PIKK-Akt signal pathway, and the phosphorylation by Akt can negatively regulate its activity. We found inhibiting GSK3β activity with LiCl or sepecial siRNA up-regulated the γH2AX level and reverted the decrease of γH2AX caused by silencing of DNA-PKcs. In addition, in the presence of fostriecin, an inhibitor of PP2A, γH2AX was significantly increased after DSBs. All of above suggest that there could be two pathways for DNA-PKcs to regulate γH2AX expression, 1) directly phosphorylates H2AX; 2) modulates γH2AX lever via the DNA-PKcs/Akt/GSK3 pathway.

keywords:DNA-PKcs;γH2AX;GSK3; PP2A ; siRNA

(PS2089) Novel chemical enhancers of heat shock increase thermal radiosensitization through a mitotic catastrophe pathway. Konjeti R. Sekhar[1], Vijayakumar N. Sonar[2], Venkatraj Muthusamy[2], Andrei Laszlo[3], Jamil Sawani[4], Nobuo Horikoshi[3], Ryuji Higashikubo[3], Robert G. Bristow[5], Peter A. Crooks[2], Joseph L. Roti Roti[3], Michael L. Freeman[1]. [1]Vanderbilt University, Nashville, TN, USA, [2]University of Kentucky, Lexington, KY,

USA, [3]Washington University, St. Louis, MO, USA, [4]University of Toronto, Toronto, NU, Canada, [5]University of Toronto, Toronto, ON, Canada.

Radiation therapy combined with adjuvant hyperthermia has the potential to provide outstanding local-regional control for refractory disease. However, achieving therapeutic thermal dose can be problematic. In the current investigation, we used a chemistry-driven approach with the goal of designing and synthesizing novel small molecules that could function as thermal radiosensitizers. (Z)-(F)-2-(1-benzenesulfonylindol-3-ylmethylene)-1-azabicyclo[2.2.2]octan-3-ol was identified as a compound that could lower the threshold for Hsf1 activation and thermal sensitivity. Enhanced thermal sensitivity was associated with significant thermal radiosensitization. We established the structural requirements for activity: the presence of an N-benzenesulfonylindole or N-benzylindole moiety linked at the indolic 3-position to a 2-(1-azabicyclo[2.2.2]octan-3-ol) or 2-(1-azabicyclo[2.2.2]octan-3-one) moiety. These small molecules functioned by exploiting the underlying biophysical events responsible for thermal sensitization. Thermal radiosensitization was characterized by biochemically and found to include loss of mitochondrial membrane potential, followed by mitotic catastrophe. These studies identified a novel series of small molecules that represent a promising tool for the treatment of recurrent tumors by ionizing radiation.

(PS2090) Inhibition of repair of radiation-induced damage by mild hyperthermia with reference to the effect on quiescent cells in solid tumors. Shin-ichiro Masunaga, Kenji Nagata, Minoru Suzuki, Genro Kashino, Yuko Kinashi, Koji Ono. Research Reactor Institute, Kyoto University, Osaka, Japan.

Purpose: We evaluated the usefulness of mild temperature hyperthermia (MTH) as an inhibitor of the repair of radiation-induced damage in terms of the responses of the total (= proliferating (P) + quiescent (Q)) and Q cell populations in solid tumors *in vivo*.

Materials and Methods: SCC VII tumor-bearing mice received a continuous administration of 5-bromo-2′-deoxyuridine (BrdU) to label all P cells. Then, they received high dose-rate γ-ray irradiation (HDRI) immediately followed by MTH or an administration of caffeine or wortmannin, or reduced dose-rate γ-ray irradiation in simultaneous combination with MTH or an administration of caffeine or wortmannin. Nine hours after the start of irradiation, the tumor cells were isolated and incubated with a cytokinesis blocker, and the micronucleus (MN) frequency in cells without BrdU labeling (= Q cells) was determined using immunofluorescence staining for BrdU. The MN frequency in the total tumor cell populations was determined using tumors that were not pretreated with BrdU. In addition, clonogenic cell survival for the total tumor cells was determined using an *in vivo-in vitro* assay.

Results: In both the total and Q cell populations, especially the latter, MTH efficiently suppressed the reduction in sensitivity caused by leaving an interval between HDRI and the assay and decreasing the irradiation dose rate, as well as the combination with wortmannin administration.

Conclusion: From the viewpoint of solid tumor control as a whole, including intratumor Q-cell control, MTH is useful for suppressing the operational repair of both potentially lethal and sublethal damage.

(PS2091) The anti-tumor effects of cisplatin-TSL with hyperthermia (HT) and radiation therapy (RT) in a human colorectal cancer xenograft. Jessica A. Tashjian, Eric A. Lee, Benjamin L. Viglianti, Yulin Zhao, Ana M. Ponce, Bruce Bondurant, Mark W. Dewhirst. Duke University, Durham, NC, USA.

The objective of this work was to determine anti-tumor effect of a novel temperature-sensitive cisplatin liposome (cis-LTSL) with hyperthermia (HT) and radiation therapy (RT) in HCT116 xenografts. Animals were stratified by tumor size and randomized to: saline i.p., free cisplatin 5 mg/kg i.p., or cis-LTSL 5 mg/kg i.v; hyperthermia (42°C) or normothermia (34°C) +/– 5 Gy irradiation x3. On the first day of treatment mice were administered drug followed by HT for 60 min (water bath). Thirty minutes later, animals were given 5 Gy RT. Two more fractions of RT were given on subsequent days. Tumor volumes and body weights were measured every other day until the tumors reached 5x treatment volume (5x TXT).

Tumor growth time data are shown in Table 1. Change in body weight for cis-LTSL was similar to that of free cisplatin independent of HT or RT.

Treatment			Median Time to 5× TXT Volume in Days (95% CI)	p-value	Overall p-value
−RT	HT	Saline	17.5 (±2.3)		**0.011**
		Free drug	20.7 (±1.4)		
		LTSL	21.4 (±2.7)		
		Cohort - overall result	*20.6 (±1.3)*		
	NT	Saline	19.1 (±2.6)		
		Free drug	22.4 (±2.4)		
		LTSL	20.9 (±2.8)		
		Cohort - overall result	*20.5 (±1.5)*		
	Strata - overall result (±HT, −RT)		***20.6 (±0.7)***	**0.024**	
+RT	HT	Saline	35.0 (±2.5)		
		Free drug	36.5 (±2.5)		
		LTSL	39.3 (±3)		
		Cohort - overall result	*36.5 (±1.3)*		
	NT	Saline	33.9 (±2.7)		
		Free drug	36.4 (±2.2)		
		LTSL	36.2 (±3.1)		
		Cohort - overall result	*35.6 (±1.0)*		
	Strata - overall result (±HT, +RT)		***36.0 (±0.9)***	**0.034**	

Table 1. Median survival time (in days) by treatment group. N = 10 for all groups except n = 11 for 'free cis/HR/-rt' and n = 9 for 'free cis/NT/-rt' and 'saline/HT/RT'. Results of log-rank (Mantel-Cox) analysis shown.

A significantly greater growth time was seen with the triple combination of cis-LTSL, HT and RT. We hope to further optimize the growth time of the triple combination by varying the sequence of therapy. Current studies are being performed to determine whether enhanced drug levels are achieved when HT is combined with cis-LTSL. Future studies will characterize anti-tumor effects with histologic endpoints focusing on markers of radioresistance.

Work supported by a grant from the NIH CA42745.

(PS2092) Localized hyperthermia combined with intratumoral Dendritic cells induces systemic antitumor immunity. Arunika Mukhopadhaya, Joseph Mendecki, XinYuan Dong, Alan A. Alfieri, Laibin Liu, Shalom Kalnicki, Madhur Garg, Chandan Guha. Albert Einstein College of Medicine, Bronx, NY, USA.

Solid malignant tumors like prostate adenocarcinoma when treated with localized tumor hyperthermia (LTH) can serve as a potential source of antigens, where dying apoptotic/necrotic cells release tumor peptides slowly over time. Additionally, LTH treated cells can release heat shock proteins that can chaperone antigenic peptides to antigen-presenting cells (APCs), ie., dendritic cells (DCs). **PURPOSE:** To discern whether sequential LTH and intratumoral DC and/or systemic GMCSF (Granulocyte Macrophage Colony Stimulating factor) would activate an antitumor immune response in a syngeneic murine model of prostate cancer, RM1 tumors, in C57BL/6 male mice, were subjected to LTH (43.7C

for 1 hr) x 2, separated by 5days. Following the second LTH treatment, animals received either PBS or DCs (10^6) intratumorally (3 days x 3fractions). Separate cohorts received systemic murine GM-CSF, (recombinant Adenovirus, $8x10^9$ particles), 1 day after LTH. Control animals received AdenoLacZ or AdenoGFP. RM1 tumors were followed by volume and antitumor immune response(s) were measured by cytokine release assays, ELISPOT and LDH release to measure T helper and CTL response of splenocytes. **RESULTS and DISCUSSION:** LTH alone demonstrated tumor growth delays when compared to untreated RM1 tumor. Intratumoral DC significantly ($p=0.007$) augmented the tumoricidal effects of LTH. Systemic AdGMCSF enhanced the tumoricidal effects of LTH+DC ($p<0.001$). Intratumoral DC injection induced tumor-specific T-helper cell activity (IFNγ ELISPOTS) and CTL activity (LDH release 17–20% vs 5%). AdGMCSF augmented the CTL response to 36.3% (range, 30–44%), indicating amplification of tumor-specific immune response with concomitant adjuvant GMCSF. The combination of LTH, AdGMCSF and intratumor DC produced strong systemic tumor-specific immune responses and enhanced local tumor control based on tumor volume growth delays. These results support an *in situ* approach, ie., cytokine activated host and autologous intra tumor DC's when combined with immune modulation by LTH as an effective treatment approach for local and systemic recurrence of prostate cancer.

(PS2093) Increased granulocyte recovery from radiation exposure following mild hyperthermia. Thomas Mace, Maegan Capitano, Adrienne Kisailus, Wainwright Jaggernauth, Elizabeth Repasky. Roswell Park Cancer Institute, Buffalo, NY, USA.

The immune system is highly sensitive to ionizing radiation and, therefore, death from secondary infections due to decreased leukocyte counts is a major threat following nuclear accidents or attacks. To protect individuals with depressed immune systems, strategies must be developed that can easily, safely, and rapidly restore the ability to fight common infections after radiation exposure. Here we tested whether elevation of body temperature to the fever-range could alter or improve the reconstitution of peripheral blood leukocytes after radiation exposure. C57BL/6 mice were given a non-myeloablative dose (3Gy) of total body irradiation (TBI) followed 2 hours later by a 6 hour whole body hyperthermia (WBH) treatment (maintained at 39.5°C). Blood and splenocytes were collected one week prior to, immediately following and at weekly intervals after TBI. Blood leukocytes were stained for B cells (B220+), T cells (CD3+), NK cells (NK1.1), and granulocytes (GR-1) and analyzed by flow cytometry. Percent cell population recovery was then calculated. Because certain cytokines are known to regulate marrow production of various leukocytes, we also measured the serum concentrations of G-CSF, GM-CSF, and MIP-2 by ELISA. In mice treated with WBH following TBI, a significant increase in recovery of peripheral blood granulocytes was observed by day 8 compared to mice that received radiation alone. WBH alone did not result in a significant change in blood leukocyte numbers on day 8. Similar changes in leukocyte populations were observed in the spleen with an increase in granulocyte numbers following WBH treatment. Furthermore, we found that G-CSF concentrations are increased two fold in the serum of radiated and heated mice associated with the increased granulocyte recovery. These data suggests a role of the thermal environment in regulation of the bone marrow compartment, resulting in increased granulocyte numbers in the blood and the spleen following TBI. Since granulocytes are a front-line defense against bacterial infection, mild hyperthermia could be useful as a therapeutic intervention for the protection against secondary infections following ionizing radiation exposure. Current work is exploring the contribution of thermally regulated, marrow stimulating growth factors responsible for immune reconstitution.

(PS2094) Selective inhibition of cyclooxygenase-2 and activation of adenosine membrane receptors - two new promising approaches for treatment of radiation-induced myelosuppression. Michal Hofer[1], Milan Pospíšil[1], Antonín Vacek[1], Jiřina Holá[1], Denisa Štreitová[1], Vladimír Znojil[2]. [1]Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic, [2]Medical Faculty, Masaryk University, Brno, Czech Republic.

Two new pharmacological approaches have been recently tested in Laboratory of Experimental Hematology, Institute of Biophysics, Academy of Sciences of the Czech Republic, in experiments on mice, namely administration of selective cyclooxygenase-2 (COX-2) inhibitors and activation of adenosine membrane receptors. Thorough analysis of the state of peripheral blood and bone marrow was used for evaluation of the efficacy of the treatments. Meloxicam, a representative of selective COX-2 inhibitors was found to enhance numbers of hematopoietic progenitor cells for granulocytes/macrophages (GM-CFC) and erythrocytes (BFU-E) when given either in a single dose before or repeatedly (four times) after exposure to a sublethal radiation dose. An induction of endogenous production of granulocyte colony-stimulating factor (G-CSF) in irradiated mice by meloxicam was described and a possible auxiliary role which might be played by meloxicam in therapy of myelosuppression with G-CSF was revealed. A synthetic agonist of adenosine A3 receptors, N6-(3-iodobenzyl)adenosine-5′-N-methyluronamide (IB-MECA), was found to stimulate proliferation of GM-CFC and BFU-E in experiments employing 5-fluorouracil as a drug killing preferentially cycling cells. Evaluation of efficacy of IB-MECA in conditions of radiation-induced bone marrow damage and experiments aimed at assessment of combined administration of meloxicam and IB-MECA are proposed for a near future. The hitherto obtained results of our studies are promising and a realistic assumption exists that at least some of them might find their continuation in clinical studies and in contingent classing of these drugs with contemporary treatment schemes of radiation-induced myelosuppression in man.

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(PS2095) Experimental studies of intestine damage caused by combined radiation-burn injury with chitosan DNA nanoparticles of [Gly2]GLP-2 and HD-5. Ai Guoping, Su Yongping, Tan Hu. Institute of Comined Injury, Chongqing, China.

Experimental Studies of intestine damage caused by combined radiation-burn injury with chitosan DNA nanoparticles of [Gly2]GLP-2 and HD-5

Objective To elucidate the influence on repopulation of intestinal epithelium and bacterial translocation from intestine by intragastric administration of chitosan DNA nanoparticles of [Gly2]GLP-2 mutant and HD-5 multigenic expression vector in the mice model of combined radiation-burn injury. **Methods** Site-specific mutagenesis of GLP-2 to prevent it inactivated by enzymolysis; cloning genes of GLP-2 mutant and HD-5 into double-gene expression vector and managing to prepare chitosan DNA nanoparticles; after oral supplementation of the chitosan DNA nanoparticles, observing index including small intestinal wet weight, height of villi, proliferation of crypt cells, endotoxin level of plasma, bacterial translocation from intestine and expression of HD-5 and GLP-2 mRNA of intestinal tissue in mice suffering combined radiation-burn injury(10GyWBI+15%III°TBSA) . **Results** Comparing with control groups, there were better situations of regeneration of intestinal epithelium, height of villi, intestinal wet weight and proliferation of crypt cells in treatment group indicating better re-establishment of intestinal mechanical barrier; at the same time there were obvious reduction of plasma endotoxin level and decreasing of bacterial translocdtion from intestine indicating better state of intestinal immune barrier; mRNA expressions of GLP-2 and HD-5 were also detected in the intestinal tissue of treatment mice by RT-PCR .**Conclusion** In vivo transfection of multigenic expression vector of GLP-2 mutant and HD-5 in the form of chitosan DNA nanoparticles is a new effective therapeutic approach to prevent intestinal injury and promote intestine recovery.

Key words: intestine damage; GLP-2; defensin 5; chitosan DNA nanoparticles; gene therapy

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(PS2096) Oxidative stress and acetylcholinesterase activation in the brain after thorium administration in swiss mice. Amit Kumar, Badri Narain Pandey, Kaushala Prasad Mishra. Bhabha Atomic Research Centre, Mumbai, India.

Thorium (232Th, IV) heavy metal radionuclide that mainly targets liver and femur has been shown to accumulate at a fraction level (~3% of total injected amount) in central nervous system in mice. The present study aimed at assessing the behavioral changes, biochemical and histological changes in brain after sub-chronic administration of thorium (10 mg/kg body weight /day for 30 days) in mice. Intra-peritoneal administration of thorium nitrate induced oxidative stress in the brain in animals. The oxidative damage was measured in terms of alterations in activity of acetylcholine esterase (AChE), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation in cerebellum (Cbl), cortex (Ctx), striatum (Str) and hippocampus (Hip) of mice brain after thorium treatment. Th treated mice showed a significant increase in lipid peroxidation (Cbl 78 %, Ctx 20 %, Str 8.5 % and Hip 65%) and AChE activity (Cbl 128%, Ctx 59%, Str 18 % and Hip 32 %) but the activity of SOD and CAT were found substantially decreased compared to control. The induced effects were reversed when mice were injected intravenously with calcium diethylenetriamine pentacetate (Ca-DTPA, 100 μM/kg body weight/day) after 1 h of thorium administration. Histological examination of thorium treated brain cerebellum showed edema and neuronal degenerative changes. Thorium was found to affect conditioned response of treated mice as studied by standard shuttle box method. The observed effects of thorium on AChE, antioxidant enzymes, lipid peroxidation and histological features were dependent on thorium localization in tissues as determined by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) method. Th localization was found to follow the order: Cbl (1.2 %)> Ctx (0.8%)> Hip (0.66%)> Str (0.4%) of the total injected Th over 30 days exposure. It is concluded that administration of thorium in mice at sub-chronic doses induced significant oxidative stress in brain as manifested in alterations in AChE, lipid peroxidation and antioxidant enzymes, which were restored to near normal level by injection of Ca-DTPA suggesting potential application of chelators in amelioration of thorium induced effects on body.

(PS2097) Chronic administration of the angiotensin II type 1 receptor antagonist (AT1RA) L158, 809 prevents radiation-induced cognitive impairment. Weiling Zhao[1], Valerie Payne[1], Mitra Kooshki[1], David Riddle[2], Judy Brunso-Bechtold[2], Mike Robbins[1]. [1]Department of Radiation Oncology, Wake Forest University School of Medicine, Winston-Salem, NC, USA, [2]Department of Neurobiology and Anatomy, Wake Forest University School of Medicine, Winston-Salem, NC, USA.

Brain irradiation for primary and metastatic brain tumors can lead to devastating functional deficits, including chronic progressive cognitive impairment several months to years after irradiation. We hypothesize that radiation-induced cognitive impairment is due, in part, to acute and chronic inflammation and oxidative stress. Angiotensin II (Ang II) is a pro-inflammatory mediator and the local renin-angiotensin system (RAS) has been identified in the brain. Inhibiting activity of Ang II using RAS blockers has proven effective in treatment of experimental radiation-induced injury in the kidney, lung and heart. We hypothesized that administration of the AT_1RA L158, 809 would modulate the severity of radiation-induced cognitive impairment. Adult male Fischer 344 x Brown Norway rats, 14–16 weeks old, received 40 Gy ^{137}Cs γ rays whole brain irradiation (WBI) delivered in 5 Gy fractions, twice/week, for 4 weeks; age-matched controls received sham-irradiation. L158,809 (20mg/L) was given in the drinking water starting 3 days prior to 40 Gy WBI and continued during the experiment. Cognitive function was assessed using the object recognition test 1 year postirradiation. Administration of L158, 809 prevented the radiation-induced cognitive impairment in the rats. Increased expression of renin and AT_1R was observed in rat brain tissue isolated 54 weeks postirradiation. Gene expression of the NADPH oxidase components, gp91phox and p22phox, were upregulated in the irradiated rat brain, compared with the non-irradiated sham controls. Previous studies have indicated that some AT_1RAs can activate the peroxisome proliferator-activated receptor γ (PPARγ). In our study, we found that mRNA levels of PPARγ and its co-activator, PGC-1α, were down-regulated in the irradiated rat brain. Administration of L158, 809 prevented this radiation-induced down-regulation. Our findings suggest that i] radiation can modulate local RAS components and gp91phox and p22phox; ii] administration of an AT_1RA can prevent radiation-induced cognitive impairment; iii] PPARγ and PGC-1α may be important mediators for the beneficial role of RAS blockade. (*Supported by NIH Grant CA122318*)

(PS2098) Growth hormone protects against lethal irradiation. Benny J. Chen, Divino DeOliveria, Nelson J. Chao. Duke University Medical Center, Durham, NC, USA.

Ionizing irradiation can cause bone marrow failure leading to death. There is currently lack of effective therapeutic agent for this condition. We and others have previously demonstrated that growth hormone promotes hematopoietic and immune reconstitution after stem cell transplantation. In this study, we investigated the ability of growth hormone to protect against lethal irradiation. The studies were performed using BALB/c mice. Recombinant human growth hormone (rhGH) was administered at a dose of 20 μ g/dose, i.v., once a day, starting within one hour after irradiation. BALB/c mice were irradiated with 7.5 Gy and treated with saline or rhGH for 35 days. In the saline control group, 3 out of 28 mice (11%) survived more than 100 days after irradiation. By contrast, 17 out of 28 mice (61%) in the rhGH-treated group survived more than 100 days after irradiation ($P<0.0001$). Similar results were obtained when the treatment course was shortened to 5 days. Moreover, the radioprotective effect of rhGH was still evident when higher dose of radiation (8.5 Gy, LD100/20) was used. These data indicate that growth hormone can protect against lethal irradiation. Because bone marrow failure is usually the primary cause of death after irradiation, we next sought to determine the effects of rhGH on hematopoietic and immune recovery. Compared with the saline control group, treatment with rhGH for 35 days on irradiated (7.5 Gy) BALB/c mice significantly accelerated the recovery of platelets in peripheral blood. Similarly, the recoveries of total white cells, CD4 and CD8 T cell subsets, B cells, and NK cells post irradiation were also significantly accelerated in the rhGH-treated mice comparing with the saline-treated mice. In addition, treatment with rhGH increased the frequency of hematopoietic stem/progenitor cells (c-Kit$^+$Lin$^{-/low}$Sca-1$^+$) in bone marrow harvested at day 14 after irradiation, suggesting the effects of growth hormone are at the stem/progenitor level. Our data demonstrate that growth hormone promotes hematopoietic and immune recovery post irradiation and protects against lethal irradiation even when administered after irradiation.

(PS2099) Redox modulation of oxidative stress by Mn porphyrin-based radioprotectors/anticancer therapeutics. The effect of charge distribution. Júlio S. Rebouças[1], Ivan Spasojevic[1], Ludmil Benov[2], Daryono H. Tajhjono[3], Ines Batinic-Haberle[1]. [1]Duke University Medical Center, Durham, NC, USA, [2]Kuwait University, Safat, Kuwait, [3]Bandung Institute of Technology, Bandung, Indonesia.

Objective. Mn-porphyrins (MnP) comprise one of the most relevant classes of redox/signaling pathway mediators for inhibiting tumor growth and enhancing radiation-based cancer therapy. The leading compounds Mn(III) tetrakis(*N*-ethylpyridinium-2-yl)porphyrin, MnTE-2-PyP^{5+} (AEOL10113) and its imidazolium counterpart MnTDE-2-ImP^{5+} (AEOL10150) are potent radioprotectors and anticancer agents, able to regulate oncogenic and inflammatory transcription factors via modulation of reactive oxygen/nitrogen species. A good understanding of the structure-activity relationships governing the scavenging of ROS/RNS by these MnP remains imperative, and particularly relevant are the effects of charge and

metal-centered redox potential ($E_{½}$). Here we evaluate the impact of charge distribution on the *in vitro* and *in vivo* properties of MnP possessing the same overall charge: Mn(III) tetrakis(*N,N′*-dimethylimidazolium-2-yl)porphyrin, MnTDM-2-ImP^{5+} (AEOL10123), its pyrazolium isomer MnTDM-3-PzP^{5+}, and the methylpyridinium complexes MnTM-2-PyP^{5+} and MnTM-4-PyP^{5+}.

Experimental. All MnP were fully characterized. Their antioxidant properties were evaluated *in vitro* by SOD activity and kinetic salt effect studies, and tested *in vivo* on the protection of SOD-deficient *E. coli* grown aerobically.

Results. Electron-withdrawing and charge distribution effects on imidazolium and pyrazolium MnP are remarkably different, resulting in >300 mV decrease in $E_{½}$ and a 240-fold loss in SOD activity on MnTDM-3-PzP^{5+} relative to MnTDM-2-ImP^{5+}. Such an effect is comparable to that on MnTM-2-PyP^{5+} and MnTM-4-PyP^{5+}. Although imidazolium, pyrazolium, and pyridinium charges are usually considered delocalized, the *in vitro* data suggest a localized charge distribution relative to the MnP core. MnTDM-3-PzP^{5+} and MnTM-4-PyP^{5+} did not protect SOD-deficient *E. coli*, in agreement with low k_{cat}. Although MnTM-2-PyP^{5+} and MnTDM-2-ImP^{5+} have similar k_{cat} approaching that of SOD enzyme, MnTDM-2-ImP^{5+} was much less protective to *E. coli* than MnTM-2-PyP^{5+}, due to its bulkier size and decreased cellular localization.

The implications of these results to the design of potent and orally bioavailable redox/signaling pathway mediators are discussed.

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(PS2100) Potent radioprotector/anticancer drug MnTE-2-PyP^{5+}: its pharmacokinetics and subcellular distribution. Ivan Spasojevic[1], Lichun Zhang[1], Yumin Chen[2], Teresa J. Noel[2], Marsha P. Cole[2], Yunfeng Zhao[2], Júlio S. Rebouças[1], Daret St. Clair[2], Ines Batinic-Haberle[3]. [1]Duke University Medical Center, Durham, NC, USA, [2]University of Kentucky, Lexington, KY, USA, [3]Duke Univ Medical Center, Durham, NC, USA.

Objective. SOD mimic, Mn(III) tetrakis(*N*-ethylpyridinium-2yl)porphyrin, MnTE-2-PyP^{5+} (AEOL10113) exerted remarkable radioprotection and anticancer action in different animal models. Decreasing levels of oxygen and nitrogen species is considered its predominant mode of action that also involves redox-regulation of signaling pathways. Its high positive charge, though essential for its *in vivo* antioxidant efficacy, may limit its ability to reach cellular compartments. Herein, we undertook a thorough pharmacokinetic study (PK) of MnTE-2-PyP^{5+}, which would help us to optimize its structure to design highly bioavailable therapeutic. Further, we analyzed whether MnTE-2-PyP^{5+} localized in mitochondria and at levels enough to protect it against superoxide or peroxynitrite-mediated oxidative stress injury.

Experimental. We have developed a sensitive method for MnTE-2-PyP^{5+} detection based on the exchange of Mn^{2+} with Zn^{2+}, followed by HPLC/fluorescence detection of ZnTE-2-PyP^{4+}. Plasma levels of MnTE-2-PyP^{5+} and its levels in liver, lungs, heart, kidneys, spleen, and brain were determined after single i.p. administration of 10 mg/kg over 48 h. In another experiment, at 4 and 7 h after single 10 mg/kg i.p. dose of MnTE-2-PyP^{5+}, mice (8 in total) were anesthesized, perfused with saline and heart mitochondria isolated.

Results. Pharmacokinetics of MnTE-2-PyP^{5+} is characterized by initial rapid tissue distribution phase (primarily into liver) ($t_{½}$ = 0.5 h), followed by a prolonged elimination phase ($t_{½}$ = 7 h) presumably rate-limited by a slow drug release from organs. Despite high charge, drug was found even in brain. Further, we found that, despite its high charge, MnTE-2-PyP^{5+} is capable of entering heart mitochondria *in vivo* at 5.1 μM (2.95 ng/mg protein) after only single i.p. administration of 10 mg/kg. *Ferrer-Sueta at al* showed with submitochondrial particles, that > 3 μM MnTE-2-PyP^{5+} protects mitochondrial electron transport chain from peroxynitrite-mediated damage. Our study complements their data in showing for the first time, that concentration of MnTE-2-PyP^{5+} in mitochondria is sufficient to exert antioxidant action.

These results justify the development of MnTE-2-PyP^{5+} as a prospective therapeutic agent.

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(PS2101) Dose-dependent effects of cranial cesium irradiation on cognition in c57bl6/j mice. Laura Villasana[1], Ken A. Jenrow[2], Steve L. Brown[2], Jae Ho Kim[2], Jacob Raber[1]. [1]Oregon Health and Science University, Portland, OR, USA, [2]Henry Ford Health Systems, Detroit, MI, USA.

The brain is exposed to irradiation under a variety of situations. Deficits in hippocampal-dependent learning and memory often characterize radiation-induced cognitive dysfunction and might be involved in effects of irradiation on neuronal precursor cells in the subgranular zone of the hippocampus. The apparent relationship between hippocampal neurogenesis and memory formation supports the hypothesis that effects of radiation exposure on hippocampal neurogenesis contributes to cognitive injury. To determine the dose dependency of the effects of cranial Cs-137 irradiation on cognitive function, two-month-old C57Bl6/J male mice (n = 7 to 8 mice/dose) were irradiated at 0, 10, or 15 Gy and behaviorally tested three months later. To minimize the effect of stress on behavioral performance, the mice were subsequently tested first for levels of anxiety and exploratory behavior in the open field, light-dark, elevated zero maze, and elevated plus maze (week 1), than for object recognition and water maze performance (weeks 2 and 3), for sensorimotor function on the rotorod (week 4), and last for emotional learning and memory in the fear conditioning and the passive avoidance tests (week 5). Following behavioral testing, the mice were perfused with BrdU, perfused, and processed for measures of neurogenesis. Cesium irradiation increased activity levels in the open field, elevated zero maze, and elevated plus maze. Mice irradiated at 15 Gy showed impairments in hippocampus-dependent spatial memory retention in the water maze and in hippocampus-dependent contextual freezing. No effects of irradiation were detected in the other cognitive tests. These data show that the water maze and fear conditioning tests are sensitive to the effects of cranial Cesium irradiation. These cognitive tests will be used to evaluate the therapeutic potential of drugs to inhibit or even prevent radiation-induced impairments in brain function. This work is supported by U19 AI067734 (NIAID).

(PS2102) Gender-related differences in radiation cataractogenesis. Mark A. Henderson[1], Shailaja Valluri[2], Colleen DesRosiers[1], Jennifer T. Lopez[1], Christopher N. Batuello[1], Andrea Caperell-Grant[3], Marc S. Mendonca[1], Eva-Marie Powers[4], Robert M. Bigsby[3], Joseph R. Dynlacht[1]. [1]Indiana University School of Medicine, Department of Radiation Oncology, Indianapolis, IN, USA, [2]Indiana University School of Medicine, Department of Ophthalmology, Indianapolis, IN, USA, [3]Indiana University School of Medicine, Department of Obstetrics and Gynecology, Indianapolis, IN, USA, [4]Indiana University School of Dentistry, Indianapolis, IN, USA.

We are currently examining the role of the major secreted estrogen 17-β-estradiol (E2) in the modulation of cataractogenesis induced by high LET (^{56}Fe ions) or low LET (γ-rays) radiation in a rat model. Recent reports suggest that exposure to even relatively low doses of space radiation may result in a reduced latent period for and an increased incidence of cataractogenesis in astronauts. Therefore, radiation cataractogenesis is an important consideration for astronauts participating in prolonged missions. We previously demonstrated that E2 may enhance cataractogenesis induced by low LET ionizing radiation (IR) in female Sprague-Dawley rats. In the current study, we compared the latent period and incidence of cataractogenesis between male and female rats exposed to low LET radiation. Placebo-implanted or E2-implanted 56-day old male rats and ovary-intact placebo-implanted 56-day old female rats were irradiated to the right eye only with 10 Gy of ^{60}Co γ-rays using the Leksell Gamma Knife. The E2-treated rats received continuous estrogen therapy beginning one week prior to irradiation. Slit-lamp biomicroscopy was used to measure lens opacification of the anterior and posterior subcapsular lenses of irradiated and control (contralateral) eyes every 2–4 weeks. Although data are still accruing, the incidence of anterior and posterior subcapsular cataracts appears to be lower in female placebo-implanted rats irradiated with 10 Gy of ^{60}Co γ -rays than for male rats irradiated with the same dose. There is also no significant difference in the incidence or rate of cataractogenesis in placebo-implanted males versus male rats that received E2 implants. We have also initiated

studies to compare the incidence and rates of cataractogenesis in male and female Sprague-Dawley rats exposed to high LET radiation at Brookhaven National Laboratory. The irradiated groups included ovariectomized placebo-implanted and ovary-intact 56-day old females, and placebo-implanted or E2-implanted 56-day old males. Rats were anesthetized and placed in a specially constructed holder which enabled the simultaneous irradiation of only the right eye of 3 rats with 1 Gy of 600 MeV ^{56}Fe ions. Data accrual from these high LET studies is ongoing.

(PS2103) Prophylactic Effect of Flaxseed Oil against Radiation-induced Hepatotoxicity in Mice. Arvind L. Bhatia. Radiation Biology Laboaratory, Jaipur, India.

Flaxseed (linseed, *Linum usitatissimum*, Linaceae) is widely used for its edible oil in many parts of the world. The present study investigates the radioprotective and antioxidative potential of flaxseed oil (FO). Swiss albino mice were administered FO orally once daily for 15 consecutive days, then exposed to a single dose of 5 Gy of gamma radiation. Lipid peroxide, reduced glutathione and total protein were estimated in the liver. Aspartate amino transferase (AST), alanine amino transferase (ALT), acid and alkaline phosphatase estimations in serum were also carried out. Radiation induced increases in the levels of lipid peroxidation (LPO), AST, ALT and acid phosphatase were significantly ameliorated by flaxseed oil pretreatment, and radiation-induced depletion in the level of glutathione (GSH) and alkaline phosphatase activities was significantly inhibited by flaxseed oil administration. Lifespan was increased in flaxseed oil treated irradiated mice in comparison to their respective control mice, with survival data showing an LD50/30 (lethal dose for 50% of animals after 30 days) of 7.1 and 10 Gy for control and FO treated irradiated mice respectively, and produced a dose reduction factor for flaxseed oil (DRF) = 1.40. Radiation induced deficits in body and organ weight were significantly reduced or prevented in flaxseed oil pretreated mice. The protection afforded by flaxseed oil may be attributed to the constituents of the oil, which include omega 3 essential fatty acids and phytoestrogenic lignans, which appear to play an important role in free radical scavenging and singlet oxygen quenching. The study does not rule out the possibility of a prophylactic potential of flaxseed oil against radiation induced degenerative changes in liver.

(PS2104) Comparison between photodynamic and sonodynamic cytotoxicities in vitro. Jhony EL Maalouf[1], Jean Louis Mestas[1], Laurent Alberti[2], Sabrina Chesnais[1], Jean Paul Steghens[3], Cathignol Dominique[1]. [1]INSERM UMR 556 Lyon France, Lyon, France, [2]INSERM U590, Lyon, France, [3]UF 21455 Laboratoire de stress oxydant, Lyon, France.

The photodynamic therapy involves administering a photosensitizing drug in the tumor followed by delivery of light of an appropriate wavelength to the target area, in the presence of oxygen. Sonodynamic therapy consists on substituting the light by ultrasound in the photodynamic therapy. Low intensity ultrasound generates cavitation bubbles probably responsible of the sonodynamic cytotoxicity. In this work Photodynamic and sonodynamic cytotoxicities with Photofrin® (PF) were compared in vitro on AT2 rat prostatic tumoral cells. The photo and sono-irradiation durations and intensities as well as the PF concentrations effect, on cell cytotoxicity, were studied. In this aim, cells were treated with 5, 10 and 20 μg/mL of PF, in Foetal Bovine Serum (FBS) supplemented or free medium, before being irradiated by a light source (day light type) or an ultrasonic transducer (focalized or plan, Frequency ~ 500 kHz). Cytotoxicity was evaluated by Propidium Iodide (PI) fluorescent flow cytometer assay. Results show that cell death increases with the rise of ultrasound or light intensities, and that longer irradiation duration enhanced the photodynamic cytotoxicity but not the ultrasonic cytotoxicity. In fact, the bubble movements (cavitation) generated in medium and detected by a passive hydrophone disappeared after 1 minute of irradiation. Higher PF concentrations induced linear increase in cell death rates for light irradiation, but, during sono-irradiation, the mortality enhancement stabilized starting 10 μg/ml PF. Results also showed that the FBS inhibited the phototoxicity and not the sonocytotoxicity. This can be explained by different cytotoxic effects induced by each technique, such as the production of singulet oxygen (mainly bound to serum proteins) for the first and the mechanical membrane fragilization for the other. In conclusion, results showed the high efficacy of the photodynamic technique in comparison with the sonodynamic technique, and pointed at the limits and possible advantages of the sonodynamic technique, with these experimental designs, being related to the non permanent cavitation phenomenon, and being independent from the extracellular medium composition, respectively.

(PS2105) Effect of epothilone B and radiation in Chinese hamster cells. Shungjun Yang, Mingliang Jiang, Hani Ashamalla. New York Methodist Hospital, Brooklyn, NY, USA.

Epothilones are microtubule-stabilizing agents, believed to induce cytotoxicity by a similar mechanism as that of taxanes. These compounds appear active against paclitaxel-resistant cells and tumors overexpressing P-glycoprotein or bearing tubulin mutations, suggestive of their potential for wide spectrum anticancer effect. Taxanes are one of the important drugs used in clinical chemo-radiation of several common cancers. Epothilones could have similar potential. Although the primary mode of action of these drugs does not involve damaging DNA, it is of interest whether pathways contributing to radiation survival, specifically non-homologous end-joining of double stranded DNA breaks, would also affect cytotoxicity of epothilone B and its possible interaction with radiation. This study examined in vitro responses to epothilone B in combination with x-rays. We used Chinese hamster CHO-K1 cells and its radiosensitive derivative xrs-5 cells, defective in Ku80 of non-homologous end-joining. Culture growth was monitored by colorimetric method and cell counts, cell cycle by flow cytometry, and cytotoxicity by colony assay. Epothilone B showed a concentration- and duration-dependent effect in both cell lines, somewhat more toxic to the repair-deficient mutant than its wild type parent. For instance, survival to epothilone B exposure at 5 nM for a 24 h period was 67% and 51% respectively of CHO-K1 and xrs-5 cells respectively. Consistent with known characteristics of these cells, xrs-5 cells were more radiosensitive, with 4.5% surviving a dose of 3 Gy as compared with 48% in the parental cells. Radiation to drug pretreated-cells had lower clonogenic survival than that of cells exposed to either agent alone, more so in the wild type CHO cells than its repair-deficient derivative. These results suggest the involvement of Ku80 in epothilone B cytotoxicity and enhancement of radiation effect. Further understanding of contributing mechanisms would help in designing optimally combined use of the two agents.

(PS2106) Enhancement of somatostatin-receptor targeted radionuclide therapy by gemcitabine pre-treatment mediated receptor up-regulation and cell cycle modulations. Tapan K. Nayak[1], Eric R. Prossnitz[1], Robert W. Atcher[2], Jeffrey P. Norenberg[1]. [1]University of New Mexico Health Science Center, Albuquerque, NM, USA, [2]Los Alamos National Laboratory, Los Alamos, NM, USA.

Introduction

Advanced clinical studies of the patients treated with somatostatin-receptor (sstr) targeted [DOTA0-Tyr3]-octreotide (DOTATOC) labeled with beta emitters have shown overall response rates in the range of 15–33%. This study evaluates the potential for combination therapy with gemcitabine in an effort to improve clinical outcomes.

Methods

Human pancreatic adenocarcinoma Capan-2, rat pancreatic cancer AR42J, and human small cell lung cancer NCI-H69 cells were each treated with 1 microgram/mL of gemcitabine. All the cell lines described have documented sstr expression. After 4 days of treatment, the media was replaced and cells were allowed to replenish for 4 additional days. Cell cycle and receptor binding studies were performed with 177Lu-DOTATOC after the total 8-day treatment as described. Cell viability and apoptosis experiments

were performed to study the effects of combination gemcitabine pre-treatment and 177Lu-DOTATOC radionuclide therapy. Parallel control studies were performed with receptor non-specific 177Lu-DOTA and non-radiolabeled DOTATOC.

Results

Cells treated with gemcitabine for 4 days showed a minor down-regulation of sstr expression and ligand binding. However, after the 4 days of replenishment the specific cellular and intracellular uptake of 177Lu-DOTATOC was 1.5–3 times higher than the untreated cells. In gemcitabine pretreated capan-2 cells, the number of cells in G2M phase was two times that of untreated cells. Due to sstr up-regulation and cell cycle modulations, synergistic effects of gemcitibine pretreatment were observed in cell viability and apoptosis assays. 177Lu-DOTATOC showed 2–3 times greater apoptosis in gemcitabine pre-treated cells than in untreated cells.

Conclusion: Gemcitabine pre-treatment up-regulates sstr expression and acts as a radiosensitizer. The rationale combination of gemcitabine and sstr targeted radiolabeled is a promising chemo-radiation therapeutic tool. This combination has a great potential to improve clinical outcomes and merits further study.

(PS2107) Chemosensitization by 2-deoxy-D-glucose in multicellular tumor spheroids results from the multiple death pathways stimulated by a combination of endogenous and induced oxidative stress. Divya Khaitan, Sudhir Chandna, Bilikere S. Dwarakanath. Institute of Nuclear Medicine and Allied Sciences, Delhi, India.

Earlier studies have shown that the glycolytic inhibitor, 2-deoxy-D-glucose (2-DG) enhances radiation induced cell death in multicellular tumor spheroids (MTS) of a human glioma cell line (BMG-1), which is 2–3 folds higher than in monolayer cultures. Since certain mechanisms of cellular responses to chemotherapeutic agents are similar to radiation responses, we investigated the chemosensitization by 2-DG in MTS. Mitochondrial status, oxidative stress, DNA and cytogenetic damage, various cell death processes, Pgp and topoisomerase II levels, and GSH/GSSG ratio were analyzed. Significantly higher constitutive TNF-α levels in spheroidal cells were coupled with a decreased GSH/GSSG ratio, although endogenous ROS level was not altered. Cellular Pgp levels increased three-fold and topoisomerase IIα translocated in the cytoplasm of MTS, predicting more resistant response of spheroidal cells to topoisomerase-II poisons. In contrast, MTS showed 10 times more sensitivity (IC_{50} 1μM) to etoposide (a topoisomerase-II poison) than monolayer cultures (IC_{50} 10μM). Etoposide-induced oxidative stress coupled with TNFα-dependent oxidative stress resulted primarily in apoptosis in these MTS, which was quenched by N-acetyl-cysteine (NAC). Under similar conditions, the sensitization by 2-DG (5mM; equimolar with glucose; present for 2 h after 6 h of etoposide treatment) was also 2–3 folds higher in MTS. A persistent oxidative stress was induced in this case, which was more effectively quenched by pyruvate than NAC. A decrease in GSH/GSSG ratio ($\sim$35%) induced by etoposide + 2-DG coupled with decrease in Pgp levels led to a five fold increase in the cell death (primarily necrosis) in spheroids as compared to monolayer cultures. These studies suggest that oxidative stress induced by etoposide alone or in combination with 2-DG synergizes with endogenous TNFα mediated oxidative stress activating multiple death pathways that ride over the pro-survival responses resulting in a profound cell death in MTS. The present study demonstrates the suitability of 3-D multicellular tumour spheroids for predicting responses of *in vivo* tumours to bioenergetics based therapeutic modalities and for providing insight into the possible mechanisms involved.

(PS2108) Chemoradiosensitization of a novel camptothecin derivative. Ge Huang[1], Huijuan Wang[1], Li-xi Yang[2]. [1]Radiobiology Laboratory, California Pacific Medical Center Research Institute, San Francisco, CA, USA, [2]St. Mary's Medical Center, San Francisco, CA, USA.

A second-generation camptothecin derivative (TLC-388) with higher efficacy and reduced toxicity has been synthesized and tested as a novel chemoradiosensitizing agent. This study is to investigate the mechanisms of chemoradiosensitizing effects of TLC388 on H23 human non-small cell lung cancer cells. Using TUNEL assay, a significantly higher percentage of apoptotic cells was observed in group treated with TLC388 plus radiotherapy than those in groups treated with drug or radiation alone. Sensitizer enhancement ratio (SER) was 1.91. Apoptosis increased with drug concentrations and radiation doses, exhibiting dose-dependent patterns. The results suggest that apoptosis would be a main mode of cell death that might underlie the increased chemoradiosensitization of TLC388. Treatment with TLC388 plus 4 Gy radiation could also produce up to 42% of necrotic cells that were measured by trypan blue exclusion assay. But with TLC388 alone or 4 Gy radiation alone 9.8% or 11.1% of necrotic cells were detected, respectively. Immunofluorescent staining method was employed to determine gamma-H2AX as a molecular biomarker of DNA double strand breaks (DSBs) in cells treated with TLC388 +/- radiation or radiation alone. After TLC388 or radiation exposure the formation of gamma-H2AX foci were observed. When cells were treated with TLC388 plus radiation at doses of 0.5 - 2 Gy, the percentage of cells containing gamma-H2AX foci increased significantly. Even more interesting, a markedly higher percentage (65.4%) of mitotic cells displayed gamma-H2AX foci after treatment with 30 nM TLC388 plus 0.5 Gy radiation, whereas only 5.9% or 26.1% of M phase cells demonstrated gamma-H2AX foci when cells treated with 30 nM TLC388 alone or 0.5 Gy radiation alone, respectively. It is suggested that mitotic cells would be very sensitive in the production of DSBs after TLC388-radiation combined treatment. These data strongly suggest that the formation of DSBs would lead to the induction of apoptosis at doses of lower than 4 Gy and some necrosis at doses of 4 Gy or above. All these results would contribute to the elucidation of the mechanisms of chemoradiosensitization of TLC388 and its development as a novel chemoradiosensitizing drug for improved radiotherapy. (This work was supported in part by a grant from UC TRDRP)

(PS2109) Intra-tumoral delivery of radio-labelled iododeoxyuridine. Shirley Lehnert[1], Abraham Owusu[1], Yongbiao Li[1], Edward Bump[2], Bill Riddoch[2]. [1]McGill University, Montreal, PQ, Canada, [2]Draximage Inc., Kirkland, PQ, Canada.

We have previously reported the use of biodegradable carrier matrices as delivery systems for drugs sensitizing tumor cells to external beam or interstitial radiation. We have now applied this approach to the delivery of the thymidine analogue 5-iodo-2′ deoxyuridine radiolabeled with ^{125}I [^{125}IUdR] which is highly toxic to cells if it is incorporated into DNA. Unincorporated ^{125}IUdR on the other hand is unstable *in vivo* and in man or mouse has a half-life of only a few minutes. This problem can be avoided if the radionuclide is released intra-tumorally and, since ^{125}IUdR incorporation is cell cycle-specific, prolonged delivery by a controlled release system optimizes the proportion of cells which incorporate the analogue.

Evaluation of ^{125}IUdR delivery by biodegradable intra-tumoral implant was done in 2 experimental tumor models; subcutaneous mouse fibrosarcoma RIF-1, and the rat glioblastoma C6 implanted intra-cranially. The delivery vehicle used was Biosyntech Hydrogel, a chitosan-based thermolabile hydrogel. The end point was tumor growth delay for RIF-1 and survival time for C6 implanted rats. It was demonstrated by HPLC analysis of tumor extracts at various times after implantation of ^{125}IUdR/hydrogel that the radiolabel remained associated with IUdR and that a significant proportion of ^{125}IUdR was incorporated into tumor cell DNA. Both tumor systems showed a response which was proportional to the extent of ^{125}IUdR incorporation into DNA. Tumor control was achieved using a lower dose of ^{125}IUdR than had previously been reported. Inclusion in the polymer delivery system of a second drug, methotrexate, an anti-metabolite intended to boost the incorporation of ^{125}IUdR by depletion of the endogenous thymidine pool increased the incorporation of ^{125}IUdR into tumor cell DNA and enhanced the tumor response to sub-optimal levels of ^{125}IUdR.

We conclude that delivery of ^{125}IUdR by intra-tumoral implant is a feasible method for the application of this modality. The Biosyntech hydrogel is particularly suited to this application because incorporation of the radioactive nuclide into the vehicle requires minimal manipulation.

(PS2110) FDG-PET predicts sensitivity of human head and neck cancer xenografts to cisplatin combined with 2-deoxy-D-glucose. Andrean L. Simons, David M. Mattson, Melissa A. Fath, Susan A. Walsh, Brian J. Smith, Richard D. Hichwa, Michael M. Graham, Kenneth J. Dornfeld, Douglas R. Spitz. University of Iowa, Iowa City, IA, USA.

Cisplatin (CIS) cytotoxicity has been shown to be significantly enhanced by the glycolytic inhibitor 2-deoxyglucose (2DG) via mechanisms involving metabolic oxidative stress in human head and neck cancer cells. Based on these data, we investigated the effects of CIS and 2DG in two head and neck cancer cell lines *in vitro* and *in vivo* and determined if glucose uptake as measured by FDG-PET imaging would predict sensitivity to this combined modality. We additionally determined if 2DG would further enhance the cytotoxicity associated with cisplatin and radiotherapy. Sensitivity to CIS and 2DG was determined in FaDu and Cal-27 human head and neck squamous carcinoma cells. Drug-induced cytotoxicity was determined using clonogenic cell survival assays, and glutathione levels were measured to determine perturbations in thiol metabolism in culture. Antitumor activity was determined in an immunodeficient mouse xenograft model. Glucose uptake was determined by FDG-PET. Treatment with CIS in combination with 2DG significantly inhibited the clonogenic survival of FaDu and Cal-27 cells compared to CIS or 2DG alone, while increasing parameters indicative of oxidative stress such as oxidized glutathione *in vitro*. The combination of CIS and 2DG significantly inhibited FaDu and Cal-27 tumor growth and increased overall survival compared to either agent alone. Animals bearing Cal-27 tumors demonstrated higher pre-treatment FDG uptake and increased survival relative to FaDu tumors treated with CIS and 2DG. Finally 2DG significantly enhanced CIS-induced radiosensitization in FaDu tumor cells. These results demonstrate the synergistic antiproliferative and antitumor activity of CIS in combination with 2DG in human head and neck cancer cells and the potential for the use of glucose uptake to predict sensitivity of cancer cells to chemotherapy. These findings provide a rationale for evaluating glycolytic inhibitors in combination with chemotherapeutic drugs and radiotherapy in the clinical setting. (Supported by R01-CA100045; P01-CA66081, P30-CA086862).

(PS2111) On the chemical yield of base lesions, strand breaks, and clustered damage generated in plasmid DNA by the direct effect of X-rays. William A. Bernhard[1], Shubhadeep Purkayastha[1], Jamie R. Milligan[2]. [1]Univ. of Rochester, Rochester, NY, USA, [2]University of California at San Diego, , California, La Jolla, CA, USA.

The purpose of this study was to determine the yield of DNA base damages, deoxyribose damage, and clustered lesions due to the direct effects of ionizing radiation, and to compare these with the yield of DNA trapped radicals previously measured in the same pUC18 plasmid. The plasmids were prepared as films hydrated in the range $2.5 < \Gamma < 22.5$ mol water/mol nucleotide. Single strand breaks (ssb) and double strand breaks (dsb) were detected by agarose gel electrophoresis. Specific types of base lesions were converted into ssb and dsb using base-excision repair enzymes, endonuclease III (Nth) and formamidopyrimidine-DNA glycosylase (Fpg). The yield of base damage detected by this method displayed a strikingly different dependency on the level of hydration (Γ) compared with that for the yield of DNA trapped radicals; the former decreased by 3.2x as Γ is varied from 2.5 to 22.5 and the later increased by 2.4x over the same range. In order to explain this divergence, we propose that ssb yields, produced in plasmid DNA by the direct effect, cannot be properly analyzed when one assumes a simple Poisson distribution consisting of, on average, one strand break per plasmid. The yields of dsb, on the other hand, are consistent with changes in free radical trapping as a function of hydration. Consequently, the composition of these clusters could be quantified. Deoxyribose damage on each of the two opposing strands occurs with a yield of 3.5 +/-0.5 nmol/J for fully hydrated pUC18, comparable to the yield of 4.1 +/-0.9 nmol/J for dsb derived from opposed damages in which at least one of the sites is a damaged base.

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(PS2112) Measurement of hydroxyl radicals and 8-hydroxydeoxyguanosine induced by high-LET heavy-ion irradiation. Takashi Moritake, Kazunori Anzai, Kailash Manda, Megumi Ueno, Mitsuko Takusagawa, Mayumi Iwakawa, Takashi Imai. National Institute of Radiological Sciences, Chiba, Japan.

Purpose:

The aim of this study was to quantitatively evaluate the generation of hydroxyl radicals ($^{\bullet}$OH) in water and 8-hydroxydeoxyguanosine (8-OHdG) in DNA during high linear energy transfer (LET) ion irradiation. In addition, the effect of oxygen on $^{\bullet}$OH and 8-OHdG generation was evaluated.

Materials and Methods:

Pure water containing 200 mM 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) and pure water containing 1.0 mg/ml salmon sperm DNA were irradiated with heavy-ion beams at dose levels of 20 Gy and 10 Gy, respectively. In order to investigate the effect of oxygen on the generation of $^{\bullet}$OH and 8-OHdG, the samples were irradiated under atmospheric and deaerated conditions. Irradiation using four ion species at five different energies (carbon, 290 MeV/n and 135 MeV/n; neon, 400 MeV/n; silicon, 490 MeV/n; and argon, 500 MeV/n) was carried out at NIRS-HIMAC in Japan. Each type of ion was irradiated at three different LET values, using PMMA plates of varying thickness to attenuate the irradiation energy: carbon (290), 20–80 keV/μm; carbon (135), 25–95 keV/μm; neon, 30–100 keV/μm; silicon, 80–300 keV/μm; and argon, 100–230 keV/μm. In order to compare the effects of high-LET heavy-ion irradiation and low-LET photon irradiation, identical samples were also irradiated with x-rays (200 kV, 20 mA; approximately 2 keV/μm) under the same conditions. Yields of $^{\bullet}$OH were measured using an electron spin resonance (ESR) spectrometer. Levels of 8-OHdG in DNA were measured by HPLC with an electrochemical detector (ECD) after each irradiation.

Results:

For each type of ion irradiation, the amounts of both $^{\bullet}$OH and 8-OHdG formed decreased logarithmically as LET (keV/μm) increased. The amounts of both species formed at each LET value tended to become greater as the atomic number of the irradiating species (carbon, neon, silicon, or argon) increased. The results obtained for aerated and deaerated samples revealed the same pattern; however, significantly smaller amounts of $^{\bullet}$OH and 8-OHdG were formed under deaerated conditions.

Conclusion:

These findings may be helpful in predicting the biological effect of $^{\bullet}$OH generated by high-LET heavy-ion irradiation under both aerated and deaerated conditions along the projection of beams for cancer therapy.

(PS2113) Deoxyribose damage is sensitive to base sequence context and end effects: the release of unaltered free base from oligodeoxynucleotides films by the direct effect of ionizing radiation. Kiarn K. Sharma, William A. Bernhard. University of Rochester Medical Center, Rochester, NY, USA.

The mechanisms for the release of unaltered free base in oligodeoxynucleotide films of d(CTCTCGAGAG) and d(GAGAGCTCTC) by the direct effect of ionizing radiation are investigated at hydration (Γ) of 2.5 mol H_2O per mol nucleotide. The yields of unaltered free base release were measured by X-irradiating at room temperature (RT), using HPLC. The sum of the free bases released, G(fbr) estimates the sugar damage and hence yield for the strand breaks, G_{total}(ssb). The yields of unaltered free bases for the d(CTCTCGAGAG) film are $G(\text{Cyt}) = 13.3 \pm 0.7$ nmol/J; $G(\text{Gua}) = 29.7 \pm 1.1$ nmol/J; $G(\text{Thy}) = 6.1 \pm 0.6$ nmol/J and $G(\text{Ade}) = 14.7 \pm 1.5$ nmol/J respectively. Similarly the yields for d(GAGAGCTCTC) film are $G(\text{Cyt}) = 20.2 \pm 3.8$ nmol/J; $G(\text{Gua}) = 21.0 \pm 3.2$ nmol/J; $G(\text{Thy}) = 11.2 \pm 1.8$ nmol/J and $G(\text{Ade}) = 6.4 \pm 1.2$ nmol/J. The G(fbr) for d(CTCTCGAGAG) and d(GAGAGCTCTC) are 63.8 ± 4.0 nmol/J and 58.7 ± 10.4 nmol/J respectively, which are same within the standard deviations. The key findings are: i) the $G(\text{Gua})/G(\text{Cyt})$ ratio decreases from 2.2 ± 0.1 for d(CTCTCGAGAG) to 1.0 ± 0.2 for d(GAGAGCTCTC) and ii) the G(Ade)/G(Thy) ratio changes from 2.4 ± 0.3 for d(CTCTCGAGAG) to 0.6 ± 0.1 for d(GAGAGCTCTC).

Finding i) suggests that there is an end effect whereby deoxyribose damage at the 3′-end is enhanced by Gua attachment

relative to Cyt attachment. Finding ii) is evidence that, independent of any end effects, deoxyribose damage is influenced by base sequence context.

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(PS2114) Photo-excitation of one-electron oxidized rna nucleosides and rna-oligomers in the near uv-vis region produces sugar radicals. Amitava Adhikary, Sean Collins, Deepti Khanduri, David Becker, Michael D. Sevilla. Oakland University, Rochester, MI, USA.

Purpose: To identify and characterize sugar radicals formed via photo-excitation of one-electron-oxidized purine (i.e., $G^{\bullet+}$ and A(-H)•) in RNA-model compounds (nucleosides, -tides and oligomers) in the near UV-visible region following our ongoing efforts in dsDNA and its model compounds [Ref. 1–5].

Materials and methods: $G^{\bullet+}$ and A(-H)• formed by one-electron oxidation by $Cl_2^{\bullet-}$ in frozen aqueous (D_2O) glasses in 7.5 *M* LiCl of (i) RNA-nucleosides (Ado and Guo) and their derivatives with selective deuteration on the sugar ring (1', 2', (5', 5')), of (ii) RNA-nucleotides, e.g., 3'-GMP, 5'-GMP, 3',5′-cGMP , 5'-AMP, 3'-AMP, 2'-AMP, 2',3′-cAMP, 3',5′-cAMP, 3′,5′-ADP, 2′,5′-ADP, and of (iii) a series of RNA-oligomers -including, UUGGUU, were photo-excited at 143 K using a photoflood lamp and the ESR spectra of these photo-excited samples were recorded at 77 K.

Results and conclusion: (i) Overall very high yields (ca. 70–90%) of sugar radical formation were observed in these samples. (ii) specific sugar radicals C3'• (quartet) and C5'• (doublet) were identified and their hyperfine couplings were characterized by the the collapse of the wings in the 2'-D-Ado with respect to the spectrum observed in undeuterated Ado and also by the collapse of the central doublet in 5′,5′-D,D-Ado and 5′,5′-D,D,-Guo. 1'-D-Ado and Ado as well as 1'-D-Guo and Guo show identical ESR spectra upon photo-excitation of A(-H)• and $G^{\bullet+}$ respectively, thereby implying that the C2'• sugar radical is not formed. Thus, we conclude that RNA and DNA nucleosides behave nearly identically, suggesting little effect of the C2'-OH group. (iii) However, both for 3′,5′-cyclic nucleotides and for the oligomers studied, we observe formation of only C2'• sugar radical. These results are explainable by the deactivation of the preferred C3'- and C5'- sites in the sugar moiety by phosphate substitution.

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(PS2115) An improved analytic description of the Bethe surface of liquid water: application to inelastic and stopping cross section calculations for low-energy electrons. Dimitris Emfietzoglou[1], Isabel Abril[2], Rafael Garcia-Molina[3], Anand Pathak[4], Hooshang Nikjoo[5]. [1]Medical Physics Laboratory, University of Ioannina Medical School, Ioannina, Greece, [2]Departament de Física Aplicada, Universitat d'Alacant, Alacant, Spain, [3]Departamento de Física – C IOyN, Universidad de Murcia, Murcia, Spain, [4]School of Physics, University of Hyderabad, Hyderabad, India, [5]USRA, Houston, TX, USA.

We present an improved analytic description of the Bethe surface of liquid water which, for the first time, is fully consistent with theoretical and experimental constraints and permits electron inelastic and stopping cross section calculations in the, as yet, experimentally inaccessible range below the Bethe limit with an accuracy comparable to the higher-energy (ICRU/NIST) values (~5%). The Bethe surface of a material provides all the information necessary for inelastic and stopping calculations for electrons within the framework of the plane-wave (1st) Born approximation (PWBA), the validity of which covers most of the electronic regime of interest. For low energy electrons the straightforward application of the Bethe theory (essentially an optical-limit theory) is problematic since the momentum-dependence of the material-specific structure functions (e.g. oscillator strengths, dielectric functions) becomes important. Although a substantial body of literature exists for inelastic and stopping calculations for low-energy electrons in liquid water, the Bethe surface characteristics of this material has so far been approximated using simplifying assumptions which are not consistent with recent experimental data. This work presents a comprehensive investigation of the Bethe surface of liquid water by critically examining the suitability of various analytic descriptions which have been most frequently represented in the literature, such as, the Lindhard dielectric function and its modification by Mermin, and various forms of the extended-Drude and δ-oscillator models. We then advance an accurate and easy-to-use analytic parameterization of the experimental data of liquid water over the whole energy-momentum plane based on the extended-Drude formalism. The model satisfies to within 1% the *f*-sum-rule for all values of momentum transfer and returns a mean excitation energy (82 eV) in good agreement with recent experimental measurements (80 eV). By means of an exchange and perturbation corrected-PWBA calculation we present near-exact (to within ~5%) values of electron inelastic cross sections and stopping powers over the radiobiologically important range from the Bethe limit (~10 keV) down to the Bragg peak (~0.1 keV). Differences from earlier calculations are, on average, at the 20–50% level.

(PS2116) An object oriented track-structure code for simulations of energy depositions from light ions. Kristin Wiklund, Anders Brahme, Bengt K. Lind. Karolinska Institute and Stockholm University, Stockholm, Sweden.

Purpose

To develop a track-structure code for the simulation of energy depositions in order to investigate the biological efficiency of different radiation qualities.

Methods

Differences in the biological effectiveness of different radiations result from different microscopic distributions of primary interactions. This is often explained by the fact that the effects are a result of a correlation mechanism between several elementary hits, and not by a single hit mechanism. It is clear that the microscopic patterns of interaction and energy depositions by radiation are crucial to any detailed understanding of the mechanism by which they induce cell killing, mutations, chromosome aberrations and carcinogenesis. Track structure analysis based on computer calculations requires accurate cross sections for all interactions considered to acquire reliable results and is therefore a weak point. The primary electron fluence is extracted from accurate Continuous Distorted Wave-Eikonal Initial State (CDW-EIS) inelastic cross sections for ions. Inelastic cross section for electrons between 10 eV-100 keV calculated within plane-wave approximation with the generalized oscillation strength,GOS, provided by J M Fernandez-Varea, is used. Elastic electron cross down to 10 eV is calculated with the program ELSEPA (Salvat et al), were we use a point nucleus as the nuclear charge distribution model and Numerical-Dirac-Fock distribution as the electron distribution model. We assume free atoms and use Furness-McCarthy exchange potential, Buckingham correlation-polarization potential and no absorption potential. In order to take the excitations into account, we use the semi-empirical approaches of Olivero et al. Even though a fine mesh of cross section data will be stored in the internal data base some further interpolation will be needed.

Results

The code structure is ready and has been tested and the results are promising, but further verifications are needed. The final cross sections are just about to be implemented in the code. Since all events and their information are stored it allows the scoring to be performed separately from the MC calculation. In order to speed up the interpolation we found that a MC interpolation is more efficient than the common linear/bi linear way of interpolation.

(PS2117) Effects of microscopic target structures on local dose distributions. Jacob A. Gersh, Michael Dingfelder, Larry H. Toburen. East Carolina University, Greenville, NC, USA.

When charged particles traverse interfaces between regions of differing atomic composition and density, interactions occurring within close proximities to the interface can cause perturbations in the amount of energy deposited. The irradiation of bone marrow in human trabecular spongiosa presents an example in which target structure can influence the local dose distributions. In trabecular spongiosa, the intricate matrix of interconnected microscopic strands of bone that define this region contribute to perturbations in the patterns of dose distributions by incident charged particle irradiation. In this work, the extent by which a charged particle's track, and the subsequent pattern of energy deposition, is influenced by the target structure during transport through the human trabecular spongiosa is explored. Particles are transported through geometric spongiosa models using a hybrid Monte Carlo radiation transport code that combines the electron transport capabilities of PENELOPE with the alpha particle transport capabilities of PARTRAC. By scoring energy depositions in microscopic "detectors" randomly placed throughout regions of inhomogeneous target tissue, quantification of inhomogeneity of dose is performed. Preliminary calculations confirm the presence of perturbations in dose across marrow cavities within the spongiosa. Dose fractions and microscopic dose will be presented for a range of electron energies and MeV alpha particles.

(PS2118) On OH radicals in water under heavy ion irradiation. Mitsumasa Taguchi[1], Atsushi Kimura[1], Gérard Baldacchino[2], Yosuke Katsumura[3], Koichi Hirota[1]. [1]Japan Atomic Energy Agency, Takasaki, Japan, [2]CEA/Saclay, Cedex, France, [3]The University of Tokyo, Tokyo, Japan.

High energetic heavy ions give unique irradiation effects on target materials, and are promising tool for the applications in chemical and biological fields. These unique irradiation effects are resulted in the high density and non-homogeneous distributions of radicals and subsequent diffusions and reactions in track. Reactions in water under heavy ion irradiation are mainly induced by OH (hydroxyl) radicals. In the present study, we used aqueous solutions to estimate the primary yield of the OH radicals as the basic knowledge for the application of heavy ions to advanced cancer therapy, nano-fabrications, and so on.

The primary yields of the OH radicals have been investigated by the 1) product analysis and 2) pulse radiolysis methods. 1) The aqueous phenol solutions were irradiated with He, C and Ne ions ranging from 2 to 18 MeV/n. The yields of irradiation products of phenol super-linearly increased with the incident energy of the ions. The yields of the OH radicals were estimated by analyzing the yields of the irradiation products of phenol. The yields of the OH radicals increased with the specific energy for each ion, but decreased with both the mass of each ion at the same specific energy and elapsed time after irradiation. 2) Highly sensitive transient absorption measurement system was developed for the direct observation of radical behaviors in water under heavy ion irradiation. A blue diode laser (50 mW, 440 nm) and Si photodiode were used as probe light and detector, respectively. A small absorbance change less than 10^{-4} was recorded after 10 pulses irradiation. The transient absorbance of $(SCN)_2^-$ formed by the reaction of OH radicals with SCN^- in aqueous KSCN solution was observed under pulsed C ion irradiation.

(PS2119) Proton induced electron emission spectra from condensed phase targets. R. A. McLawhorn[1], S. L. McLawhorn[1], G. W. Kalmus[2], L. H. Toburen[1], E. L. B. Justiniano[1], J. L. Shinpaugh[1]. [1]East Carolina University, Department of Physics, Greenville, NC, USA, [2]East Carolina University, Department of Biology, Greenville, NC, USA.

Understanding the effects of charged particle radiation in biological material requires knowledge of the initial spatial patterns of energy deposition. This is important because the initial patterns of energy deposition can heavily influence final biological outcomes. The patterns of energy deposition from charged particle radiation can be modeled using event-by-event Monte Carlo track structure codes. These codes rely on theoretically determined interaction cross sections for the charged particles with the material under consideration. Interaction cross sections in condensed phase materials cannot be directly measured. Therefore, to test the accuracy of Monte Carlo electron transport codes, we have measured doubly differential electron emission yields $\gamma(\varepsilon,\theta)$ as a function of electron energy ε and the emission angle θ from condensed phase targets following fast proton impact. These electron emission yields can be compared with simulated yields from Monte Carlo transport codes as a test of these codes in modeling the production and transport of secondary electrons in condensed phase materials. Electron emission spectra from thin (1 μm) foil targets were measured for emission angles from 15° to 155° with respect to the incident beam using electron time-of-flight energy analysis. The time-of-flight technique allows accurate measurement of low-energy electrons which dominate the emission spectrum. Al, Cu, and Au targets have been studied to provide a database of homogeneous materials from which comparisons of theoretical and computational models can be made. Published measurements of single-differential electron emission yields $\gamma(\varepsilon)$ and total electron emission yields γ are available for these materials, allowing benchmark testing of the experimental system. The present work on homogeneous materials will be extended to complex biological targets, such as thin (5–10 μm) tissue sections. Results from tissue will provide a more biologically relevant target to test Monte Carlo track structure simulations.

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(PS2120) Foliar absorption pathway and use efficiency of ^{45}Ca and ^{32}P radioisotope tracer technique on sweet persimmon. Md. Belal N. Hossain. College of Life & Environment, Daegu, Republic of Korea.

Abstract

Foliar experiments were conducted to identify the absorption pathway and use efficiency of ^{45}Ca and ^{32}P radio isotopes containing phosphatic fertilizers with dorsal and ventral leaf surface during 2, 5, 10, 24, 48 and 96 hours after application on sweet persimmon. The absorption capacities were analyzed using bio-imaging analyzer and scintillation counter. The highest absorption was 2.064, 0.994 and 2.401, 1.823 μCi per gram dry weight of leaf for ^{45}Ca and ^{32}P radio isotopes containing incalgyun and seopung both lower and upper leaf surface, respectively. The lowest absorption was obtained 0.034 and 0.074; 0.152 and 0.200 μCi both ^{45}Ca and ^{32}P incalgyun and seopung with ventral and dorsal parts of leaf within two hours after application of fertilizers, respectively. The higher absorption capacity was found in dorsal part of leaf surface than the ventral part. Phosphorus absorption was higher than calcium due to higher concentration of phosphorus. It means that absorption was differently influenced on calcium and phosphorus containing compounds. Incalgyun fertilizer is better than seopung fertilizer as a foliar application. The absorption capacity was increased with the increase of time. Correlation studies revealed that ventral and dorsal parts with different fertilizers and between fertilizers positively correlated with various time intervals after application of fertilizer. It is concluded that stomata can be an important pathway for the uptake of foliar applied substances. For rapid and complete uptake of foliar incalgyun fertilizer is necessary to spray both the dorsal and ventral leaf surface as evenly as possible to get optimum yield and improve the quality of fruit.

Key words: ^{45}Ca and ^{32}P isotopic fertilizers, abaxial and adaxial leaf surface, duration, bio-imaging, liquid scintillation counting

(PS2121) A study of anomalous behavior of radon in groundwater and soil gas for earthquake prediction. Sandeep Mahajan. Guru Nanak Dev University, Amritsar, Amritsar, India.

In the present study, radon monitoring is carried out in groundwater and soil gas using Alpha-Scintillometry and track-etch (LR-115) techniques respectively at Guru Nanak Dev University, Amritsar (India) from July 2004 to March 2005. The analysis of radon data in light of earthquake prediction studies has been made.

The effect of seasonal changes on the concentration of radon in soil gas and groundwater is very well taken into consideration. The radon anomalies, both positive and negative, in the data are correlated with micro seismic events recorded along Main Boundary Thrust (MBT) and Main Central Thrust (MCT) of NW Himalayas in the grid (28 - 34° North, 72 - 79° East). About 140 seismic events (as reported in IMD Catalogue) with intensity > 1 M are recorded during this period. The anomalous change in the radon concentration before an event suggests that continuous radon monitoring in a grid pattern can serve as a productive tool in earthquake prediction studies.

(PS2122) A novel analytical approach for estimating terrestrial cosmic-ray dose for anywhere in the world. Tatsuhiko Sato[1], Hiroshi Yasuda[2], Koji Niita[3], Akira Endo[1], Lembit Sihver[4]. [1]Japan Atomic Energy Agency, Tokai, Ibaraki, Japan, [2]National Institute of Radiological Sciences, Inage, Chiba, Japan, [3]Research Organization for Information Science and Technology, Tokai, Ibaraki, Japan, [4]Chalmers University of Technology, Gothenburg, Sweden.

People are subjected to be exposed to various kinds of natural radiations, and it is estimated that roughly 20% of their doses are attributed to the terrestrial cosmic-ray exposure. However, the cosmic-ray dose rates significantly depend on various parameters such as altitude and solar modulation potential, and none of the existing models are able to predict the values with satisfactory accuracy. Furthermore, radiation protection for aircrews against the terrestrial cosmic-rays has been intensively discussed since the publication of ICRP60 in which the aircrew exposure is recognized as occupational one. Development of aircrew-dose calculation code is the one of the most important issues in the discussion.

With these situations in our mind, we performed Monte Carlo simulations for estimating the terrestrial cosmic-ray spectra using our developing code PHITS. Excellent agreements were observed between the calculated and measured spectra for almost all particles that contribute to the cosmic-ray doses for a wide altitude range even at the sea level. Based on a comprehensive analysis of the simulation results, we proposed analytical functions that can predict the cosmic-ray spectra for anywhere in the world except for the locations with altitudes over 20 km. The functions coupled with the fluence to dose conversion coefficients enable us to calculate the cosmic-ray dose rates in a short computational time. This analytical model will be incorporated into the Japanese Internet System for Calculation of Aviation Route Doses JISCARD.

The details of the results for the cosmic-ray neutron spectra had already been published in our previous paper (1). We therefore focus on describing the results for other particles - proton, He nucleus, muon, photon, electron and positron - at the meeting. The world-wide contour map of the cosmic-ray dose rates at the ground level, which were calculated by the analytical model coupled with a global relief data, is also given in the presentation.

(1) T.Sato and K. Niita, Radiat. Res. 166, 544–555 (2006). See also http://www3.tokai-sc.jaea.go.jp/rphpwww/radiation-protection/expacs/expacs-eng.html

(PS2123) Assessment of space radiation risk for future lunar missions. Myung-Hee Y. Kim[1], Artem Ponomarev[2], Bill Atwell[3], Francis Cucinotta[4]. [1]Wyle Laboratories, Inc., Houston, TX, USA, [2]Universities Space Research Association, Houston, TX, USA, [3]The Boeing Company, Houston, TX, USA, [4]NASA Johnson Space Center, Houston, TX, USA.

In design of lunar exploration missions, assessments of radiation risk are required in support of resource management decisions, operational planning, and go/no-go decisions. Such assessments require the understanding of future space radiation environments. The future galactic cosmic radiation (GCR) flux was estimated as a function of interplanetary deceleration potential, which was coupled with the estimated neutron monitor rate from the Climax monitor using a statistical model. A probability distribution function for solar particle event (SPE) occurrence was formed from proton fluence measurements of SPEs that have occurred during the past 5 solar cycles (19–23). Large proton SPEs, which were identified from impulsive nitrate enhancements in polar ice for the fluences greater than 2×10^9 protons/cm^2 for energies greater than 30 MeV, were also combined to extend the probability calculation for high levels of proton fluence. The probability with which any given proton fluence level of a SPE will be exceeded during a space mission of defined duration was then calculated. Analytic energy spectra of SPEs at different ranks of the integral fluences were constructed over broad energy ranges extending to GeV, and representative exposure levels at those fluences were analyzed. For the development of an integrated strategy for radiation protection on lunar exploration missions, effective doses at various points inside a spacecraft were calculated with detailed geometry models representing proposed transfer vehicle and habitat concepts. Various configuration concepts of radiation shelter in exploration-class spacecraft were compared with respect to preliminary assessments of radiation risk from SPEs and GCR inside the spacecraft.

(PS2124) Verification of the PHITS-based analytical model in application to dosimetry of cosmic radiation exposure in aircraft. Hiroshi Yasuda[1], Tatsuhiko Sato[2], Masashi Takada[1], Takashi Nakamura[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Japan Atomic Energy Agency, Tokai-mura, Japan.

Although it is important to know accurately energy spectra of energetic neutrons (>10MeV) for radiological protection of aircraft and spacecraft crew, measurement of neutrons at aviation altitude is still difficult because signals from charged particles such as protons and heavy ions are mixed with those from neutrons. We thus try to develop a portable neutron detector which can automatically measure energetic cosmic neutrons in the high-energy range separately from charged particles and gamma rays.

For testing the feasibility of our concept, experiments of cosmic radiation measurements were performed in a jet aircraft using various detectors (scintillation counters, dose-equivalent counters and an ionization chamber). The aircraft flew on two routes (north/south) over the Japan sea in March, 2007; the flight duration was about 2h each. The measured data were compared to model calculations using the PHITS-based analytical model newly developed for dosimetry of atmospheric cosmic radiation[1)]. As preliminary results, the average dose-rate value obtained from an extended-range dose-equivalent counter in the north flight (at 10.4km in altitude) was 0.85μSv h^{-1} and that in the south flight (at 9.5km) was 0.56μSv h^{-1}. These values show fairly good agreement with the model calculations, indicating the importance of separating contribution of protons in neutron measurements at high altitude. Results of ongoing further data analyses will be presented.

1) T.Sato and K. Niita, Radiat. Res. **166**, 544–555 (2006).

(PS2125) Modulation of the growth of pulmonary tumor colonies in mice after single or fractionated low-level irradiations with X-rays. Jolanta Wrembel-Wargocka, Ewa M. Nowosielska, Aneta Cheda, Marek K. Janiak. Military Institute of Hygiene and Epidemiology, Dept. of Radiobiology and Radiation Protection, Warsaw, Poland.

Epidemiological and experimental data indicate that exposures to low doses of low-LET ionizing radiation may trigger the activity of natural anti-tumor immune mechanisms and inhibit tumor growth**.** Natural killer (NK) cells and activated macrophages play important roles in the anti-tumor defence of the host. In view of this, the aim of the present study was to correlate the tumor-inhibitory effect of low doses of X-rays with the activities of NK cells and macrophages.

In the experiments, BALB/c mice were whole body-irradiated (WBI) with single or fractionated doses of 0.1, 0.2, or 1.0 Gy X-rays and then intravenously injected with L1 sarcoma cells; 14 days later, tumor colonies were counted on the lungs' surface. Immunogenicity assay was used to check if L1 cells were immunogenic for BALB/c mice. Cytotoxic activities of NK cells and macrophages were estimated using the classical ^{51}Cr-release and [^{3}H]thymidine-uptake assays, respectively. The anti-asialo GM_1 antibody (Ab) and

carrageenan (CGN) were intraperitoneally injected to block the NK cell- and macrophage-mediated activities *in vivo*, respectively.

The results indicate that L1 sarcoma cells are not immunogenic for the BALB/c mice (index of stimulation below 3.0). Single and fractionated WBI of mice with 0.1 or 0.2 Gy of X-rays led to a reduction in the number of the pulmonary tumor colonies, the effect being accompanied by the enhanced cytotoxic activities of both NK lymphocytes and macrophages. Treatment of mice with Ab or CGN abrogated the tumor-inhibitory effects of the low-level exposures to X-rays.

The obtained data suggest that suppression of the development of pulmonary tumor colonies by single or fractionated irradiations of mice with the two low doses of X-rays may result from stimulation of the natural anti-tumor defence reactions mediated by NK cells and/or cytotoxic macrophages.

(PS2126) Enzymatic alterations in rats' brain exposed to low level microwave radiation. Paulraj Rajamani, Jitendra Behari. Jawaharlal Nehru University, New Delhi, India.

Abstract: Enzymes are essential for many life processes. They are usually present in small quantities in living organism, but damage by any agent can cause a large scale perturbations in living beings. Of the enzymes investigated to date two enzymes, which are responsible for cellular growth, have received increasing attention. We have selected two growth related enzymes such as ornithine decarboxylase (ODC), performs a rate-limiting step in the synthesis of polyamines and protein kinase C (PKC) is a key enzyme involved in the transduction of signals conveyed from membrane receptors to the intra-cellular region of action of hormones, growth factors and cytokines. Present work describes the effect of low level continuous microwaves (2.45 GHz) on developing rat brain. 35 day old Wistar rats were used for this study. The animals were exposed 2 hr/day for 35 days at a power density of 0.34mW/cm^2 (Specific absorption rate 0.1 W/kg) in a specially made anechoic chamber. After the exposure, the rats were sacrificed and the brain tissue was dissected out and used for enzymatic assays. A significant increase in ornithine decarboxylase activity was observed in the exposed group as compared to the control. Correspondingly, a significant decrease in the calcium dependent protein kinase activity was observed. These results indicate that this type of radiation affects the membrane bound enzymes, which are associated with cell proliferation and differentiation, thereby pointing out its possible role as a tumor promoter.

(PS2127) Activation of interleulin-9 receptor and downstream STAT3/5 in primary T-lymphomas in vivo in susceptible B6 and resistant C3H mouse. Yi Shang[1], Shizuko Kakinuma[1], Yoshiko Amasaki[1], Mayumi Nishimura[1], Yoshiro Kobayashi[2], Yoshiya Shimada[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Toho University, Chiba, Japan.

Deregulation of cytokine receptor expression and downstream signal transduction cascade play important roles in lymphoma development. It is reported that lymphoma and leukemia occasionally express both IL-9 and IL-9 receptor (IL-9R) and proliferate in response to IL-9 in vitro. In the present study, we investigated expression and biochemical characteristics of IL-9Ralpha and activation of downstream Jak-Stat signal transduction in primary T lymphoma (TL) arising in TL-prone B6 mice and resistant C3H. The results indicated that *IL-9Ralpha* and IL-9Ralpha protein is markedly expressed in B6 TL-cells. Three isoforms of IL-9Ralpha proteins with different glycosylation level were found in TL. The less glycosylated IL-9Ralpha was inclined to be the more phosphorylated, being an inverse relationship between glycosylation and phosphrylation. Normal thymocytes expressed the largest isoform of IL-9Ralpha only slightly. Phosphorylation of downstream Stat3 and Stat5 was also detected, but Stat1 activation was not. Overall phosphorylation of IL-9Ralpha and activation of downstream Stat3 and Stat5 was more elevated in B6 than C3H-TL, suggesting a link between IL-9R activation and radiation susceptibility to TL induction. In parallel with Stat5 phosphorylation, expression of cyclin D1 increased in B6-TL cells, while expression of Socs3, a negative regulator of Stat5, increased in C3H. These findings indicate that IL-9Ralpha and downstream Jak-Stat signal transduction were more activated in B6 than C3H TL cells, and that target genes of downstream Stat5 differed between these two strains.

(PS2128) Dose and dose rate dependency in radiation-induced mutation in liver and spleen of gpt-delta mice. Tetsuya Ono[1], Naohito Okudaira[1], Yoshihiko Uehara[1], Tsuneya Matsumoto[2], Youichi Oghiso[2], Kimio Tanaka[2], Kazuaki Ichinohe[2], Shingo Nakamura[2], Satoshi Tanaka[2], Nao Kagawa[3], Kazuo Fujikawa[3], Akira Ootsuyama[4], Toshiyuki Norimura[4], Takehiko Nohmi[5]. [1]Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan, [2]Institute for Environmental Sciences, Rokkasho, Aomori, Japan, [3]Kinki University, Osaka, Japan, [4]University of Occupational and Environmental Health, Kitakyushu, Japan, [5]National Institute of Health Sciences, Tokyo, Japan.

Since mutation plays a key role in carcinogenesis, it would be important to understand the characteristics of mutation induced by radiation in each tissue of a body. Dose dependency and dose rate effects of radiation-induced mutation in germ line cells in mice were elucidated by extensive studies of Russell's group (W.L. Russell and E.M. Kelly, Proc. Natl. Acad. Sci. USA 79 (1982) 539–541), but little is known for somatic tissues. Here we have studied dose and dose rate effects in liver and spleen of mouse using a transfected gene red-gam as a marker to monitor mutation. The mice were gpt-delta strain (T. Nohmi and K.I. Masumura, Adv. Biophys. 38 (2004) 97–121) at 2 months of age. They were irradiated with gamma-rays at dose rates of 920 mGy/min, 1 mGy/min and 12.5 uGy/min. The total doses were 4 Gy and 8 Gy. After irradiation, DNA was extracted from liver and spleen, treated with packaging extract to separate red-gam gene from mouse genome and was subject to determination of mutant frequency by Spi(-)assay. The assay detects the deletion type mutation which is the major type of mutation induced by radiation.

In liver, the dose response curves under 920 mGy/min and 1 mGy/min were linear but the slopes were different; about 1/2.5 at 1 mGy/min. The irradiation of mice at 12.5 uGy/min for 483 days (22 hrs/d, the total dose of 8 Gy) revealed a slight but significant elevation of mutant frequency when compared to the mutant frequency in age-matched control mice. The rate of induction was comparable to that observed at 1 mGy/min. Thus, the dose and dose rate dependencies in liver for radiation-induced mutation seem to be similar to those reported in germ cells by Russell et al.

In spleen, 1 mGy/min gave a linear dose response. The slope, however, was higher than that in liver. At 12.5 uGy/min, a slight but not significant increase was observed. These results indicate a presence of tissue-specificity in the radiation-induced mutations. It suggests that the DNA repair system working on radiation-induced DNA damage varies among the tissues.

(PS2129) Reduction of the background mutation by a low dose X-irradiation of *Drosophila* spermatocytes at a low dose-rate. Takao Koana[1], Mikie O. Okada[1], Keiji Ogura[2]. [1]Central Research Institute of Electric Power Industry, Tokyo, Japan, [2]Institute of Research and Innovation, Kashiwa, Chiba, Japan.

A sex-linked recessive lethal mutation assay was performed in *Drosophila melanogaster*. DNA repair proficient immature spermatocytes and spermatogonia were irradiated with X-rays at a high or low dose-rate. Mutation frequency in the sperms irradiated with a low total dose (0.2Gy) at a low dose-rate (0.05Gy/min) was significantly lower than that in the sham-irradiated group whereas irradiation with a high dose (10Gy) at the same dose-rate resulted in a significant increase in the mutation frequency. It was obvious that the dose-response relationship was not linear, but U-shaped. A low dose irradiation at a high dose-rate (0.5Gy/min) did not cause a significant reduction in mutation frequency. Mutation in the high dose, high dose-rate group was more frequent than in the high dose, low dose-rate group. A dose-rate effect was evident.

When mutant male flies defective in DNA excision repair function were used instead of wild type flies, a low dose irradiation at a low dose-rate did not cause the reduction in the mutation

frequency. These observations suggest that the dose-response relationship is dependent on the DNA repair function. It is inferred that error-free DNA repair functions were activated by a low dose of low dose-rate irradiation and this repaired spontaneous DNA damage rather than the X-ray induced one, thus forming a practical threshold.

(PS2130) Influence of p53 on the induction of mouse skin tumors by repetitive beta-irradiation. Akira Ootsuyama, Ryuji Okazaki, Toshiyuki Norimura. Univ. of Occup. and Environm. Health, Japan, Kitakyushu, Japan.

Introduction: In wildtype ($p53^{+/+}$) mice, p53-dependent and -independent DNA repair mechanisms restore damaged DNA. Irreparably damaged cells are then effectively removed by p53-dependent apoptosis after low dose rate radiation (LDR). Therefore, the teratogenic rate is the same as that of non-irradiated controls. In contrast, only the p53-independent DNA repair mechanism works in knockout ($p53^{-/-}$) mice; p53-dependent DNA repair and apoptosis do not work. So the teratogenic rate does not decrease to a control level in $p53^{+/+}$ mice even at low dose rate irradiation. DNA damage after LDR is thought to be caused by mechanisms similar to those involved in the processes of carcinogenesis and teratogenesis. This study examined whether p53 can remove damage not only via a DNA repair mechanism but also by apoptosis during a radiation-induced carcinogenic process. In $p53^{+/+}$ mice, cancer might not occur if DNA repair and apoptotic removal of damaged cells are efficiently carried out during repetitive LDR. However, the occurrence of cancer in $p53^{-/-}$ mice under the same experimental conditions could reveal a radiation threshold dose in the carcinogenesis process.

Method: The backs of seven-week-old mice ($p53^{+/+}$, $p53^{+/-}$, and $p53^{-/-}$) were irradiated with beta-rays three times a week until a tumor appeared or throughout the life of the mice. A ^{90}Sr-^{90}Y disk delivered a beta ray source of 1.85 GBq at 15 Gy/min. Group I received 2.5 Gy/day, while Group II received 5 Gy/day. The *p53* gene from each tumor was analyzed for mutations and loss of heterozygosity (LOH).

Results: No tumors appeared in any of the $p53^{-/-}$ animals. It seems that life of this mouse will be too short to observe radiation carcinogenesis. However, tumors did occur in the $p53^{+/-}$ mice, with an incidence of 8/21 in Group I and 25/45 in Group II. Tumors were also found in the $p53^{+/+}$ mice; 8/28 in Group I and 6/33 in Group II appeared about 150 days later than those of the $p53^{+/-}$ mice. Of 26 heterozygote tumors examined, 17 exhibited LOH of the *p53* gene but no mutations. In $p53^{+/+}$ mice, 7/9 of tumors had mutations and 1/9 had LOH.

Conclusion: The status of a *p53* gene obviously affects the incidence and time of tumor formation. One of the reasons may be that the types of radiation induced abnormality of p53 gene vary with the status of a *p53* gene.

(PS2131) High relative biological effectiveness of carbon ion radiation on induction of rat mammary carcinoma and its lack of H-*ras* and *Tp53* mutations. Tatsuhiko Imaoka[1], Mayumi Nishimura[1], Shizuko Kakinuma[1], Yukiko Hatano[1], Yasushi Ohmachi[1], Akihiro Kawano[2], Akihiko Maekawa[3], Yoshiya Shimada[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]National Federation of Agricultural Co-operative Associations, Yamaguchi, Japan, [3]National Institute of Technology and Evaluation, Tokyo, Japan.

Purpose: The high relative biological effectiveness (RBE) of high linear energy transfer (LET) heavy-ion radiation has enabled powerful radiotherapy. The potential risk for later onset of secondary cancers, however, has not been adequately studied. We undertook the present study to clarify the RBE of therapeutic carbon ion radiation and molecular changes that occur in the rat mammary cancer model.

Methods and Materials: Seven- to eight-week-old rats of ACI, F344, Wistar and Sprague-Dawley strains were observed until one year of age following irradiation (0.05–2 Gy) with either 290 MeV/u carbon ion radiation with a spread-out Bragg peak (LET 40–90 keV/μm) generated from the Heavy-Ion Medical Accelerator in Chiba (HIMAC) or ^{137}Cs γ-rays.

Results: Carbon ion irradiation significantly induced mammary carcinomas in Sprague-Dawley rats but less so in Wistar, ACI and F344 strains. The dose-effect relationship for carcinoma incidence in Sprague-Dawley rats was concave downward, providing an RBE of 2 at a typical therapeutic dose per fraction, whereas >10 should be considered for radiation protection at low doses. Immunohistochemically, 14 of 18 carcinomas were positive for estrogen receptor α. All carcinomas examined were free of common H-*ras* and *Tp53* mutations. Importantly, lung metastasis (7%) was characteristic of carbon ion-irradiated rats.

Conclusions: We found clear genetic variability in susceptibility to carbon ion-induced mammary carcinomas. The high RBE for carbon ion radiation further supports the importance of precise dose localization in radiotherapy. Common point mutations in *Tp53* and H-*ras* were not involved in carbon ion induction of rat mammary carcinomas.

(PS2132) Combined effects of ionizing radiation and *N*-ethyl-*N*-nitrosourea in murine thymic lymphoma. Shizuko Kakinuma, Yoshiko Amasaki, Kazumi Yamauchi, Mayumi Nishimura, Tatsuhiko Imaoka, Yoshiya Shimada. National Institute of Radiological Sciences, Chiba, Japan.

We are living in the environment with numerous natural and man-made radiation and chemicals. Cancer development in human is considered as a result of interaction with these factors. But, the quantitative assessment and mechanistic understanding of combined effects of radiation and chemical carcinogens are still insufficient. The aim of this study is to elucidate the mode and mechanism of the combined effect of X-rays with *N*-ethyl-*N*-nitrosourea (ENU) on mouse thymic lymphoma, especially at low or threshold dose range.

We previously showed that the dose-response curve for X-ray- or ENU-induced thymic lymphoma had threshold dose. X-ray-induced thymic lymphoma was characteristic for frequent inactivation of *Ikaros* caused by transcriptional silencing, unusual splicing, point mutation and small insertion, most of which were associated with loss of heterozygosity (LOH). In contrast, ENU-induced tumors had point mutations of *Ikaros* without LOH. In combination, X-irradiation followed by ENU exposure resulted in the synergistic effect at high dose, while the effect was antagonistic at low or threshold dose. When the order of exposure was reversed, i.e., ENU followed by X-rays, the mode of combined exposure was additive at any doses. Molecular analysis demonstrated that *Ikaros* mutation in thymic lymphoma after X-irradiation followed by ENU at high doses was predominantly point mutation without LOH (ENU-type). But, after reverse treatment, mutation spectrum of *Ikaros* was similar to that observed in thymic lymphoma after X-ray exposure alone. It is concluded that the mode of combined effect is dependent upon the treatment order and the dose of carcinogens.

(PS2133) Effect of simultaneous of X-rays and *N*-ethyl-*N*-nitrosourea on lymphomagenesis in B6C3F1 mice. Yoshiko Amasaki[1], Shinobu Hirano[2], Shizuko Kakinuma[1], Kazumi Yamauchi[1], Mayumi Nishimura[1], Tatsuhiko Imaoka[1], Yoshiro Kobayashi[2], Yoshiya Shimada[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Department of Biomolecular Science, Faculty of Science, Toho University, Chiba, Japan.

Radiation carcinogenesis in human is considered as a result of the combined effect of radiation and environment factors. We previously showed that the combined effect on the induction of thymic lymphoma after exposure to X-rays and *N*-ethyl-*N*-nitrosourea (ENU) was dependent upon dose of carcinogen and treatment order. Considering our real life, the simultaneous exposure of radiation and chemical carcinogen is the most common case. The aim of this study is to elucidate the mode and mechanism of the combined effect on thymic lymphoma development after simultaneous exposure of X-rays and ENU.

Four-weeks old female B6C3F1 mice were exposed to X-rays (0.2 to 1.0 Gy per week) for 4 consecutive weeks, and the same mice were simultaneously treated with ENU (0, 50, 100, 200 ppm)

in drinking water. Combination of low dose of X-rays (0.2 and 0.4Gy x4) and ENU (50 ppm) did not induce thymic lymphoma. However, in combination of higher dose of X-rays (0.8 and 1.0 Gy x4) and ENU (100 and 200 ppm), the thymic lymphoma development was synergistically enhanced. Molecular analysis demonstrated that the frequency of point mutations of *Ikaros* in the thymic lymphoma after simultaneous exposure was increased (46%) compared with that after single exposure of X-rays (27%) or ENU (24%). Mutation spectrum after simultaneous exposure was not only X-ray-induced or ENU-induced type but also additional new one. It is concluded that combined effect of simultaneous exposure is ascribed to the increased point mutation of *Ikaros*.

(PS2134) Promoter methylation of *Slc* family genes in rat mammary tumors induced by gamma rays or carbon ions. Mayumi Nishimura[1], Tatsuhiko Imaoka[1], Shizuko Kakinuma[1], Yu Yamaguchi[1], Yasushi Ohmachi[1], Satoshi Yamashita[2], Toshikazu Ushijima[2], Yoshiya Shimada[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]National Cancer Center Research Institute, Tokyo, Japan.

Aberrant methylation of CpG islands, which are CpG dinucleotide rich areas located in the promoter region of many genes, serves as a mechanism for inactivation of tumor suppressor genes in many cancers. It is reported that lung tumors induced by plutonium and, to a lesser extent, X-rays showed higher frequency of estrogen receptor (ER) promoter methylation than those induced by NNK, suggesting a close link between radiation exposure and promoter methylation. Recently, *Slc* family is suspected as a new tumor suppressor gene in human colon and breast cancers. In the present study, we examined the status of expression and promoter methylation of *Slc* family genes in radiation (gamma-rays or carbon ions) -induced rat mammary tumors comparing them with spontaneous and chemically (MNU or PhIP) induced ones.

We found up-regulation of *Slc7a1*, *7a5*(*Lat1*) and *16a13* and down-regulation of *Slc1a5*, *7a8*(*Lat2*), *7a10*, *28a2* and *28a3*, suggesting an unbalance of transport of neutral amino acids, purine and pyrimidine. Promoter of *Slc7a10* was found heavily methylated in chemically induced tumors, but, unexpectedly, less methylated in spontaneous and radiation-induced ones. It is suggested that promoter methylation of *Slc7a10* is dependent on the carcinogens in rat mammary tumors. Other *Slc* genes, which contain CpG islands in their promoter, are now under investigation.

(PS2135) Methylation of *SOCS3* and *p15* in carbon-ion-induced thymic lymphomas of B6C3F1 mice. Yoshiya Shimada[1], Shigeko Ebishima[2], Yu Yamaguchi[2], Yoshikazu Kuwahara[1], Shizuko Kakinuma[1], Yoshiko Amasaki[1], Mayumi Nishimura[1], Tatsuhiko Imaoka[1], Yoshiro Kobayashi[2], Yuichi Sato[3]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Department of Biomolecular Science, Faculty of Science, Toho University, Chiba, Japan, [3]Chiyoda Technol Co., Tokyo, Japan.

Transcriptional suppression of genes by promoter hypermethylation is an important mechanism of inactivation of tumor suppressor genes. The aim of this study was to elucidate the distribution of methylation pattern of the genes, *p15* and *Socs3*, which are involved in proliferation of T-lymphocytes, in mouse thymic lymphomas (TLs) induced by high LET-carbon-ions. TLs were induced by carbon-ion irradiation with spread-out Bragg peak with LET of 40–90 keV/um (HIMAC in Chiba) of five-old-female B6C3F1 mice once a week for 4 consecutive weeks. Distribution of methylation was determined by bisulfite treatment followed by sequence analysis. It was found that *p15* was not methylated in normal thymocytes, while it was methylated in 30% of TL cells. The frequency of methylation and expression of *p15* showed inverse correlation. Methylation pattern of *Socs3* in normal thymocytes exhibited high methylation in the 3'-region from starting codon. Methylation status of *Socs3* in TLs was heterogeneous; some TLs showed dense methylation throughout promoter region, while the other TLs showed hypo-methylation. The correlation of methylation pattern and expression pattern was not observed. These results suggest that methylation status differs among genes in TLs, and that it does not always correlate with the expression.

(PS2136) Radioprotection effect and anti-tumor immunity by yeast-derived β-glucan in mice. Yeunhwa Gu. Suzuka University of Medical Science, Suzuka City, Mie, Japan.

Intraperitoneal injection of β-glucan greatly reduces mortality of mice exposed to whole body X-ray radiation and tumor growth in tumor bearing mice. Since the leukocyte and lymphocyte number was increased by a single dose of β-glucan, the radioprotective effect of β-glucan is probably mediated at least in part by a hemopoietic action in irradiated mice. In addition, both of the NK and LAK activity increased significantly by repeated dose of β-glucan. Augmented immunological activity as seen in increased NK and LAK activity by β-glucan seems to play a role in preventing secondary infections associated with irradiation, and to contribute probably to attenuated tumor growth in tumor-bearing mice through enhanced anti-tumor immunity. From these, β-glucan is expected to be promising for the treatment of cancer patients receiving radiotherapy.

(PS2137) Polydrug: A novel concept for mitigation of radiation injury. Mukut Sharma. Medical College of Wisconsin, Milwaukee, WI, USA.

Ionizing radiation causes global injury and certain tissues including the kidneys are especially radiosensitive. Total body irradiation [TBI] results in proteinuria, hypertension and high blood urea nitrogen after 4–6 weeks that lead to end-stage renal disease in a rat model of radiation nephropathy. We have recently identified increased *in vitro* glomerular permeability [P_{alb}] as an early effect of radiation on the kidney. We have used *in vitro* P_{alb} as a test for initial screening of agents for mitigation of radiation injury and to study the early cellular and molecular changes after irradiation.

Our long term goal is to identify the initial effects of radiation that lead to cumulative changes in cellular structures and signaling pathways culminating in organ failure. We have shown that pretreatment with inhibitors of cyclooxygenase [COX], or superoxide dismutase [SOD] mimetics prevents the radiation-induced increase in P_{alb} and, that a nitric oxide [NO] donor protects the glomerular filtration barrier against superoxide. We hypothesize that a combination of these drugs, termed Polydrug, can mitigate increased P_{alb}.

Rats received sham treatment [Control], TBI with a single dose of 10 Gy [Rad] or TBI followed by COX inhibitor indomethacin [5 mg/kg, 1 dose, i.p.], SOD mimetic Tempol [100 mg/kg, 7 doses, i.p.] and NO donor DETA-NONOate [0.04 mg/kg, 7 doses, i.p.] administered once daily [QD] [Rad+Drug]. P_{alb} was determined using an *in vitro* assay.

Polydrug mitigated the effect of TBI on P_{alb}. TBI increased P_{alb} within one hour [Rad 0.64 ± 0.07 *vs.* Control 0.1 ± 0.06, $P<0.001$]. Administration of the Polydrug within 30 minutes after TBI preserved P_{alb} at 1 hour [Rad 0.64 ± 0.07 *vs.* Rad+Drug 0.05 ± 0.07, $P<0.001$] and at 1 day [Rad 0.75 ± 0.08 *vs.* Rad+Drug 0.18 ± 0.08, $P<0.001$]. QD treatment with Tempol and DETA-NONOate [days 2–6] prevented the TBI-induced increase in P_{alb} on day 7 [Rad 0.75 ± 0.09 *vs.* Rad+Drug 0.14 ± 0.12, $P<0.001$].

These preliminary findings demonstrate that a combination of pharmacological agents, each targeting a specific molecular event/pathway, may evolve into a Polydrug to mitigate the effects of TBI on kidney. Such comprehensive approach will permit inclusion of additional drugs as new targets are identified.

(PS2138) Recombinant human epidermal growth factor accelerate the proliferation and migration of the irradiated human fibroblasts in vitro. Sang-wook Lee, Soo Young Moon, Eun Kyung Choi. Asan Medical Center, University of Ulsan, College of Medicine, Seoul, Republic of Korea.

Purpose: To explore the effect of recombinant human epidermal growth factor (rhEGF) on the proliferation and survival of human fibroblasts and keratinocytes following irradiation

Methods and Materials: Fibroblast was originated human skin and primary cultured. The trypan blue stain assay and MTT assay were used to study the proliferative effects of EGF on human fibroblast cell lines *in vitro*. An incubation of fibroblasts with rhEGF for 24 hours immediately after irradiation was counted everyday. Cell cycle distributions were analyzed by FACS analysis.

Results: Number of fibroblast was significantly more increased rhEGF (1.0 nM, 10 nM, 100 nM, 1000 nM) treated cell than control after 8 Gy irradiation. Most effective dose of rhEGF was at 100nM. These survival differences were maintained at 1week later. Proportion of S phase was significantly increased on rhEGF treated cells. Conclusions: rhEGF cause increased fibroblast proliferation following irradiation. We expect that rhEGF was effective for radiation induced wound healing.

(PS2139) A novel somatostatin analogue, SOM230 (pasireotide), increases survival after total body irradiation. Qiang Fu[1], Herbert Schmid[2], Marjan Boerma[1], Xiaohua Qiu[1], Junru Wang[1], Martin Hauer-Jensen[1]. [1]Arkansas Cancer Research Center, Little Rock, AR, USA, [2]Novartis Institutes of Biomedical Research, Basel, Switzerland.

Background: Intraluminal factors, notably exocrine pancreatic secretions, influence the severity of intestinal injury and lethality after total body irradiation (TBI). Surgical removal of the pancreas, pancreatic duct-occlusion, inhibition of intraluminal pancreatic enzymes, and inhibition of the exocrine pancreas with octreotide, a somatostatin receptor 2-selective analogue, reduce post-radiation lethality after abdominal irradiation and/or structural intestinal injury after localized irradiation. We investigated whether a novel somatostatin analogue with broader somatostatin receptor affinity and greater metabolic stability compared to octreotide reduced TBI-induced lethality in mice.

Methods: CD2F1 mice were exposed to uniform TBI in a cesium irradiator. Various doses of SOM230 were administered twice daily by subcutaneous injection, beginning either 2 days before TBI or 4 hrs after TBI. Overall survival, median survival time, hematological parameters, intestinal crypt colony assay, mucosal surface area, and circulating cytokine levels were assessed for up to 30 days.

Results: Administration of SOM230 (480μg/kg bid), starting 2 days before TBI, significantly improved 30-day survival ($p=0.001$) and extended median survival time ($p=0.0006$). While SOM230 administration did not influence hematopoietic injury or recovery or initial intestinal crypt survival, SOM230 administration was associated with highly significant preservation of the mucosal surface epithelium compared to vehicle-treated controls ($p<10^{-6}$). The levels of interleukin 12 were significantly lower in SOM230-treated mice than in controls ($p=0.005$). Importantly, SOM230 administration commencing 4 hrs after TBI also significantly increased survival ($p=0.0004$) and prolonged median survival time ($p=0.0001$).

Conclusion: SOM230 may be a useful medical countermeasure against the intestinal effects of TBI, both in the pre-irradiation situation, as applicable to first responders, rescue workers, and cleanup workers, as well as in the post-irradiation mass exposure mitigation setting.

(PS2140) Influence of endothelin-1 receptor inhibition on functional, structural and molecular changes in the rat heart after irradiation. Marjan Boerma, Junru Wang, Ashwini Kulkarni, Kerrey A. Roberto, Xiaohua Qiu, Martin Hauer-Jensen. University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Background: Radiation-induced heart disease (RIHD) is a potentially life-threatening side effect after radiation therapy of thoracic and chest wall tumors. Mast cells play a predominantly protective role during development of RIHD. Moreover, recent evidence suggests that the endothelin (ET) system mediates the protective effect of mast cells in certain disorders. This study, therefore, examined the effects of combined inhibition of the two ET-1 receptors (ETA and ETB) on molecular, structural and functional aspects of the cardiac radiation response in a rat model of RIHD.

Methods: Male mast cell-deficient (Ws/Ws), mast cell-competent (+/+) and Sprague-Dawley (S-D) rats received localized heart irradiation with a single dose of 18 Gy. Left ventricular mRNA levels of ET-1 and its receptors were measured with real-time PCR in Ws/Ws and +/+ rats 1 week and 3 months after irradiation. S-D rats were treated with bosentan, a combined ETA/ETB antagonist, from 2 weeks before until 6 months after irradiation. At 6 months, cardiac changes were assessed with the Langendorff perfused rat heart preparations, (immuno)histochemistry, and real-time PCR.

Results: There were no differences between Ws/Ws and +/+ hearts in terms of baseline ET-1, ETA, and ETB transcript levels. In contrast, both 1 week and 3 months after irradiation, +/+ hearts exhibited up-regulation of ET-1 and ETA, whereas Ws/Ws hearts did not. Long term administration of bosentan to S-D rats was associated with reduced left ventricular diastolic pressure, developed pressure, and $+dP/dt_{max}$, but did not significantly affect radiation-induced adverse cardiac remodeling or upregulation of TGF-β.

Conclusions: Localized radiation exposure up-regulates ET-1 and ETA in mast cell-competent rats, but not in mast cell-deficient rats, supporting the notion of pathophysiologically significant interactions between mast cells and the cardiac endothelin system in RIHD. Because of the known opposing effects of the two ET-1 receptors, further studies are needed with specific inhibitors of ETA and ETB.

(PS2141) Heat-killed mineral yeast as a potent post-irradiation radioprotector. Kazunori Anzai[1], Nobuo Ikota[1], Megumi Ueno[1], Minako Nyuui[1], Makoto Akashi[1], Tsutomu V. Kagiya[2]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Health Research Foundation, Kyoto, Japan.

Total-body ionizing irradiation of living body causes various deleterious effects depending on the magnitude of the dose. When irradiated at 7.5 Gy, most of mice will die within 30 days post irradiation as a mode of bone marrow death. Since we possibly receive radiation exposure by radiation therapy, nuclear accident, and so on, finding a new protecting agent is desired. In the present study, in vivo radioprotection activity of Zn-, Mn-, Cu-, or Se-containing heat-treated Saccharomyces serevisiae yeast (Zn-yeast, Mn-yeast, Cu-yeast, Se-yeast, respectively) was examined.

The mineral-yeast powder was suspended in a 0.5% methylcellulose solution. The suspension was administered i.p. to C3H mice before or after 7.5 Gy whole body X-irradiation. The 30-day survival of mice without injection of mineral-yeast was about 7%. The survival of mice to which mineral-yeast was injected at 30 min before irradiation was 65–90%. The survival of mice was about 75%, when Se- or Mn-yeast was injected immediately after the irradiation, while it was more than 90% when Zn- or Cu-yeast was injected. Even the yeast sample containing no mineral showed significant radioprotection (70–80%). The Zn-yeast showed radioprotection when injected at 60 min post-irradiation (>80%) and even at 24 h post-irradiation (about 30%). The DRF of Zn-yeast (100 mg/kg, i.p. immediately after irradiation) was about 1.2. When Zn-yeast suspension was separated to supernatant and pellet by centrifugation, the sedimented fraction resuspended again in a 0.5% methylcellulose solution showed high radioprotection activity administered immediately after irradiation. The supernatant fraction also showed radioprotection but the activity was less than the sedimented fraction. Endogenous spleen colony assay showed that injection of Zn-yeast after 7.5 Gy irradiation significantly increased the number of spleen colony at 11 days after irradiation.

Bio-modulation is a possible mechanism for the radioprotection because of following reasons: 1) yeast without mineral is effective, 2) it is effective for the radiation dose of bone marrow death, 3) it is effective when administered post-irradiation. In addition to the bio-modulation, other mechanisms may be involved since Zn- or Cu-yeast showed higher radioprotection than the yeast without mineral.

(PS2142) Phosphorylation and sub-cellular localization of MAPK p38 in the bone marrow cells irradiated *in vivo* and the role of amifostine in these processes. Helena R. Segreto, Celina T. Oshima, Maria Regina R. Silva, Mizue I. Egami, Priscilla B. Carvalho, Vicente P. Teixeira, Roberto A. Segreto. Universidade Federal de São Paulo, São Paulo, Brazil.

The purpose of the present study is to evaluate the activation and sub-cellular localization of MAPK p38 during apoptosis signaling in the bone marrow cells irradiated *in vivo* and the role of amisfotine in these processes.

The animals were assigned in 4 groups: G1 - received physiological saline solution (PSS) intraperitoneally (ip), G2 - amifostine (ip), G3 - PSS + whole body radiation dose of 7 Gy and G4 - amifostine (ip) 30 min before irradiation. All animals were euthanized at 0.5, 1, 2 and 4h after treatment.Bone marrows were submitted to ultra-structural and immunohistochemical study.

It was observed: 1. a better preserved cellularity, less frequency of apoptotic cells, and less frequency of granulocytes (g) and megakaryocytes (m) immunopositive to cleaved caspase-3 in the irradiated amifostine-treated marrows (G4) compared to the untread ones (G3); 2. strong expression of p38 protein in the nucleous and cytoplasm of (g) and (m) in all groups (G1,G2,G3,G4); 3. undetected or faint expression of phosphorylated p38 (p-p38) in the cytoplasm of few (g) and (m) in the non irradiated marrows (G1, G2), strongly positive expression of p-p38 in the cytoplasm of (g) and (m) in irradiated marrows (G3) and negative or weak positivity in the cytoplasm of (g) and (m) in the irradiated amifostine-treated marrows (G4).

Taken together, data showed that: 1. p38 protein is expressed in physiological conditions in the bone marrow cells and that radiation and amifostine did not change its expression or sub-cellular localization; 2. radiation induced p38 phosphorylation and amifostine strongly reduced this activation; 3. p38 activation occurs during apoptosis signaling in the bone marrow cells *in vivo* after a whole body high radiation dose.

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(PS2143) Protective effects of a new herbal composition (HemoHIM) against a gamma-radiation and anticancer drugs. Sung-Kee Jo[1], Hae-Ran Park[1], Uhee Jung[1], Sung-Ho Kim[2], Sung-Tae Yee[3]. [1]Advanced Radiation Technology Institute, Jeongeup Campus of KAERI, Jeonbuk, Republic of Korea, [2]Chonnam National University, Gwangju, Republic of Korea, [3]Sunchon National University, Sunchon, Republic of Korea.

HemoHIM, a herbal composition of three edible herbs (*Angelica gigas* Nakai, *Cnidium officinale* Makino, *Paeonia japonica* Miyabe), was developed to protect the immune, hematopoietic, and intestinal tissues against the radiation. In this study, we investigated the protective effects of HemoHIM in irradiated or cyclophosphamide-treated mice and performed a clinical trial on an alleviation of leukopenia in cancer patients during a cancer therapy. The radioprotective activities were evaluated for whole-body gamma-irradiated mice. The hematopoietic stem cell survival (endogenous spleen colony) and intestinal crypt survival were increased by the HemoHIM administration ($p<0.05$). The number of lymphocyte ($p<0.05$), and the T cell activity ($p<0.05$) and NK cell activity ($p<0.05$) were also restored by the HemoHIM administration. HemoHIM reduced the fatigue level of the irradiated mice in a weight-loaded swimming test ($p<0.05$). The radioprotective properties of HemoHIM were finally evidenced by a 2-fold increase of the 30-day survival rate and by a decrease of tumor incidence at 60 weeks after an irradiation. Also, the protective effects on the immune system in the cyclophosphamide-treated mice were investigated. The administration of HemoHIM restored the white blood cell and lymphocyte numbers ($p<0.05$) as well as the NK cell function ($p<0.05$). HemoHIM also showed normalizing effects for the Th1/Th2 immune balance. In a preliminary clinical study, 85 patients diagnosed as breast or uterine cervix cancer were administered with HemoHIM (6 g/day) for 12 weeks during radiation and/or chemotherapy. In the HemoHIM adminstration group, fewer cases of severe leukopenia (< 3000 leukocytes/mm^3) were shown when compared with the control group, and it was more evident in the breast cancer patients. In conclusion, HemoHIM reduced the self-renewal tissue damage conferred by the radiation or cyclophosphamide in the mice and produced some relevant results from a preliminary clinical study. Based on these results, HemoHIM was approved as an immunomodulator for a Health Dietary Supplement by the Korea FDA. Further clinical studies are planned for the application of HemoHIM as an adjunctive agent for cancer therapy.

(PS2144) Histone deacetylase inhibitors reduce lethality following total body irradiation. Stephen L. Brown, Andrew Kolozsvary, Jianguo Liu, Jae Ho Kim. Henry Ford Hospital, Detroit, MI, USA.

These studies were designed to experimentally assess the potential of histone deacetylase inhibitors, HDACi, to reduce lethality from total body irradiation. Groups of Balb/c mice were exposed to 6.5 or 7.0 Gy alone or in combination with HDACi, either valproic acid (400 mg/kg i.p. administered either 24h before or 1h after radiation exposure) or trichostatin A (0.5 mg/kg, i.p. administered 1h after radiation exposure). Survival was significantly increased in groups of mice receiving HDACi ($p<0.05$). Particularly compelling was the increase in survival when HDACi followed the radiation exposure by 1h. Acetylated histone H3 was confirmed using Western blot analyses in samples of blood and spleen from mice treated with valproic acid and trichostatin A. Leukopenia and erythropenia were significantly improved in groups of mice receiving HDACi in combination with radiation compared to that in mice receiving radiation alone. Similarly, spleen weights in mice that were reduced following radiation alone exposure were significantly increased ($p<0.05$) in mice receiving HDACi plus radiation. Because HDACi have been shown to enhance the self-renewal of bone marrow hematopoietic stem cells, we examined the effect of HDACi on radiation-induced stem cell colony formation using an indigenous spleen colony forming assay. Ten days after sublethal irradiation plus HDACi (400 mg/kg valproic acid, i.p. 1h after x-rays), the number of spleen colonies was 7 fold higher compared to a similar group of mice exposed to radiation alone. The results indicate that HDACi is a new class of radiation protector against total body irradiation capable of significant radiation mitigation even when administered after the exposure.

(PS2145) Radioprotective effects of recombinant human epidermal growth factor (rhEGF) in C3H/HeJ mice. Hae Jin Oh, Won Woo Kim, Sook In Chung, Jinsil Seong. Department of Radiation Oncology, Brain Korea 21 Project for Medical Science, Yonsei University Medical College, Seoul, Korea.

Object

In order to investigate radioprotective effects of recombinant human epidermal growth factor (rhEGF) on radiation-induced mucosal damage in C3H/HeJ mice.

Materials and Methods

The radiation damage model was established that C3H/HeJ mice expose to a single dose whole body irradiation of 8 Gy, 10 Gy. The groups of treatment model were divided into 4 groups: control, 10 Gy irradiation alone group, rhEGF alone group, combination group of rhEGF and radiation. The rhEGF was administered 100 μg/kg intraperitoneally on Day 1, 2, 3 and 3.5. Histologic examination was performed with H&E stain in jejunal mucosa. Radiation-induced apoptosis was determined in each group with the Apoptag kit: DNA terminal transferase nick-end labeling method. Tissue sections were evaluated for PCNA expression by immunohistochemical stain.

Results

In the radiation damage model, the 8 Gy irradiated groups statistically had less weight loss compared to the 10 Gy irradiated group. The number of crypt cells was greatly decreased at 24h after 10 Gy in jejunum crypt by H&E stain. Apoptosis index of jejunum crypt in 10 Gy irradiated group was significantly increased at 24h after irradiation. In the treatment model, the combination group was showed significantly improvement the reduction of weight loss and the number of radiation-induced apoptosis compared with 10 Gy irradiated group.

Conclusion

Our results suggest that rhEGF protects radiation-induced mucosal damage in C3H/HeJ mice. Improved recovery of normal tissue and reduced number of radiation-induced apoptosis may be possible mechanism of radioprotective effect of rhEGF.

(PS2146) Keratinocyte growth factor (Palifermin) accelerates the radiation-induced up-regulation of integrin linked kinase in oral mucosa (mouse) during daily fractionated irradiation. Bettina Habelt, Margret Kuschel, Wolfgang Doerr. Med. Faculty Carl Gustav Carus, Dresden, Germany.

The early radiation response of oral mucosa is a severe and often dose-limiting side effect of radiotherapy for advanced head-and-neck tumours. Repopulation, involving a complex reorganisation of the proliferative processes in the epithelium, occurs in response to daily fractionated irradiation with a delay of about 1 week after the first fraction. These processes result in an increase in mucosal radiation tolerance with increasing overall treatment time. The present study was initiated to determine changes in the expression of Integrin Linked Kinase (ILK) during daily fractionated irradiation, and their modulation by administration of Keratinocyte Growth Factor. ILK links integrins with growth factor receptors and thus modulates intracellular signal transduction. Changes in ILK hence may contribute to the regulation of the repopulation processes.

Daily fractionated irradiation with 5×3 Gy/week (200 kV X-rays) was given to the snouts of the animals over a total of 2 weeks. In an additional experimental arm, Palifermin (*delta*N23-KGF) was administered as a single injection of 15 mg/kg at the day before the first fraction. Groups of 3 mice per day were sacrificed from day 0 to 16, and the tongues were processed for immunohistochemistry. ILK expression was analysed semi-quantitatively using an arbitrary score for the staining signal.

Compared to un-irradiated controls, an increase in the expression of ILK was found at the end of the first treatment week, i.e. in coincidence with the onset of repopulation. Administration of Palifermin on day −1 resulted in an almost immediate increase in ILK expression already on day 0, which remained elevated during the entire first week of irradiation, before a return to control values at the beginning of week 2.

In conclusion, the expression of ILK in oral mucosa increases during fractionated irradiation. These changes are seen at the time of onset of effective repopulation. This indicates a regulatory role of this protein in the mucosal regeneration response. The earlier stimulation of ILK expression by KGF suggests that this growth factor modulates the intracellular signal transduction via this pathway, eventually resulting in increased mucosal tolerance to fractionated irradiation.

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(PS2147) Mitochondrial targeting of a catalase transgene product further increases radioresistance induced by MnSOD overexpression in 32Dcl3 murine hematopoietic progenitor cells. Michael W. Epperly[1], J Andres Melendez[2], Xichen Zhang[1], Darcy Franicola[1], Tracy Smith[1], Joel S. Greenberger[1]. [1]University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA, [2]Center for Immunology & Microbial Disease, Albany Medical School, Albany, NY, USA.

Mitochondrial localization of the radioprotective MnSOD transgene in hematopoietic cells dismutates irradiation induced superoxide to hydrogen peroxide which is converted to water and oxygen by catalase or glutathione peroxide. Increased concentration of this hydrogen peroxide product can be cytotoxic. We hypothesized, that increased mitochondrial localized catalase to remove hydrogen peroxide would further increase radioresistance. The human catalase transgene was cloned into a pSVZeo plasmid. To localize the transgene product catalase to the mitochondria, the mitochondrial localization sequence of MnSOD was cloned and attached to the catalase transgene (mt-catalase) and then cloned into a pSVZeo plasmid. The plasmids were electroporated into 32Dcl3 and MnSOD transgene overexpressing clonal cell line 2C6 and subclones of each expressing the non-targeted catalase or mt-catalase were selected by growing the cells in neomycin. The clonal cell lines were shown to express either catalase or mt-catalase by RT-PCR using specific primers. Then 32Dcl3, 32D-cat and 32D-mt-catalase cells were irradiated to doses ranging from 0 to 8 Gy, plated in methycellulose, incubated at 37°C for seven days, and colonies of greater that 50 cells counted. The data was analyzed by linear quadratic and single-hit, multi-target models. The 32D-mt-cat cells were more radioresistant than 32D-cat as seen by an increased shoulder on the survival curve ($n = 10.3 + 0.5$ or $5.9 + 0.2$, respectively, $p = 0.0025$). Both 32D-mt-cat and 32D-cat were more resistant compared to 32Dcl3 cells ($n = 2.9 + 1.1$, $p = 0.0196$ or 0.0479). Next, 2C6 cells were transfected with the same catalase or mt-catalase plasmids, subclones selected, and irradiated. Cells from 2C6 transfected with mt-catalase, but not catalase, showed increased radioresistance increasing the Do from $0.979 + 0.1$Gy for 2C6 to $1.171 + 0.1$ Gy for 2C6-mCat cells. Thus, overexpression of both MnSOD and mt-catalase transgenes is superior to one alone for radioprotection.

(PS2148) Radioprotective mechanisms by a new chemical entity ON01210 (Ex-Rad™) in HUVEC cells. Sanchita P. Ghosh[1], Michael W. Perkins[1], Kevin Hieber[1], Stephen C. Cosenza[2], M.V. Ramana Reddy[2], E. Premkumar Reddy[2], Manoj Maniar[3], Alan Alfieri[4], Thomas Seed[5], K. Sree Kumar[1]. [1]AFRRI,USUHS, Bethesda, MD, USA, [2]Fels Institute for Cancer Research and Molecular Biology, Philadelphia, PA, USA, [3]Onconova Therapeutics Inc., Lawrenceville, NJ, USA, [4]Albert Einstein College of Medicine and Montefiore Med. Ctre, Bronx, NY, USA, [5]RERF, Hiroshima, Japan.

A new chemical entity, ON01210 (Ex-Rad), which was synthesized by Onconova Therapeutics Inc., significantly protected mice against lethal gamma irradiation when administered subcutaneously 24 h before and 15 min before irradiation at a dose of 500 mg/kg of body weight. *In vitro* experiments with primary human umbilical vein endothelial cells (HUVEC) were done to examine the role of apoptosis in radiation injury and protection by Ex-Rad. Exponentially growing HUVEC cells were incubated with various concentrations of Ex-Rad. Twenty-four hours later they were irradiated to ^{60}Co at doses ranging from 2 to 10 Gy at a dose rate of 0.6 Gy/min. Cytotoxicity was evaluated by WST assay and Ex-Rad was found non-toxic at all concentrations studied (10–100 μM). Ex-Rad was found to protect irradiated cells as determined by clonogenic assay. Fourteen days after irradiation, surviving colonies were stained, counted and compared to unirradiated cells. Apoptotic cell death was determined by Annexin V/propidium iodide double staining and subsequent fluorescence activated cell sorting (FACS) analysis. Ex-Rad protected HUVEC from γ-ray induced apoptosis 72 h after radiation. To determine the radioprotective mechanism, expression of several genes including p53, p21, p73, Bax, Gadd45a, and NFkappaB were studied by western blot. After irradiation, p53, the tumor suppressor protein, is activated, this, in turn activates p73. Both p53 and p73 activate a number of genes, such as p21 and Gadd45, that trigger protective cell cycle arrest and DNA repair. When the damage is irreparable, apoptosis occurs through transactivation of Bax, and the subsequent release of cytochrome C from mitochondria. Whole cell extracts were made 4, 24, and 72 h post radiation from Ex-Rad treated and untreated cells. Maximum expression was found 24 h post radiation at 10 Gy for most of the genes, and expression was found to be reduced in Ex-Rad treated cells. The aggregate of these results suggest that Ex-Rad radioprotects, at least in part, through inhibition of apoptosis.

(PS2149) Carbon monoxide protects the immature mouse hippocampus from radiation-induced apoptosis. Glenn T. Gobbel[1], Kotaro Nakaya[2], Sait Sirin[1], Leo E. Otterbein[3], John C. Flickinger[1]. [1]University of Pittsburgh, Pittsburgh, PA, USA, [2]Tokyo Women's Medical University, Tokyo, Japan, [3]Harvard University, Boston, MA, USA.

Cognitive deficits are common following therapeutic brain irradiation and are particularly severe in children. The exact pathophysiologic basis of these deficits is unknown, and there is a need for animal models to investigate the underlying cause of such deficits and to develop methods to ameliorate the injury. In the present study, we delineated the effects of irradiation on the immature mouse brain and tested whether a novel intervention, pre-treatment with carbon monoxide (CO), could inhibit radiation-induced injury. Postnatal day (PND) 1, 7, 14, and adult (PND 30–60) mice were treated with 0 or 8 Gy of whole brain irradiation, and their brains were examined for the presence of apoptotic cells 9 hrs later by the TdT-UTP nick end-labeling (TUNEL) method. In the hippocampus, a brain region with a key role in learning and memory, there was extensive apoptosis within the CA1, CA3, and dentate gyrus regions of the PND1, 7, and 14 animals but not in the adults. Evaluation of animals 2 weeks after 8 Gy of whole brain irradiation on PND14 revealed an ~35% decrease in the volume of the dentate gyrus relative to unirradiated controls, showing that radiation damage results in prolonged effects on the hippocampus. CA1 and CA3 regions were unchanged. CO was then evaluated for its ability to inhibit radiation-induced hippocampal apoptosis in vivo. Preliminary studies had shown that exposure to CO could block radiation-induced apoptosis of cells growing in vitro. Immature mice were placed in a chamber containing well-tolerated levels of CO (250 ppm) for 2h followed immediately by treatment with 0–8 Gy of whole brain irradiation. Pre-treatment with CO significantly reduced apoptosis throughout the hippocampus by 30–60%. These results suggest that the immature hippocampus is particularly sensitive to radiation. The hippocampal sensitivity is prolonged during postntatal development in comparison to other brain regions, such as the neocortex, in which levels of apoptosis following irradiation are very high in PND1 animals but much lower at PND7 and beyond. Methods that effectively block apoptosis, such as administration of CO, will help to determine whether radiation-induced apoptotic cell death contributes to long-term hippocampal damage and cognitive deficits.

(PS2150) The role of gap junctions on irradiated htori3-llu cells +/- radioprotector. Virginia G. Serra[1,2], Leticia Ortloff[1], Pinal Pandya[1], Anil Kulkarni[3], Lora Green[1]. [1]Loma Linda University, Loma Linda, CA, USA, [2]Radiation Genome & Stabitlity Unit Medical Research Council, Didcot Harwell, United Kingdom, [3]The UT Medical School at Houston, Houston, TX, USA.

We addressed the question of how gap junction competence at the time of irradiation would affect the efficacy of a radioprotector. In this particular study our investigational endpoints for judging the radioprotector (Nucleotide T; NT) were; chromosomal damage, apoptosis and clonagenic survival under defined conditions of gap junctional status in human thyroid cells (HTORI3-LLU) plus/minus gamma-irradiation.

The formation of micronuclei, apoptosis and survival plus/minus the optimum concentration of NT (12μM) were measured at times appropriate for the various endpoints following exposure to doses of γ-rays that ranged from 0 to 7 Gy. The micronucleus assay was conducted using cytochalsin B and scoring a minimum of 500 binucleated cells per condition in duplicate. Cell survival was assayed by two different methods: cells were plated either at a low or high confluence to yield gap junction-incompetent/competent cells, respectively, and colonies counted. Both survival methods were conducted with and without added NT and Heptanol (3mM), the latter used to down-regulate gap junctions prior to irradiation.

Micronuclei and apoptosis levels increased linearly as a function of radiation dose. The cells treated with NT were protected with respect to having a decreased frequency of micronuclei, although the apoptotic levels were unaffected by the addition of NT. However, at 7Gy there was no sparing effect from the added NT. There was measurable toxicity from the combined treatment such that damage at 7Gy outweighed any protection the presence of NT could impart. When cells were cultured to be gap junction incompetent (down-regulated junctions), either as a result of an initial low plating density, or when at a high density treatment with 3mM heptanol, survival rate was higher than gap-junction competent cells. This data suggests that in these cells decreasing cell-cell communication was protective in terms of clonagenic cell survival.

In conclusion, we have found that this radioprotector can reduce chromosomal damage, but did not protect cellular survival in gap-junction incompetent cells. We are currently conducting experiments to reassessing these endpoints using different ways to modulate gap junctional status.

(PS2151) Synthetic FGF2 peptide mitigates gastrointestinal radiation damage. Lurong Zhang[1], Weimin Sun[1], Louis Pena[2], Jianjun Wang[1], Shanmin Yang[1], Shanmin Yang[1], Hengshan Zhang[1], Wei Wang[1], Mei Zhang[1], Chaomei Liu[1], Paul Okunieff[1]. [1]Department of Radiation Oncology, Rochester, NY, USA, [2]Brookhaven National Laboratory, Upton, NY, USA.

In a previous study, we found that the native full-length FGF2 (fibroblast growth factor 2) used before or 4 hour after radiation could prevent/mitigate the gastrointestinal radiation damage. However, the full-length protein is unstable and expensive. We have synthesized a functional fragment of FGF2 (namely, FGF-P), which is very stable in various severe conditions. A panel of studies has been carried out to determine the utility of this novel biological agent with an emphasis on mitigation (use after radiation exposure).

Results: (**1**) FGF-P bound to FGF receptors with a high affinity and triggered the down-stream signal pathways in a manner similar to native protein. (**2**) When delivered by ip, po, or im routes, im injection was practical and effective. (**3**) Among a range of FGF-P doses tested, the lowest effective dose could be 0.5mg/kg. (**4**) When subtotal body radiation was given BALB/c at 10.5 Gy, 80–100% mice treated with vehicle alone died within 7 days while 66–83% mice that had treated with 0.5mg/kg FGF-P for 5 doses (10 min, day 1, 2, 3, and 4 after radiation) survived. And (5) the crypt assay and *in vivo* BrdU incorporation assay assessed at 3.5 days post-radiation indicated that FGF-P stimulated GI stem cell proliferation.

Conclusion: FGF-P is stable and can be delivered intramuscularly after irradiation exposure leading to long-term survival of some animals that would otherwise die of a GI syndrome.

(PS2152) Single injection of novel radioprotectant CBLB502 significantly increases survival of lethally irradiated non-human primates. Vadim Krivokrysenko[1], Farrel Fort[1], Eugenia Strom[1], Andrei Osterman[2], Ludmila Burdelya[3], Thomas Tallant[3], Natalia Tararova[1], Ratan Maitra[1], Joseph DiDonato[3], Andrei Gudkov[1], Elena Feinstein[1]. [1]Cleveland Biolabs, Cleveland, OH, USA, [2]Burnham Institute, LaJolla, CA, USA, [3]Cleveland Clinic Foundation, Cleveland, OH, USA.

Protection of the human organism from ionizing radiation (IR) is a key problem in nuclear safety, radiation therapy, and space travel. Toxicity of ionizing radiation is associated with induction of massive apoptosis in radiosensitive organs. We tested the ability of novel compound, CBLB502, to protect mice and monkeys from lethal irradiation. CBLB502 is a rationally designed derivative of *Salmonella enterica* flagellin that promotes cell survival through interaction with toll-like receptor 5. In a large series of experiments in mouse models of hematopoietic and gastrointestinal radiation injury, it demonstrated strong radioprotection potency with very low toxicity, displaying DMF (dose modifying factor) of 1.5–1.8. The drug is also effective when administered at least up to 2 hours following lethal irradiation. While protecting mice from lethal irradiation, CBLB502 had no effect on radiosensitivity of tumor cells *in vitro* and *in vivo*, suggesting its use for reducing side effects of cancer treatment.

Single-dose i.m. injection of 0.04 mg/kg CBLB502 as a monotherapy increased survival of rhesus monkeys irradiated with 6.5 Gy (LD_{70}) dose of whole-body gamma irradiation from 25% in vehicle control group (n=8) to 64% (n=11). CBLB502 reduced the severity of damage of spleen, thymus and bone marrow as well as reduced the scale and duration of thrombocytopenia and neutropenia. We conclude that CBLB502 is a potent and nontoxic radiation countermeasure that has great potential as a life-saving treatment.

(PS2153) Radioprotective effect of hypothermia on the blood system cells in mammals. Andrei V. Rodionov, N.A. Karnaukhova, L.A. Sergievich, D.A. Ignat'ev, V.N. Karnaukhov. Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Russia.

The aim of this investigation was to study the radioprotective effect of hypothermia on the immune and hemopoietic systems.The functional (synthetic) activity of blood lymphocytes, thymocytes and bone marrow nucleated cells of rats cooled to 17–18°C under hypoxia-hypercapnia has been investigated by fluorescent microspectrometry. The rats were exposed to acute γ-irradiation at a dose of 8Gy. The blood was taken in 2 hours, 24 hours and on day 4 after irradiation. The total numbers of leukocytes, lymphocytes, erythrocytes and bone marrow nucleated cells were estimated. The irradiation caused a heavy form of the hemopoietic syndrome: the functional inactivation and cell death were observed. The lymphocyte synthetic activity (parameter alpha) decreased by 28% in normothermic and by 12% in hypothermic rats on day 1. The thymus in normothermic rats was completely depleted on day 4. In hypothermic animals the depletion was lower, cells with a high synthetic activity were found. 2 hours after irradiation, the parameter alpha decreased sharply by 30% in the bone marrow nucleated cells of normothermic rats and by 18% in hypothermic rats; at the same time the total number of nucleated cells reduced by 8% and 5%, respectively. At day 4 after irradiation, less than 10% bone marrow cells remained in both animal groups, but in hypothermic rats the total number of nucleated cells was higher by a factor of 4. The data indicate that the hypothermia is a radioprotective factor in blood system cells thus enhancing the resistance of organism to radiation. The radioprotective effects of hibernating ground squirrels (natural hypothermia) and hypothermic rats (artificial hypothermia) are compared. The results of the study may be of importance for experimental practice and medicine, radiotherapy, cosmic biology.

(PS2154) Radiation protection by toll like receptors (TLR) ligands and small molecules. Damodar Gupta, Andrei Gudkov. Cleveland Clinic, Cleveland, OH, USA.

Introduction: The development of radioprotectors has been the subject of intense research in view of their potential in a radiation environment such as radiotherapy, space exploration and even nuclear war or accidents. However, no ideal radio-protectorsare available to date. Therefore a systematic screening approach was used to identify compounds (from microbes and small molecules) which alter mitochondrial functions and activate NFkβ. These agents were further used to study radioprotection in both *in vivo* and *in vitro* model systems.

Methods: Radioprotection by TLR ligands (CBLB 601 for TLR 2/6, CBLB 502 for TRL5) or small molecule (RPT5–2) was measured *in vivo*. To study the mechanism of radiation protection by these agents, *in vitro* studies were performed on immortalized urothelial (NUAA) or normal kidney epithelial (NKE) cells. Cells were exposed to increasing doses of γ-radiation (dose rate 245R/min) and %survival was measured with respect to control. Time kinetics (−15min to −90min) studies were done for measurement of radioprotection by different agents following LD90 radiation dose (NKE; 3Gy and NUAA; 5.45Gy). Changes in superoxide generation and mitochondrial membrane potential (MMP) were measured using a flow cytometer with dihydroethidium and DiOC6(3) dyes respectively.

Results: Pre-irradiation treatment of NKE cells with non toxic concentrations of either CBLB 601 (−15min) CBLB 502 (−45min) or RPT5–2 (−30min) protects over 65%, 55% and 75% cells respectively with a 15–30 min time window. For NUAA cells, it was over 45%, 40 and 55% in case of either CBLB 601 (−15 to 45min) CBLB 502 (−45 to 60min) or RPT5–2 (−30 to 45min). Cells treated with CBLB 502 showed the maximum decrease in MMP and superoxide radical generation at 45min followed by a gradual increase with time that reached normal levels after 3 hrs, which suggests the importance of mitochondrial activity inhibition in offering radio-protection by the CBLB 502.

Conclusion: Alterations in the mitochondrial redox balance using TLR ligands or RPT5–2 played an important role in radiation protection. However, further *in vitro* and *in vivo* studies are needed to clarify their mechanisms of action and possible interactions. This technique can also be used to screen and identify the time window and concentrations of agents for their radio-protective efficacy.

(PS2155) Postirradiation dynamism of intratumoral HIF-1 activity; Balance of degradation and hyper-translation of HIF-1α protein. Hiroshi Harada, Satoshi Itasaka, Shinae Kondoh, Masahiro Hiraoka. Kyoto University, Kyoto, Japan.

Tumor microenvironment is dramatically altered after ionizing radiation. Hypoxia-inducible Factor-1 (HIF-1), in response to the alteration, promotes tumor radioresistance by inducing various gene expressions. To better understand the postirradiation dynamism of intratumoral HIF-1 activity and underlying molecular mechanism, we established a subcutaneous HeLa tumor xenograft with a novel *5HREp-ODD-luc* reporter gene, by which HIF-1 activity was monitored as bioluminescence in real-time. The molecular-imaging analyses revealed that, after irradiation, intratumoral HIF-1 activity was dramatically decreased in response to improved oxygen supply to hypoxic cells (re-oxygenation). The HIF-1 activity turned to increase, although the tumor was still re-oxygenated. The increase was suppressed by administration of PI3K/Akt/mTOR pathway-inhibitors, LY294002 or rapamycin, or of a non-metabolizable glucose analog, 2-deoxy-D-glucose. *In vitro* studies confirmed that addition of glucose just at re-oxygenation treatment up-regulated HIF-1α translation in PI3K/Akt/mTOR-dependent manner. These results suggest that, after irradiation, intratumoral HIF-1 activity once decreases *via* re-oxygenation-induced HIF-1α degradation, and then increases *via* glucose-dependent HIF-1α hyper-translation through PI3K-AKT-mTOR pathway. This is the first report indicating that postirradiation alteration of glucose- as well as hypoxia-microenvironment affects intratumoral HIF-1 activity.

(PS2156) Cytotoxic and cytoprotective signaling pathways mediated by reactive oxygen and nitrogen species. Takanori Katsube, Masahiko Mori, Hideo Tsuji, Tadahiro Shiomi, Makoto Onoda. National Institute of Radiological Sciences, Chiba-shi, Japan.

Reactive oxygen and nitrogen species (RONS) are generated within cells by ionizing radiation via primary ionizing events as well as through secondary amplification systems including metabolic synthesis. Their high reactivities to a variety of macromolecules within cells not only cause dysfunction of the target molecules but also modulate intra- and inter-cellular signal transduction pathways. Hence, RONS are implicated directly and/or indirectly to play a crucial role in biological effects of ionizing radiation. Extensive studies concerning the inflammatory responses have shown that cellular effects of RONS are much complicated and either detrimental or protective, probably depending on the amount, duration, and site of generation as well as the type of species. To elucidate the molecular mechanisms of RONS in both inflammatory and radiation responses, we examined cellular responses to the relatively low concentrations (lower than 100 μM) of exogenous H_2O_2 and NO.

A human intestinal epithelial cell line, Caco-2, were grown on permeable supports and exposed to H_2O_2 and/or NO donors, such as NOC5 and NOC12. A H_2O_2 treatment induced tyrosine phosphorylation of numerous cellular proteins including ZO-1, E-cadherin, and ß-catenin, components of cell-cell junctions, and exhibited several remarkable features of cell-cell junctional dysfunction, such as an increase in paracellular permeability and disturbance of morphological architecture of cell-cell junctions. On the other hand, a combined treatment with H_2O_2 and an NO donor suppressed the protein tyrosine phosphorylation and relieved the damage to the cell-cell junction. These findings suggest the presence of two distinct intracellular signalling pathways for detrimental and protective events mediated by H_2O_2 and NO, respectively. The latter pathway somehow might attenuate the former.

We will also show the latest findings with regard to the effects of H_2O_2 on radiosensitive mutant cell lines. Targeted disruption of repair genes, such as XRCC4 and Artemis, for DNA double-strand breaks (DSBs) sensitized a human intestinal cancer cell line,

HCT116, to ionizing radiation as well as H_2O_2. Mechanisms underlying DSBs induction by relatively low concentrations of H_2O_2 (around 10–40 μM) are now under investigation.

(PS2157) Radiation-induced oxidative stress in lungs of mice knocked-out for genes involved in inflammatory processes. Carine Laurent[1], Wen-Chen Yeh[2], Richard P. Hill[2]. [1]DSM/CEA-IN2P3/CNRS, LARIA-CIRIL-GANIL, Caen, France, [2]Ontario Cancer Institute, Toronto, ON, Canada.

The purpose of the study was to investigate the involvement of IL-1 and TNF-α pathways in radiation-induced lung damage via the production of oxidative stress in mouse lung. Mice C57BL6/J knocked-out for specific genes (TNFR1, MyD88, TRIF, IRAK-4) involved in the induction of inflammatory processes were exposed to X-rays to the whole thorax. Right and left lungs were removed and dissociated. Micronuclei (MN) levels, antioxidant enzymes activities, lipid peroxidation products were assessed as a function of time up to 24 weeks after irradiation (PI). TNF-alpha and TGF-beta protein content were measured. MN levels were increased early in IRAK-4, TRIF and TNFR1 deficient mice. Wild-type (WT) mice showed a persistent high MN frequency before decreasing from 12 weeks PI without reaching control value at 24 weeks PI. TNFR1 knocked-out mice demonstrated a higher level of MN until 8 weeks PI. Lipid peroxidation products were not increased. SOD activity was strongly increased at early times only in left lung of WT mice. At later times, SOD was decreased in WT mice whereas it increased in TNFR1 KO mice. Catalase activity was not significantly changed, only a slight increase was observed with time following the pattern of lipid peroxidation products. TNF-alpha content was increased in left lung of WT mice whereas TGF-beta was increased in right lung of both types of mice. Genistein treatment (12 mg/kg) given immediately after irradiation resulted in a strong decrease in MN frequency that persisted to at least 1 week PI. The exposure of mouse lungs to radiation led to a prolonged expression of high levels of DNA damage (MN). Much of this damage was produced at early times PI as shown by the effect of genistein treatment, although these studies do not rule out regeneration of DNA damage at greater than 1 week PI. The results with the knock out mice imply a role for other pathways in the induction of the DNA damage although the increased damage associated with the TNFR1 knock out suggests that this gene product may provide a protective effect. Further studies are required to address this issue. Interestingly no change in lipid peroxidation was observed over time (24 weeks PI) despite the results for DNA damage. The left lung appears to play a more important role in inflammatory processes as demonstrated by the increase in TNF-alpha content and in SOD activity.

(PS2158) Gene expression profiles of MCF-7 cells under hypoxic condition. Chin-yu Lin[1], Mong-Hsun Tsai[2], William DeGraff[3], James B. Mitchell[3], Eric Y. Chuang[4]. [1]Medical department of National Taiwan university, Taipei, Taiwan, [2]Institute of Biotechnology, National Taiwan university, Taipei, Taiwan, [3]Radiation Research Branch, NIH, Bethesda, MD, USA, [4]Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei, Taiwan.

Hypoxia is well known as a consequence of the development of a solid tumor. Besides, hypoxia also promote cancer survival by exerting direct effects on the expression of genes related to processes such as angiogenesis, glycolysis, apoptosis, cell-cycle control, and signaling transduction which are central to the survival of a tumor cell population in hypoxia. To understand the regulation of molecular mechanisms and functional pathways resulting from hypoxia is essential when elucidate how solid tumor survives in hypoxia and might also provide the necessary infrastructure to develop novel ways to treat tumors. In this study, we investigated gene expression profiles of MCF-7 (breast cancer cell line) cells under hypoxic condition by using cDNA microarray analysis. In our experiment, total RNA samples were collected at 0, 4, 8, 24, 48 hour after exposed to hypoxia (1% O_2, 5% CO_2, and balanced N_2). Human 8k cDNA chips were used for microarray analysis. After unsupervised hierarchical clustering, 629 genes were identified with significant changes over the time course (> two-fold changes). Based on gene ontology analysis, these genes were further classified into several biochemical pathways and functional categories. Most of our results were compatible with previous studies. Moreover, we found genes which were seldom published with association to hypoxia. These genes include up-regulated genes involved in anti-NK cell cytotoxicity (HLA-A, HLA-B) and actin-filament-related gene (FSCN1), and down-regulated genes involved in NK cell cytotoxicity (CD48, TNFRSF10C, IL12A), WNT pathway (WNT2, WNT11, SMAD2, CCND2), and tumor suppressor genes (SFN, WOX1, NGFR, PPP2R1B, etc). Our preliminary results may suggest that MCF-7 cells under hypoxia can escape from NK cell cytotoxicity through inducing HLA expression. Hypoxia can down regulate tumor suppressor genes to help MCF-7 survival. Finally, FSCN1 overexpression and down-regulation of β-catenin in hypoxic condition may promote the ability of metastasis in MCF-7 cells.

(PS2159) Regulation of mRNA translation is a major contributor to hypoxia regulated gene expression. Twan van den Beucken, Marianne Koritzinsky, Michael Magagnin, Renaud Seigneuric, Philippe Lambin, Bradly G. Wouters. University of Maastricht, Maastricht, The Netherlands.

The majority of tumors contain poorly oxygenated areas. This is important because hypoxia is associated with poor prognosis and implicated in promoting malignancy. The cellular effects of hypoxia are mediated in large part by changes in gene expression, which are thought to arise primarily from a robust transcriptional response. However, we and others have shown that hypoxia can also affect protein expression by regulating mRNA translation. Translation is rapidly inhibited by hypoxia due to activation of the unfolded protein response (UPR) and phosphorylation of eIF2α. After longer exposure, the UPR subsides but translation remains suppressed due to disruption of the mRNA cap binding complex eIF4F. At present, it is unclear to what extent regulation of translation contributes to overall changes in gene expression during hypoxia. We addressed this by analyzing both transcriptional and translational changes on a genome wide scale in human carcinoma cell line DU145. Affymetrix gene chips were used to track changes in total mRNA and in the subset of actively translated mRNA associated with polysomes following exposure to 1, 2, 4, 8, 12, 16, 24 hours of hypoxia. This allowed determination of the transcriptome (mRNA abundance), translatome (efficiently translated mRNA) as well as the translation efficiency of all hypoxia regulated genes as a function of time. We found that the translatome consists of a set of 2714 up-regulated and 1898 down-regulated genes. In comparison, the transcriptome consists of a substantially smaller set of 1467 induced and 913 repressed genes. The contribution of changes in translation efficiency to the translatome varies strongly with time and is maximal at early time points where more than 40% of the top 200 induced genes exhibit a >2-fold increase compared with the global average. Preliminary sequence analysis demonstrated enrichment of mRNAs containing upstream open reading frames in these transcripts. Together, our data show that regulation of mRNA translation contributes significantly to differential gene expression and may be especially relevant during acute hypoxia. The presence of a common molecular mechanism underlying preferential translation offers potential therapeutic interventions for manipulating the hypoxic response.

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(PS2160) Activation of HIF-1 after mild hyperthermia and its downstream effect on tumor angiogenesis and metabolism. Eui Jung Moon, Ines Batinic-Haberle, Mark W. Dewhirst. Duke University Medical Center, Durham, NC, USA.

Hyperthermia (HT) is a strong adjuvant cancer treatment because of its cell killing and radiosensitizing effects on both normoxic and hypoxic cells. Mild hyperthermia (<43°C), in

particular, has been shown to induce tumor reoxygenation that results in improved response to radiotherapy. However, the underlying molecular mechanisms how mild HT improves tumor oxygenation is not clearly defined.

Under hypoxia, cancer cells exhibit adaptive mechanism via hypoxia-inducible factor-1 (HIF-1), a transcription factor that activates variety of genes involved in tumor progression. In prior studies our group has demonstrated that radiation-induced reoxygenation activates HIF-1 through reactive oxygen species (ROS), and inhibition of HIF-1 leads to the enhanced anti-tumor effects. Thus, we hypothesize that alteration of HIF-1 following HT-induced tumor reoxygenation is involved in radiosensitization.

In this study we treated human cervix cancer (SiHa) and breast cancer cells (MCF7, MCF7-Her2, and inflammatory breast cancer (IBC), SUM149 and SUM159) at a range of temperatures for 1 hour and measured HIF-1 levels with ELISA. Interestingly, at mild hyperthermic temperatures, HIF-1 levels in SiHa were significantly increased and were the highest immediately after treatment at 42°C. To determine the possible role of ROS in HIF-1 activation, we have treated cells with SOD mimetic, a small molecule that scavenges ROS, and the treatment of SOD mimetic abolished the upregulation of HIF-1. This indicates that ROS played a role in HIF-1 activation in SiHa.

In contrast to SiHa, in Her2 overexpressing MCF7-Her2, the levels of HIF-1 decreased after 1-hour HT at 42°C. We have also observed decreased Her2 expression in response to HT. In wild type MCF7 and two Her2 negative IBCs, SUM149 and SUM159, HIF-1 levels were not significantly altered after HT. Since Her2 is known to upregulate HIF-1 activity in oxygen independent way, our data suggests that Her2 might be a key factor for HIF-1 regulation in breast cancer after HT.

In our future study, we will determine the HT-induced HIF-1 regulation and downstream effect on tumor angiogenesis and metabolism *in vivo*. Therefore, our study will provide better perception about the role of hyperthermia as an adjuvant therapy, and help conduct the therapeutic design for greater clinical outcome.

(PS2161) TRC8 regulates the chromosomal passenger protein, survivin. A potential role in hypoxia/reperfusion-induced G2 arrest and chromosome integrity. Md Ashraful Islam, Wayne S. Zundel. University of Louisville, Louisville, KY, USA.

TRC8 is a multi-membrane spanning protein containing a RING-H2 finger originally identified in renal cancer. TRC8 has previously been implicated as a potential regulator of hypoxic responses via its reported association with pVHL. Here we show that TRC8 is highly expressed in a panel of cell lines but its expression is relatively low in non-transformed cells. In response to hypoxia, there is a down regulation of TRC8 in all cell lines we have investigated and this is associated with multiple molecular mass shifts potentially due to regulated proteolysis or other post-translational modification.

To further investigate the molecular architecture underlying TRC8 function, we conducted yeast-2-hybrid screens and identified Survivin from multiple clones. Survivin is a chromosomal passenger protein aberrantly expressed in transformed cells. Interestingly, targeted depletion of TRC8 by RNAi showed a significant loss of Survivin expression but no reciprocal change of expression in TRC8 when Survivin was targeted by RNAi. Interestingly, TRC8 associates with survivin in normoxic but not hypoxic conditions suggesting that only certain 'processed' forms of TRC8 can interact with Survivin. TRC8 as well as Survivin were highly expressed during S and G2/M phase of the cell cycle and knockdown of TRC8 revealed cellular arrest of growth predominantly in G2. As Survivin is known to be modulated by ubiquitylation at centromeres during cell division at G2/M, this suggests that TRC8 may serve as the relevant RING finger in an Ub E3 ligase controlling either Survivin or the chromosome passenger ubiquitylation, assembly and function. These preliminary results also suggest that TRC8 and Survivin could be the mechanism underlying the poorly understood G2 block induced by hypoxia/ischemia and potentially influence chromosome instability during acute hypoxia and tumorigenesis.

(PS2162) Role of the Mitochondria-K^+ Channel Axis in the Response of Glioblastoma Cell Lines to Hypoxia. Jason M. Derry, Joan Allalunis-Turner. Department of Oncology, University of Alberta, Cross Cancer Institute, Edmonton, AB, Canada.

Our previous work has shown that glioblastoma multiforme (GBM) cell lines differ in their ability to adapt to and survive under hypoxic conditions, and that differences in hypoxia tolerance are linked to significant differences in mitochondrial function. Recently, evidence of an altered mitochondria-reactive oxygen species (ROS)-K^+ channel axis involving the voltage-gated $K_v1.5$ potassium channel has been demonstrated for cancer cells, including GBM (Bonnet *et al*., Cancer Cell 11:37–51, 2007). The purpose of this study is to test the hypothesis that redox-sensitive K^+ channels function as effectors of the initial response of tumor cells to hypoxia, and ultimately, contribute to development of an aggressive tumor phenotype. To test this, we are using gene expression profiling (real time PCR) to probe GBM cell lines ($n = 6$) for: (1) differences in the aerobic *vs*. hypoxic expression of voltage-gated ion channels that have been previously linked to oxygen sensing (*e.g*. $K_v1.2$, $K_v1.5$, $K_v2.1$ and BKCa channel subunits); and (2) the expression of individual channel subunits that have been identified previously as having oncogenic potential. Results indicate that GBM cell lines have unique patterns of K^+ channel expression under aerobic *vs*. hypoxic conditions; however, among hypoxia-tolerant cell lines, the magnitude of these differences is modest. In contrast, the hypoxia-sensitive M010b GBM cell line showed a ~8–20 fold reduced transcript expression of the SK3 small-conductance calcium-activated K^+ channel compared to hypoxia-resistant lines under both aerobic and hypoxic conditions. Additionally, under hypoxic conditions, an oxygen-sensitive splice variant of the BKCa K^+ channel (STREX) showed the largest increase in expression in the M010b line as compared to that of hypoxia-tolerant cell lines. Studies are currently underway to confirm these observations at the level of protein expression. To further explore K^+ channel function in GBM, we will use shRNA to knockdown, and dichloroacetate (DCA) to induce, respectively, K^+ channel expression. Changes in expression profiles will be correlated with development of hypoxia tolerance in tumor cells (cell survival *vs*. apoptosis), mitochondrial function (mitochondrial membrane potential, ROS production), and GBM cell motility and invasion.

(PS2163) NADPH oxidase mediates radiation-induced oxidative stress and inflammation in brain endothelium. J Racquel Collins-Underwood, Weiling Zhao, Mike E. Robbins. Wake Forest University School of Medicine, Winston-Salem, NC, USA.

Progressive dementia occurs in up to 50% of brain tumor patients who are long-term survivors after treatment with brain irradiation. The need to both understand and minimize the side effects of brain irradiation is heightened by the ever-increasing number of patients with brain metastases that require treatment with large field or whole brain irradiation (WBI); some 200,000 cancer patients/year receive large field or WBI. At the present time, there are no successful treatments for radiation-induced brain injury, nor are there any known effective preventive strategies. Data support a role for the renin-angiotensin system (RAS) in radiation-induced late effects in kidney and lung; both angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II (Ang II) receptor antagonists (ATRA) have proved effective. However, the pathogenic mechanism(s) involved remains unknown. Ang II signaling is mediated by generation of reactive oxygen species (ROS) via activation of nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase. NADPH oxidase is a multi-subunit enzyme localized to cell membranes whose activation requires the translocation of cytosolic subunits $p47^{phox}$, $p67^{phox}$, and Rac1. Activated NADPH oxidase converts molecular oxygen (O_2) to the superoxide anion ($O_2^{\cdot-}$). We hypothesize that brain irradiation leads to activation of the intrinsic RAS and the subsequent induction of proinflammatory mediators regulated via the downstream NADPH oxidase signaling pathway. We report that ionizing radiation upregulated components of NADPH oxidase, namely Nox-4 and $p22^{phox}$. We further report that ionizing radiation increased i] generation of intracellular ROS, ii] expression of the proinflammatory transcription factor NFκB and iii] expression of the proinflammatory mediators ICAM-1 and PAI-1. This increase appeared to be NADPH oxidase-dependent;

pharmacologic (apocynin) inhibition of NADPH oxidase blocked the radiation-mediated upregulation of these proinflammatory mediators. These results suggest that the brain RAS may play a role in radiation-induced brain injury through activation of NADPH oxidase and subsequent upregulation of proinflammatory mediators.

(PS2164) Reactive oxygen species modulate CDK4/cyclinD1 in differentiation of PLB-985 cells. Wakako Hiraoka, Yoshihiro Ando. Department of Physics, Meiji University, Kawasaki, Japan.

We have studied the role of reactive oxygen species (ROS) in monocitic differentiation of myelomonoblastic PLB-985 cells. Exposure to low concentration (50 *nM*) of phorbol *12*-myristate *13*-acetate (PMA) for 4–5 days lead the cells to acquire the monocitic features such as phagocytogenesis and adhesiveness. To elucidate the function of ROS generated from NADPH oxidase on the progress of differentiation, wild-type cells and CGD-mutant cells, NADPH oxidase $gp91^{phox}$ negative mutant, were employed. Quantification of ROS in cells was performed by chemiluminescence with *2*-methyl-*p*-methoxy phenylethylimidazopyrazinone (MPEC). Qualilification of ROS was assayed with the method combining ESR and spin-trapping with *5*-diisopropoxyphosphoryl-*5*-methyl-*1*-pyrroline *N*-oxide (DIPPMPO). Cell cycle and the expression of adhesion molecules were monitored with flow cytometer. Phosphorylation of pRb was assayed with western blotting.

Immediately after activation with PMA, hydroxyl radical and superoxide were detected in wild-type PLB-985 cells. Three days later, the expression of Mac-1α/CD11b was detected in not CGD-mutant but wild-type cells. Furthermore catalase diminished Mac-1α during monocitic treatment. In such condition which hydroxyl radical and superoxide were generated, hydrogen peroxide should be produced easier in the cells. Our results indicate the importance of hydrogen peroxide for the expression of Mac-1α. Cell cycle of wild type PLB-985 treated with PMA for 5 days was almost arrested in G1, whereas the rate of CGD-mutant in G1 phase after PMA treatment was lower than that of wild type cells. Co-incubation with PMA and CDK4/cyclin D1 inhibitor, Fascaplysin, showed the lower rate of cells in G1 phase than that of no inhibitor. Fascaplysin also reduced the adhesiveness of PMA-treated wild-type PLB-985 cells. CDK4/cyclin D1 process is proved to be essential to G1 arrest during PMA-induced monocytic differentiation containing expression of Mac-1α/CD11b. ROS generated from NADPH oxidase should be trigger of activation of CDK4/cyclin D1 in this process.

(PS2165) Assessment of hypoxia after pulmonary irradiation in rats with exogenous and endogenous hypoxia markers (EF5, CA9, HIF1α). Katharina C. Fleckenstein[1,2], Benjamin M. Gauter-Fleckenstein[1,3], Zahid Rabbani[1], Thies Schroeder[1], Zeljko Vujaskovic[1]. [1]Duke University Medical Center, Durham, NC, USA, [2]Department of Radiation Oncology, Mannheim Medical Center, University of Heidelberg, Germany, [3]Department of Anaesthesiology, Mannheim Medical Center, University of Heidelberg, Germany.

Purpose:

To assess hypoxia in rat lungs after radiation (RT) using immunohistochemical studies with the exogenous marker EF5, endogenous Carboanhydrase 9 (CA9) and HIF1α.

Materials and Methods:

Female Fisher rats were irradiated to the right hemithorax with 28Gy. Rats were harvested before and from 1 day to 20 weeks after RT. One hour before sacrificing, rats were injected with EF5 i.v. Tissue was snap frozen and processed for immunofluorescence evaluation of hypoxia.

For quantitative assessment of tissue oxygenation, maximal EF5 binding in "cube references" from 3 separate non-irradiated rats was measured as described by Koch in 2001.

Multiple staining with cell specific markers (ED1, MUC1, CD31) was performed to differentiate between the pulmonary cell types. Histopathology was assessed with H&E staining.

Results:

EF5 revealed a significant number of hypoxic cells at 1 week after RT. With time after RT, EF5 signal intensity and number of hypoxic cells increased. 95% of the EF5 signal was at or below 17% of maximal binding, suggesting moderate to severe hypoxia as defined by Koch in 2001. Preliminary results indicate hypoxia to be present mainly in pneumocytes type II and endothelial cells. There was almost no co-localization of EF5 with ED1, a marker for activated macrophages, which were found in increasing numbers at later times after RT.

The endogenous hypoxia marker CA9 correlated with the EF5 signal at early times up to 8 weeks after RT. 20 weeks after RT there were additional CA9 positive cells which did not show any EF5 signal. Staining for HIF1α showed an increased expression 1 week after RT. A distinct co-localization was seen between HIF1α and CA9 expression. However, there were additional HIF1α positive cells which did not express CA9.

Conclusion:

We established a valid and quantifiable staining protocol for EF5 in non malignant lung tissue. We could prove moderate to severe hypoxia in lung tissue after RT before histopathological damage was seen. Hypoxia was mainly seen in pneumocytes type II and endothelial cells. At early times after RT the exogenous hypoxia marker EF5 correlated with the endogenous hypoxia marker CA9. At later times after RT we found additional CA9 positive areas, which also stained positive for HIF1α, suggesting an expression not driven by hypoxia.

(PS2166) ATM-dependent signaling in response to ionizing radiation - a proteomic approach. Amrita K. Cheema, Sung A. Lee, Lihua Zhang, Rency Verghese, Habtom Ressom, Anatoly Dritschilo, Mira Jung. Georgetown University, Washington, DC, USA.

Ataxia-telangiectasia (AT) is a human genetic disease characterized by exquisite radiation sensitivity, neurological degeneration, immune deficiencies, genetic instability and predisposition to cancer. ATM gene that is mutated in patients with ataxia telangiectasia is one of major gene products that exhibit multifaceted functions involved in various signaling pathways in response to genotoxic insults. Previously, a clonal cell line, AT5BIVA/CL8, has been established by introducing a vector carrying the full-length wild-type *ATM* cDNA in AT5BIVA cells, which are SV40 immortalized fibroblast derived from an AT group D patient. AT5BIVA/CL8 cells exhibited a recovery of cellular radiation sensitivity to normal levels.

To study ionizing radiation-induced signaling events that are ATM dependent, we used a proteomic approach. The time course of differential protein expression was determined in ATCL8 cells in response to exposure to ionizing radiation. After exposure to 5Gy of ionizing radiation, whole cell extracts were prepared at 30minutes, 1hr, 3hrs and 24hrs. The proteins were then resolved by 2D gel electrophoresis. The differences in the protein expression profiles of ATCL8 cells which were exposed to radiation as compared to the control cells (untreated) was determined using an image analysis software (Dymension). Mass spectrometry analysis of these spots was performed in order to identify these proteins. We have successfully identified several interesting proteins which are up and downregulated as well as those which are uniquely expressed in the treated and control cells, with high confidence. Studies are underway to validate the MS results. The role of the differentially expressed proteins in context of radiation resistant phenotype of the ATCL8 cells will be discussed.

(PS2167) Mechanisms of ATM regulation by TGFβ. Jenny Paupert, Mary-Helen Barcellos-Hoff. Lawrence Berkeley National laboratory, Berkeley, CA, USA.

DNA double strand breaks is the principle lesion induced by ionizing radiation (IR). The cellular response to IR require the phosphorylation and the activation of the kinase activity of a nuclear protein kinase ataxia telengiectasia mutated (ATM). Our previous study show that the absence of transforming growth factor beta 1 (TGFβ1) decreased ATM phosphorylation and activity correlates

with increased radiosensitivity of epithelial cells (Kirshner et al. Cancer Res, 2006). A large panel of TFGβ inhibitors is currently in clinical development (antibodies, antisense oligonucleotide and small molecule inhibitors) We propose that identifying TGFβ inhibitors that regulate ATM activity could permit their use to radiosensitize epithelial tumors.

We have used non-malignant MCF10-A epithelial cells to investigate the molecular mechanism leading to decreased ATM phosphorylation in the absence of TGFβ1 and to define precisely which proteins in the TGFβ signaling pathway are involved in the regulation of ATM phosphorylation. TGFβ1 signaling is complex involving several receptors and downstream effectors. TGFβ1 binds to the constitutively active type II TGFβ receptor kinase (TBRII) and recruits the type I receptor (TBRI) which it then phosphorylates, which leads to the phosphorylation and activation of SMAD proteins. In most cell types, ALK-5 is the predominant TBRI and activated ALK-5 phosphorylates SMAD2 and 3 that in turn complex with SMAD-4, which translocates to the nucleus where it regulates gene expression. To determine which TBR1 and which downstream effectors are involved in the regulation of ATM activity, we inhibited TGFβ1 signaling at differents steps. We found that either antagonism of TGFβ1 binding to the receptor with blocking antibody and intracellular inhibition of ALK-5 with small-molecule inhibitors in resulted in decreased ATM phosphorylation after IR exposure. These data suggest that ALK-5 is the TBRI involved. We are currently confirming this result by using others ALK-5 small inhibitors. The second step is to determine if the SMAD proteins are involved by overexpressing SMAD-7, which prevents SMAD2 and 3 phosphorylation by ALK-5, and appears to inhibit the DNA damage response.

(PS2168) ATM, MOF and DNA repair. Tej K. Pandita. Washington University School of Medicine, St Louis, MO, USA.

Chromatin modifications are important for all cellular processes that involve DNA metabolism, including transcription, replication and DNA DSB repair. Chromatin can be modified by the addition of adducts to histone tail residues or by nucleosome remodelling, which requires ATP-dependent chromatin-remodelling complexes. Although the role of these mechanisms in transcription is well studied, their impact on DNA DSBs repair has only recently become evident. One crucial chromatin modification is phosphorylation of histone H2AX, which links with the recruitment of histone modifiers and ATP-dependent chromatin-remodelling complexes to sites of DNA damage. H2AX modification is dependent on the action PI3-Kinases like ataxia telangiectasia mutated (ATM), AT-related (ATR) and DNA-dependent protein kinase (DNA-PK). ATM and ATR kinase activities are responsible for the formation of megabase-sized, phospho-H2AX-containing regions around sites of DSBs. However it is unclear how the phosphorylation of H2AX at DNA DSB is regulated. Recently, we identified a chromatin-modifying factor "hMOF", the human ortholog of the *Drosophila MOF* gene (**M**ales absent **O**n the **F**irst) which interacts with ATM. hMOF has histone acetyltransferase activity and its deficiency results in marked reduction of acetylation of histone H4 at lysine 16 but does not influence the trimethylation status of histone H3 at lysine 4, although both represent euchromatin markers for relaxed chromatin. Acetylation of histone H4 at lysine 16 disrupts higher-order chromatin structure, changes the functional interactions between chromatin-associated proteins and serves as a switch for changing chromatin from a repressive to a transcriptionally active state in yeast as well as humans. hMOF inactivation or over expression of isoforms of heterochromatin protein 1 results in enhanced IR-induced chromosome aberrations in G1-, S- and G2-phases of the cell cycle. Recently, histone ubiquitinations, acetylations, and methylations have been implicated in the DNA repair pathways. We will specifically discuss how hMOF coordinates with ATM to regulate the phosphorylation of H2AX and thus affect the repair of DNA DSBs.

(PS2169) Persisted formation of phosphorylated ATM and 53BP1 foci and radiation induced permanent cell cycle arrest examined by single-cell based assay. Yasuyoshi Oka, Keiji Suzuki, Masao Tomonaga. Nagasaki university, Nagasaki, Japan.

ATM-p53 pathway plays a critical role in maintaining genomic stability through their participation in multiple cell cycle checkpoints. Immediately after irradiation, ATM protein kinase is activated and autophosphorylated on Ser 1981 at the sites of DNA double strand breaks, and phosphorylates many downstream proteins, such as H2AX, 53BP1, Chk2 and p53. Phosphorylated ATM, phosphorylated H2AX, and 53BP1 form the initial foci in the region flanking DNA double strand breaks. Subsequently, the number of foci decreases concurrently with DNA double strand break repair, while some fraction of foci remain, for a long time. Thus, it can be hypothesized that the persisted foci formation of DNA damage checkpoint factors is involved in ATM-p53-dependent permanent cell cycle arrest observed in normal human diploid cells. In the present study, we established single-cell based assay to test the hypothesis. Normal human diploid cells unirradiated, or irradiated with 2 Gy of X-rays were plated onto cover slips at low density (3 cells / cm^2). After incubation for 3 days, cells were analyzed by immunofluorescence staining with anti-Ser1981 phosphorylated-ATM and anti-53BP1 antibodies. In this experimental setting, 40% of control cells and 12% of irradiated cells form micro-colony, which consists of more than eight cells after 3 day-incubation. We observed a single or two cells in 76% of the irradiated populations, while such cells were observed in 28% of the control populations. Phosphorylated ATM and 53BP1 foci were detected in about 75% of an irradiated single and two cells, and in about 24% of a control single and two cells. The average number of foci was 2 in a single cell, and 1 in two cells. In the micro-colonies, 25% of irradiated cells, and 10% of control cells had foci. These results suggest that a few ATM and 53BP1 foci persisted for a long time after irradiation are sufficient for permanent cell cycle arrest of normal human diploid cells.

(PS2170) ATM/NF-κB-mediated adaptive radioresistanace in human keratinocytes. Kazi Mokim Ahmed[1], Ming Fan[1], Danupon Nantajit[1], Junran Zhang[2], Jian Jian Li[1]. [1]Purdue University, West Lafayette, IN, USA, [2]Washington University School of Medicine, St. Louis, MO, USA.

Mammalian cells exposed to a certain low dose of ionizing radiation (LDIR; ≤10 cGy) can induce a temporary but significant tolerance to subsequent insults of relative high dose radiation. The molecular mechanism underlying the defensive response is poorly understood. Elucidating the mechanism of LDIR-mediated adaptive radioresistance will provide insights about the defending system in cells and is important to re-sensitize therapy-resistant tumor cells. The present study reveals that pre-exposure of immortalized human HK18 keratinocytes to LDIR (10-cGy x-ray) enhanced cell growth and clonogenic survival against the cell death induced by a challenge high dose γ-irradiation. DNA-damage response protein ATM, stress kinases MEK/ERK, and the transcription factor NF-κB were co-activated. Consistent with our hypothesis that ATM, MEK/ERK and NF-κB is in the same pathway of LDIR-induced adaptive radioresistance, inhibition of ATM blocked the activation of MEK/ERK and NF-κB. Inhibition of MEK/ERK abolished NF-κB activation, and LDIR-induced radioresistance was blocked when either ATM or NF-κB was inhibited. In addition, blocking ATM, NF-κB or MEK/ERK inhibited LDIR-induced expression of chaperon proteins 14-3-3s (σ/ζ), and cyclins (D1/B1). Interestingly, 14-3-3ζ and cyclin D1 formed complex in un-irradiated cells and the complex was inhibited by LDIR, resulting a significant increase of cyclin D1 in the cytoplasm. In contrast, no significant reduction in 14-3-3ζ/cyclin D1 complex, or cytoplasmic cyclin D1 accumulation was detected after high dose radiation (5-Gy γ-ray). Together, our data provide the first direct evidence that a co-operative function of ATM and NF-κB signaling networks, which up-regulate and accumulate cyclin D1 in the cytoplasm via 14-3-3ζ regulation, is critical for signaling LDIR-induced adaptive radioresistance.

(PS2171) The γ-ray irradiation induced lyGDI cleavage, cell apoptosis and its nuclear signal function. Xinwen Zhou[1], Shiho

Suto[2], Fumio Suzuki[3], T Ota[4]. [1]Department of Radiation Medicine and Public Health, Suzhou, China, [2]Department of Molecular RadiobiologyResearch Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan, [3]Department of Molecular Radiobiology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan, [4]Division of Cancer Research, Medical Research Institute, Kanazawa Medical University, Ishikawa, Japan.

LyGDI has vital role to regulate the RhoGTPases activity in lymphocyte by blocking GDP dissociation from Rho GTPases, thus keep Rho GTPase proteins in active state, however, its role is not fully known in IR-irradiation induced apoptosis. Our results demonstrated that truncation of LyGDI followed ionizing radiation (IR) in mouse thymic 3SB cells, and this cleavage of LyGDI was found to be p53-dependent. A 23-kDa fragment of LyGDI, resulting from activated caspase-3-induced cleavage at an N-terminal consensus site following the Asp18 residue, accumulated at peak quantities between 5 and 12 hours following IR-irradiation, after which the fragment could no longer be detected. Cleavage of LyGDI was inhibited by the Caspase inhibitor (Z-VAD-fmk). Subcellular fractionation revealed the truncated 23-kDa fragment of LYGDI within the nuclear fraction of IR-irradiated 3SB cells, whereas full-length LyGDI was found only in the cytoplasmic fraction. Immunofluorescence confirmed this observation. Translocation into nuclear of cleavage LyGDI probably had some signal function to apoptosis in lymphocyte. In order to confirm the apoptotic function for this nuclear translocation, the wild type and D19 mutant (equal to caspase-3 cleavage) were expressed in Hela cell. We found that exdogenous full length LyGDI located in the cytoplasm parts, D19 LyGDI accumulated into nuclear. Moreover, this exdogenous lyGDI expression also improvedγ- irradiation induced apoptotic rate in Hela cell

(PS2172) Role of Bcl-2/Bax ratio in predicting radiotherapy response in patients with local advanced non-small lung cancer. Wei-Dong Wang[1], Rong Li[2], Zheng-tang Chen[1]. [1]Cancer Institute of PLA, Xinqiao Hospital, Chongqing, China, [2]Institute of Combined Injury, College of Preventive Medicine, Third Military Medical University, Chongqing, China.

Objective: Markers predictive of clinical responsiveness of patients with lung cancer to radiotherapy may have significant implications in optimizing effective therapeutic regimes to those patients in whom it will be preferentially effective. Because apoptosis plays a critical role in tumor response to radiotherapy, the present study investigates whether there is a correlation between the ratio of bcl-2 oncoprotein and bax expression in lung cancer tissue and the clinical response to radiotherapy in patients with local advanced non-small lung cancer.

Methods: A retrospective review of records of 37 non-small lung cancer patients (stage IIIB, age 53$\pm$9.7) who underwent external beam radiotherapy (60 Gy) for lung cancer was conducted. On the basis of post-treatment computed tomography image of lung cancer, the cancers of 11 patients were classified as radiation nonresponders and 26 as radiation responders. Immunohistochemical study, using the streptavidin-biotin complex technique and monoclonal antibody to bcl-2 and polyclonal antibody to bax protein was used on paraffin sections. Cases were considered positive if at least 5% of tumor cells displayed cytoplasmic staining for bcl-2 or bax. In each tumor, the bcl-2/bax ratio was calculated dividing the percentage of bcl-2-positive cells by the percentage of bax-positive cells.

Results: bcl-2 immunoreactivity was significantly higher in lung tumors not responsive to radiotherapy (46.2 $\pm$ 4.5), compared with the radiation responders (22.4 $\pm$ 3.7) ($P < 0.001$). In contrast, expression of bax protein was less in non-responders (26.3 $\pm$ 4.2) compared with responders (45.8 $\pm$ 5.5). The bcl-2/bax ratio was greater in non-responders (2.1 $\pm$ 0.2) compared with responders (0.57 $\pm$ 0.11), and was correlated with poor therapeutic responsiveness of lung cancer to radiotherapy. This correlation ($r = 0.89$) was independent of age, pathological classification, and performance status.

ConclusionThis study supports a role for the bcl-2/bax ratio as a potential predictive marker of therapeutic response to radiotherapy in patients with local advanced non-small lung cancer. The degree of relative risk for a specific bcl-2/bax ratio remains to be determined from large-scale, randomized, prospective trials.

(PS2173) Survivin expression is not altered during UV-B induced apoptosis in SCL-II cells. Ralf Kriehuber[1], Marcus Unverricht[2], Nicole Busch[2], Dieter G. Weiss[2]. [1]Forschungszentrum Jülich, Jülich, Germany, [2]Universität Rostock, Rostock, Germany.

Survivin is a member of the inhibitors of apoptosis and has been implicated in both the regulation of cell division and the suppression of apoptosis. Whereas over-expression of survivin correlates with an unfavourable prognosis in many malignant tumours, siRNA against survivin causes a reduction of cell proliferation, the induction of apoptosis and an enhanced radiosensitivity of squamous cell carcinoma cell lines and other tumour cell lines.

The survivin expression levels in the squamous cell carcinoma derived SCL-II cell line were assessed by Western/Immunoblotting after exposure to ionizing radiation (IR) and ultraviolet (UV) radiation. Apoptosis was assessed by PARP cleavage and by flow cytometry (Annexin-V/ PI assay).

SCL-II cells show little induction of apoptosis after exposure to IR but a strong apoptotic response after exposure to UV-B (25% apoptotic cells 5 h after exposure) which correlates with PARP cleavage and characteristic changes in cell morphology. Survivin expression is reduced after exposure to IR (^{60}Co, 4 Gy) 8 h to 24 h post-irradiation but remains constant after UV-B (0.45 J/cm^2) and UV-C (28 mJ/cm^2) exposure when compared to controls. One of the used survivin antibodies detects a subband (28 kDa) which positively correlates with the apoptotic state of SCL-II cells. Cycloheximide treatment does not inhibit survivin expression but does suppress the expression of the 28 kDa protein in UV-B exposed cells.

Conclusions: The expression of survivin is inversely correlated with the apoptotic state of SCL-II cells. UV-B and UV-C triggered apoptosis in SCL-II cells is not abolished by survivin.

(PS2174) Protein serine/threonine phosphatase type 2a regulates IR-induced apoptosis. James M. Larner, Jun Mi. University of Virginia, Charlottesville, VA, USA.

In response to IR, p53 plays a critical role in regulating both DNA repair and apoptosis. Although p53 contains multiple phosphorylation sites, the phosphorylation of the serine 46 site is thought to promote apoptotic cell death through mitochondrial outer-membrane permeabilization (MOMP) and subsequent activation of Caspase 7-PARP apoptotic signaling. We therefore investigated which phosphatase regulates Ser 46 following IR reasoning that the responsible phosphatase would be a rational target for a radiosensitizer. Since our previous work has shown that both PP1 and PP2A are regulated by IR in an ATM-dependent manner, we concentrated on these two phosphatases. Our data demonstrate that selective inhibition of PP2A (but not PP1) by the cell permeable inhibitor Calyculin A following IR promotes 1) prolongation of the Ser 46 phosphorylation of p53 and 2) IR-induced Caspase 7 and PARP cleavage. Furthermore, siRNA-mediated PP2A knock-down of either catalytic subunit or B55α regulatory subunit enhance IR-induced Ser 46 phosphorylation, cleaved Caspase 7 and cleaved PARP. We conclude that PP2A regulates IR-induced apoptosis possibly through dephosphorylation Ser 46 of p53.

(PS2175) The DNA repair protein Nbs1 suppresses ionizing radiation-induced apoptosis. Friederike Eckardt-Schupp, Daniel Sagan, Simone Moertl, Hedda Eichholtz-Wirth. GSF-National Research Center for Environment and Health, Neuherberg, Germany.

Mutations in the *NBS1* gene lead to the *Nijmegen Breakage Syndrome* (*NBS*) in humans, characterized by immunodeficiency,

increased cancer predisposition and enhanced radiosensitivity of the patients. Enhanced radiosensitivity is also a hallmark of cell lines derived from *NBS* patients. The Nbs1 protein, together with the proteins Mre11 and Rad50, builds the trimeric MRN complex which recognizes DNA double strand breaks (DSB). Commonly, enhanced radiosensitivity is explained by an impaired processing of ionizing radiation (IR)-induced DSB.

Recently, we have described a new function of the NBS1 protein in suppressing IR-induced apoptosis. *NBS1*$^{-/-}$ lymphoblasts show 2–3 fold enhanced IR-induced apoptosis as compared to consanguineous *NBS1*$^{+/-}$ cells. In *NBS1*$^{-/-}$ cells, IR-induced apoptosis is p53-independent; it is due to enhanced clustering of the death receptor CD95 caused by reactive oxygen species which are generated by ionizing radiation. In *NBS1* proficient cells, clustering of the death receptor CD95 is suppressed by the PI3-kinase (PI3-K)/AKT pathway which is defective in *NBS1*$^{-/-}$ cells (D. Sagan et al. (2007), Apoptosis DOI 10.1007/s10495–006-0021–0).

Our new data indicate a disturbed regulation of some members of the family of SRC-kinases (SFK), which are involved in PI3-K activation by phosphorylation of the PI3-K subunits. We present studies on the role of SFK for PI3-K activation in *NBS1*$^{-/-}$ and *NBS1*$^{+/-}$ lymphoblasts, in order to prove that the observed lower expression of two members of this family (LCK and HCK) in *NBS1*$^{-/-}$ cells is indeed the cause for the disturbed activation of the PI3-K/AKT pathway.

This is the first study to describe an anti-apoptotic function for Nbs1 in human cells which is p53- independent. Our data provide evidence for a regulatory function of the Nbs1 protein for the ligand-independent activation of CD95 after ionizing irradiation. Our model explains enhanced radiosensitivity of *NBS* cells by IR-induced CD95-dependent apoptosis as one putative cause. So far, enhanced radiosensitivity of *NBS* cells has been explained by impaired repair of radiation-induced DSB, although the function of NBS1 for DSB repair is not discussed without controversy in the literature.

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(PS2176) Possible role of synergistic interaction of ionizing radiation with other detrimental agents for radiation accident consequences. Vladislav G. Petin[1], Jin Kyu Kim[2]. [1]Medical Radiological Research Center, Obninsk, Russian Federation, [2]Korea Atomic Energy Research Institute, Jeongeup, Republic of Korea.

The combined effects of radiation and other detrimental agents are recognized as an important research field, but the data evidencing the importance of the synergistic interaction for radiation protection are limited. The main scope of this study was to demonstrate a possible significance of synergistic interaction of ionizing radiation and other detrimental factors for strengthening the Chernobyl and other radiation accident consequences. Based on a numerous experimental data, some universal manifestations of synergism were derived, which might be independent of the agents applied, the biological objects and the effects observed. First of all, there is a range of acting agents within which the synergistic interaction can be displayed. Second, inside this range there is an optimal value providing the highest synergistic effect. And third, the synergy depends on the intensity of the agents employed: at a smaller intensity of ionizing radiation or other physical factors (or the concentration of the chemical agents), one has to reduce the intensity of another factor (for instance, the acting temperature) to preserve the highest synergistic effect. These data, in principle, indicate a potential significance of synergistic interaction at low intensity of adverse factors encountered in the natural environment. Some practical examples of the significance of synergistic interaction of various agents for strengthening radiation accident consequences are presented.

(PS2177) Ionising radiation exposure of the eyes of patients during X-ray examinations. Jaroslaw Jazwinski[1], Maria A. Staniszewska[2], Agnieszka Kowalska[1], Magdalena Zabicka[3], Radoslaw Rozycki[4], Ewa M. Nowosielska[1], Marek K. Janiak[1]. [1]Military Institute of Hygiene and Epidemiology, Dept. of Radiobiology and Radiation Protection, Warsaw, Poland, [2]Medical University, Institute of Radiology, Lodz, Poland, [3]Military Medical Institute, Dept. of Radiology, Warsaw, Poland, [4]Military Medical Institute, Dept. of Ophthalmology, Warsaw, Poland.

Introduction

Diagnostic procedures with use of ionising radiation are associated with radiation risk to the patient. Although the whole body effective dose during an examination is usually small, some organs and tissues may incur harmful doses of radiation. One of the most sensitive tissues to radiation is the reproductive layer of the lens' capsule. Hence, the aim of the present study was to estimate the level of exposure of the patient's lenses and eyeballs to ionising radiation during routine radiological examinations and to determine which examinations pose the highest radiation risk to the patient's eyes.

Material and Methods

Exposures to ionising radiation were performed from the X-ray diagnostic source at the Department of Radiology, Military Medical Institute in Warsaw. For estimations of the absorbed doses thermo-luminescent dosimeters, a dosimeter reader, and a human body phantom were used. The dosimeters were previously calibrated for the X-ray energies emitted by the 80 and 120 kV X-ray equipment. The measurements were carried out during computed tomography, vascular procedures, conventional chest and skull radiography and dental examinations. From the obtained data values of the equivalent doses received by the eyes during each type of the examination were determined.

Results

As indicated by the analysis of the results of the measurements and calculations the applied experimental method allows for precise and reliable estimation of the radiation risk to the eyes of a patient subjected to radio-diagnostic tests.

Conclusion

From the obtained results the following conclusions can be drawn:

- depending on the X-ray procedure, the patient's lenses can be exposed to doses ranging from natural background to several mSv per one examination;
- exposure to the patient's eye can be decreased by modifying the X-ray equipment parameters and use of protective screens;
- physicians referring their patients to X-ray tests should be aware of the radiation risk to the eyes associated with the given procedure;
- the obtained results can be used for estimation of the radiation risk to patients subjected to various types of X-ray examinations.

(PS2178) Modeling effects of atomic bomb radiation on disease outcomes with radiation-influenced risk factors. Lori A. Williams[1], Wan-Ling Hsu[2], Kenneth J. Kopecky[1]. [1]University of Washington, Seattle, WA, USA, [2]Radiation Effects Research Foundation, Hiroshima, Japan.

Exposure to atomic bomb radiation is associated with a dose-related increase in risk of death from heart disease and stroke (Preston et al, Radiation Res 160:381, 2003). This effect could be direct or could be mediated through an intermediate variable such as hypertension. If radiation exposure directly affects cardiovascular mortality, hypertension would be considered a potential confounder. In traditional analysis of longitudinal data (e.g., using Cox proportional hazards regression models) analysts adjust for variables that are potential confounders, but not for variables that are intermediates. When pathways of interest involve variables, such as hypertension, that could be intermediates or confounders, novel analysis methods may provide additional insight into the complex relationships. To evaluate one novel analysis method - marginal structural models (MSM) - we used data from the Radiation Effects Research Foundation's Adult Health Study (AHS). The AHS is a longitudinal study of survivors of the atomic bombings of Hiroshima and Nagasaki, conducted to investigate the late health effects of their exposures to ionizing radiation from the bombings. Almost 17,000 survivors have been examined at least once at biennial AHS medical examinations offered since 1958, with an

average of 9 examinations through 2002. The mortality data, using the Japanese family registration system (koseki), were complete through 2003. We investigated using an MSM to evaluate the effect of radiation on cardiovascular mortality accounting for hypertension. We accounted for hypertension using weights that represent each person's probability of having been exposed to a particular radiation dose given their current hypertension status. Our analysis is an assessment of the feasibility of using MSMs for analyzing AHS data and it provides an estimate of the effect of radiation on cardiovascular mortality through all possible pathways, including the pathway through hypertension. By comparing our results to the traditional modeling results, we may gain insight into the role of hypertension in the pathway between radiation and cardiovascular death. Agreement between the two methods would confirm the results of traditional modeling and differences between the two methods could suggest new ways of thinking about this pathway.

(PS2179) An "Effective functional subunit size" model for the dose response of rat spinal cord paralysis. Magdalena Adamus-Górka[1], Panayiotis Mavroidis[2,1], Anders Brahme[1], Bengt K. Lind[1]. [1]Department of Medical Radiation Physics, Karolinska Institutet & Stockholm University, Stockholm, Sweden, [2]Department of Medical Physics, Larissa, Greece.

Background Radiobiological models for normal tissue complication probability (NTCP) are more and more commonly used in order to estimate the clinical outcome of radiation therapy. A normal tissue complication probability model to be considered a good and reliable one should fulfill the following two requirements: (a) it should predict the sigmoid shape of the dose-response curve as well as possible and (b) it should duly handle the volume effect. In the work from 2005 (IJROBP 61(3):892–900, 2005) P. van Luijk et al. suggest that none of the existing NTCP models is able to describe the volume effects present in the rat spinal cord during irradiation with small proton beams and they indicate the need for developing such new models.

Methods We have used the experimental data from H. Bijl et al. (IJROBP 52(1):205–211, 2002) to try explaining the change in the fifty percent effective dose (ED50) for different field sizes. We initiated this study to evaluate whether the induction of white matter necrosis in rat spinal cord after irradiation with small proton beams could be explained independent of used NTCP model. We therefore introduced a new concept of effective FSU dose, where a convolution of the original dose distribution with a function describing the effective size of a single FSU results in the average doses in a functional subunit. Such procedure allows determining the ED50 in an FSU of a certain size, within the irradiation field. We have also looked at non uniform dose distributions to see whether using a similar method we can explain the so called "bath and shower experiments" (IJROBP 57(1): 274–281, 2003).

Results Using the least square method to compare the effective doses for different sizes of functional subunits with the experimental data we observe the best fit for about 8 mm length. It seems that this length could be understood as an effective size of functional subunits in rat spinal cord, explaining what is otherwise interpreted as a volume effect. For the non uniform dose distributions an effective FSU length of 5 mm gives the optimal fit with the Probit dose-response model.

Conclusions The concept of an effective FSU length seems to explain at least part of the effects seen when small portions of the rat spinal cord are irradiated. The most likely FSU length for the shower and bath experiments is 5 mm according to these calculations.

(PS2180) PRIME I: a phase III randomised trial assessing the impact of adjuvant breast radiotherapy on quality of life in low risk older patients following breast conserving surgery. Ian H. Kunkler[1,2], Robin J. Prescott[2], Linda J. Williams[2], Celia C. King[1]. [1]Western General Hospital, Edinburgh, United Kingdom, [2]University of Edinburgh, Edinburgh, United Kingdom.

Background: Breast cancer in older patients represents an increasing health care burden. There is a dearth of data on the impact of adjuvant radiotherapy on quality of life in older patients, in whom there are competing risks of death from morbidity. The PRIME I trial presents the first level I data on the impact of breast radiotherapy in such patients.

Patients and methods: After breast conserving therapy with clear margins, women =/>65 years with histologically node negative (T0–2,N0,M0) unilateral breast cancer receiving adjuvant endocrine therapy were randomised to receive whole breast radiotherapy (40–50 Gy in 15–25 fractions) or no further treatment. Primary endpoints were quality of life, anxiety and depression and cost effectiveness. Quality of life (QoL) was measured on four occasions over 15 months by the EORTC QLQ C30 and BR23 modules. Mental state was assessed by the Hospital Anxiety and Depression Scale and Philadelphia Geriatric Center Morale Scale, and physical functioning by the Barthel Activity of Daily Living Index and Clackmannan Scale.Cost-effectiveness was measured with the EuroQol scale for the calculation of QALYs.

Results: 255 patients were randomised in the trial. The expected improvements in QoL with the omission of radiotherapy were not identified in the EuroQol assessment or in the functionality or symptom domains of the EORTC scales. RT was associated with increased breast symptoms and fatigue but with less insomnia or endocrine side effects. The Barthel Index indicated a small but significant fall in functionality in the RT compared to the no RT arm. Costs to the National Health Service with post-operative radiotherapy were calculated to be of the order of [pound]2000 ($3774) per patient. On follow up to 15 months, there were no recurrences and quality of life utilities from EuroQol were almost identical.

Conclusions: Although there are no global differences in quality of life scores between patients treated with and without RT, there are several dimensions which show significant gains from the omission of radiotherapy. Within this time frame, no radiotherapy is the cost-effective choice. Extrapolations from these data suggest RT may not be a cost effective treatment unless it results in a recurrence rate of at least 5% lower in absolute terms than those treated without radiotherapy.

(PS3001) Operational issues influencing dose assessment by the dicentric assay: The effect of blood transport temperature and cell culture type. Maria Moroni. AFRRI, Bethesda, MD, USA.

Introduction. The dicentric assay is the current "gold standard" method for biodosimetry. Application of this assay for triage dose assessment after radiation mass casualties is envisioned. Harmonized laboratory protocols and International Standardization Organization guidelines are available. However, operational issues influencing dose assessment, blood transport temperature and cell culture type (whole blood or isolated lymphocytes), are not well defined. Here we studied the effects of simulated blood transport temperature conditions and cell culture types (whole blood versus isolated lymphocyte cultures) on radiation-induced dicentric yields.

Experimental procedures. Peripheral blood was obtained by phlebotomy into vacutainers from healthy donors after informed consent and immediately subjected to a 3-Gy dose from a ^{60}Co gamma source. Following irradiation, blood was subjected to different temperature/shipment conditions before short-term culturing of either whole blood or isolated lymphocytes. A temperature data logger was used to monitor temperature. The cells were cultured in Marrowmax (Invitrogen, CA, USA) for 48 hours. First-division metaphase spreads were harvested after a 24-h colcemid block using a metaphase harvester and a spreader (Hanabi, Japan) and were stained in 4% Giemsa in PBS. Dicentric frequency was estimated based on 500 metaphase spreads analysis.

Results. Immediate exposure of samples to simulated refrigerated shipment conditions, following irradiation, significantly increased dicentric yield. Therefore, shipment of blood under refrigerated conditions, immediately after radiation exposure should be avoided, as it may result in dose overestimation. The mechanism of dicentric formation under refrigerated conditions is being studied. Corrugated shipment boxes did not provide adequate long-term insulation. Dicentric yield was identical in isolated lymphocyte and whole-blood cultures; isolated lymphocyte cultures provide better quality metaphase spreads.

Conclusions. Blood transport conditions must be standardized. Whole blood culture method can be used for dose assessment in mass casualties to increase throughput.

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(PS3002) Clonal structure of human lymphocyte pool predicts frequent presence of normal clones: Possible impact on cytogenetic biodosimetry several decades after radiation exposure for retrospective biodosimetry. Yoshiaki Kodama, Mimako Nakano, Kazuo Ohtaki, Asao Noda, Nori Nakamura. Radiation Effects Resesarch Foundation, Hiroshima, Japan.

We have previously found that the probability of detecting clonal chromosome aberrations, defined as identical stable-type aberrations found in at least three cells, is predictable (inversely proportional to the clone size) by scoring 500 cells as our ordinary biodosimetric studies in A-bomb survivors. To extend the clonal structure model of the lymphocyte pool, here we scored 5,000 cells from each of five survivors with FISH painting of chromosomes 1, 2, and 4. After screening of translocations involving the painted chromosomes, all the slides were re-stained for the Q-banding to determine the unpainted, counterpart chromosomes and the break-points involved in the translocations to identify clonal aberrations (i.e., identical aberrations carried by at least three cells). The minimum clone size detected was 3/5,000 or 0.06%, and we found 31 such clones (clone sizes of 3–5 cells/5,000), and progressively less number of clones with the increase of the clone sizes, as predicted by the model. The results indicate that the clonal structure holds not only to the clone size level of 0.06% but even lower levels. By contrast, we already know two extremely rare cases of the opposite direction; namely, the two survivors bore clonal translocations in more than 30% of the blood lymphocytes. Overall results strongly indicate that the clonally derived lymphocytes, whether chromosomally marked or not, are ubiquitous among the blood lymphocytes. Therefore, a fraction of normal cells must be clonally derived, and they can lessen the frequency of non-clonal translocations, which is the bio-indicator of radiation dose, by as much as 50% in some rare cases. Collectively, the clonally derived non-aberrant cells would reduce the mean translocation frequency by 5–10%.

(PS3003) Chromosome aberrations in the progeny of human lymphocytes exposed to energetic heavy ions. Kerry George[1], Marco Durante[2], Todd Elliott[1], Francis Cucinotta[3]. [1]Wyle Laboratories, Houston, TX, USA, [2]University Federico II, Naples, Italy, [3]NASA, Houston, TX, USA.

Radiation health risks cannot be fully assessed and effective countermeasures cannot be fully developed until the early events that lead to carcinogenesis and other types of late health effects are more clearly understood. We exposed human peripheral blood lymphocytes to either energetic heavy ions or γ-rays, and analyzed chromosomes in the progeny of these cells using both multi-color fluorescence banding (RxFISH) and multi-color fluorescence *in situ* hybridization (mFISH) techniques. Results showed that the relative biological effectiveness (RBE) of iron ions (energy 1 GeV/nucleon) for the induction of interchanges is much less for the daughters of irradiated cells than for the population originally exposed. However, the RBE is highly dependent on the type of chromosome aberration. Yields of insertions and complex- and simple-type chromosome exchanges were compared for each analysis technique. Telomere probe analysis verified that high-energy heavy ions induce true terminal deletions of chromosomes, and that chromosomes missing a telomere can be transmitted through the cell cycle. To investigate possible variations in individual radiation sensitivity, we assessed samples from three different blood donors. High-energy heavy ions generate a substantial health risk for human space exploration, and results of these studies may help in understanding the induction of late effects from this type of radiation exposure.

(PS3004) Variable sensitivity of chromosomes 2, 8 and 14 in human peripheral blood lymphocytes exposed to 480 MeV/n ^{12}C-IONS. Marta Deperas-Kaminska[1], Gennady N. Timoshenko[2], Eugene A. Krasavin[2], Andrzej Wojcik[3]. [1]Swietokrzyska Akademy, Kielce, Poland, [2]Joint Institute for Nuclear Research, Dubna, Russian Federation, [3]Institute of Nuclear Chemistry and Technology, Warszawa, Poland.

Purpose: To investigate by FISH the distribution of radiation-induced chromosomal aberrations in chromosomes 2, 8 and 14 of 3 donors.

Methods: Irradiation of blood from 3 healthy donors (two male and one female) was performed at the Nuclotron accelerator at the Joint Institute for Nuclear Research in Dubna (Russia). Whole blood samples were irradiation with 1.1, 2.3 and 3.5 Gy of ^{12}C - ions. At the position of the sample the beam energy was 480 MeV/n and LET=10.6 keV/μm. Chromosomes 2, 8 and 14 were painted in different colors and aberrations scored with the help of an image-analysis system.

Results: Chromosome 2 was generally less sensitive than expected on the basis of DNA-content and the extent of inter-donor variability was minimal. A higher than expected frequency of aberrations was found in chromosome 14 and 8 of all donors.

Conclusion: Chromosome 2 appears to be less sensitive to heavy ions than chromosomes 8 and 14. This result is in line with recent results of a study on the sensitivity of chromosomes 2, 8 and 14 to gamma rays.

(PS3005) Chronological changes of chromosomal translocation rates in spleen cells from mice continuously exposed to low dose-rate gamma-rays. Atsushi Kohda[1], Takuo Toyokawa[2], Kazuaki Ichinohe[1], Yoichi Oghiso[1], Kimio Tanaka[1]. [1]Institute for Environmental Sciences, Rokkasho, Aomori, Japan, [2]Tohoku Nuclear Co., Rokkasho, Aomori, Japan.

It is well known that high dose-rate radiation-induced chromosomal translocation persists for a long time in lymphocytes and bone marrow cells in a dose-dependent manner. However, the fate of translocations after chronic low dose-rate radiation exposure remains to be clarified. In the present study, we examined translocation rates in spleen cells from SPF C3H/HeN female mice chronically exposed to gamma-rays at a dose rate of 20 mGy/22 hr/day from the age of 8 weeks for a maximum of about 400 days. Spleen cells from mice received the total accumulated doses of 0.5, 1, 2, 4, and 8 Gy, were cultured for 48 hrs with LPS, Con A, and 2-ME to make chromosome preparations. Exchange-type aberrations such as translocation were analyzed using multiplex-fluorescence *in situ* hybridization (M-FISH). While translocation rates were not increased before 456 days in the non-exposed control mice, those were increased in the irradiated mice, depending on total doses, and were about 25-times higher at the total dose of 8 Gy than those of the age-matched control mice, accompanied by more complex aberrations involving more than three chromosomes. These results indicate a possibility that translocations induced by chronic low dose-rate radiation could persist in lymphocytes, and accumulate in proportion to the total dose. (This work was supported by a grant from Aomori Prefecture, Japan.)

(PS3006) Experience with biological dosimetry. Horst Romm, Ursula Oestreicher. Federal Office for Radiation Protection, Oberschleissheim, Germany.

Chromosome analysis is the method of choice in case of Biological dosimetry, used for the quantification of exposures to ionising radiation. The advantage and disadvantage of dicentric chromosomes and symmetrical translocations are described. In general, confounding factors on the lower detectable dose limit and scoring criteria for symmetrical translocations are discussed.

In case of acute exposures scoring of dicentric chromosomes will be preferred, when the blood sample can be taken shortly after the exposure. The dicentric chromosome is characteristic for ionizing radiation and the spontaneous frequency is very low in healthy general population (~1 dicentric/1000 cells) and may be

influenced by smoking. After whole body exposure with low LET radiation doses down to about 100 mGy are detectable.

In case of chronical exposure or if the exposure happened years before blood sampling, the analyses of symmetrical translocations in stable cells will be favoured. In contrast to dicentric chromosomes, which are fading with time, symmetrical translocations can persist, being transmitted from blood forming tissue to the peripheral blood. The background frequency of symmetrical translocations show a clear age dependency, resulting in a ~10 fold higher spontaneous frequency and in higher individual variation. Using appropriate calibration curves whole-body doses of about 300 mGy for individuals <40 years of age and about 500 mGy for individuals >40 years of age can be detected.

(PS3007) Micronucleus (MN) versus nucleoplasmic bridge (NPB) assessment for radiation biodosimetry in human lymphocytes. Irena A. Nowak, Ollivier Hyrien, Yuhchyau Chen. University of Rochester, Rochester, NY, USA.

Purpose: The cytokinesis-block micronucleus assay (CBMN) is an established cytogenetic method for the measurement of chromosome loss and breakage in lymphocytes. Both micronucleus (MN) frequency and nucleoplasmic bridge (NPB) frequency increase after genotoxic insults, yet the validity of MN and NPB assay for radiation biodosimetry remains to be defined. **Methods**: Peripheral blood mononuclear cells (PBMN) in healthy donors were isolated and irradiated using a Cs-137 source at a dose rate of 3.2 Gy/min. Three independent cultures were set up for each radiation dose in RPMI 1640 medium supplemented with 10% FBS and Phytohemoagglutinin A for 72 h. Cytochalasin B was used to inhibit cytokinesis and slides prepared for microscopy. For each radiation dose, 1000 binucleated cells were scored for MN and for NPBs. A nonparametric Kruskal-Wallis test was used to assess the relationship between the dose of irradiation and both the frequency of MN and NPBs ($p < 0.0001$). The Hill model was fitted to the radiation dose-response curves for each human subject. Mixed models were used to account for inter- and intra-subject variations when describing the distribution of the percentage of MN at the different doses of irradiation. Overdispersed Poisson regression models were considered to describe the distribution of counts of NPBs at various radiation doses. The sensitivity of each biomarker for detecting radiation exposure was deduced from these models. **Results:** We observe an increase of both MN and NPB frequency with increasing radiation doses. The sensitivity of MN assay for detecting radiation exposure is estimated as 58%, 84%, 98%, 99.9%, 100%, and 100% for radiation doses of 0.5 Gy, 1 Gy, 2 Gy, 4 Gy, 6 Gy, and 8 Gy, respectively. The sensitivity of NPBs for detecting radiation exposure is 33%, 62%, 92%, 99.9%, 100%, and 100% for the same doses of radiation, respectively. **Conclusions**: Our data support that both MN frequency and NPB frequency are potential assays for radiation biodosimetry. The sensitivity of both markers approach 100% for radiation doses above 4 Gy, and are above 90% for radiation dose of 2 Gy. For radiation doses less than 2 Gy, the MN scoring appears better in terms of sensitivity than NPB frequency. However, the NPB scoring appears more specific than the MN scoring in the control group without in vitro radiation.

(PS3008) Optimizing cytogenetic analysis for radiation-induced chromosomal aberration in C57Bl/6 mice. Ying Tsai[1], Catherine Ferrarotto[2], Nancy Wang[1], Ruth Wilkins[2], Yuhchyau Chen[1]. [1]University of Rochester, Rochester, NY, USA, [2]Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa, ON, Canada.

Purpose: The dicentric chromosome assay (DCA) is the standard for radiation biodosimetry and is essential for the validation of novel assays. Although DCA in humans has been studied extensively, little work has been published on mice in recent years. As mouse models are commonly used for *in vivo* radiation research, mouse protocols optimized for DCA using modern culture conditions and reagents are essential for research in this area. **Method:** C57Bl/6 mice at 8 –9 weeks-old were exposed to 0 to 3 Gy of total body irradiation (TBI) using a Cs-137 source. At 43 to 46 hr post-TBI, mice were anesthetized and blood was drawn by cardiac puncture according to the institutional animal protocol. Blood was cultured using various conditions and time intervals in RPMI 1640 with 10% FBS and different combinations of lipopolysaccharide (LPS) and phytohemagglutinin (PHA). Bromodeoxyuridine (BrdU) was added to the culture medium for visual differentiation of cells in 1st (M1), 2nd (M2) and 3rd (M3) mitoses. Colcemid was added 3 hr before cells were harvested at various culture intervals from 32 to 50 hr. The concentration of potassium chloride for hypotonic treatment and pre-fixation conditions were also optimized. Cells were stained using a Fluorescence Plus Giemsa protocol. Slides were scored for M1/M2/ M3 ratios per 200 metaphases. Dicentrics were scored at 32 and 44 hr. **Results:** There was a 50% contamination of M2 and M3 cells in samples cultured for longer than 38 hr. The least contamination was observed in 32 hr cultures showing less than 20% M2 and M3 cells. Dose response curves for 32 and 44 hr cultures showed an increase of dicentrics with increasing dose. The dose response curve was exponential for the 32 hr culture, but not for 44 hr culture. We found no major increase in M2 and M3 contamination with increasing doses up to 3 Gy. **Conclusions:** Many variables influence the outcome of DCA in C57Bl/6 mice, including reagents, concentrations of mitogens and colcemid, and the culture intervals. We found that LPS from Sigma and PHA from Irvine Scientific produce reliable metaphases. Lymphocytes harvested at 32 hr resulted in more metaphase spreads and the least M2 and M3 contamination. In addition, a 32 hr culture time is adequate for all doses with no adjustment for radiation induced cell-cycle delay required.

(PS3009) Relative biological effectiveness of low energy alpha particles in the survival of V79 hamster cells. Bliss L. Tracy[1], Mark A. Hill[2], David L. Stevens[2], Dudley T. Goodhead[2]. [1]Radiation Protection Bureau, Ottawa, ON, Canada, [2]Medical Research Council, Harwell, United Kingdom.

As alpha particles slow down in their passage through matter, the linear energy transfer (LET) rises, reaching a maximum value (the Bragg peak) at an alpha energy of about 0.7 MeV, and thereafter decreases rapidly as the alpha energy approaches zero. This study was undertaken to shed light on how the relative biological effectiveness (RBE) of alpha particles varies along the entire track of the particle as it passes through living cells.

The endpoint chosen was the clonogenic survival of V79 Hamster cells - a well characterized cell line. Alpha particle irradiations were delivered by the Medical Research Council plutonium-238 irradiator, which allows the irradiation of a cell layer with an essentially parallel beam of nearly monoenergetic alpha particles. By varying the depth of helium through which the alpha particles pass, the energy can be varied from 4.2 MeV down to zero, with covers a range of LET values from 102 to 223 keV/μm. For each LET value, clonogenic survival as a function of radiation dose was measured 8 days after irradiation. The relative biological effectiveness (RBE) for each LET value was calculated by comparing the alpha survival curve with that from 250 kVp X-rays.The RBE values were found to remain nearly constant throughout the entire range of LET studied. For example, the RBE for 1% survival varied only from 3.4 to 2.9 as the LET changed from 110 to 220 keV/μm. This is contrary to what one would expect from the literature, which indicates that the RBE should reach a maximum at about 110 keV/μm and then decrease beyond this value. The results are discussed in terms cell damage mechanisms.

(PS3010) Comparison of BAC FISH with specific telomeres and centromere probes and chromosome painting on detection of radiation induced chromosome translocation and dose reconstruction. Qing-Jie Liu[1], Xue Lu[1], Xiao-Wei Wang[2], Jiang-Bin Feng[1], Xiao-Ning Chen[3], Julie R. Korenberg[3], De-Qing Chen[1]. [1]National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention, Beijing, China, [2]Chinese National

Human Genome Center, Beijing, Beijing, China, [3]Cedars-Sinai Medical Center, UCLA, Los Angeles, CA, USA.

To compare the efficiency of fluorescence *in situ* hybridization using bacteria artificial chromosome (BAC FISH) established in our lab and conventional chromosome painting (PAINT) on detection of radiation-induced chromosome translocation and dose reconstruction. BAC clones specific for telomeres and centromeres of chromosomes 1, 2 and 4 were selected, and an BAC FISH (Chromosome 1, 2 and 4 stained with green color, red color and yellow color) method was to be established. Normal human peripheral blood samples were irradiated with 0~5.00Gy ^{60}Co γ-rays. Then the chromosome translocations in these samples were detected with BAC FISH and PAINT (probes from Cytocell Technologies) using probes of chromosomes 1, 2 and 4. The genome translocation rates were calculated with observed chromosome translocation rates, and the dose-response curves of two methods were established. The peripheral blood samples of two individuals, who were accidentally exposed ionizing radiation 3 years before and had the biodosimetry data shortly after the accident, were collected and chromosome translocation analyzed. The absorbed doses were estimated with two methods according to the dose-response curves. **Results** An BAC FISH method, using BAC clones specific telomeres and centromeres of chromosomes 1, 2 and 4, labeled with biotin-14-dATP and/or digoxigenin-11-dUTP, was established. The 3 pairs of chromosomes could be easily distinguished. The genome translocation rates induced by 0~5.00Gy ^{60}Co γ-rays were increased with absorbed dose. The observed translocation rates with BAC FISH were higher than that with PAINT on each dose level. The dose-response curve were Y= $0.043D^2$ +0.0008D +0.0048 for BAC FISH and Y= $0.043D^2$ + 0.006D + 0.0027 for PAINT. The genome translocation rates of the two individuals who were accidentally exposed to radiation were calculated, and the exposed dose levels were reconstructed. The estimated dose levels were comparable. **Conclusion** The BAC FISH established in this study could be used in dose reconstruction for previous ionizing radiation exposure.

(PS3011) Radiation-induced chromosome instability and bystander effect in human peripheral blood lymphocytes in delayed terms following Chernobyl accident. Maria A. Pilinskaya, Sergey S. Dibskiy, Olena W. Shemetun, Yelena B. Dibskaya, Oksana A. Talan, Ludmila R. Pedan. Research Centre for radiation medicine, Kiev, Ukraine.

In delayed terms following Chernobyl accident evaluation of late cytogenetic consequences of human radiation exposure - hidden, delayed, transmissible chromosome instability and "bystander effect" became actual. For investigation of radiation-induced chromosome instability two methods had been applied - provocative mutagenesis assay (G_1-dimatyph or G_2-bleomycin tests used for persons differed on absorbed radiation doses*)* and two-termed (during 48 and 144 hours) cultivation of peripheral blood lymphocytes received from progeny of irradiated parents. In children born to irradiated parents as well as in clean-up workers with low radiation doses¨adaptive response£ had been detected; in high-doses patients recovered from acute radiation sickness increased chromosome sensitivity (hidden chromosome instability?) to additional mutagenic exposure had been revealed. In progeny of irradiated parents increased frequency of chromosome aberrations (single fragments and abnormal monocentrics) had been established especially in long-termed cultures. Induction of chromatid breaks confirmed possibility of expression of delayed chromosome instability in consequent mitosis; appearance of stable aberrations can consider as biomarker of transmissible chromosome instability in progeny of irradiated parents. In proposed by us model system - "mixed human lymphocytes culture" consisted of cells differed by cytogenetic sex markers - interaction between X-irradiated *in vitro* (in doses 250 and 1000 mGy) and intact cells had been discovered. The difference between spectrum of aberrations in exposed and intact cells had been established - in targeted cells specific cytogenetic markers of irradiation dominated, in "bystander" cells chromatid types of aberrations (chromatid breaks and terminal chromosome deletions - cytogenetic indicators of chromosome instability) mainly induced.

(PS3012) Radiation induced bystander studies in human prostate tumor cells. Vered Anzenberg, Jeffrey A. Coderre. Massachusetts Institute of Technology, Cambridge, MA, USA.

The purpose of this study was to characterize the ability of irradiated tumor cells to produce bystander effects in co-cultured cells. Human DU145 prostate carcinoma cells were grown on a 1.4 μm-thick mylar membrane in specially constructed cell culture dishes for irradiation with alpha particles (average energy 3.14 MeV) from a ^{241}Am source, or in 6-well plates for irradiation with 250 kVp x-rays. Bystander cells (either DU145 prostate carcinoma cells or AG01522 human fibroblasts) were grown on inserts and placed into the medium above the irradiated DU145 cells immediately after the irradiation and co-incubated for 4 hrs. The bystander effect endpoints measured were micronucleus (MN) formation and γH_2AX double strand break repair foci. A 1.5–2.0-fold increase in MN formation was observed in both DU145 and AG01522 bystander cells after either alpha particle or x-ray irradiation of the DU145 target cells. When the general radical scavenger dimethyl sulfoxide (DMSO), present in the medium during the irradiation and co-incubation of the bystander cells, all MN formation bystander effects were completely eliminated. The nitric oxide scavenger PTIO blocked MN formation in DU145 and AG01522 bystander cells after x-ray irradiation of the target DU145 cells. In contrast, after direct alpha particle irradiation of DU145 cells, PTIO blocked MN formation in AG01522 bystander cells but not in bystander DU145 cells. A 1.5-fold γH_2AX bystander increase was only detected in AG01522 cells, and only after x-ray irradiation of target DU145 cells. Both DMSO and PTIO, when present during the x-ray irradiation of the DU145 cells, completely eliminated the γH_2AX bystander signal in AG01522 cells. Alpha particle irradiation of the target DU145 cells produced no γH_2AX bystander effect in either cell line. DU145 cells showed no increase in γH_2AX bystander signal under any conditions. This study has focused on bystander effects produced by irradiated tumor cells and has detected differences between alpha particles and x-rays in: 1) the ability of scavengers to block the bystander effect, and 2) the ability of AG01522 cells to respond to the medium-mediated signal.

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(PS3013) Radiation-quality dependence of genomic instability in mutation induced by the pre-treatment with low-fluence heavy ions. Masao Suzuki, Chizuru Tsuruoka, Yukio Uchihori, Hisashi Kitamura. National Institute of Radiological Sciences, Chiba, Japan.

A central paradigm in radiation biology has been that only a cell "hit" by a track of radiation would be affected to induce radiobiological effects, and a cell "not hit" should not be. This paradigm is the basis for the current system for risk estimation from radiation. However, it recently has been challenged by so called non-targeted effects, including bystander effect and genomic instability, and such radiation-induced non-targeted effects may have important implications for risk evaluation of low dose / low dose rate radiations. In this study we have investigated cellular responses in normal human fibroblasts induced with low dose / low dose rate irradiations of qualitatively different radiation types, such as gamma rays, neutrons and high-LET heavy ions. Cells were pre-treated with low-fluence irradiation (~1mGy/7–8h) of ^{137}Cs gamma rays, ^{241}Am-Be neutrons, helium ions (LET=2.3keV/μm) and carbon ions (LET=13.3keV/μm) before following irradiation with a 200kV X-ray challenge dose. The helium- and carbon-ion beams were produced by the Heavy Ion Medical Accelerator in Chiba (HIMAC) at National Institute of Radiological Sciences in Japan. There was observed no difference in X-ray induced cell-killing effects, which was detected with the colony-forming assay, when using pre-treatment with low-dose irradiations of gamma rays, neutrons, helium ions and carbon ions. For mutation induction at *hprt* locus detected as 6-thioguanine resistant clones, there was no difference in X-ray-induced mutation frequency at 1.5Gy of X-ray challenge dose between un-pretreated and gamma-ray pre-treated cells. In the case of the pre-treatment with high-LET ions, mutation frequency was around 4.0 times higher in carbon-ion pre-treated cells and 1.9 times higher in helium-ion pre-treated cells than in un-

pretreated cells. On the contrary, it was reduced in neutron pre-treated cells, when comparing to un-pretreated cells. Furthermore, the enhancement of X-ray-induced mutation frequency in cells pre-treated with helium and carbon ions was reduced at the control level, when using a specific inhibitor of gap-junction mediated cell-cell communication (40μM lindane). There is evidence that gap-junction mediated cell-cell communication play an important role of inducing genomic instability by pre-treatment with heavy ions.

(PS3014) Analysis of heavy-ion induced bystander effect using microbeam irradiation. Tomoo Funayama[1], Seiichi Wada[2], Takehiko Kakizaki[1], Nobuyuki Hamada[2], Yuichiro Yokota[1], Tetsuya Sakashita[1], Yasuhiko Kobayashi[1]. [1]Japan Atomic Energy Agency, Takasaki, Japan, [2]Gunma University, Maebashi, Japan.

Heavy-ion radiation is known to induce higher extent of biological effect on irradiated biological organisms than other lower-LET radiations. However, because of a spatial distribution of ion hit, cell population exposed to a low dose of heavy-ion radiation contains limited number of hit cells, and rests are non-hit. In such situation, contribution of bystander effect may be important to assess a total effect on cell population.

Therefore, we have developed a system of heavy-ion microbeam, which enables targeting and irradiating individual cells with precise number of heavy-ion particles. As our system contains tracking system of each individual cell in post-irradiation period, it is capable of tracking and analyzing changes of non-hit bystander cell individually even long after irradiation. Using the system, we exhibited that an argon ion hit on cell nucleus causes growth inhibition of CHO-K1 cells, and simultaneously the cells located nearby hit-cells also shows significant growth inhibition by bystander effect.

To explore a mechanism underlying this bystander growth inhibition, we focused on DNA double strand breaks (DSBs), which are known as a trigger of various radiation response of direct hit cells. We inoculated approximately 2,500 cells on microbeam dish, thereafter selected 25 cells are targeted and irradiated by 5 count of $^{40}Ar^{13+}$ ion microbeam (11.5 MeV/u, LET=1260 keV/μm). After irradiation, cells were fixed and induction of DSBs were visualized by immunostaining of phospohlylated histone H2AX (γH2AX), which is known as a molecular marker of DSBs. Thereafter, the frequency of γH2AX positive cell nuclei was scored.

The samples with the postincubation time longer than 30 minutes showed increased frequency of γH2AX positive cells compared with medium-irradiated control sample in a range of 3–4%. This result suggested that the heavy ion irradiation induced bystander effect on the phosphorylation of histone H2AX in CHO-K1 cells. As γH2AX is a molecular marker of DSBs, the result suggests that heavy-ion radiation induces DSBs not only on direct hit cells, but on bystander cells. Further analysis will be needed to elucidate mechanisms of how DSBs are induced in bystander cells, however, it is clear that DSBs are a key player of heavy-ion induced bystander response as well as direct hit effect.

(PS3015) New in vitro micronucleus assay to investigate bystander effect in artificial human 3D tissue system following low LET irradiation. Giuseppe Schettino[1], David J. Brenner[2]. [1]Gray Cancer Institute, Northwood, United Kingdom, [2]Columbia University, New York, NY, USA.

At Columbia University, we have developed a novel in vitro assay to investigate micronuclei formation in 3D artificial human tissues following partial exposure to low LET protons. The EpiDerm tissues (MatTek) consist of fully differentiated cell layers resembling the normal human epidermis and cultured on collagen coated cell culture inserts. The irradiation is performed using 4.5 MeV protons (10 keV/um entrance LET) from the new RARAF accelerator (5 MV Singletron) and slit masks (50 um) to expose only a narrow strip of cells. Following the irradiation, the samples are incubated in Cytochalasin-B for 48h when are then cut into narrow slices (200–400 um) parallel to the irradiation line. Each slice is then individually incubated in 0.1% EDTA trypsin till obtaining a single cell solution. Cells are finally fixed on slides where are scored for micronuclei after being stained with Acridine Orange. The enzymatic dissociation provides ~2.5x10^5 cells/sample (>90% viable by trypan blue exclusion test). The average number of dissociated cells is in agreement with the estimated number of cells/sample given by MatTek, indicating that almost all cells of interest are collected. After 48h in Cytochalasin-B, the binucleation frequency is ~60% and it does not increase significantly after 72h incubation. Using a custom developed microtome, the tissue samples can be cut down to 50 um stripes although 200 um stripes are easier to handle and provide a more statistically relevant number of cells. Micronuclei are scored for each individual stripe and the fraction of micronucleated cells is reported as a function of the stripe distance from the irradiation line. Micronuclei induction significantly higher than background level (2-fold increase) has been measured in samples irradiated with as little as 0.1 Gy. A bystander response was measured up to 3 mm away with no clear dose dependency. The technique described allows the investigation of the bystander effect in a complex 3D environment which closely mimics the human skin. Although the method has been applied to micronuclei induction, it can easily be used for chromosomal aberrations. The data indicate that low LET radiation may trigger significant bystander effect in 3D tissue systems over a considerable distance of a few millimetres.

(PS3016) Cellular response in imrt:three types of bystander effects. Natalka Suchowerska[1,2], E. Claridge[1,2], J. Bewes[2], M. Zhang[1], D. R. McKenzie[2], M. Ebert, M. Jackson[1], C. Milross[1] [1]Royal Prince Alfred Hospital, Sydney, Australia, [2]University of Sydney, Sydney, Australia.

IMRT promises improved patient outcomes by precisely conforming the radiation dose to the tumour volume. The spatial modulation of dose, inherent to IMRT, makes the expression of Bystander Effects more apparent. There is a growing body of evidence to show that in IMRT, the local dose is an inaccurate predictor of tissue response. The distribution of dose, both spatially and temporally, has a major influence. We report on cell survival following exposure to spatially modulated beams, as created by intensity modulated radiotherapy (IMRT), using in vitro experiments with malignant melanoma cells (MM576). Cell survival in modulated fields was compared with cell survival in a uniform control field. Three different spatial modulations of the field were used: a control "Open" field in which all cells in a flask were uniformly exposed; a "Quarter" field in which 25% of cells at one end of the flask were exposed; and a "Striped" field in which 25% of cells were exposed in three parallel stripes. The cell survival in both the shielded and unshielded regions of the modulated fields, as determined by a clonogenic assay, were compared to the cell survival in the Open field. We have found three distinct ways in which the cell survival is influenced by the fate of nearby cells. The first of these (Type I effect) is the classical Bystander Effect whereby cell survival is reduced when nearby cells receive a high radiation dose, but some survive. The Type II effect is an increase in cell survival when nearby cells receive a lethal dose. The Type III effect is an increase in survival in cells receiving a high dose of radiation when nearby cells receive a low dose of radiation. Our observation of these Bystander Effects highlights a need for improved radiobiological models, that include communicated effects.

(PS3017) How do experimental conditions and radiation affect cytokine signals? Angelica Facoetti[1], Daniele Alloni[2], Francesca Ballarini[2], Andrea Mairani[2], Luca Mariotti[2], Rosanna Nano[1], Andrea Ottolenghi[2]. [1]Dipartimento di Biologia Animale, Università di Pavia e INFN, sezione di Pavia, Pavia, Italy, [2]Dipartimento di Fisica Nucleare e Teorica, Università di Pavia e INFN, sezione di Pavia, Pavia, Italy.

In the field of radiobiology, considerable efforts are presently directed towards non targeted effects following exposure to ionizing radiation in cells that have not seen radiation but have been affected by intercellular signals from irradiated cells. In particular, one of the

major challenges remains in understanding the mechanisms underlying the bystander signal transmission.

Theoretical models and simulations can be of help for improving our knowledge of the mechanisms, and for investigating the possible role of these effects in determining deviations from the linear relationship between dose and risk which is generally assumed in radiation protection. Our group has developed a model for the reproduction of bystander data with sparsely seeded cells, based on the diffusion of cytokines in the medium, and several questions raised concerning the cytokine release process.

In the present work the influence of several experimental conditions such as cell density and medium volume on cytokine release will be investigated by means of ELISA in non irradiated AG01522 fibroblasts.

Then, the influence of radiation quality will be evaluated by the use of microarray and ELISA analysis of medium collected after low (gamma rays) and high (alpha particles and carbon ions) LET radiation.

A number of experimental approaches exist for the study of the bystander signal molecule(s) but the transfer of culture medium from irradiated cells to unirradiated cells remains the most diffuse, although controversial results were reported. One explanation for the differences found between laboratories is that there might be several experimental factors affecting the results.

In the present work several crucial points of medium transfer experiments, such as filtration and stocking of samples, cell densities and medium amounts, will be investigated measuring IL-6 concentrations in medium harvested from AG01522 fibroblasts.

In conclusion, one way to solve these doubts is the identification of a reproducible model system that involves intercellular signalling and that represents a good candidate for studying perturbation by ionizing radiation.

Acknowledgments

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(PS3018) Initiation and manifestation of genomic instability in irradiated and bystander populations. Ryonfa Lee, James W. Kelly, Kim L. Chapman, Munira A. Kadhim. Medical Research Council, Harwell, United Kingdom.

Genetic instability (GI) is defined as a persistent elevated rate in the accumulation of new genetic alterations in cells and is regarded as a characteristic of tumourigenic progression. Previous studies from our group have demonstrated the expression of GI response in both irradiated and bystander cells measured by chromosome aberrations. The initiation and perpetuation of GI in irradiated cells directly involve radiation-induced DSBs; however, in bystander populations, the immediate contribution of DNA lesions to the formation of chromosome aberrations is still unclear. Therefore, this study aims to reveal the earliest detectable chromosome aberrations using a chemically induced premature chromosome condensation (PCC) technique. Moreover, the process to the manifestation of GI is assessed using the same biological endpoints in irradiated and bystander cells.

Human foetal lung fibroblasts (HF19), in which GI has been previously observed, were exposed to 0.01 - 1 Gy X-rays and co-cultured with unexposed cells for 1 or 18 hr to allow communication between the irradiated and bystander populations by media-borne factors. Cells were then separated from the two vessels for either immediate analysis or the transfer into long-term culture. For the detection of cytogenetic damage, excess chromosome fragments and chromatid breaks were analysed in prematurely condensed G_2-cells (G_2-PCCs) following calyculin A treatment.

An elevated number of chromosome fragments was detected in exposed samples collected at 18 hr post-irradiation, although no pronounced increase was observed in bystander cells co-cultured for both 1 and 18 hr. Chromosome fragment yields in both irradiated and bystander cells were comparable to the control level following long-term culture. However, an increase in the number of chromatid breaks was found in both directly exposed and bystander populations following further cell cultivation. The investigation of chromosomal damage in G_2-PCCs shows that chromatid-type rather than chromosome-type aberrations are the main type of lesions contributing to GI in both irradiated and bystander cells following low-LET and low-dose exposure. The analysis of apoptosis and accelerated senescence are currently ongoing and will be presented with cytogenetic results.

(PS3019) Study on bystander cell death inV79 cells using SR X-ray microbeam. Munetoshi Maeda[1], Masanori Tomita[2], Noriko Usami[1], Katsumi Kobayashi[1]. [1]High Energy Accelerator Research Organzaition, KEK, Institute of Materials Structure Science, Tsukuba-shi, Ibaraki, Japan, [2]Central Research Institute of Electric Power Industry, Low Dose Radiation Research Center, Komae-shi, Tokyo, Japan.

In cell populations exposed to ionizing radiation of low dose, the biological effects appear in cells which have not been traversed by charged particles. This phenomenon is termed the bystander effect. Detailed mechanism of the bystander effect is not yet clear, although the important role of intracellular and/or intercellular signaling is suggested. In irradiation experiments using a broad radiation field, we cannot distinguish individual cellular responses of bystander type. Therefore, the microbeam cell irradiation system, with which we can observe cellular responses of both non-irradiated individual cells and individual cells irradiated by microbeams, becomes a powerful tool to elucidate the mechanisms of the bystander effect. We have been studying the biological effects of low-dose radiation using a synchrotron X-ray microbeam irradiation system developed at the Photon Factory, KEK. This system is able to provide a 5.35 keV X-ray microbeam of arbitrary size larger than 5 microns square. In this research, by using this advantage, we have compared bystander cell death of V79 cells irradiated with X-ray microbeams of different sizes. To avoid interference between the colonies originating from single cells, we plated 2,000 individual V79 cells on specially designed Mylar-based dishes, and irradiated 5 cells located on the center of the scanned area (6 mm square) in the dish in two ways: one is to aim at nucleus with 10 microns square X-ray beam, and the other, entire-cell with 50 microns square X-ray beam. Survival fractions of bystander cells in the scanned area were measured by using single-cell clonogenic assay. Interestingly, in the case of nucleus-irradiation, survival fraction of bystander cell decreased down to 90% around 1 Gy and increased back to 96% in high dose region, whereas in the case of entire-cell-irradiation, it decreased monotonously to 92%. Dose-dependent enhancement of bystander cell death was observed only in nucleus-irradiated case. We speculate that the energy deposition in cytoplasm might induce some kind of intracellular signaling and reduce the bystander signal secretion. The relationship between cell death and intracellular energy-deposited sites will be discussed.

(PS3020) Low dose radiation-induced bystander effects in the spleen. Benjamin J. Blyth[1], Edouard I. Azzam[2], Roger W. Howell[2], Pamela J. Sykes[1]. [1]Flinders University and Medical Centre, Adelaide, South Australia, Australia, [2]University of Medicine and Dentistry of New Jersey, Newark, NJ, USA.

Bystander effects triggered by 'hit' cells after low dose radiation exposure have been repeatedly demonstrated using a variety of biological endpoints in many *in vitro* systems. To date, work to corroborate these observations *in vivo* has been limited. We have been developing and optimising an adoptive transfer system in C57BL/6J mice to analyse the response of bystander cells to low dose irradiated cells *in vivo*.

Lectin-stimulated splenic T lymphocytes are radiolabelled with ^{3}H-thymidine (<1 mBq/cell). The low-energy β-particles emitted by the DNA-incorporated ^{3}H chronically irradiate the labelled lymphocytes. Five-hundred thousand irradiated donor cells (or control cells sham-treated with thymidine), fluorescently labelled with a rhodol-based intracellular-bound dye are then injected intravenously into syngeneic recipient mice. After 20 hours, adoptively transferred donor cells identified by the tracking-dye (confirmed by microautoradiography) are found lodged in the spleen. The absorbed dose and the number of donor cells lodged in the spleen can be altered to perform dose/dose-rate studies. Using multi-coloured fluorescence microscopy, apoptosis, proliferation and transforming growth factor-β expression are presently being assessed in bystander cells in proximity to irradiated or unirradiated

control donor cells. These endpoints represent effector and signalling pathways that have been observed by others *in vitro* and implicated in propagating bystander effects.

The results from this study will assess tissue responses *in vivo* to non-uniform dose distributions and determine whether low-dose irradiated cells communicate either damage or protection to their unirradiated neighbours.

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(PS3021) Bystander response in human lymphocytes and leukemic cells by irradiated conditioned medium from human leukemic cells exposed to low and high dose of gamma radiation. Badri N. Pandey, Amit Kumar, Lori Rastogi, Kaushala P. Mishra. Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India.

Recent investigations have shown the higher radio-sensitivity of many types of tumor cells towards radiation doses <50 cGy. This emerging phenomenon called low dose 'hyper radio-sensitivity' has recently gained clinical attention to treat patients with low-grade Non-Hodgkin's Lymphoma by whole body low dose irradiation. In addition, evidences have been accumulated to show the interaction of irradiated cells with their un-irradiated neighbors (radiation-induced bystander effect) and thus modifying the cellular radio-sensitivity. The present investigation is aimed to study the gamma-radiation effects in human promyelocytic leukemic cells (HL-60) exposed to either low dose 5 cGy (0.2 cGy/min.) or a dose of 1 Gy (1.6 Gy/min.). Furthermore, we have studied the effect of irradiated conditioned medium (ICM) from HL-60 cells on un-irradiated tumor cells (HL-60) and normal cells (human peripheral blood lymphocytes) to understand the bystander response under the irradiation conditions. Compared to controls, an increase in apoptosis was observed in HL-60 cells exposed to 5 cGy (27 %) or 1 Gy (45 %) measured by annexin-V and TUNEL method after 24 h of incubation. The magnitude of apoptosis was further enhanced (10–20 %) with increase in post-irradiation incubation (up to 48 h). The un-irradiated HL-60 cells cultured in ICM obtained after 24 h of irradiation of HL-60 cells either with 5 cGy or with 1 Gy showed 15 and 25 % increase in apoptosis, respectively. However, it was interesting to observe that increase in cellular apoptosis was 5 % in lymphocytes cultured in ICM obtained from HL-60 cells irradiated after low dose (5 cGy), which was significantly higher(25 %) in lymphocytes cultured with ICM from HL-60 cells after 1 Gy. Under these irradiation conditions, the magnitude of apoptosis in tumor and normal lymphocytes was found correlated with increase in level of intracellular reactive oxygen species (ROS) and alterations in mitochondrial membrane potential measured by fluorescence probes, 2,7-dichloro dihydro fluorescein diacetate (DCH-FDA) and DiO6, respectively. The results suggest release of factor(s) from irradiated tumor cells, which seems to show differential bystander response towards neighboring tumor and normal cells depending on the applied dose of radiation.

(PS3022) Bystander effect in normal human fibroblast cells induced by very low-doses of X-ray irradiation. Mitsuaki Ojima, Nobuhiko Ban, Michiaki Kai. Oita University of Nursing and Health Sciences, Oita, Japan.

DNA damage, such as DNA double strand breaks, is generally accepted to be the most biologically significant lesion by ionizing radiation. The generation of radiation-induced DNA damages in cells has been recently examined by enumeration of phosphorylated ataxia telangiectasia mutated (ATM) foci. In this study, to understand the cellular effect of very low-dose radiation, we examined the formation of phosphorylated ATM foci in non-dividing primary normal human fibroblasts cells (MRC-5) irradiated to very low-doses of X-rays. Non-dividing confluent cells on slide glass were irradiated with 1.2~100 mGy of X-rays and incubated for 0.05 h. After incubation, the formation of phosphorylated ATM foci was investigated by fluorescein antibody technique of phosphorylated ATM. When the dose of X-ray increased from 1.2 mGy to 20 mGy, the number of phosphorylated ATM foci increased with a slope larger than when the dose of X-ray increased from 20 mGy to 100 mGy. The existence of radiation-induced bystander effect, damage occurs in cells that were not traversed by radiation but were in receipt of signals from irradiated cells, is now accepted. A characteristic of the radiation-induced bystander effect is thought to enhance cell damage, and so we thought that the lower the radiation dose, the higher the number of phosphorylated ATM foci, suggesting the existence of radiation-induced bystander effect. In the recent study, gap junctional intercellular communication (GJIC) has been thought to be relevant to radiation-induced bystander effects. We further investigated whether low-dose sensitivity observed in cells irradiated with doses ranging for 1.2 mGy to 20 mGy is canceled by Lindane, an inhibitor of GJIC. This result showed that no low-dose sensitivity was observed when cells were treated with 40 μM Lindane and that the induction of phosphorylated ATM foci produced a linear dose-response relationship with doses ranging for 1.2 mGy to 100 mGy. Thus, this study suggested that a dramatic increase of initial DNA damage in cells irradiated with doses ranging for 1.2 mGy to 20 mGy resulted from a radiation-induced bystander effect. These results may have implications for understanding the radiation risk of low dose.

(PS3023) Biochemical regularities of post-radiation recovery in animal spermatozoa. Kateryna Andreychenko[1], Alla Klepko[2], Nataliya Nurischenko[1]. [1]Taras Shevchenko National University of Kyiv, Kyiv, Ukraine, [2]Scientific Center for Radiation Medicine, Kyiv, Ukraine.

Animal spermatozoa are highly differentiated sexual cells which are specializing in storage and delivery hereditary material to the egg cells for fertilization and syngamy. The present research aimed elucidating biochemical mechanisms of post-radiation recovery in rat spermatozoa. The experiments were performed on epididymal and isolated spermatozoa irradiated in saline by gamma-rays of ^{60}Co with dose input 0,2 Gy/sec in the wide dose range up to 1000 Gy at 20°C. The activities of four enzymes, namely superoxide dismutase (SOD), catalase (Cat), glutathione reductase (GR) and glutathione peroxidase (GP) were identified both in isolated spermatozoa and in epididymises containing spermatozoa according to the commonly used protocols. DNA repair was evaluated with the aid of DNA unscheduled synthesis technique.

The experiments have shown the obvious dose dependent loss of motility and velocity by isolated spermatozoa upon gamma-irradiation. Meanwhile the epididymal spermatozoa proved to be more radioresistant and persisted more severe irradiation. The biochemical identification of enzymatic activities in isolated and epididymal spermatozoa has shown that the activities of SOD, GR, GP were approximately the same. Cat in epididymal spermatozoa was tenfold more active than in isolated ones, the enzyme being fully located in the epididymal tissue but not in spermatozoa. Upon gamma-irradiation high activity of epididymal Cat significantly contributed to peroxide inactivation thus preventing chain reactions development for lipid peroxidation in plasma membranes. In contrast to that, in isolated spermatozoa intensive peroxide formation caused rapid lipid peroxidation followed by plasma membranes rupturing. Therefore the epididymal spermatozoa were able to recover from sublethal and potentially lethal damages and to retain space movements and tail swings due to antioxidative defense of their membranes triggered by Cat from epididymises. However the DNA lesions remained unrepaired both in isolated and epididymal spermatozoa. In this connection an association of genome damaging with the preservation of moving characteristics in epididymal spermatozoa may be valuable for biotechnological purposes, e.g. gene transfer and parthenogenesis induction.

(PS3024) Cranial irradiation as a non-invasive tool to specifically alter adult hippocampal neurogenesis and induce hippocampal-dependent cognitive deficits. Nada Ben Abdallah[1], Robert K. Filipkowski[2], Piotr Jaholkowski[2], Leszek Kaczmarek[2], Martin Pruschy[3], Lutz Slomianka[1], Hans-Peter Lipp[1]. [1]University of Zurich, Zurich, Switzerland, [2]Department of Molecular and

Cellular Neurobiology, Nencki Institute, Warsaw, Poland, [3]University Hospital of Zurich, Zurich, Switzerland.

Background

X-rays are used to non-invasively alter and clarify the function of neurogenesis in the adult rodent hippocampus. Previously, this approach showed a role of hippocampal neurogenesis in learning and memory, particularly with the Morris water-maze and fear-conditioning. It has been argued, however, that such behavioral impairment arises from a combined effect of neurogenesis and other radiation-induced brain changes

Methods

To address this question, we investigated the effects of low doses of X-rays in two independent experiments. In experiment 1 we analyzed the time course of adult hippocampal proliferation, neurogenesis, and cell-death changes induced by a 4Gy dose in C57Bl/6j mice (n=48), at 16 hours, 48 hours, one week, and one month after radiation

In experiment 2 we studied the specificity of a 10Gy dose in damaging hippocampal neurogenesis by analyzing hippocampal cognitive performance. For this experiment, we used a genetic mouse model [Cyclin D2 knockout mice] displaying no adult neurogenesis in the subgranular zone of the hippocampus. As primary behavioral test we used the Morris water-maze

Results

Briefly, experiment 1 showed significant ($p<0.0001$) changes in hippocampal proliferation and differentiation, followed by complete restoration one month after treatment. Changes in cell-death were also obvious, and likewise recovered after one month

Experiment 2 revealed the absence of long-term memory retention of mice genetically lacking adult neurogenesis, regardless of whether or not they had received radiation. Interestingly, exposure to radiation significantly reduced long-term memory retention in WT-mice ($p<0.0001$) to levels similar to those of KO-mice arguing against claims that radiation effects are not specific to adult neurogenesis

Conclusions

These observations show (experiment 1) reversibility of radiation effects on adult neurogenesis, and (experiment 2) similarity between cognitive changes induced by genetically and radiation-induced ablation of adult neurogenesis, suggesting a phenomenological specificity of the effects of radiation on hippocampal neurogenesis and, thus, performance

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(PS3025) X-ray sensitivity of endothelial stem/progenitor cells does not correlate with induction of apoptosis or absence of checkpoints. Marc S. Mendonca, Helen Chin-Sinex, Ryan Dhaemers, Laura Mead, Merv C. Yoder, David A. Ingram. IU School of Medicine, Indianapolis, IN, USA.

Endothelial stem/progenitor cells are thought to play a role in normal tissue injury repair and perhaps tumor response after radiation and/or chemotherapy treatment. We have isolated a novel hierarchy of endothelial progenitor cells from human peripheral and umbilical cord blood, and vessel walls. All of these high proliferative potential cell lines were CD31, 141, 105, 146, and 144 positive and CD45 and 14 negative, therefore devoid of any hematopoietic cell contamination. We characterized the radiation response of three endothelial progenitor cell isolates from cord blood (CBM4, CBF10, and CBM12) and two from adult peripheral blood (EPC060805 and LM2). The plating efficiencies (PEs) and population doubling times (Tp) were varied but all of cell isolates formed large visible colonies ten days after single cell plating. Irradiation with 160 kVp X-rays revealed these endothelial progenitor cells to be quite radiation sensitive with SF-2Gy of 0.26 ± 0.07 and shoulderless exponential X-ray survival curves with D0s of 1.00 ± 0.1 Gt. However, fractionation studies (5 Gy versus 2.5 Gy $+\Delta T + 2.5$ Gy) clearly demonstrate split dose repair capacity. In addition, despite their high radiation sensitivity, irradiation of either bone marrow or adult endothelial stem progenitors with 3 Gy resulted in very low induction of apoptosis. Furthermore, after 10 Gy of X-rays the cord blood endothelial progenitors induced substantial apoptosis 48 hrs of 20 ± 5 % but irradiation of the adult endothelial progenitors resulted no induced apoptosis above control levels of 5%. Western analysis revealed a stabilization of p53 and increase in proapoptoic Bax protein after 10 Gy in the cord blood endothelial progenitors. Cell cycle and additional Western studies reveal induction of p21waf1/cip1 dependent cell cycle checkpoints in the cord blood endothelial progenitors, which have not been observed in other embryonic stem cells studies. The data indicate that while endothelial stem/progenitor cells appear very radiation sensitive as a group their radiobiology is complex with the induction of apoptosis being both dose and cell type dependent. We are now investigating the molecular basis of these differences in order to better understand the role of endothelial stem/progenitors in radiation-induced tumor response and normal tissue injury.

(PS3026) Effect of irradiation on labeling retaining cell population of the mouse mammary gland. Irineu Illa Bochaca, Rodrigo Fernandez-Gonzalez, Markus C. Fleisch, Mary Helen Barcellos-Hoff. Laurence Berkeley National Laboratory, Berkeley, CA, USA.

Our principal goal is to study how ionizing radiation, which is a known breast carcinogen, affects the stem cell population in mammary gland. The mammary gland epithelium consists of a layer of cells in contact with the lumen and a basal layer of myoepithelial cells with contractile capacity. These two cell types have a common progenitor or putative adult stem cell (ASC). Since ASC intermittently cycle, this characteristic is thought to contribute to the accumulation of damage and neoplastic transformation. However in some tissues like the small intestine and brain subventricular zone, ASC are exquisitely sensitive to radiation-induced apoptosis, while in others, like the colon and in brain cancer, they appear to be resistant. There is little data to date on the relative sensitivity of mammary ASC. The mammary gland undergoes ductal morphogenesis induced by ovarian hormones during puberty (approximately three weeks of age in mice), during which the ASC population expands. Because there are no in situ ASC markers in the mammary gland, we used bromodeoxyuridine (BrdU) to mark label retaining cells (LRC) following a classic pulse-chase labeling protocol. During the chase period the transient amplifying cells divide and lose the BrdU labeling, while the LRC populations is composed of slow cell cycling stem cells, quiescent cells and differentiated cells.

Three-week old wild type and p53 null Balb/c mice were sham-irradiated or exposed to 1Gy γ-radiation. Forty-eight hours afterwards, osmotic pumps containing BrdU were implanted subcutaneously into all mice. After two weeks of constant dose release, the pumps were removed. The mammary glands were dissected after 10 weeks. We used immunofluorescence and multiscale image analysis to determine the frequency and distribution of LRC. Preliminary results indicate significantly fewer LRC in the irradiated compared to the sham irradiated mice, which could be explained by increased proliferation in that population or by increased radiation sensitivity. These data will be discussed and the alternatives tested in ongoing studies.

This project was carried out as part of the NIEHS/NCI Bay Area Breast Cancer and the Environment Research Center.

(PS3027) Application of flow cytometry for the assessment of spermatozoid quality after ionizing irradiation. Denys Vatlitsov[1], Ksenia Igrunova[2], Sergiy Andreychenko[1]. [1]Scientific Center for Radiation Medicine Academy of Medical Sciense of Ukraine, Kyiv, Ukraine, [2]National Medical Academy of Postgraduate Education named after P. L. Shupyk, Kyiv, Ukraine.

Evaluation of spermatozoid viability used to be made on the basis of microscopic observations of spermatozoid morphology and motility in accordance with the World Health Organization's criteria. However, frequently such an analysis is not capable to discriminate between different states pertinent to spermatozoa. In this connection auxiliary approaches are introduced. For example,

application of flow cytometry (FC) makes possible to identify the different stages of apoptosis in spermatozoa, to measure mitochondrial membrane potential (MMP) which is responsible for spermatozoa mobility, to establish the presence of reactive oxygen species (ROS) whose activity commonly causes the loss of membrane integrity and to quantify the DNA ploidy in various groups of spermatozoa as well.

The experiments were performed on epididymal rat spermatozoa that had been gamma-irradiated by ^{60}Co in the dose range 1 - 100 Gy with dose input 0,02 Gy/sec. Immediately after irradiation the spermatozoa were rescued from epididymices and transferred to saline at 37°C. Motility, morphology and concentration of spermatozoa were determined under light microscope MBI-6 (Russia).

The other parameters, namely apoptosis development (AD), $\Delta\Psi$m - MMP and ROS production were identified on flow cytometer PAS (Partec, Germany). A minimum of 20000 cells per sample was analyzed. AD was followed by Annexin V - Apoptosis detection Kit I (BD Pharmingen, USA) including Annexine V-fluorescein isothiocyanate which visualizes phosphstidylserine translocation from the inner to the outer leaflet of the plasma membrane. MMP was measured by means of Rhodamin 123 dye. ROS production was detected through dihydroethidine oxidation to ethidium bromide. DNA ploidy was quantified using intercalating fluorescent agent - propidium iodide staining.

The data received have shown the existence of specific correlations between the radiation dose and the quantitative distribution of irradiated spermatozoa on subpopulations of apoptic, necrotized, immobile and viable cells. Furthermore, the DNA ploidy in spermatozoa changed depending on the corresponding state of spermatozoon.The present research has shown that the combination of FC with microscopic analysis will be of great use for assessing fertility potential of normal and irradiated spermatozoa.

(PS3028) Comparison of radiation sensitivity of rat respiratory tract epithelial cells. Yutaka Yamada, Akifumi Nakata, Yoshiya Shimada. National Institute of Radiological Sciences, Chiba, Japan.

Lung model of ICRP publication 66 describes that cells at risk in respiratory tract tissues are secretory and basal cells in bronchial airways, and Clara and Type II cells in alveolar interstitial region. It is, however, unclear whether there is a difference in radiation sensitivities between the target cells. The purpose of this study is to compare the dose-response relationships of radiation-induced cell death and transformation in primarily cultured rat tracheal epithelial (RTE) and rat lung epithelial (RLE) cells.

The RLE cells were isolated from lung of female Wistar rats (4–6-week-old) by enzyme digestion and gradient centrifugation. Tracheae were filled with enzyme solution, and then the RTE cells were rinsed from inside the tracheae. The RLE and RTE cells were cultured in serum free medium including epidermal growth factor and other necessary factors. The cells were irradiated using an alpha source of 238Pu (3.6MeV, 0.8Gy/min) or a gamma source of 137Cs (8.2Gy/min). The cytotoxic responses of the cells to irradiations were determined in colony formation assay. Transformants formed dense colonies in 2% serum containing and growth factor-free selective medium, and transformation frequencies (TF) were calculated from number of the colonies.

The irradiation caused similarly an exponential decrease in survival in the RLE and RTE cells, and D37 of alpha particle and gamma ray were 0.65 Gy and 3.6 Gy, respectively. Relative biological effectiveness (RBE) for cell killing was 5.5 in the both types of cell. TF for the RLE and RTE were 3.7x10-3E and 2.4x10-3E at 2 Gy of alpha particle, respectively. At 7.5 Gy of gamma ray, TF for the RLE and RTE increased to 8.0x10-3E and 7.1x10-3E, respectively. The RBE for transformation of RLE was 1.7, and that of RTE was 1.3. These results indicate that there is no difference between the radiation sensitivities of the RLE and the RTE cells in culture condition. This primary epithelial cell culture system of rat lung will be useful for analysis of radiation sensitivity among the different target cells and mechanistic studies of early changes in radiation-induced carcinogenesis.

(PS3029) Effect of ionizing radiation on differentiation of human embryonic stem cells in culture. Irina V. Panyutin, Eleanore J. Chuang, Igor G. Panyutin, Ronald D. Neumann. NIH/CC, Bethesda, MD, USA.

The characteristic feature of human embryonic stem cells (hESC) is their pluripotency, i.e., their ability to differentiate into any cell type. Pluripotency is manifested by expression of several hESC specific markers such as Oct 3/4, SSEA, Tra-1–80 and Alkaline Phosphatase (AP). Differentiation can be induced by a variety of specific, as well as non-specific, factors. We studied whether exposure to ionizing radiation affects the pluripotency of hESC. The cell line BGO1V was exposed to 1 Gy and 5 Gy of gamma radiation from a Cobalt-60 source, and expression of pluripotency markers was measured by immunochemical staining using specific antibodies. The level of the expression of the markers was quantitatively assessed by flow cytometry. As a control for the response of hESC to ionizing radiation, cells were stained with antibody to phosphorylated histone H2AX ($\tilde{I}^3$-H2AX). Cell cultures were grown in the presence of either Fibroblast Growth Factor (FGF) or one of the three neurotrophins: NT3, NT4 or BDNF, that were shown to promote survival of hESC. Immediately after 1 Gy of irradiation we observed a decrease in the expression of the pluripotency markers when hESC were grown in standard conditions, i.e., in the presence of FGF. In contrast, when growing in the presence of neurotrophins, hESC showed an increase or the same level of the expression of the pluripotency markers. Irradiation with 5 Gy resulted in decreased pluripotency in all growth conditions. However, 13 days after both 1 and 5 Gy irradiation, we observed an increase in the expression of the pluripotency markers in FGF-containing media; while in the neurotrophin-containing media, cells showed the same or slightly decreased expression of the pluripotency markers. We conclude that ionizing radiation can affect the pluripotency of hESC, but the effect depends on the growth conditions and the time after irradiation.

(PS3030) Intraesophageal manganese superoxide dismutase plasmid/liposome (MnSOD-PL) administration before irradiation increases engraftment of intraveneously injected esophageal stem cells. Yunyun Niu, Michael W. Epperly, Hongmei Shen, Joel Greenberger. University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA.

We hypothesized that following irradiation the esophageal microenvironment would have increased reactive oxygen species production and that antioxidant gene therapy would decrease this ROS resulting in increased engraftment of esophageal stem cells. We tested whether increased expression of MnSOD following intraesophageal administration of MnSOD-PL increased engraftment of esophageal stem cells. Female C57BL/6NHsd mice which received MnSOD-PL (100 μg plasmid DNA) intraesophageally 24 hrs earlier were irradiated along with control mice to 29 Gy to the esophagus. The mice were injected intravenously with bone marrow from male ROSA (LacZ, G418-r transgenic mice) five days later (time of greatest esophageal basal cell apoptosis). Fourteen days later the mice (first generation) were sacrificed, esophagus removed, single cell suspensions prepared, stained with Hoescht dye and sorted by flow cytometer into side population (SP) and nonside population (NSP) cells. Half of the SP and NSP cells isolated from the MnSOD-PL treated mice were injected I.V. into a second generation of recipient MnSOD-PL treated as well as control irradiated mice. The remaining cells were grown in 96 well plates and selected in neomycin. Second generation recipient mice were sacrificed 14 days after transplant and the SP and NSP cells isolated and grown in well plates as described above. In the first generation recipient mice, those pre-treated with MnSOD-PL had increased expression of LacZ+, G418-r in SP and NSP cells per esophagus (38.4 + 4.2% and 21.8 + 5.5% , respectively) compared to the control irradiated mice (21.4 + 4.4%, p=0.0105, and 5.2 + 2.4%, p=0.0277, respectively). Second generation MnSOD-PL treated recipient mice, transplanted with SP cells from MnSOD-PL treated mice had more LacZ+, G418-r SP cells per esophagus (47.9 + 3.5%) than that detected in mice injected with NSP cells from the same donors (22.3 + 2.4%, $p < 0.0001$), control irradiation only SP cells (22.3 + 2.0, $p = 0.0001$) or control irradiation only NSP cells (9.6 + 2.5%, $p < 0.0001$). More LacZ+ colony forming cells were

recovered from both first and second generation recipient mice pretreated with MnSOD-PL than control irradiated mice. Therefore, swallowed MnSOD-PL pretreatment decreased irradiation induced ROS and improved engraftment of esophageal stem cells.

(PS3031) A single dose of gamma or proton radiation rapidly compromises skeletal structure of adult mice. Hisataka Kondo[1,2], Jonathan Phillips[2], Charles L. Limoli[1], Eduardo A.C. Almeida[2], David John Loftus[2], Wenonah Vercoutere[2], Emily Morey-Holton[2], Rose Mojarrab[2], Munroop K. Atwal[2], Ruth K. Globus[2], Nancy D. Searby[2]. [1]Radiation Oncology, University of California Irvine, Irvine, CA, USA, [2]NASA Ames Research Center, Moffett Field, CA, USA.

Ionizing radiation can cause substantial tissue degeneration, including effects on bone. During growth when the skeleton is rapidly modeling, irradiation of mice leads to a significant reduction in bone mass, measured at 3 months, relative to age-matched controls. To determine if a single dose of radiation rapidly compromises structural integrity of cancellous bone in the adult remodeling skeleton, and to define the time- and dose-dependence of the response, adult mice were exposed to either 1 GeV proton (2–4 Gy) or ^{137}Cs gamma (1–2 Gy) irradiation. Bone structure was analyzed by microcomputed tomography (μCT) either 3 days or 10 days post irradiation. We found that proton irradiation at 2, 3 or 4 Gy caused approximately 45% reduction in the tibial cancellous bone volume fraction (BV/TV) in 19 wk old female mice 10 d following irradiation compared to non-irradiated, age-matched controls. A reduction in trabecular number and an increase in trabecular spacing were observed. Connectivity, which impacts bone strength, was reduced 45% compared with controls for 2 Gy protons and 65% for 4 Gy protons. Similar effects on bone were observed in 18 wk old male mice that were gamma irradiated. That is, 2 Gy caused a 15% net decline in cancellous BV/TV 3 days post irradiation compared with basal controls. Ten days following 1 Gy gamma irradiation, BV/TV was reduced 30% compared with controls and 45% after 2 Gy. Connectivity was 30% lower in mice irradiated with 1 Gy gamma rays and 55% lower in mice irradiated with 2 Gy gamma rays. At the same dose and time post-irradiation (2 Gy and 10 d), proton irradiation resulted in a slightly higher loss of BV/TV and connectivity than gamma rays. In conclusion, radiation leads to rapid tissue degeneration in adult, remodeling bone, and is likely to increase fracture risk. This work was supported by NASA grant NNH04ZUU005N/RAD2004-0000-0110.

(PS3032) Impact of ^{56}Fe ion radiation on Human Neural Stem Cell Differentiation. Yongjia Yu, Yuanyuan Gao, Ping Wu. University of Texas Medical Branch, Galveston, TX, USA.

Space radiations such as heavy ions pose a risk of damaging the central nerve system (CNS) of astronauts. Human neural stem cells (hNSCs) are of particular interest since functional NSCs in adult brain are potential targets of radiation damage. To study the impact of heavy ion radiations on the differentiation of NSCs *in vitro*, we examined the response of a human fetal neural stem cell line (K048) to ^{56}Fe ion irradiation. The K048 cells retain self-renewal capability and can differentiate into neurons and astrocytes *in vitro* and *in vivo*. K048 cells were irradiated with a single dose of 1000 MeV/n ^{56}Fe ions at the dose range of 0.1 to 2.0 Gy. At various time points post irradiation, survived stem cells were induced to differentiate, and the percentage of neurons and astrocytes generated from these cells were determined by immunostaining. A dose-dependent inhibition effect of ^{56}Fe radiation on neuronal differentiation was observed, similar to that in -irradiated cells. However, in contrast to -irradiation that has no apparent impact on astroglial differentiation, ^{56}Fe-irradiation increased the percentage of astrocytes. These effects of ^{56}Fe ions diminished gradually at doses below 2 Gy, but persisted for at least four cell passages (approximately 6 weeks) at 2 Gy. The molecular mechanisms underlying these findings remain unclear and are currently under investigation.

(PS3033) Valproic Acid significantly radiosensitzes MCF-7 cells in 2D, adherent clonogenic assays, but does not radiosensitize MCF-7 cells grown in 3D, self-renewing non-adherent mammosphere culture. Wendy A. Woodward, Jessica Li Li. UT MD Anderson Cancer Center, Houston, TX, USA.

Background: Tumor initiating cells from breast cancers can be propagated in vitro as mammospheres in non-adherent serum-free conditions. We and others have demonstrated that progenitor cells from the breast cancer cell line MCF-7 are relatively resistant to radiation. In mouse models we have demonstrated this resistance is mediated by increased baseline beta-catenin signaling in progenitors and subsequent upregulation of the down-stream effector survivin in response to radiation in progenitor cells. We hypothesized the histone deacetylase inhibitor, Valproic Acid (VA) may be a non-specific inhibitor of beta-catenin in progenitor cells and examined the role of VA as a radiation sensitizer in MCF-7 cells grown in clonogenic assays on plastic (2D), in non-adherent mammosphere culture (first passage, 3D-P0), and in passaged mammosphere culture (self-renewing cell enrichment, 3D-P1).

Methods: Expression and localization of activated beta-catenin in MCF-7 mammospheres was examined by immunofluorescence using an antibody to unphosphorylated beta-catenin. Response to radiation in the presence and absence of VA was measured using clonogenic assays in adherent and non-adherent conditions. Clonogenic survival curves were generated using Sigmaplot 8.0.

Results: In clonogenic assays of untreated cultures, 3D-P1 mammospheres are significantly more resistant to radiation than 3D-P0 mammospheres which are more resistant than 2D MCF-7 cells after fractionated and single fraction radiation (i.e. surviving fraction (SF) after 2Gy x 5: 3D-P1 42% [CI 39–52], 2D 1.7% [CI 1.5–2.0], difference 40.3%). VA dramatically abrogates nuclear and cytoplasmic beta-catenin staining in MCF-7 mammospheres. VA is a radiosenistizer of MCF-7 cells grown on plastic radiated in both single doses (SF2 22% [CI 17–28] vs. 12% [CI 11–14] and fractionated doses (SF after 2Gy x 5, 1.7% [CI 1.5–2.0] vs. 0%). VA has no radiosensitizing effect on P0 or P1 mammospheres and is radioprotective in some growth conditions (3D-P0 SF2 90% [CI 56–100] vs. 50% [CI 45–57].

Conclusions: Radiosensitization by VA varies within subpopulations of MCF-7 cells. Although VA inhibits beta-catenin in 3D MCF-7 culture, this was not sufficient to radiosensitize these cells, likely due to the non-specific effects of VA as a histone deacetylase inhibitor in these cells.

(PS3034) Accumulation and persistence of mutations induced in somatic stem cells of mice during irradiation with low dose-rate gamma rays for 483 days. Kazuo Fujikawa[1], Nao Kagawa[1], Tetsuya Ono[2], Isamu Hayata[3]. [1]Kinki Univ., Higashiosaka, Japan, [2]Tohoku Univ., Sendai, Japan, [3]National Institute of Radiological Sciences, Chiba, Japan.

To determine whether mutations continually induced by low-level radiation persist and accumulate in somatic stem cells, mice heterozygous at the Dlb-1 locus (Dlb-1^{b}/Dlb-1^{a}) were irradiated at a dose rate of 0.01 mGy/min or lower for 483 days from 8 to 78 weeks of age to attain a total dose of 0.02, 0.42 or 8.0 Gy. Small intestines were sampled from control and irradiated mice 2 weeks after the completion of irradiation and subjected to the Dlb-1 mutation assay in which evidence of mutations at the Dlb-1 locus in small intestinal stem cells was detected as mutant clones on the surface of villi, and the frequency (F) and the size (S) of mutant clones were measured. More than 10000 villi were observed for each of mice. F was defined as the number of clones detected per 10000 villi observed. S was determined as area occupied by mutant clone relative to whole villus surface.

F values with SD in the low, the middle and the high dose groups were 20.0±5.4 (N=18), 22.4±5.0 (16), 29.4±10.9 (17), respectively, the last of which was significantly higher than control frequency of 21.2±4.2 (19). Thus, frequency of induced mutant clones (f) in the 8 Gy irradiated group was 8.3±2.2. As compared at the same dose level, this value is equal within experimental errors to a f of 7.3±2.5 that had been obtained for young mice at 8 to 9 weeks of age after chronic gamma-irradiation at a dose rate of 1 mGy/min; but, it is considerably lower than a f of 50.4±10.1 recorded for young mice after acute gamma-irradiation at 1 Gy/min.

The mean S values of induced mutant clones (i.e., values corrected for the sizes of spontaneously arisen clones) did not show significant variation that could be ascribed to the age of mice irradiated and the dose-rate of gamma rays used. We thus may conclude that (1) mutations continually induced by low-level radiation accumulate in somatic stem cells and persist for prolonged time; (2) susceptibility of stem cells to radiation mutagenesis does not change with age.

(PS3035) Low dose irradiation inhibits BMP-induced osteodifferentiation with low LET X-rays and high LET ^{56}Fe-HZE particles. Paban K. Agrawala[1], Xinhua Lin[2], Louis A. Pena[1]. [1]Brookhaven National Laboratory, Upton, NY, USA, [2]BioSET Inc, Rockville, MD, USA.

Objectives: Radiation toxicity is widely known but sublethal radiation effects are less well understood. This study examined the effect of low dose radiation in an *in vitro* model of differentiation, namely Bone Morphogenic Protein-2 (BMP-2) induced osteodifferentiation. Prior studies showed inhibition of osteodifferentiation at doses well into the range associated with clonogenic and apoptotic toxicity (8–20 Gy). Our work focuses on doses 10–100 times lower. Procedures: Osteodifferentiation was induced by BMP-2 or Fibroblast Growth Factor-2 (FGF-2, bFGF) in C2C12 and C3H10T½ cells grown on 96-well plates in reduced serum. Cultures were exposed to 5 or 25 cGy low LET X-ray (100 kVp) or ^{56}Fe HZE particles (1–25 cGy-equiv, 1 GeV/n) at the NASA Space Radiation Laboratory (NSRL) with appropriate dosimetry considerations. Irradiation was performed either 10' before or 30' after growth factor treatment. Alkaline phosphatase (ALP) induction, an early biomarker of osteodifferentiation, was bioassayed 5–7 days after irradiation using pNPP substrate. ALP values were normalized to cell number by total protein measured by BCA assay or by cell number measured by XTT assay. Results: ALP activity was induced by BMP-2 in a concentration dependent manner in C2C12 by >15-fold, and by both BMP-2 and FGF-2 in C3H10T½ cells by 3–4 fold. At radiation doses greater than 100 cGy, cytotoxicity was observed in both cell types. But 5 or 25 cGy X-irradiation *prior* to BMP-2 resulted in reduced ALP induction at ~11 and ~5 fold compared to unirradiated C2C12 controls. Radiation *after* BMP-2 was less effective in impairing ALP induction. Similar data were obtained for C3H10T½ cells. Analogous results were obtained with ^{56}Fe HZE irradiation of C3H10T½ which led to ~45% decrease in ALP activity as compared to ~18 % for the equivalent X-ray dose. Conclusion: Unlike prior studies that failed to observe impaired osteodifferention at radiation doses below 800 cGy, we measured impairment following doses as little as 5 cGy with low or high LET radiation. Further, irradiation prior to triggering the differentiation program has a greater effect than irradiation after differentiation has already been initiated. Finally, ^{56}Fe HZE had a higher RBE than X-ray using this experimental endpoint. (Support: U.S. Dept. Energy grant KP-1401020/MO-079)

(PS3036) Systolic blood pressure and systolic hypertension in adolescence of in utero exposed atomic-bomb survivors. Eiji Nakashima[1], Masazumi Akahoshi[2], Kazuo Neriishi[1], Saeko Fujiwara[1]. [1]Radiation Effects Research Foundation, Hiroshima, Japan, [2]Radiation Effects Research Foundation, Nagasaki, Japan.

Fetal origin of adult disease (FOAD) hypothesis holds that intrauterine insults predict adult diseases such as hypertension, coronary heart disease, and type II diabetes. The aim of this study is to elucidate the relationship between adolescent systolic blood pressure (SBP) and radiation dose for in utero exposed A-bomb survivors and to show that the analysis results do not contradict the FOAD hypothesis.

Annual medical examinations were undergone during adolescence by the prenatally exposed A-bomb survivors of Hiroshima and Nagasaki, and SBP and several anthropometric measurements were recorded during these examinations. For 1014 in utero exposed persons, two types of longitudinal data analysis were used for a total of 7029 observations (6.93 observations per subject) of SBP (continuous data) and systolic hypertension (binary data) for persons from ages nine to 19 years. We took into consideration age effect and effects of body weight and/or body mass index (BMI) in the longitudinal analyses using the mixed effects model for SBP data and the generalized estimating equation model for systolic hypertension prevalence data.

For the SBP measurements, common dose effect was 2.08 mmHg per Gy and was significant ($p = 0.017$). Dose by trimester interaction was suggestive ($p = 0.060$). Significant radiation dose effect was found in the second trimester ($p = 0.001$) with an estimated 4.17 mmHg per Gy, but in the first and third trimesters, radiation dose effects were not significant ($p > 0.50$). For systolic hypertension prevalence, radiation dose effect was significant ($p = 0.009$); the odds ratio at 1 Gy was 2.23 (95% Confidence Interval (CI): 1.23, 4.04), and dose by trimester interaction was not significant ($p > 0.50$). Dose response of systolic hypertension had no threshold, with point estimate 0 Gy (95% CI: <0.0, 1.1 Gy).

This dose-dependent increase of hypertension prevalence in adolescent A-bomb survivors exposed in utero supports the FOAD hypothesis. SBP dose response was most pronounced at the middle trimester among the three trimesters, at which time the organogenesis of blood pressure relevant organs is most active.

(PS3037) Radiation effects on noncancer diseases among the prenatally exposed atomic bomb survivors. Yoshimi Tatsukawa, Eiji Nakashima, Michiko Yamada, Sachiyo Funamoto, Masazumi Akahoshi, Saeko Fujiwara. Radiation Effects Research Foundation, Hiroshima, Japan.

Purpose: To examine relationships between incidence of noncancer diseases and radiation dose among the prenatally exposed atomic bomb survivors

Methods: Longitudinal clinical examinations among the prenatally exposed atomic bomb survivors began in 1978 at the Radiation Effects Research Foundation and consisted of history taking, physical examination, and laboratory tests. A total of 589 participants (≥1Gy 2.2%) were followed in this study until 2006 through biennial health examinations. Cox regression models were used to estimate the effects of radiation dose on incidence of noncancer diseases

Results: Body mass index (BMI) tended to decrease with increasing radiation dose, although dose effects were statistically not significant. During the follow-up period, 236, 264 and nine subjects developed hypertension, hypercholesterolemia and cardiovascular disease (stroke or myocardial infarction), respectively. Significant radiation dose effects were not detected. After adjusting for BMI, relative risk at 1Gy and confidence intervals (CIs) for incidence of hypertension, hypercholesterolemia and cardiovascular disease were 0.77 (95% CI, 0.44–1.33, *P*=0.34), 1.27 (95% CI, 0.71–2.28, *P*=0.42) and 0.38 (95% CI, 0.01–22.5, *P*=0.65), respectively. We also analyzed difference in dose effects on these diseases between the prenatally exposed atomic bomb survivors and young survivors who were exposed at younger than 10 years of age (≥1Gy 23.6%). No significant difference was observed between the two groups, although significant radiation effects for hypertension and cardiovascular disease were found among the young survivors

Conclusion: This is the first report to examine radiation effects on the incidence of noncancer diseases using longitudinal clinical data among the prenatally exposed atomic bomb survivors, although these results are still preliminary. Significant radiation dose effects were not detected, which might have been due to the small number of subjects or cases. Further follow-up is required to verify the dose effects on these diseases among the prenatally exposed atomic bomb survivors

(PS3038) A review of epidemiological associations between low and moderate doses of ionizing radiation and late cardiovascular effects, and their possible mechanisms. Mark P. Little[1], E Janet Tawn[2], Ioanna Tzoulaki[1], Richard Wakeford[3], Guido Hildebrandt[4], Francois Paris[5], Paul Elliott[1]. [1]Imperial College Faculty of Medicine, London, United Kingdom, [2]Westlakes

Research Institute, Moor Row, United Kingdom, [3]University of Manchester, Manchester, United Kingdom, [4]University of Leipzig, Leipzig, Germany, [5]INSERM U 601, University of Nantes, Nantes, France.

The link between high doses of ionizing radiation and damage to the heart and coronary arteries is established. In this paper, we review the epidemiological evidence for associations between low and moderate doses (<5 Gy) of ionizing radiation exposure and late occurring cardiovascular disease. Risks per unit dose in epidemiological studies vary over at least two orders of magnitude, possibly a result of confounding factors. An examination of possible biological mechanisms indicates that the most likely causative effect of radiation is damage to endothelial cells and subsequent induction of an inflammatory response, although it seems unlikely that this would extend to low dose and low dose-rate exposure. However, a role for somatic mutation has been proposed that would indicate a stochastic effect. In the absence of a convincing mechanistic explanation of epidemiological evidence that is, at present, less than persuasive, a cause-and-effect interpretation of the reported statistical associations cannot be reliably inferred, although neither can it be reliably excluded. Further epidemiological and biological evidence will allow a firmer conclusion to be drawn.

(PS3039) Relationship between radiation exposure and age at menopause. Ritsu Sakata[1], Yukiko Shimizu[1], Nobuo Nishi[1], Hiromi Sugiyama[1], Fumiyoshi Kasagi[1], Hiroko Moriwaki[1], Mikiko Hayashi[1], Manami Konda[1], Midori Soda[2], Akihiko Suyama[2], Kazunori Kodama[1]. [1]Radiation Effects Research Foundation, Hiroshima, Japan, [2]Radiation Effects Research Foundation, Nagasaki, Japan.

Purpose

Early studies regarding female A-bomb survivors noted that the number of women experiencing menopause immediately after exposure was significantly higher in the proximally exposed group in comparison to the distally exposed group, with average menopausal age tending to be significantly younger particularly among those with acute radiation syndrome. However, it remains speculative whether the effects upon a lower menopausal age are caused by physical injury and mental shock immediately after A-bomb exposure, or a direct effect of radiation. In this study, we examined the relationship between age at menopause occurring several decades after exposure, radiation exposure, and other factors based upon the Life Span Study (LSS) population of the Radiation Effects Research Foundation (RERF).

Methods

RERF has thus far conducted three mail surveys (1969, 1978, and 1991) on the female subjects of the LSS population. About 18,000 subjects among those with available information on radiation exposure dose replied to the question about menopausal age in any of these three surveys. The women who reached menopause within 5 years after bombing were excluded from analyses in an effort to exclude the potential effects of physical injury and mental shock followed by A-bomb exposure. Regression analyses were used for examining the relationships between age at menopause and ovary dose or the following factors that may affect age at menopause: birth cohort, smoking history, age at menarche, and other factors. The radiation effect on age at menopause seemed to have threshold, therefore we also performed an analysis that accounted for threshold.

Results

Significant relationships were observed between age at menopause and radiation dose, birth cohort, delivery, and smoking history. Age at menopause was significantly younger with increased radiation dose, after adjusting for other factors. The results of an analysis considered at threshold suggested that the threshold level around 0.5Gy.

Conclusions

Age at menopause was significantly younger with increased radiation ovary dose after adjusting for city, birth cohort, smoking history and age at menarche. In addition, this relationship seemed to have a threshold around 0.5Gy.

(PS3040) Circulatory disease mortality in atomic bomb survivors, 1950–2003. Yukiko Shimizu, Kazunori Kodama, Nobuo Nishi, Fumiyoshi Kasagi, Akihiko Suyama, Midori Soda, Hiromi Sugiyama, Ritu Sakata, Hiroko Moriwaki, Mikiko Hayashi, Manami Konda, Roy Shore. Radiation Effects Research Foundation, Hiroshima, Japan.

The present report examined radiation risk of circulatory disease mortality in the Life Span Study (LSS) cohort followed by the Radiation Effects Research Foundation. Primary analysis was based on 19,054 circulatory disease deaths (9,622 cerebrovascular disease, 8,463 heart disease, 969 other circulatory disease) among 86,611 LSS cohort members with DS02 dose estimates. Poisson regression methods for grouped survival data were used.

Elevated risks were seen for circulatory disease as a group (ERR/Gy = 0.11, 95% CI 0.05, 0.17) or for the broad-based disease categories, cerebrovascular disease (ERR/Gy = 0.09, 95% CI 0.01, 0.17) and heart disease (ERR/Gy = 0.13, 95% CI 0.05, 0.21), but not for all subcategories of disease. We hypothesized that radiation acts, at least in part, by causing or promoting atherosclerosis. However, contrary to our expectation, increases of the risk were uncertain for Ischemic heart disease (IHD) or cerebral infarction, which are most representative of atherosclerotic changes, though radiation effects on these diseases could not be ruled out. The risk of IHD increased only in the higher dose categories, and the linear increase was not significant. Stronger associations were observed between radiation and other heart diseases, such as hypertensive heart disease (ERR/Gy = 0.37, 95% CI 0.08, 0.72), rheumatic heart disease(ERR/Gy = 0.86, 95% CI 0.25, 1.72), and other heart disease(ERR/Gy = 0.16, 95% CI 0.03, 0.30). Though the data were not inconsistent with linearity over the range, there was considerable uncertainty about the shape of the dose response in the low-dose range, in particular the evidence of risk below about 0.5 Gy was unclear for circulatory disease, cerebrovascular disease, and heart disease.

(PS3041) Therapeutic advantage of GRID therapy for a single high dose fraction using a multileaf collimator. Kai Dou, John Ashburn, Prakash Aryal, Ellis Lee Johnson, Robert Zwicker. University of Kentucky, Lexington, KY, USA.

Purpose: Film dosimetry of megavoltage spatially fractionated (GRID) radiation therapy delivered using a multileaf collimator (MLC GRID) was carried out. Its therapeutic advantage was assessed based on dosimetric measurements using a modified linear quadratic model. Great therapeutic advantages for MLC GRIDs were demonstrated.

Materials and Methods: A Varian Clinac 2100EX linear accelerator equipped with an MLC was used for the MLC GRID therapy. The two MLC GRID blocks were produced, denoted by MLC5 and MLC10, representing 5×5 and 10×10 mm openings projected at isocenter, respectively. A Cerrobend GRID block was also used for comparison with MLC GRIDs. A linear-quadratic (LQ) model was used to calculate the survival fraction (SF) of tumor and normal tissues. Therapeutic gain was obtained by the SF ratios of normal tissues under an MLC GRID to that under equivalent open field.

Results: 5×5 mm (MLC5) and 10×10 mm (MLC10) GRIDS were created by an MLC using 6 MV and 18 MV x rays. Their dose distributions were measured at different depths using a film dosimetry. The peak-to-valley dose ratios at the depth of maximum dose at 100cm SSD were found to be 19% for MLC5 and 17% for MLC10. Therapeutic ratios varied from 0.9 to 45 for a wide range of tumor sensitivities at single fraction doses of up to 30 Gy. MLC10 GRID therapy showed a higher therapeutic gain than MLC5 and an 8mm Cerrobend GRID block.

Conclusion: With high, single-fraction doses, MLC GRID radiotherapy exhibited a significant therapeutic advantage over the open field radiotherapy when the tumor cells were more radioresistant. Dosimetric properties of MLC GRIDs allowed for therapeutic evaluation using a modified LQ model. One of the GRIDs investigated, MLC10 showed a great increase in therapeutic benefit compared to MLC5 and a Cerrobend GRID block with 8mm apertures.

(PS3042) Cancer cure for a common man: experiences in delivering radiation treatment in rural India. Mudundi R. Raju. Los Alamos National Laboratory, Los Alamos, NM, USA.

The gulf between scientific and technological developments and the life of a common man, even in getting basic needs, is widening rapidly. This is especially true in rapidly developing countries like India. This concern, along with a hope to develop a small bridge with the help of like-minded scientists, led the author to take voluntary retirement from the Los Alamos National Laboratory in 1993 after three decades of research work in the field of particle radiobiology and radiotherapy. The purpose of this presentation is to make a plea to the members of the Society to initiate efforts that would help a common man in receiving the benefits of modern developments in radiation oncology. In addition, it is important to educate the common man about the benefits, not just the dangers, of ionizing radiation. Dr. Raju has established the Mahatma Ghandi International Cancer Center, a radiation treatment center in a rural area near the town of Bhimavaram in Andhra Pradesh, India. The objective is to develop a working model center to provide good quality radiation treatment using appropriate science and technology, working together with equipment manufacturers and the respective local and national governments. More than 75% of population in India lives in rural areas and most of the cancer centers are located in faraway cities and beyond the reach of a common man. To date, more than 300 cancer patients have been treated, nearly one third of them cervical cancers. The author will present his experiences in implementing this center and discuss the need to develop other such centers. Since most scientists are altruistic by nature, socially conscientious scientists can play an important role in developing these much needed cancer centers to provide cancer care for the common man. Dr. Raju will discuss this example of socially conscientious science in the context of a historical perspective on the interface between science and the common good.

(PS3043) Mathematical modeling of breast irradiation protocols - treatment success and failure. Heiko Enderling[1], Alexander R. Anderson[2], Mark A. Chaplain[2], Jayant S. Vaidya[3], Lynn Hlatky[1], Philip Hahnfeldt[1]. [1]Tufts University School of Medicine, Center of Cancer Systems Biology, Caritas St. Elizabeth's Medical Center, Boston, MA, USA, [2]University of Dundee, Division of Mathematics, Dundee, United Kingdom, [3]University of Dundee, Division of Surgery and Molecular Oncology, Dundee, United Kingdom.

Adjuvant radiotherapy reduces local recurrence rates after early breast cancer treatment by two thirds compared to treatment with breast conserving surgery alone. However, it fails to provide total tumor control in about 10% of patients. It has been hypothesized that fields of morphologically normal, yet mutated cells in the tumor bed could be the source for the persisting rate of local recurrence. Such fields could arise due to clonal expansion from pre-malignant stem cells in the breast during puberty and pregnancy. We have developed a mathematical model of breast tumor initiation, progression and radiotherapy, focusing on stem cell fractions and tumor suppressor gene dynamics as well as breast specific tissue compositions. Cells are implemented as individual entities with inherited genetic material, and we describe spatial tumor-microenvironment dynamics and enzyme diffusion with continuous partial differential equations. Increased fitness and selective advantage following mutative hits are implemented as an increase in proliferation and reduction in spatial constraints. Our model of tumor dynamics and irradiation survival shows that novel treatment strategies of fewer but larger fractions have the potential to provide similar tumor control as achieved with conventional 25x2Gy treatment. However, without a boost to the tumor bed pre-malignant sister clones from the original stem cell in the vicinity of the primary tumor are likely to survive the treatment and hence represent a likely source of local recurrence. With our model we predict that a boost to the tumor bed, such as delivered in single-dose targeted intraoperative radiotherapy, can eradicate these mutated cells in the tumor bed and thus provide a longer relapse-free survival.

(PS3044) Pulsed dose rate brachytherapy for carcinoma of cervix: experience at our institute. DN Sharma, GK Rath. All India Institute of Medical Sciences, New Delhi, India.

Introduction: The worldwide experience with pulsed dose rate brachytherapy (PDR) brachytherapy is limited. The aim of present study was to analyze the results of PDR in patients with cervical cancer.

Materials and Methods: From September 2003 to September 2005, 48 patients with cervical cancer treated with PDR intracavitary radiotherapy (ICRT). FIGO stage distribution of the patients was as follows: Stage IB, 3; IIA, 1; IIB, 15; IIIB, 25; IVA, 1; and unknown stage, 3. Six patients underwent surgery followed by postoperative radiotherapy (RT) and remaining 42 patients were treated by definitive RT with or without chemotherapy. Radiotherapy consisted of whole pelvis external beam RT (EBRT) with a dose of 40 Gy in 22 fractions in 4.5 weeks followed by 10 Gy/5F/ 1 week with midline shielding. After an interval of 1–2 weeks, a single session of standard ICRT application was done (intravaginal ovoids in postoperative cases) to deliver a dose of 27 Gy to point A by PDR brachytherapy. The hourly pulse of 70 cGy to point A was given. The pulse time ranged from 8–15 minutes.

Results: Follow up period ranged from 5 months to 30 months. Seven patients (3 in stage II and 4 in stage III) had recurrent/residual disease in the pelvis. Overall one year recurrence free survival (RFS) rate was 85%. Stage II and stage III RFS rate were 81% and 84% respectively. One patient developed RTOG grade IV urinary toxicity (vesicovaginal fistula) and one developed grade III proctitis. Thus overall grade III/IV toxicity rate was 4% (2 out of 48 patients).

Conclusion: Our results reveal that PDR brachytherapy + EBRT provides excellent pelvic disease control and low radiation related morbidity rate in the patients with cervical carcinoma.

(PS3045) Stationary magnetic filed from MRI: A study on the exposed subjects. Arnav Bhatia[1], Alka Kumar[2], Atul Kumar[2]. [1]Jaipur Engineering College and Research Centre, Jaipur, India, [2]Anil Hospital and Research Centre, Jaipur, India.

The effects of extremely low frequency electromagnetic and radio-frequency radiation on our health warrant careful evaluation before the recommendation on the prudent avoidance of equipment which generates non-ionizing radiation. Usually the intensity of exposure is low in the general population but can be greatly increased in the workplace. MRI spectroscopy is a special MRI method that identifies certain medical problems by looking for specific chemicals in body tissues. Widely used as a noninvasive diagnostic tool in the medical community, MRI is used to detect the early evidence of many ailments of soft tissues such as brain abnormalities, heart disease, coronary artery diseases and disorders of the ligaments, etc. The magnetic field used is 30,000 + times that of the earth's magnetic field. Its effect on the body, however, is claimed to be harmless and temporary. A regional survey of the personnels carried out with the help of local diagnostic laboratories (fitted with a field of 1.5 T) show mild and sporadic effects viz. skin or eye irritation due to iron pigments in tattoos or tattooed eyeliner, respectively causing a burn with some local patches, a slight risk of an allergic reaction if contrast material is used during the MRI. However, most reactions happened to be mild and could be treated by the local application of medicine. The reduced eye hand coordination and visual contrast sensitivity were also some of the observed effects which might be thought more psychological than somatic. On the other hand, some beneficial effects in elderly people suffering from gouts, euphoria and muscle relaxation had been also recorded. Though MRI provides image with better quality than regular x-rays and CAT scans for soft-tissues inside the body and uses very powerful magnet, however, no known alarming harmful effects were noticed with regrad to WBC, RBC, MCV, MCH, MCHC etc. On the contrary a slight rise in WBC reflects on the magneto-therapeutic aspect of MRI.

(PS3046) A unified framework for biologically conformal radiation therapy (BCRT) treatment planning. Yong Yang[1], Lei

Xing[2]. [1]University of Pittsbugh, Pittsburgh, PA, USA, [2]Stanford University, Stanford, CA, USA.

The spatial biology distribution in most tumors and sensitive structures is heterogeneous. Recent progress in biological imaging is making the mapping of this distribution increasingly possible. At the same time, intensity modulated radiation therapy provides an effective technology alowing us to produce an arbitrarily shaped 3D dose distribution. In this work we establish a theoretical framework to quantitatively incorporate the spatial biology data into IMRT inverse planning. In order to implement this, we first derive a general formula for determining the desired dose to each tumor voxel for a known biology distribution of the tumor based on a linear-quadratic (LQ) model. The desired target dose distribution is then used as the prescription for inverse planning. An objective function with the voxel-dependent prescription is constructed with incorporation of the nonuniform dose prescription. The functional unit density distribution in a sensitive structure is also considered phenomenologically when constructing the objective function. Two cases with different hypothetical biology distributions are used to illustrate the new inverse planning formalism. For comparison, treatments with a few uniform dose prescriptions are also planned. The biological indices, TCP and NTCP, are calculated for both types of plans and the superiority of the proposed technique over the conventional uniform dose escalation scheme is demonstrated. Our calculations revealed that it is technically feasible to produce deliberately nonuniform dose distributions with consideration of biological information. Compared with the conventional dose escalation scheme based on the delivery of a uniform dose to the target volume, the new technique is capable of generating biologically conformal IMRT plans that significantly improve the TCP while reducing or keeping the NTCPs at their current levels. Biologically conformal radiation therapy (BCRT) incorporates patient specific biological information and provides an outstanding opportunity for us to truly individualize radiation treatment.

(PS3047) Analysis of differential transcriptional and proteome response of human lung derived cells exposed to single and multiple doses of gamma-rays. Daniela Trani[1,2], Marco Cassone[1], Chiara Lucchetti[1,3], Marco Durante[4], Mario Caputi[2], Antonio Giordano[1,3]. [1]Temple University, Philadelphia, PA, USA, [2]Seconda Universite degli Studi di Napoli, Napoli, Italy, [3]University of Siena, Siena, Italy, [4]University of Naples, Napoli, Italy.

Introduction. Several malignant tumors, like non-small cell lung cancer (NSCLC), are known to be radio-resistant compared to normal cells and tissues, but still, little is known about the genetic basis of this behavior. In the past, most studies employed acute exposure to very high doses and high dose rates to elucidate the molecular mechanisms of radiation response. The present study has been designed to investigate the effects of dose fractionation in normal and cancer lung cells, in order to gain better insight on the molecular basis of the response of normal tissue and tumors to radiation treatment.

Material and Methods. Asynchronously proliferating cultures of the human epithelial cell lines NL20 (normal) and H358 (non small cell lung cancer, NSCLC) were exposed to a single dose of 4 Gy or to 3×4 Gy (over 48 hours) of γ-radiation from a ^{137}Cs source (100 cGy/min). After irradiation, all samples were incubated at 37 °C for 4, 12, 24, 48, 72 or 96 hours. For each time point, cells were collected and a cell viability assay, FACS analysis, DNA laddering and cDNA Microarray were performed. Proteomics approach was also employed.

Results. NL20 and H358 display, over time, different cellular and molecular responses to radiation: NSCLC cells are more resistant to radiation compared to NL20. NL20 show higher proliferative ability, lower apoptosis and different cell cycle progression when dose fractionation is employed. Gene and protein expression profiles resulting from microarray and proteome analyses indicate that single and fractionated exposure might activate specific genes and/or pathways, although under our experimental conditions a quantitative dose response is predominant.

Conclusions. Acute and fractionated exposures differently affect the ability of normal lung cells to recover and to survive the radiation-induced injuries. Normal and lung cancer cells respond to radiation by modulating key molecular pathways in a very distinctive manner. Qualitative and quantitative information collected from these types of studies will contribute both to cancer therapy development and the implementation of radio-protective countermeasures.

(PS3048) Study for genetic effects of atomic-bomb radiation by using of a DNA microarray-based comparative genomic hybridization (array-CGH) method. Norio Takahashi[1], Yasunari Satoh[1], Keiko Sasaki[1], Mieko Kodaira[1], Yoshiaki Kodama[1], Keiko Sugita[2], Naohiro Tsuyama[3], Hiroaki Katayama[2]. [1]Dept. Genetics, Radiation Effects Research Foundation, Hiroshima, Japan, [2]Dept. Information Technology, Radiation Effects Research Foundation, Hiroshima, Japan, [3]Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan.

[Objective] We have studied the effects of A-bomb radiation on human germ cells, that is, whether the mutation rate has increased significantly in the offspring of A-bomb survivors compared with controls. For feasibly conducting the study, it is essential to use methodology which will be able to efficiently collect a large volume of genetic information from a sufficient number of samples. Moreover, we should use the methodology which can reliably detect mutations appearing as a change of two copies of DNA fragment to one, since radiation-induced mutations seem to be predominantly a deletion type. To identify these mutations genome-wide, we have introduced DNA-microarray based comparative genomic hybridization (array CGH) method. Preliminary experiments demonstrated that deletion-type and amplification-type variations with the size of 50 kb or more could be detected. Before launching a large-scale study using the array-CGH, the feasibility of this technique was validated in a pilot study. [Experiment] We used an array with about 2,240 Bac-clones. These target clones were distributed across human autosomes at an interval of about 1.2 Mb. We examined genome samples obtained from 40 offspring of A-bomb survivors and 40 controls. [Results] In the pilot study, a total of 253 variants with different copy numbers were detected. Among them, 14 were "rare variants," each of which was detected in only one person in this population. Out of these 14, eight variants were amplifications and six were deletions. The remaining 239, which were "common variants" observed in two or more persons, were identified on 16 kinds of targets. Furthermore, a study of the parental genomes in terms of the rare variants showed that all of the variants were inherited from one of the parents. [Conclusion] From our data, we consider that array CGH is one of the most useful techniques for our research purposes. Now, we are planning a larger population study.

(PS3049) Systemic effects of low-dose and low-dose-rate irradiation in C57BL/6 mice. Hee-sun Kim, Seung-yeon Song, Suk-chul Shin, Shin-hye Oh, Cha-soon Kim, Meeseon Jeong, Kwang-hee Yang, Seon-yong Nam, Ji-young Kim, Chong-soon Kim. Radiation Health Research Institute, Seoul, Republic of Korea.

ICRP 60 is supported by linear non-threshold (LNT) hypothesis that was based on the studies on survivors of the atomic bombs in Japan to interpret the influence of low-dose (LD) radiation on the human body [1]. Therefore, it had limits when explaining the various defense systems of the human body. In other words, not only were the existing rationalization system based on LNT hypothesis and the creditability of its theoretical support, but it also caused the distorted understanding of the public on the hazards of radiation. Therefore, international leveled studies on the influence of LD radiation on human bodies have been triggered. Recently, hormesis effects have been expanded as an opposition to the LNT hypothesis [2]. In fact, a report has said that human bodies have defensive systems such as the abilities to repair DNA damage, to activate immunity and to respond to adaptation and that these systems act as resistance against radiation [3]. ICRP has profoundly recognized the necessity to update the concept of the protection system, and therefore has been supplementing and supporting the ideas of radiation protection by Dr. J. Clark from 1999 [4]. Following this trend, many countries have started their studies on

LD radiation. They have established LD irradiation facilities and have implemented studies on the biological effects of radiation. Although Korea currently increased radiation related facilities and usefulness of radiation in our life, it has a relatively weak in study for effects of LD radiation. Therefore, we have realized the necessity to participate in this trend of studies to establish the concept of LD ($\leq$0.2Gy) and low-dose-rate (LDR, $\leq$6mGy/hr) irradiation based on UNSCEAR [5]. Moreover, a facility for the studies on the influence of LD and LDR irradiation on animals and cells has been constructed on December 2004. In this congress, I summarized the systemic protective effects of LD (0.2Gy) and LDR (0.7mGy/hr) γ-irradiated mice include immunology

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(PS3050) Polymorphisms in XRCC1 and XRCC3 genes as predictors of individual radiosensitivity. Selena Palma[1], Tommaso Cornetta[2], Renata Cozzi[2], Tommaso Poggioli[2], Donatella Tirindelli[1], Antonella Testa[1]. [1]ENEA C.R. Casaccia, Roma, Italy, [2]Department of Biology, "Roma TRE" University, Roma, Italy.

Introduction: Enhanced sensitivities and variability in processing the induced DNA damage can be responsible of both higher risk of developing cancer and elevated normal tissue adverse reactions. It has been hypothesized that these complex traits are influenced by genetic factors, in particular by "minor genetic determinants" [1], depending on the interplay of several gene products. In view of the importance of DNA repair in cell and tissue response to radiation, genetic variants in genes involved in DNA damage repair pathways are suitable candidates in search for the genetic basis of radiosensitivity. The purpose of this study was to examine the association of polymorphisms in XRCC1 and XRCC3 with cellular radiosensitivity expressed by DNA primary damage and chromatid breaks.

Materials and Methods: Polymerase chain reaction-restriction fragment length polymorphism assay was performed to detect XRCC1 (Arg399Gln) and XRCC3 (Thr241Met) variant alleles; among the cytogenetic test systems on peripheral blood lymphocytes, the G2 assay can be considered as the gold standard of chromosomal radiosensitivity [2], while the comet assay is a suitable tool to study direct DNA damage at single cell level. We used the G2 and the Comet assays to measure radiosensitivity in blood cells from twenty-two healthy subjects after *in vitro* X-ray (0.4 Gy) exposure.

Results: Preliminary results indicate that, after irradiation, subjects bearing XRCC1 wild type genotype show an higher level of chromatid type aberrations than those carrying at least one XRCC1 variant allele. As far as Comet Assay parameters, no difference was detected comparing wild type and variant XRCC1 genotypes. ***Conclusions:*** Our results suggest that the presence of XRCC1 Arg399Gln variant could influence the individual radiosensitivity. The combination of genotype analysis with DNA damage sensitivity assays is a useful approach to screen individuals for DNA repair deficiencies to predict health risk.

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(PS3051) Combined effects of ionizing radiation and cadmium ions on DNA damage and gene expression in cultured medaka fish cells. Dmytro Grygoryev, Oleksandr Moskalenko, John Zimbrick. Colorado State University, Fort Collins, CO, USA.

Ionizing radiation-induced (IR) formation of genomic DNA damage and cellular gene expression changes can be modulated by nearby chemical species such as heavy metal ions. Many of these ions are cytotoxic and carcinogenic. Cadmium (Cd) is a toxic transitional metal recognized as a carcinogen by the International Agency for Research on Cancer. We studied the formation of a product of DNA base oxidation, 8-hydroxyguanine (8-OHG), double strand breaks (DSB) and changes in steady-state mRNA levels of several genes caused by the simultaneous action of Cd and gamma radiation. We performed our studies in cultured medaka fish (*Oryzias latipes*) fibroblast cells by HPLC-ECD, PFGE and RT-PCR methods. We studied combined effects of Cd and IR on cell survival by colony formation assay methodologies. Our data show that a 24 hour incubation of cells in media containing Cd ion concentrations higher than 10 uM leads to a significant decrease in the fraction of surviving cells. Cd ions also increase the sensitivity of cells to IR. Thus, 24-hour incubation with 30 uM Cd prior to irradiation produces a 4-fold reduction in the radiation-induced LD_{50} of medaka cells. Incubation with Cd prior to irradiation leads to nonlinear increases in radiation-induced yields of 8-OHG and DSB. Dose-yield plots of these lesions exhibit an S-shaped curve with a sharp increase in the yield of lesions in the 10–20 uM range of Cd concentrations. IR and Cd treatment-induced changes in transcript levels of several medaka orthologs of human, mouse, and yeast genes participating in major DNA damage response-related pathways such as Base Excision Repair (BER), NHEJ, and cell cycle arrest have also been detected. Our data show that the combined action of ionizing radiation and cadmium leads to an increase in DNA damage levels compared to the effects of the radiation or cadmium alone and can induce DNA repair and cell cycle-related gene expression changes. These results are consistent with the hypothesis that the presence of Cd modulates the efficiency of DNA repair systems thus causing increases in radiation-induced DNA damage levels and decreases in cell survival.

(PS3052) Effects of gamma irradiation on the viability of *Cryptosporidium parvum* measured by real-time PCR. Mikyo Joung[1], Sooung Lee[1], Woo-Yoon Park[2], Jae-Ran Yu[1]. [1]Dept. of Environmental and Tropical Medicine, College of Medicine, Konkuk University, Chungju, Republic of Korea, [2]Dept. of Radiation Oncology, College of Medicine, Chungbuk National University, Cheongju, Republic of Korea.

Cryptosporidium parvum is protozoan present over the environment, a challenge to the water industry and threat to public health. It is critical to develop improved detection methods as well as disinfection methods for the protection from cryptosporidiosis. In this study, we investigated the viability of *C. parvum* after gamma irradiation using real-time PCR method. *C. parvum* oocysts contained in 1.5-ml tubes were Co-60 gamma-irradiated with doses of 1 kGy, 5 kGy, 10 kGy and 25 kGy. Real-time PCR was done with cDNA made from extracted total RNA from oocysts directly or HCT-8 cells infected with excysted *C. parvum* oocysts after gamma irradiation. For the absolute quantification using real-time PCR, novel CP2 gene was used as a target gene. Preliminary data showed that the lower detection limit of real-time PCR using CP2 primer was ten oocysts. Oocyst viability was not affected by gamma irradiation under 5 kGy when the viability was checked as soon as irradiation was finished. However oocysts infectivity on HCT-8 cells decreased 2^5 fold compared to unirradiated oocysts even at 1 kGy irradiation. Cell infectivity was much more damaged when oocysts were irradiated with 5 kGy to 10 kGy but still some oocysts seemed to be maintained infectivity. It was appeared that both oocyst viability and infectivity were fatally damaged by 25 kGy. Although oocyst viability was seemed to be recovered through the time passage until 5 days after irradiation, cell infectivity was not appeared to be recovered. The real-time PCR assay with CP2 gene as a target for the measuring of viability and infectivity of *C. parvum* was tried for the first time in this study and the results ware proved to be quite reliable. And in this study, we confirmed that some proportion of *C. parvum* oocysts have resistance to gamma irradiation under 10 kGy. These findings were similar results with those we have published already with animal study.

Key Words: *Cryptosporidium parvum; Gamma Irradiation, Viability, CP2*

(PS3053) Genomic instability induced in the descendants of normal human fibroblasts surviving heavy-ion irradiation. Nobuyuki Hamada[1], Takamitsu Hara[1], Tetsuya Sakashita[2], Tomoo Funayama[2], Sakura Sora[1], Yasuhiko Kobayashi[2]. [1]21st Century COE Program and Dept Quantum Biol, Gunma Univ Grad Sch Med, Gunma, Japan, [2]Microbeam Radiat Biol Gr, Japan Atomic Energy Agency, Gunma, Japan.

There is a great deal of evidence demonstrating that ionizing radiations persistently destabilize the genome and induce delayed reproductive death and other phenotypes in the descendants at delayed times after irradiation of the parental cells. Whereas the relative biological effectiveness (RBE) in irradiated cells has been known to differ with the linear energy transfer (LET), the LET dependence of the manifestation of radiation-induced genomic instability is not completely understood heretofore. To address this, we have analyzed the delayed effects arising in the progeny of normal human fibroblasts surviving high-LET heavy-ion or low-LET irradiation.

First, delayed loss of clonogenicity was examined as an endpoint of delayed reproductive death. Confluent cultures exposed to ^{60}Co γ-rays (0.2 keV/μm) or six different beams of heavy ions (16.2–1610 keV/μm) were plated for primary colony formation. Thereafter, cells harvested from primary colonies were plated for secondary colony formation. While the RBE based on the primary 10% survival dose as well as that on the secondary 80% survival dose consistently peaked at 108 keV/μm (220 MeV ^{12}C ions), there was very little difference in the RBE based on secondary survival at the primary 10% survival dose. This suggests that delayed reproductive death arising only during secondary colony formation is independent of the LET and may depend upon initial damages having been fixed during primary colony formation.

Second, morphological variation within each primary colony stemmed from single progenitor cells was hence examined as an endpoint of clonal heterogeneity. Irradiation facilitated the induction of cells that exhibit an enlarged morphology in colonies, for which 220 MeV ^{12}C ions were more effective than γ-rays. Collectively, these enlarged cells induced in primary colonies were considered to account for the late-arising loss of clonogenicity observed when primary colonies underwent secondary colony formation. The mechanisms that may initiate and perpetuate genomic instability following high- or low-LET irradiation need to be further studied.

(PS3054) Analysis of Common Deletion (CD) and a novel deletion of mitochondrial DNA induced by ionizing radiation. Ai Kurihara[1], Lu Wang[1], Yoshikazu Kuwahara[1], Taisuke Baba[1], Koji Ono[2], Manabu Fukumoto[1]. [1]Tohoku University, Sendai, Japan, [2]Kyoto University, Kumatori, Osaka, Japan.

Purpose: In order to identify supportive evidence of radiation exposure of cells, we analyzed the relationship between exposure to ionizing radiation and the induction of deletions in mitochondrial DNA (mtDNA).

Materials and methods: Using a human hepatoblastoma cell line, HepG2 and its derivatives, HepG2-A, −89 and −400, established after long term exposure to X-rays, mtDNA deletions were analyzed by polymerase chain reaction (PCR) and real-time PCR after cells were subjected to radiation and genotoxic treatments.

Results: The most abundant large-scale deletion reported in mtDNA is called a 'common deletion' (CD). CD is a 4,977-bp deletion specifically occurring between two 13-bp repeats from nucleotide position (nt)8,470 to nt13,446 in the mtDNA sequence and can be used as a marker of oxidative damage to mtDNA. In this study, it was induced within 24 hours after exposure to 5 Gray of X-rays and was associated with replication of mtDNA. CD became undetectable several days after the exposure due to the death of cells containing mitochondria within which CD had been induced. Furthermore, we found a novel mtDNA deletion that consisted of a 4,934 base-pair deletion between nt 8,435 and 13,368. Compared with CD induction, a lower dose of ionizing radiation was required to induce the 4,934 base-pair deletion and this deletion was independent of the quality of radiation used and was not induced by treatments with H_2O_2 and other genotoxic reagents including bleomycin.

Conclusion: CD is induced by ionizing radiation, however, the amount of CD detected at a certain point in time after radiation exposure is dependent on the initial frequency of CD induced and the death rate of cells with mtDNA containing CD. The novel mtDNA deletion found in this study, will be used to determine whether cells were exposed to ionizing radiation or not.

(PS3055) Chromosome model reveals dynamic redistribution of DNA damage into nuclear sub-domains. Sylvain V. Costes[1], Artem L. Ponomarev[2], James Chen[1], Francis A. Cucinotta[2], Mary Helen Barcellos-Hoff[1]. [1]Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [2]NASA JSC, Houston, TX, USA.

Several proteins involved in the response to DNA damage (i.e. 53BP1, phosphorylated ATM and γH2AX) form microscopically visible nuclear domains, or foci, after exposure to ionizing radiation. Radiation-induced foci (RIF) are believed to be located where DNA damage is induced. To test this assumption, we analyze RIF spatial distribution in cells irradiated with high linear energy transfer (LET) radiation. Since energy is randomly deposited along high-LET particle paths, RIF along these paths should also be randomly distributed. The probability to induce DNA double strand breaks (DSB) can be derived from DNA fragment data measured experimentally by pulsed-field gel electrophoresis. This probability is used in Monte Carlo simulations to predict DSB locations in synthetic nuclei geometrically described by a complete set of human chromosomes, taking into account microscope optics from real experiments. Although simulations produce DNA-weighted random (Poisson) distributions, experimental RIF distributions obtained as early as 5 min after exposure to 1 GeV/amu Fe are non-random. This deviation from the expected DNA-weighted random pattern can be further characterized by "relative DNA image measurements". This novel imaging approach introduced here shows that RIF locate preferentially at the interface between high and low DNA density regions, and in regions with slightly less DNA than predicted. This deviation from random behavior is more pronounced within the first 5 min following irradiation for phosphorylated ATM RIF, while γH2AX and 53BP1 RIF show very pronounced deviation up to 30 min after exposure. The combination of quantitative image analysis with models of radiation-induced DNA damage demonstrates that the biological responses to DSBs include the formation of nuclear sub-domains with specific DNA density patterns. These findings are an important first step towards the elaboration of mechanistic computational models to predict the risk of mutations and cancer that can pose radiation to humans.

(PS3056) Reproductive and genetic toxicity in male mice after chronic oral exposure to low level of depleted uranium. Rong Li, Yanbing Leng, Yongping Su. Institute of Combined Injury,Third Military Medical University, Chongqing, China.

Objective: Depleted uranium (DU) munitions have been widely used on the battlefield since the Gulf War in 1991. This constitutes a risk of future DU contamination of groundwater and drinking water. The general population may be exposed to low levels of uranium through the diet. The present study is to investigate the effects on male reproductive and genetic toxicity after chronic oral exposure to low levels of DU.

Methods: Male and female rats were exposed to DU in food at doses of 0, 0.4, 4 or 40 mg/kg/d for 160 days. After 160 days, concentration of testosterone (T), luteinizing hormone (LH) and follicle stimulating hormone (FSH) in serum were detected. Mature male rats were mating with mature virgin female rats exposed to the same doses for 21 days. Pregnant rate and normal labor rate in F_0 rats were calculated. So did the survival rate and weight of filial generation (F_1) rats within 21 days after birth. Thereafter, the deformity rate of rat sperm and the micronucleus rate of bone marrow cells were observed. Single-cell gel electrophoresis(comet)-assay was used to detected the DNA injury of rats sperm.

Results: No adverse effects of DU on fertility were evident at any dose for 160 days in F_0 rats. The body weight and reproduction of parental generation, and the growth and development of filial generation were not different between the rats exposing to DU and

the control group ($P > 0.05$). The sexual hormone such as T and FSH in F_0 rats exposed increased, but LH content decreased($P \leq 0.05$). After DU feeding,increases were not found when measuring the deformity rate of rat sperm and Comet tail length and percentage of tail DNA at 0.4mg/kg/d. But the deformity rate of rat sperm, the micronucleus rate of bone marrow cells, comet tail length and percentage of tail DNA increased at 4and 40mg/kg/d($P<0.01$). In exposed group, the percentage of tail DNA in rat sperm increased which appeared dose-dependent ($R^2 = 0.98$). Histopathologic examination of the testes in rats exposed for 160 days did not reveal significant difference between controls and exposed animals, with the exception of an increase in Leydig cells vacuolization at 4 and 40mg/kg/d.

Conclusions: After exposure for 160 days, low levels of DU can cause genetic toxic effect, but may not cause reproductive toxic effect.

(PS3057) Cytogenetic instability in peripheral blood T lymphocytes cultured in vitro from A-bomb survivors. Kanya Hamasaki[1], Yoshiaki Kodama[2], Yoichiro Kusunoki[1], Eiji Nakashima[3], Norio Takahashi[2], Nori Nakamura[2], Kei Nakachi[1]. [1]Radiation Effects Research Foundation Department of Radiobiology/Molecular Epidemiology, Hiroshima, Japan, [2]Radiation Effects Research Foundation Department of Genetics, Hiroshima, Japan, [3]Radiation Effects Research Foundation Department of Statistics, Hiroshima, Japan.

[Purpose] Genetic instability has been suggested as one of the causes for the development of late effects following radiation exposure, whereas human data are limited. Our recent study on lymphocyte bearing clonal chromosome aberrations in A-bomb survivors did not indicate the presence of the instability in vivo (i.e., frequency of additional aberrations among the clonal cells was used as the indicator). In the present study, we examined if the instability is observable during in vitro propagation of clonally derived peripheral T lymphocytes from A-bomb survivors using the same indicator as used for the in vivo study.

[Study subjects and Methods] Two A-bomb survivors (estimated doses of >1Gy, cases 1 and 3) and two control subjects (<5mGy, matched by age and sex, cases 2 and 4) were studied. Blood T cells were clonally propagated in vitro using standard methods, and metaphases were prepared for multi-color FISH (M-FISH) analysis. One hundred cells were examined for each clonal cell population.

[Result and Discussion] We obtained 22 clonal cell populations from case 1 (9 colonies with normal karyotype, 8 with clonal translocations observed in vivo, and 5 with different aberrations), and 18 populations from case 2 (all normal karyotype). Similarly, we established 14 populations from case 3 (5 with normal karyotype, 5 with clonal translocations observed in vivo, and 4 with different aberrations), and 12 populations from case 4 (all normal karyotype except for one). In the survivors exposed to >1Gy (cases 1 and 3), total 39 additional exchange-type aberrations [translocation (t) and derivative chromosome (der)] were found among 3,600 cells (1.1%), and the corresponding value in the control subjects (case 2 and case 4) was 0.6% (17/3,000). Although the aberration frequency in exposed cases seemed somewhat higher than that in the controls, it was not significant (p=0.101 by Wald test using quasi-likelihood method). Further, we compared the frequency of all additional structural aberrations (t, der, duplication, deletion, fragment) between the exposed and control subjects, and the difference in the two sets of data was not statistically significant (p=0.136).

[Conclusion] There was no clear evidence suggesting the presence of chromosome instability among the clonally expanded lymphocytes in vitro from A-bomb survivors.

(PS3058) Permissible dose limit based on the analysis of stable chromosome aberrations in the lymphocytes. Isamu Hayata. NIRS and CRIEPI, Chiba and Komae, Japan.

Permissible dose limit for the public is one of the most important subjects in the regulation of radiation. We have analyzed chromosome translocations in the peripheral lymphocytes of people who live in normal living circumstances. The number of cells analyzed was over 7000 cells per child and over 4000 cells per adult. The results are reported by Wang et al. at this congress (ICRR 2007). Average genomic frequencies of translocations in 1,000 cells in 20 residents (61.2 year-old on average) in a large city and in 16 residents (64.4 year-old on average) in a remote village and in 8 children (12.3 year-old on average) in the remote village were 9.6, 8.4, and 3.2, respectively. Their standard deviations were 5.0, 3.1 and 2.0. Since exposed dose can be estimated by the frequency of chromosome aberrations, we converted those frequencies of translocations shown above to radiation doses. Lloyd et al. (1983) reported that the induction rate of dicentrics by chronic low dose gamma irradiation is about 2.5 in 10,000 cells per cSv. Since translocations and dicentrics are induced by radiation in about equal frequency (Zhang and Hayata, 2004), the induction rate of translocation is considered as 2.5 in 10,000 cells per cSv. In case of acute exposure the frequency follows the dose response shown as: $Y = 2.31 \times 10^{-4} D + 6.33 \times 10^{-6} D^2$ (Sasaki et al., 2000), where Y is the frequency of dicentrics per 1,000 cells, and D is the dose in cSv. If we assume all the translocations observed in our study mentioned above are induced by chronic irradiation, the calculated doses for 9.6 (adult in a large city), 8.4 (adult in a remote village) and 3.2 (child in a remote village) in 1000 cells become 384 mSv, 336 mSv, and 128 mSv. In case of acute irradiation the calculated doses for them become 248 mSv, 225 mSv, and 107 mSv. Standard deviations were 200 mSv, 124 mSv and 80 mSv in chronic exposures, and 153 mSv, 104 mSv and 72 mSv in acute exposures. It is not possible to distinguish the cohort if the difference is within the standard deviation of the control. Therefore, our findings suggest that it is not possible to detect any effects of radiation to be caused on the human body at least up to124 mSv in adult and up to 80 mSv in child in chronic exposures, and up to 107 mSv in adult and up to 72 mSv in child in acute exposures. Permissible dose limit for the public will be discussed.

(PS3059) Effect of smoking reflected in the stable chromosome aberrations in the lymphocytes of the residents in the areas exposed to different environmental mutagens including radiation. Wei Zhang[1], Chunyan Wang[1], Masako Minamihisamatsu[2], Luxin Wei[1], Tsutomu Sugahara[3], Isamu Hayata[4]. [1]National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention, Beijing, China, [2]National Institute of Radiological Sciences, Chiba, Japan, [3]Health Research Foundation, Kyoto, Japan, [4]National Institute of Radiological Sciences (NIRS) and Central Research Institute of Electric Power Industry (CRIEPI), Chiba and Komae, Japan.

Smoking is the most influential factor among the environmental mutagens to increase cancer incidence. Previously we reported that the effect of smoking on the induction of chromosome aberration in the residents in high background radiation area (HBRA) was larger than that of the elevated level of natural radiation in China (Zhang et al., 2004). In order to know how environmental mutagens affect the induction of translocations caused by smoking we analyzed the translocations in the lymphocytes of smokers in a large city, Beijing, and compared them with those in a high background radiation area (HBRA) and in its control area (CA), remote villages, in South China. The studied residents in Beijing have lived there for longer than 40 years. The level of natural radiation in HBRA is 3 - 5 times higher than those in CA and in Beijing. The residents in remote villages (HBRA and CA) mainly smoke the shredded tobaccos through a water pipe instead of the cigarette which is common in Beijing. Individual radiation dose was measured with a pocket dose meter (Aloka PDM-10) put on the body for 24 hours. Peripheral blood was obtained from 5, 10, and 7 smokers in Beijing, HBRA and CA, respectively. Their ages were around 60 years old. Peripheral lymphocytes were cultured for 48 hours with PHA and colcemide, and harvested according to our standard method for biodosimetry. Chromosomes #1, #2, and #4 were painted for the analysis of translocations. The number of metaphases analyzed was totally 90,267 (4,103 per resident). Statistical analysis was done by Mann-Whitney's U test. Genomic frequencies of translocation in 1,000

lymphocytes per smoker are 8.7±3.0, 11.1±3.6 and 13.4±3.4 in Beijing, HBRA and CA, respectively. Standard deviation is similar among three groups. CA group shows the highest average value among three groups. There was a statistically significant difference ($p>0.05$) in the frequencies of translocation between the smokers in Beijing and those in CA, but no other possible comparisons between groups showed significant difference. The effect of smoking seems to be suppressed by the environmental mutagens including the elevated level of natural radiation in HBRA. Further study is in progress to see if such suppression effect is statistically significant or not.

(PS3060) Stable chromosome aberrations in the lymphocytes of the residents in different areas: A large city, and a high background radiation area and its control area in China. Chunyan Wang[1], Wei Zhang[1], Masako Minamihisamatsu[2], Luxin Wei[1], Tsutomu Sugahara[3], Isamu Hayata[4]. [1]National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention, Beijing, China, [2]National Institute of Radiological Sciences, Chiba, Japan, [3]Health Research Foundation, Kyoto, Japan, [4]National Institute of Radiological Sciences (NIRS) and Central Research Institute of Electric Power Industry (CRIEPI), Chiba and Komae, Japan.

We have reported in our previous papers that the frequencies of dicentrics and rings significantly increase in the high background radiation area (HBRA) in China, but the increase of the frequencies of translocations (Tr) is invisible (Hayata et al., 2004) . The level of natural radiation in HBRA is 3 - 5 times higher than that in control area (CA). Present study includes new data of the analysis of Tr in the residents in a large city, Beijing, where people are exposed to a lot of environmental mutagenic stresses including air polluting factors. The frequency of the Tr in the lymphocytes of non-smokers who live in Beijing for longer than 40 years is compared with those of non-smokers in HBRA and CA, remote villages, in China. The blood was taken from 20, 16, and 16 non-smokers in Beijing, HBRA and CA, respectively. Their average ages were 61.2, 64.1 and 64.4 years old, respectively. The blood of children in HBRA (12.3 years old on average) and in CA (12.5 years old on average) were also collected. Individual radiation dose was measured with a pocket dose meter (Aloka PDM-10) put on the body for 24 hours. The lymphocytes were cultured for 48 hours with PHA and Colcemide, and harvested according to the standard method. Tr were analyzed by the chromosome painting method using whole chromosome painting probes for #1, #2, and #4. Total number of metaphases analyzed was 213,876 (4,113 per resident). Statistical analysis was done by the Mann-Whitney's U test. Genomic frequencies of Tr in 1,000 cells per adult were 9.6±5.0, 12.8±6.4 and 8.4±3.1 in Beijing, HBRA and CA, respectively. The frequencies in child were 3.8±1.1 in HBRA and 3.2±2.0 in CA. Individual variation was large in adult and small in child. It is demonstrated that the frequencies of Tr in the lymphocytes well reflect the individual difference in the amount of mutagenic factors taken into each human body. There is a statistically significant difference ($p<0.05$) in the frequencies of Tr between child and adult, and between HBRA and CA in adult, but there is no significant difference between other comparable groups. Thus the effect of the elevated level of natural radiation in HBRA becomes obscure when the frequencies of Tr are compared between the residents in HBRA and those in a metropolis where people are incessantly exposed to a lot of environmental mutagenic stresses including air pollutants.

(PS3061) Transmission of genomic instability from a single irradiated human chromosome to the progeny of unirradiated cells. Seiji Kodama[1], Naoki Mukaida[2], Hisakatsu Nawata[1], Kentaro Ariyoshi[1], Sanae Watanabe[1], Kazunori Shiraishi[1], Keiji Suzuki[3], Mitsuo Oshimura[4], Masami Watanabe[5]. [1]Radiation Biology Laboratory, Frontier Science Innovation Center, Organization for University-Industry-Government Cooperation, Osaka Prefecture University, Sakai, Osaka, Japan, [2]Nuclear Power Engineering, Quality and Safety Management Department, Tokyo Electric Power Company, Tokyo, Japan, [3]Division of Radiation Biology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan, [4]Department of Molecular and Cell Genetics, School of Life Science, Faculty of Medicine, Tottori University, Yonago, Japan, [5]Division of Radiation Life Science, Research Reactor Institute, Kyoto University, Sennan-gun, Osaka, Japan.

Ionizing radiation induces chromosome instability that is transmitted over many generations after irradiation in the progeny of surviving cells, but it remains unclear why this instability can be transmitted to the progeny. There is lack of evidence to demonstrate that the unstable nature is conferred upon an irradiated chromosome itself by direct irradiation. If the unstable nature initiated by the targeted effect of ionizing radiation can be retained in the irradiated chromosome, then this would explain why the instability is transmitted to the progeny of surviving cells. To know the transmissible nature of genomic instability, we transferred an irradiated human chromosome into unirradiated mouse recipient cells by microcell fusion and examined the stability of the transferred human chromosome in the microcell hybrids by fluorescence in situ hybridization (FISH) using a fluorescent probe specific for the whole human chromosome. A transferred chromosome was stable in all of six microcell hybrids in which an unirradiated human chromosome had been introduced. In contrast, the transferred chromosome was unstable in four out of five microcell hybrids in which an irradiated human chromosome had been introduced. The aberrations included changes of the irradiated chromosome itself and rearrangements with recipient mouse chromosomes. To examine the possibility that residual radiation lesions remain in the irradiated human chromosome, we investigated the number of foci of phosphorylated histone H2AX (gamma-H2AX) in interphases of microcell hybrids containing the irradiated human chromosome. However, we failed to show a difference in the number of foci of gamma-H2AX between microcell hybrids with the unirradiated chromosome and those with the irradiated chromosome, indicating that there were no long-lived foci at times after exposure to ionizing radiation, presumably due to repair of such lesions by these late times. Thus, the present study demonstrates that genomic instability can be transmitted to the progeny of unirradiated cells by a chromosome exposed to ionizing radiation, implying that the memory of the radiation event is retained in the exposed chromosome, and therefore, the effect of ionizing radiation in inducing delayed chromosome instability is non-targeted.

(PS3062) LET and ion-species dependence for cell-killing effect, mutation induction and chromosome aberration in normal human fibroblasts. Tsuruoka Chizuru, Suzuki Masao, Furusawa Yoshiya, Okayasu Ryuichi, Anzai Kazunori. National Institute of Radiological Sciences, chiba-shi, Japan.

Recent studies showed that the sharp of the LET-RBE curves of various biological effects was different, when using different ion species even if similar LET values. For explaining the LET dependence of biological effects it is necessary to investigate the effects using the same ion species with different LET values. However, the data concerning the LET dependence of biological effects using same ion species is very limited. In this study, we investigated LET and ion-species dependence of biological effects, such as cell-killing effects, mutation induction and chromosome aberration. Normal human fibroblasts were irradiated with heavy ion beams, such as carbon (135 and 290 MeV/n), neon (230 and 400 MeV/n), silicon (490 MeV/n) and iron (500 MeV/n), generated by Heavy Ion Medical Accelerator in Chiba at National Institute of Radiological Sciences. The LET values were ranging from 13 to 98 keV/μm for carbon, from 30 to 184 keV/μm for neon, from 55 to 214 keV/μm for silicon and from 200 to 400 keV/μm for iron ion beams. For a comparison, we used X rays (200kV, 20mA) with 0.5-mm aluminum and 0.5-mm copper filters. Cell-killing effects were detected as a reproductive cell death using a colony formation assay. Mutation induction in HPRT locus was detected to measure 6-tioguanine (6-TG) resistant colonies. The 6-TG resistant colonies were randomly isolated and extracted genomic DNA, and the mutation spectrum of deletion patterns of HPRT exsons was analyzed by multiplex PCR. Chromosome aberration was counted as a fragmentation of prematurely condensed chromosome using a

technique of premature chromosome condensation (PCC). To prevent the fast rejoining of PCC breaks, cycloheximide was added in the PCC samples of initially measured breaks during chromatin condensation. The LET-RBE curves of induction of initially measured PCC breaks were not dependent on LETs and ion species. On the other hand, the curves of cell-killing effects, mutation induction and induction of non-rejoining PCC breaks detected after 24h-postirradiation induction were changed, depending on LETs and ion-species. Furthermore, the deletion pattern of exons was different in induced mutants among different ion-species. These results suggest the LET and ion-species dependent biological effects could be affected by repair process of induced chromatin breaks.

(PS3063) Long term transmissibility and stability of chromosome rearrangements in human cells exposed to ionizing radiation. Richard Eberle, Bradford Loucas, Michael Cornforth. University of Texas Medical Branch, Galveston, TX, USA.

Radiation-induced chromosome aberrations are typically studied at the first mitosis following exposure. These include both asymmetrical exchange products, such as dicentrics, which are nontransmissible (lethal), as well as symmetrical exchanges, such as reciprocal translocations, which are transmissible. For the purpose of conducting future molecular analysis of transmissible aberrations, we have begun to isolate and cytogenetically characterize a series of independent cell clones that survive exposure to radiation. In the mean time, this effort has provided us some insight into the processes of transmissibility and genomic stability.

Human fibroblast cultures previously immortalized with the catalytic subunit of telomerase (hTERT) were irradiated with 4 Gy of ^{137}Cs gamma rays, before being plated at low density for clonal growth. Surviving clones were randomly isolated and grown up for cytogenetic analysis using 24-color multiplex FISH (mFISH). Ten cells were karyotyped from each clone.

We have characterized some 50 independent radiation-exposed clones, and an approximately equal number of unexposed clones. The background frequency of translocations in 7 unirradiated control clones, for which more extensive scoring was done, was less than 1%. For clones deriving from irradiated cultures, 21/49 (43%) were homogeneous for a unique stable rearrangement that was present in 10/10 karyotypes. Most of the recovered rearrangements were simple reciprocal translocations. However, of the 21 clones containing stable rearrangements, 4/21 (19%) were complex, involving three chromosomes. No evidence of radiation-induced chromosomal instability, in the form of jumping translocations, was observed. We also examined the frequency of symmetrical translocations in an *uncloned* population of irradiated cells at their first mitosis following exposure. Interestingly, this frequency was virtually identical to the frequency of viable clones containing these transmissible exchanges – *irrespective* of the fact that the translocation-bearing cells at the first mitoses frequently also contained asymmetrical exchange products that would be lethal to the cell. Results underscore the significance of both simple and complex exchanges in the transmissibility of DNA misrepair products.

(PS3064) Single break driven chromosome instability in human cells. Laure M. Sabatier. CEA, Fontenay aux Roses, France.

Low dose radiation effects remain an open question, mainly due to the lack of models that permit to address it. Using human cells tagged with a plasmid integrated on end of a "marker" chromosome, we demonstrated that a single break at the telomere has dramatic consequences on the genome stability, inducing the panel of chromosomal instability detected in cancer cells including gene amplification and large chromosome imbalances (LOH up to 100 Mb), Sabatier et al, Mol Cancer Res 2005. The chromosome instability involves specific chromosomes suggesting the preferential association of certain chromosomes. The role of the proximity of chromosome territories is tested via 1) Induction of local damage and in the identification of co-damaged chromosomes, 2) Analysis of the position of the chromosomes by confocal microscopy and 3D FISH. Preliminary results seem to confirm this hypothesis.

One of the consequences of the chromosome instability induced by a single telomere loss is to let emerge cells with high proliferative advantages. An increase of the tumorigenicity of these cells is observed when transplanted on nude mice.

Such cell models would be very informative to characterize the role of the modulation of DNA damage repair (NHEJ and HR) in the consequence of single chromosome break. First we tested the feasibility of using replicative small interfering vectors for efficient and long term silencing in human cells. We silenced NHEJ protein - DNAPK, LigIV, and XRCC4- and HR protein -Rad51/Rad52/Rad54- in Hela cells. Our data suggested that the major effect of NHEJ on telomere maintenance is indirect via misrepaired breaks in subtelomeric and telomeric whereas the major effect of HR on telomere maintenance is direct via telomere replication and telomere recombination. We are working on the invalidation of DNA repair genes in telomere tagged models.

Having demonstrated that a single break near telomere could have such consequences we addressed the question of radiation-induced chromosome breaks that will occur at low doses (25 mGy - $>$ 1DSB per cell). It is observed an increase of clonogenic survival and the emergence of resistant clones due to chromosome breakage. Thus we hypothesis that irradiation at very low doses could not be without consequences on the genome stability of those human cells.

(PS3065) Induction of genome aneuploidization and nuclear DNA loss in gamma-irradiated rat spermatozoa. Veronika Bulavytska. Scienific Center for Radiation Medicine of AMSU, Kyiv, Ukraine.

Spermatozoon is a highly differentiated sexual cell which according to established radiobiological regularities may be assumed to persist after drastic ionizing irradiation. The present research aimed in elucidation the sequence of events taking place in the course of radiation damage development in animal spermatozoa.

The experiments were performed on the epididymal spermatozoa of rats. After extirpation the isolated epididymices were placed in saline and gamma-irradiated by ^{60}Co in the wide dose range 10–1000 Gy with dose input 0,2 Gy/sec. Then irradiated spermatozoa were rescued from epididymices and incubated in saline at 37°C. For scanning electron microscopy the drop of spermatozoa suspension was fixed in 2,5% glutaric aldehyde, covered with 100 Å layer of gold and examined under "JSM-35C" electron microscope (Japan). Nuclear DNA cytophotometry was made on the automatic cytophotometer "MFTX-2M" (Russia) linked with computer at the wavelength of 546 nm after spermatozoa staining with basic fuchsine. Determination of free DNA concentration in spermatozoa suspensions was done after spermatozoa removal and DNA staining with diphenylamine in Burton's modification on spectrophotometer "SP-46" (Russia) at the wavelength of 598 nm.

The experiments have revealed that gamma-irradiation enhanced the mobility of surface matrix in spermatozoa which began gradually to slip down in nutrient solution upon dose increase. The denudation of spermatozoon body was accompanied by cytoskeleton disintegration. At higher radiation doses the cytoskeleton disruption proceeded in necrosis-like manner causing spermatozoid nuclear DNA loss. According to the data of DNA cytophotometry, irradiation resulted in the aneuploidization of nuclear genome. Furthermore the appearance of exogenic DNA in cultivating medium due to spermatozoa rupturing and spermatozoid DNA release induced the additional hydrolysis of spermatozoid nuclear DNA, likely, because of surface nuclease activation, the latter being allocated in spermatozoa heads.

Thereby the investigations fulfilled have shown the complexity and multistage for radiation lesions development in spermatozoa that were gamma-irradiated in the wide dose range, from low to moderate and sublethal doses.

(PS3066) Potential evidence of radiation-induced genomic instability under chronic radiation exposure in man. Galina

Veremeyeva, Tatyana Varfolomeyeva, Alexander Akleyev. Urals Research Center for Radiation Medicine, Chelyabinsk, Russian Federation.

In spite of a large number of experimental data indicative of radiation-induced genomic instability, conclusive data on the occurrence of this phenomenon after chronic radiation exposure in man are still insufficient. In this context, studies of molecular-cellular processes in residents of the Techa riverside villages are of special interest.

Exposures of the Techa riverside population started in 1949 and resulted from the Mayak PA activities. An important specific feature of the exposure is its two-component nature: external and internal exposure due to ^{90}Sr. Because of the osteotropic properties of ^{90}Sr, and high radiosensitivity of bone marrow, the hemopoiesis is regarded as a critical system in Techa River cohort. In view of this fact, our study focused on the state of blood cells.

It has been revealed that 55 years after the onset of exposure erythrocytes from exposed individuals exhibited a significantly increased level of malon dialdehid following 24-hour incubation: 33.2±1.9 versus 27.8±1.8 in the controls.

A significantly increased frequency of CD3-CD4+ lymphocytes was observed in the study group: 2.3±0.11 versus 1.7±0.14 in the control (x 10^4 cells).

The exposed group manifested a significantly increased level of apoptosis (morphological criteria) of peripheral blood lymphocytes (12.30±0.57 ‰), compared to the controls (9.11±0.79 ‰). We are treating this finding as a compensatory reaction to high-intensity mutagenesis.

The data obtained are in good agreement with the earlier results of cytogenetic studies which pointed to a significant increase in the frequency of stable- and unstable- chromosome aberrations, in exposed individuals.

The assumption that the changes observed are of a secondary nature and originate de novo decades after reduction in dose rates to background levels, is confirmed by the fact that a portion of them (CD3-CD4+ cells, dicentrics) are of unstable nature and can be rapidly eliminated.

The data obtained correlate well with the high relative risk of leukemia mortality estimated for the Techa River cohort since it is assumed that the significant role of genetic instability in the origin and development of malignancy is a proven fact.

Therefore, the data available render support to the hypothesis about the existence of genomic instability in members of the Techa River cohort.

(PS3067) Cell killing and genomic instability in mutation induction on long-term CHO cells cultures irradiated with 290MeV/u carbon ions. Xiao Wang[1], Yoshiya Furusawa[2], Masao Suzuki[2], Ryoichi Hirayama[2], Yoshitaka Matsumoto[2], Ying Qin[2]. [1]China Institute of Atomic Energy, Beijing, China, [2]National Institute of Radiological Sciences, Chiba, Japan.

The study of radiation induced mutation and genomic instability is relevant to the estimates of the risk of secondary malignancies associated with radiation therapy and the carcinogenic effects of space environmental radiation.

HPRT locus has been the most commonly used as a target gene for mutation detection studies. In this study, we investigated the generation expression dependence of mutation induction on HPRT locus in CHO cells irradiated with carbon ions.

Chinese hamster ovary (CHO) cells were irradiated with graded doses of carbon ions (290 MeV/u, LET: 13 kev/um) accelerated with the Heavy Ion Medical Accelerator in Chiba (HIMAC) at the National Institute of Radiological Sciences (NIRS). The survival of cells plated immediately after irradiation was measured by means of cell colony formation assay. After irradiation, cells were continuously reseeding and cultures for long-term proliferation. Cell samples were collected at 6, 12, 18, 24, 30, 37 and 44 days post incubation. Mutation induction of cell samples at the HPRT locus was detected to measure 6-thioguanine-resistant colonies.

The survival fraction of the cells irradiated by carbon ions was lower than sparsely ionizing (low LET) radiations. It was also lower in high dose-rate (~1.5Gy/min) than in low dose-rate (~0.008Gy/min) irradiation at the same dose. Furthermore, high dose (D10 dose) induced high mutation fraction. Low dose and low dose-rate showed the few mutation fractions and different dose rate induced different mutation fraction even at the same dose. Mutation fraction had lower value before 6 days, the higher level between 12 to 24 days after irradiation, and then the value went down. The results suggest that heavy ion radiation may cause higher mutation induction. Different dose rate also may induce different mutation affection. The heavy ion radiation may cause the long-term mutation and genomic instability of cells.

(PS3068) M-BAND analysis of chromosome aberration in human epithelial cells exposed to γ-ray and secondary neutrons of low dose rate. Megumi Hada[1,2], Premkumar B. Saganti[3], Bradford Gersey[3], Richard Wilkins[3], Francis A. Cucinotta[1], Honglu Wu[1]. [1]NASA Johnson Space Center, Houston, TX, USA, [2]Universities Space Research Association, Houston, TX, USA, [3]Prairie View A&M University, Prairie View, TX, USA.

High-energy secondary neutrons, produced by the interaction of galactic cosmic rays with the atmosphere, spacecraft structure and planetary surfaces, contribute a significant fraction to the dose equivalent measurement in crew members and passengers of commercial aviation travel, and astronauts in space missions. The Los Alamos Nuclear Science Center (LANSCE) neutron facility's "30L" beam line is known to generate neutrons that simulate the secondary neutron spectrum of the Earth's atmosphere at high altitude. The neutron spectrum is also similar to that measured onboard spacecraft like the MIR and the International Space Station (ISS). To evaluate the biological damage, we exposed human epithelial cells *in vitro* to the LANSCE neutron beams with an entrance dose rate of 2.5 cGy/hr or γ-ray at 1.7cGy/hr, and studied the induction of chromosome aberrations that were identified with multicolor banding *in situ* hybridization (mBAND). With this technique, individually painted chromosomal bands on one chromosome allowed the identification of inter-chromosomal aberrations (translocation to unpainted chromosomes) and intra-chromosomal aberrations (inversions and deletions within a single painted chromosome). Compared to our previous results with γ-rays and 600 MeV/nucleon Fe ions of high dose rate at NASA Space Radiation Laboratory at Brookhaven National Laboratory (NSRL), the neutron data from the LANSCE experiments showed significantly higher frequency of chromosome aberrations. However, detailed analysis of the inversion type revealed that all of the three radiation types in the study induced a low incidence of simple inversions. The low dose rate γ-rays induced a lower frequency of chromosome aberrations than high dose rate γ-rays, but the inversion spectrum was similar for the same cytotoxic effect. The distribution of damage sites on chromosome 3 for different radiation types will be presented and discussed.

(PS3069) Chromosomal aberrations in hypoxic cells with Cu-ATSM induced by Cu-K shell ionization. Kaoru Takakura[1], Ayaka Shimmi[1], Yoshirou Kaji[1], Katsumi Kobayashi[2], Noriko Usami[2], Munetoshi Maeda[2], Yasuhisa Fujibayashi[3], Takako Furukawa[4], Hitoshi Imazeki[4], Hiroyuki Iso[4], Takahiro Ishikawa[4], Ryuichi Okayasu[4]. [1]International Christian University, Tokyo, Japan, [2]High Energy Accelerator Organization, Ibaraki, Japan, [3]Fukui University, Fukui, Japan, [4]National Institute of Radiological Sciences, Chiba, Japan.

This study aims to clarify the enhancement effect on chromosomal aberrations via selective energy absorption by Cu atoms in GM05389 normal cells and HSG tumor cells with non-labeled Copper (II) - diacetyl - bis(N^4-methylthiosemicarbazone) (Cu-ATSM) and monochromatic X rays. The X rays having the energy of the Cu K-shell absorption edge arising from synchrotron radiation (KEK-PF) was used for radiation source. On the view point of cancer therapy, Cu-ATSM has an outstanding character which is up-taken more easily into hypoxic cells than into normoxic cells. The amounts of Cu-ATSM taken into cells in the condition of hypoxic and normoxic were detected with the method of PIXE and it is clarified that the amount of Cu atoms is two or three times higher in hypoxic cells comparing to the cells in normoxic

condition. The distribution of Cu atoms between cytoplasm and nucleus in cells are also detected by the analysis with radioactive ^{64}Cu-ATSM and it was shown that about 10% of all Cu atoms were up-taken into nucleus of each cell of GM05389 and HSG. The irradiation with X rays having energy just above the Cu K-shell absorption edge (CuK-H X rays) on hypoxic GM05389 and HSG cells caused much enhancement of chromosomal aberrations, comparing to the X rays having energy just before the Cu K-shell absorption edge (CuK-L X rays). After cells were treated with 3000 nM Cu-ATSM in hypoxic condition, the enhancement ratio of the initial yield of breaks/gaps caused by CuK-H X-ray irradiation to that by CuK-L X rays was about 1.4 for GM05389 cells. High yield of exchange induction by CuK-H X rays were also obtained.

(PS3070) Relative biological effectiveness of 30 kV x-rays for micro-nucleated reticulocyte induction in mice, *in vivo*. Lindsay Churchley, Jennifer Lemon, Fiona McNeill, Douglas Boreham. McMaster University, Hamilton, ON, Canada.

Low energy x-rays have become more prevalent in diagnostic imaging, particularly for mammography . Currently, the ICRP assigns an RBE value of 1 for all photon energies. Mammography requires x-rays, in the energy range of 25 to 30 kV, in order to create useful images. However, it has been suggested that these low energy x-rays may be more damaging than conventional x-rays (200 to 250 kV) due the generation of higher LET secondary electrons. Past studies, which have been predominately *in vitro*, have shown a wide range of relative biological effectiveness (RBE) values for low energy x-rays for broad range of endpoints such as chromosomal abberations and apoptosis. In addition, many of these experiments used high doses in order to see biological effects. This may not be so relavent since high doses are not of concern for mammography.

In this study, the RBE of low energy x-rays was investigated by measuring the induction of micro-nucleated reticulocytes (MN-RET) *in vivo,* at low doses. Mice were exposed to 30 kV x-rays and Cs-137 gamma rays with doses ranging from 0 to 100 mGy. The blood was harvested 44 hours after irradiation, and analyzed for MN-RET production. The x-ray source was a Molybdenum x-ray tube with a 50μm Molybdenum filter. The results presented an RBE of 0.802 ± 0.034. These findings suggest that low doses of low energy x-rays may not be more damaging than conventional photons in terms of MN-RET induction and that mammograms may not be as harmful as some have previously suggested.

(PS3071) Genetic instability for Fruit Fly in the Terms of chronic irradiation. Irene A. Kozeretskaya[1], Zhanna A. Omeltchenko[2], Alexandra P. Kravets[3]. [1]Taras Shevshenko National University, Kiev, Ukraine, [2]Institute of cell biology and Genetic Engineering, Kiev, Ukraine, [3]Institute of Cell Biology and Genetic Engineering, Kiev, Ukraine.

The estimation of biological efficiency of the protracted exposure is the urgent task of the current radiobiology. Decision this problem is extremely needed both for understanding of living process self-organization and for the decision of practical tasks of dosimetry and prognostication of radiation effects risk.

The work is devoted to the study of genetic instability and fertility for the different genotypes of *Drosophilae melanogaster* in the conditions of chronic irradiation.

According to the got results, for males and females of the first generations opposite reaction on the irradiation are observed. Considerable activation of P-element, which shows up in phenotype as reduction of gonads, is observed for males.·Medical£ effect, or decline of activity of P-mobile - element is observed for females.Both these reactions are very dynamic. An effect goes down in subsequent generations and to 8–9 generation approximately identical output of effect for males and females is observed. This new stationary level of gonad reduction output as for males so for females insignificantly exceeds a control level for the same generations. The estimation of biological efficiency of the prolonged irradiation shows monotonous decline of this index for males, in other words growth of radioadaptation is observed.

The change of radiosensitivity has more complex dynamics for females. The sign of effect and its level change consistently. Gradually a¨medical£ effect goes down and there is the transition in the swaying mode to the new stationary level. This level exceeds a control and testifies to fixing in the generations of more high level of gonad reduction.

It is shown for experimental model that efficiency of the prolonged irradiation is dynamic characteristic; it can be positive and negative and to change by absolute value at the dose accumulation. On some stages of exposure biological efficiency of the protracted irradiation can exceed biological efficiency of acute irradiation.

Interpretation of these non - monotonous dose curves requires the use of pictures of complex structure of system-formatted radiation response and integration of its separate component.

(PS3072) Distribution of micronuclei in human fibroblasts across the Bragg curve of light and heavy ions. Megumi Hada[1], Shareen Lacy[2], Daila S. Gridley[3], Adam Rusek[4], Francis A. Cucinotta[1], Honglu Wu[1]. [1]NASA Johnson Space Center, Houston, TX, USA, [2]Texas Southern University, Houston, TX, USA, [3]Loma Linda University, Loma Linda, CA, USA, [4]Brookhaven National Laboratory, Upton, NY, USA.

The space environment consists of energetic particles of varying mass and energy, and understanding the "biological Bragg curve" is essential in optimizing shielding effectiveness against space radiation induced biological impacts. The "biological Bragg curve" is dependent on the energy and the type of the primary particle, and may vary for different biological endpoints. Previously, we studied the induction of micronuclei (MN) across the Bragg curve of energetic Fe and Si ions, and observed no increased yield of MN at the location of the Bragg peak. However, the ratio of mono- to bi-nucleated cells, which indicates inhibition of cell progression, was found higher at the Bragg peak location in comparison to the plateau region of the Bragg curve. Here, we report the induction of MN in normal human fibroblast cells across the Bragg curve of incident protons generated at Loma Linda University. Similar to Si and Fe ions, the ratio of mono- to bi-nucleated cells showed a clear spike as the protons reached the Bragg peak. Unlike the two heavy ions, however, the MN yield also increased at the Bragg peak location. These results confirm the hypothesis that severely damaged cells at the Bragg peak of heavy, but not light ions are more likely to go through reproductive death and not be evaluated for micronuclei.

(PS3073) Low-dose radioadaptive response of mouse blood and brain tissue to DNA damage. Thomas Ernst Schmid, Francesco Marchetti, Sandhya Bhatnagar, Andrew Julius Wyrobek. Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

Mammalian cell types vary in their sensitivity to the effects of high-dose ionizing radiation, but little is known about variability in their responses to low-dose exposures. The aim of this study was to evaluate whether tissues that differ in high-dose radiation sensitivity (e.g., brain versus white blood cells) also differ in their ability to elicit an adaptive response. Young adult male mice (B6C3F1) were treated with 5 cGy of X-rays (priming dose) followed 6 hours later by a challenge dose of 2 Gy. Fifteen minutes after the challenge dose, cells from cerebellum and white blood cells were collected and analyzed using the Comet assays under neutral conditions for detecting double-stranded DNA breakage and under alkaline conditions for detecting alkali-labile sites and single-strand DNA breaks. Neutral Comet analysis identified a 21% reduction in DNA damage in white blood cells and a similar 25% reduction in brain cells after adapting conditions versus single high-dose exposures ($p<0.01$). However, these tissues differed significantly in their adaptive response under alkaline conditions, which showed a 65% reduction in DNA damage in white blood cells versus a 22% reduction in brain cells ($p<0.01$). Our findings indicate that both brain and blood cells show radioadaptation after whole-body radiation exposure, but that the magnitude of the effect may vary with the DNA damage endpoint measured. Experiments are in

progress to further investigate tissue differences in the radioadaptive response in vivo and to identify the underlying biochemical mechanisms. *Supported by the DOE low dose program and the LBL LDRD.*

(PS3074) Comparison of initial chromosome break repair in cells irradiated with high and low LET radiation. Emiko Sekine, Maki Okada, Dong Yu, Miho Noguchi, Akira Fujimori, Ryuichi Okayasu. National Institute of Radiological Sciences, Chiba-shi, Japan.

The aim of this research is to determine the earliest biological effects at the chromosome level by high LET heavy ion radiation compared to those by X-rays in normal human cells (HFL III). We measured the number of chromosome breaks by the fusion-induced premature chromosome condensation (PCC) assay. This assay is a cytogenetic method which can visualize chromosomes in interphase cells. As radiation sources, we used x-rays, carbon ions (70 keV/μm), neon ions (70 keV/μm, 200 keV/μm), silicon ions (70 keV/μm, 200 keV/μm), and iron ions (200 keV/μm). For all the PCC experiments, plateau cells (G0/G1 phase) were irradiated at 2Gy. In the process of fusion-based PCC induction, cells have to be incubated at 37°C, and the repair of DSBs can occur during this period. In order to detect chromosome breaks induced by irradiation at the earliest time point, we applied wortmannin (WM) to inhibit most of the DNA-PK dependent NHEJ type DSB repair during the incubation.

In the case of X-rays about one half of the initial chromosome breaks were repaired, while almost no rejoining of initial breaks was observed in cells irradiated with silicon and iron particles. A correlation can be observed between the initial repair and the actual energy of the incoming particles, especially when the LET was 70 KeV/μm. When the LET was around 200 KeV/μm, the chromosome/DSB repair was almost fully inhibited irrespective of the kind of nuclides and the incoming energy.

Our present study seems to substantiate the idea of complex or "dirty" DSB induced by high LET radiation. Although the number of induced chromosome breaks is low, the complex DNA damages would lead to substantially impaired initial part of NHEJ repair and eventual severe biological consequences.

Our results provide useful insight on the initial stage of DNA DSB/chromosome damage and repair induced by high LET heavy ions.

(PS3075) Mutagenic potential of clustered DNA damage site in *Escherichia coli*. Naoya Shikazono[1], Colin Pearson[2], John Thacker[2], Peter O'Neill[2]. [1]Japan Atomic Energy Agency, Tokai-mura, Japan, [2]Medical Research Council, Harwell, United Kingdom.

Clustered DNA damage induced by a single radiation track is a unique feature of ionizing radiation. Recent *in vitro* studies have shown that the repair of lesions within clusters may be retarded, but less is known about the processing and the mutagenic effects of such clustered damage *in vivo*. Using a bacterial plasmid-based assay, we have investigated the mutagenic potential of bistranded clustered damage sites which consist of 8-oxo-7,8-dihydroguanine (8-oxoG) and dihydrothymine (DHT) at defined separations. We found a significantly higher mutation frequency for the clustered DHT + 8-oxoG lesions than that for either a single 8-oxoG or a single DHT in wild-type and in glycosylase-deficient strains of *E. coli*. From these results and similarities with the mutability of respective 8-oxoG + AP clusters, it is suggested that removal of 8-oxoG within clustered damage site is retarded, probably reflecting the preferential excision of DHT initially. For a certain fraction of clusters, 8-oxoG may be initially removed from the cluster. To gain further insights on the processing of the DHT + 8-oxoG cluster, several potential intermediates after 8-oxoG removal were assessed for their mutability. For instance, DHT + AP or DHT + Gap containing cluster, but not AP + AP or Gap +AP clusters, has a relatively low mutation frequency. Further, AP + AP or Gap + AP cluster had a reduced transformation efficiency. These results led us to suggest that, when either 8-oxoG or DHT is initially excised from a cluster containing 8-oxoG and DHT, the base remaining within the resulting damage will not be further converted to an AP site or to a single strand break *in vivo*.

(PS3076) Generation and characterization of DNA double-strand break repair gene deficient human cell lines. Masahiko MORI, Takanori KATSUBE, Naoko SHIOMI, Tadahiro SHIOMI, Makoto ONODA. National Institute of Radiological Sciences, Chiba-shi, Chiba, Japan.

DNA double strand breaks (DSBs) can arise from multiple sources including ionizing radiation (IR), and is the most serious DNA damage. Non-homologous end joining (NHEJ), which simply pieces together the broken DNA ends, can function in all phases of the cell cycle and is the predominant repair pathway in mammalian cells. In the current study, we carried out the generation and characterization of NHEJ-related gene deficient human cell lines to clarify the biological roles of NHEJ-related genes on DNA damage induced by IR.

We have produced cells that bearing a disrupted NHEJ-related gene, such as XRCC4, Artemis and MDC1, by using gene-targeting technique in human colon tumor cell line (HCT116). For clonogenic survival experiments, cells were exposed to X-rays (~ 5 Gy), plated for colony formation assay immediately after irradiation, and processed for a visualization and count of colonies 2 weeks after plating. Enumerating discrete nuclear foci of gamma-H2AX visible by immunofluorescence was performed to evaluate the ability for repairing DSBs induced by IR (~ 2 Gy)

Proliferation rates were slightly slower in all of the cell lines deficient for NHEJ-related genes than in the wild type cells, although any morphological difference was not observed between the cell lines. The highest survival rate was exhibited in the wild type cells (D10 = 3.9 Gy) and the lowest was in XRCC4-/- cells (D10 = 1.2 Gy). Artemis-/- cells (D10 = 2.2 Gy) took a middle position between the wild and XRCC4-/- cells. Formation of gamma-H2AX foci increased in a dose dependent manner of X-rays and peaked at 30 min after X-ray-exposure in all cell lines. A remarkable recovery from the DNA damage was observed in the wild type cells and the number of gamma-H2AX foci returned to the basal level within 4 hr, whereas a slower disappearance of gamma-H2AX was shown in XRCC4-/- and MDC1-/- cells, indicating a delay of repairing DSBs induced by IR. These results suggest that deficiencies of NHEJ-related genes causes a deterioration of DNA DSB repair function and DNA repair deficiencies constitute a significant component of the radiosensitivity of these cells. The NHEJ-related gene deficient human cell lines generated in this study could contribute to further development and understanding of basic research of DNA damage and repair in radiation-biology.

(PS3077) Effect of thiol-antioxidants, selenium-antioxidants and p53 inhibitor on ionizing radiation induced micronucleus formation in human lymphocytes. Prabha Tiwari, Balakrishnan Sreedevi, S Kannan, H. S. Kushwaha, Kaushala Prasad Mishra. Bhabha Atomic Research Centre, Mumbai, India.

Biological consequences of ionizing radiation are mediated through reactions of generated free radicals with DNA, membrane, protein and other cellular macromolecules. The magnitude of cellular damage can be modulated by treating cultured cells or animals with antioxidants. In view of the potential for practical applications, a variety of compounds are being tested for their radioprotective activities. Among these, aminothiols and selenium containing antioxidants hold a great promise. Present study was aimed to investigate the effect of thiol antioxidants i.e. NAC, GSH and thioproline, in modulating the DNA damage induced by gamma radiation in human peripheral blood lymphocytes (PBL). The induced damage was measured by the cytokinesis blocked micronucleus assay in the blood lymphocytes. PBL treated without or with antioxidants for 30 min. were exposed to different doses of gamma radiation (0–4 Gy), washed with culture medium and cultured for 72 h. Cytochalasin B (5 microgram/ml) was added in the samples after 44 h. A significant decrease in frequency of micronuclei was observed in lymphocytes pretreated with antiox-

idants as compared to un-treated controls. The percentage of micronucleated lymphocytes was significantly reduced with the increasing concentrations of antioxidants (100–300 micromolar). Comparative efficacy of other thiol antioxidants varied, which differed in their free radical scavenging capacity and in the cellular uptake. Results have shown that these antioxidants protected lymphocytes in dose dependent manner. Similar experiments were conducted with selenium antioxidants. Experiments were performed to study the role of p53 in modulating the frequency of radiation induced micronuclei in human peripheral blood lymphocytes by using p53 inhibitor pifithrin-alpha. Results will be presented on the role of p53 in micronuclei frequency modulation in cultured lymphocytes in vitro. It is speculated that the presence of antioxidants in culture medium accelerated the kinetics of repair of radiation-induced DNA damage offering protection to human lymphocytes.

(PS3078) Aneuploidy and G1 checkpoint activation in human cells with reduced homologous recombination activity. Mari Katsura, Yoshitaka Tomoda, Kiyoshi Miyagawa. The University of Tokyo, Tokyo, Japan.

Aneuploidy is assumed to be one of the major causes in cancer formation. While various factors contribute to the formation of aneuploidy, centrosomal abnormality is one of the most important factors. If abnormal numbers of centrosomes lead to multi-polar mitosis, aneuploid cells are generated. Duplication of centrosome is restricted once per one cell cycle. It is strictly synchronized to the cell cycle progression. When DNA is damaged, the cell cycle checkpoint is activated, and various signals suppress normal duplication of centrosomes. On the other hand, loss of centrosome integrity is also reported to activate the cell cycle checkpoint. Here we show the centrosomal abnormality and aneuploidy in human cells with reduced homologous recombination (HR) activity. HR works to preserve genomic integrity by error free DNA repair for double strand breaks and stalled replication forks. Rad51 is recognized as a major player in HR. Rad51 paralogs (Rad51B, Rad51C, Rad51D, XRCC2 and XRCC3) share sequence homology with Rad51, and are assumed to play a role in HR, whereas their exact roles in HR are unknown. Previously we generated XRCC3$^{-/-}$ and Rad51B$^{+/-/-}$ HCT116 cells by gene targeting and found an increase in aneuploidy. Here we have also generated Rad51C$^{+/-/-}$ HCT116 cells. The cells showed sensitivity to ionizing radiation and inter-strand cross linking agents. The cells also showed an increase in centrosomal abnormality and aneuploidy. In Rad51C+/-/-/- HCT116 cells, p21-dependent G1 checkpoint activation was observed. We will discuss the relationship between checkpoint activation and formation of aneuploidy in these cell lines.

(PS3079) Response of human peripheral lymphocytes to DNA damage caused by fractionated irradiation *in vivo* an *in vitro*. Martina Rezacova[1], Jirina Vavrova[2], Doris Vokurkova[3], Emilie Lukasova[4], Karel Vodrazka[5]. [1]Dept. of Biochemistry, Faculty of Medicine in Hradec Kralove, Charles University in Prague, Hradec Kralove, Czech Republic, [2]Dept. of Radiobiology, Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic, [3]Dept. of Clinical Immunology and Allergology, Faculty of Medicine in Hradec Kralove and Faculty Hospital, Charles University in Prague, Hradec Kralove, Czech Republic, [4]Institute of Byophysics, Czech Academy of Sciences, Brno, Czech Republic, [5]Dept. of Oncology and Radiotherapy, Faculty of Medicine in Hradec Kralove and Faculty Hospital, Charles University in Prague, Hradec Kralove, Czech Republic.

Objective: Upon irradiation the sites of double-strand breaks of DNA (DSB) are marked by phosphorylation of histone H2A.X and irradiation induced foci (IRIF) are formed. We studied effect of fractionated irradiation on formation of IRIF both *in vivo* and *in vitro*.

Results: We observed that phosphorylation of H2A.X at human lymphocytes irradiated *in vitro* by single dose of γ-radiation exhibits in dose range 0–5 Gy dose-dependence 1 h after the irradiation. We evaluated γH2A.X and its colocalization with 53BP1 during a course of fractionated radiotherapy at patient undergoing treatment for carcinoma of cervix uteri (2 Gy/fraction, up to 38 Gy). Fractionated radiotherapy caused at peripheral lymphocytes massive formation of IRIF 1 h after *in vivo* irradiation. However, no apparent dose-dependence can be observed, total dose of 4 Gy induced the same effect as total dose of 30 Gy.

In the second part we studied effect of fractionated irradiation *in vitro*. Human peripheral lymphocytes were irradiated by the dose of 2 Gy (1st fraction) and after 24 h they were irradiated again by the dose of 2 Gy (2nd fraction). Induction of γH2A.X foci was observed 1 h after both fractions. 24 h after the first fraction all cells repaired induced damage and the IRIF disappeared, while 24 h after the second fraction 52% of lymphocytes retained γH2A.X foci. Similar effect was observed at lymphocytes isolated from patient undergoing radiotherapy in pelvic area. 1 h after the irradiation of relatively small body volume by the 1st fraction (2 Gy) 10% of isolated lymphocytes contained IRIF. The same result was observed 1 h after the irradiation by the 2nd fraction (2 Gy). When the lymphocytes isolated 1 h after the *in vivo* irradiation were cultivated in vitro for 24 h, we observed significantly higher number of cells with IRIF after the second irradiation. While 24 h after the 1st fraction the damage was repaired, 24 h after the 2nd fraction big IRIF were still detectable.

Conclusion: Results show that dose of 2 Gy induces IRIF formation and colocalization of γH2A.X and 53BP1 1 h after both, *in vitro* and *in vivo* lymphocyte irradiation. Induced DNA damage is repaired within 24 h. Following irradiation by the 2nd dose of 2 Gy does not increase IRIF formation 1 h after irradiation, but 24 h after the repair is incomplete and lymphocytes with big IRIF persist.

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(PS3080) DSB repair kinetics after the exposure to high and low-LET conventional and microbeam radiation. Rasa Ugenskiene[1], Kevin M. Prise[2,3], Melvyn Folkard[2], Janusz Lekki[4], Zbigniew Stachura[4], Wojciech M. Kwiatek[4], Monika Zazula[1], Jerzy Stachura[1]. [1]Jagiellonian University, Medical College, Sw. Anny 12, 31–008 Krakow, Poland, [2]Gray Cancer Institute, Mount Vernon Hospital, HA6 2JR Northwood, United Kingdom, [3]Centre for Cancer Research and Cell Biology, Queen's University, BT9 7AB Belfast, United Kingdom, [4]Henryk Niewodniczanski Institute of Nuclear Physics PAN, Radzikowskiego 152, 31–342 Krakow, Poland.

Exposure to ionizing radiation causes a spectrum of DNA damage including double strand breaks (DSBs), which are the most dangerous threats for cells. The outcome depends on the extent and complexity of radiation-induced damage and efficiency of DNA repair system. The study aimed to analyze the effect of high and low linear energy transfer (LET) of different radiation qualities on DSB repair kinetics in normal human skin fibroblasts (AGO1522) cells culture. Samples were exposed to 240 kV broad field X-ray or helium (^{3}He) particles or protons (^{1}H) from the Charged Particle Microbeam at the Gray Cancer Institute. DNA repair events were followed from 30 min up to 24 h after the irradiation. DSBs were detected via immunofluorescent staining with antibodies against 53BP1 (p53 binding protein 1) and in some cases ATM (ataxia telangiectasia mutated). The DSB foci were quantified manually using a fluorescent microscope. After X-ray irradiation, the majority of DSBs (55%) were repaired during the first three hours. At the 24 h time point approximately 1.5% of un-repaired DSBs were left following the X-ray exposure. There were small differences in the rate of disappearance between the 53BP1 and ATM foci. The DNA repair kinetics after ^{1}H irradiation was similar to that of X-ray and resulted in 55% and 4% of un-repaired DSBs after 3 h and 24 h respectively. There was a clear difference in DNA repair kinetics following ^{3}He irradiation in comparison to X-rays and ^{1}H. The repair after ^{3}He irradiation was significantly slower, as there were 81% of un-repaired DSBs after 3 h and 33% after 24 h left. The difference in DNA repair kinetics may be related to physical properties of radiation quality, namely the density of ionization.

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(PS3081) Mutation induction in mammalian cells by 30 kV X-rays. Juergen Kiefer[1], Hermann Witzenberger[2]. [1]University Giessen, Wettenberg, Germany, [2]University Giessen, Giessen, Germany.

Mutations were studied at the HPRT-locus in MGHU-1 (human male bladder carcinoma) cells after exposure to filtered 30 kV X-rays and ^{60}Co gamma rays. Mutant frequencies were found to increase in a linear-quadratic fashion with dose in both cases. If only the low dose part is analysed: a RBE of 2.05+0.46 is obtained. PCR analysis was also here performed. There is a clear tendency that the fraction of deletions is increased with lower photon energies but the effect is not significant. Translocations of the q-arm of the X-chromosome where the HPRT-gene is located were also determined. With 30 kV X-rays 17% out of 53 analysed mutant clones showed translocations (95% confidence interval: 9..30). The respective numbers for gamma-induced mutants (25 clones) are 12 (confidence interval: 4.6..31). Although the lower photon energy induces more translocations the difference is again not significant. The low fraction with translocations leads to the conclusion that only a minor part of mutants with no detectable deletions can be attributed to translocations.

(PS3082) Role of DNA crosslinks and DNA monoadducts in the toxicity of the mitomycins to Fanconi anemia cells. Sara Rockwell[1], Maureen Gilmore-Hebert[1], Yanfeng Liu[1], Maria Tomasz[2]. [1]Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT, USA, [2]Department of Chemistry, Hunter College of CUNY, New York, NY, USA.

The unusual sensitivity of Fanconi Anemia (FA) Cells to Mitomycin C (MC) has been assumed to reflect defects in the repair of DNA crosslinks. However, this has not been conclusively proven. Our past studies showed that MC forms a spectrum of interstrand crosslinks, intrastrand crosslinks and monoadducts and that the relative abundance of the different alkylation products varies under aerobic and hypoxic conditions. We also showed that Decarbamoyl Mitomycin C (DMC), which has often erroneously been assumed to form DNA monoadducts but not DNA crosslinks, is actually metabolized in living cells to a crosslinking agent. While DMC produces many more DNA monoadducts than MC in cells, it also produces large numbers of DNA crosslinks. Differences in the response of FA cells to MC and DMC therefore cannot be used to assess the relative importance of defects in crosslink and monoadduct repair in these cells. We therefore compared the response of control fibroblasts and FA cell lines from complementation groups A and C to MC, DMC, and 2,7 Diaminomitosene (DAM), a mitomycin analog that forms very large numbers of monoadducts, but no crosslinks. FA-A cells showed extreme hypersensitivity to MC, as reported previously. FA-C cells were less sensitive to MC than FA-A cells, but markedly more sensitive than control cells. The toxicities of MC and DMC were similar in control cells and in FA-A cells, but FA-C cells were more sensitive to MC than to DMC. In marked contrast, control cells, FA-A cells and FA-C cells were all extremely resistant to the monoadduct-forming analog DAM. These studies with DAM provide evidence that the repair defects in FA-A and FA-C cells do not increase the cytotoxicity of the DNA monoadducts produced by the mitomycins, and therefore support the hypothesis that the sensitivity of FA-A and FA-C cells to MC and DMC reflect deficiencies in the repair of DNA crosslinks. These studies were supported by USPHS grant R01 CA28681.

(PS3083) Distinct temporal associations between human RAD51, RAD52, and BCCIP after ionizing radiation and replication fork stalling. Justin W. Wray[1], Jingmei Liu[2], Jac Nickoloff[1], Zhiyuan Shen[2]. [1]University of New Mexico, Albuquerque, NM, USA, [2]The Cancer Institute of New Jersey, Robert Wood Johnson Medical School, New Brunswick, NJ, USA.

RAD51, or its homologue, promotes strand invasion in eukaryotes during homologous recombination (HR) repair of DNA double strand breaks (DSBs), which is facilitated by mediator proteins. In *S. cervisiae*, Rad52 is thought to be the RAD51 mediator, involved in early HR. In contrast, in mammalians, RAD52 has recently been shown to be involved late in the repair process of ionizing radiation (IR) induced DNA damage. It has been proposed that BRCA2 may have evolutionarily replaced the yeast Rad52 in the repair of IR induced DSBs. BCCIP is a BRCA2 and CDKN1A (p21, Cip1 and Waf1) Interacting Protein and has been implicated in HR by its role in modulating BRCA2 and RAD51 focus formation after IR, presumably through an interaction with BRCA2.

Previously, we showed that RAD51 associates with both BCCIP and RAD52, but at separate nuclear locations and times after IR. We then proceeded to examine the responsiveness of the RAD52 protein to replication fork stress via hydroxyurea (HU). We show here that there is a more robust RAD52 focus formation in HU treated cells than in IR treated cells, even though the IR treatment induced ten-fold higher lethality. We further employed Fluorescence Recovery After Photobleaching (FRAP) to show that an EGFP-RAD52 fusion protein displays a lowered mobility and diffusion constant after HU treatment as compared with IR treated and an untreated control. RAD52 over-expression is also protective against HU, but not IR as determined by survival assay. Finally, RAD52 tightly co-localizes with RAD51 after HU treatment. Taken together, these data indicate that RAD52 may play an important role in the restart of collapsed replication forks, specifically in S-phase, via loading of RAD51, separate from BRCA2.

(PS3084) Lysine63 poly-ubiquitination protects against endogenous mutations. Chantal Ramaekers, Roland K. Chiu, Philippe Lambin, Bradly G. Wouters. University of Maastricht, Maastricht, The Netherlands.

Cells have evolved DNA damage tolerance (DDT) mechanisms to facilitate bypass of replication fork blocking lesions. In yeast, this is accomplished either by homologous recombination associated damage avoidance (error-free) or through recruitment of specialized translesion synthesis (TLS) polymerases (error-prone). As many of the yeast genes involved in the DTT pathway and their mammalian homologs encode enzymes involved in ubiquitination, we investigated the role that ubiquitination (Ub) plays in this process in human cells. We recently reported that modification of PCNA with non-canonical polyubiquitin chains linked through lysine 63 (K63polyUb) promotes error-free DDT following UV treatment through a pathway that competes with TLS. To further characterize this pathway we assessed induction of spontaneous mutations at the HPRT locus in cells compromised in their ability to form K63polyUb chains. Our data indicate that these cells become highly genetically unstable in the absence of exogenous damage. This instability occurs in a TLS independent manner, and is manifested exclusively by an increase in spontaneous large scale rearrangements. We hypothesized that a recombination pathway may be involved as it has been shown that replication fork collapse can trigger homologous recombination (HR). This was investigated by treating cells with the topoisomerase I inhibitor Camptothecin (CPT), which has been reported to act as a suitable surrogate for studying endogenous damage as it also results in the conversion of single strand breaks to double strand beaks at the replication fork. Following treatment we observed an increased mutation frequency in K63polyUb defective cells that was further enhanced by pre-treatment with the ATR inhibitor caffeine. Preliminary studies also suggest that the rate of repair of CPT induced damage is reduced in UbK63R cells as measured by loss in γH2AX foci. To further assess the role of K63polyUb in regulating recombination following endogenous and CPT induced lesions, we are quantifying HR frequencies and the resulting recombination spectra. Together, our data indicate an important role for K63polyUb chains in the DDT pathway that contributes to genomic stability by suppressing endogenous mutagenesis.

(PS3085) Induction and processing of oxidative clustered DNA lesions in the human breast cell lines MCF-7, MCF-10A, and HCC1937. Jessica M. Hair, Prakash Peddi, Dave Francisco,

Brittany Flood, Angela Cecil, Alexandros Georgakilas. Biology Department, East Carolina University, Greenville, NC, USA.

DNA is constantly exposed to oxidative stress as a result of both endogenous and external sources. Genomic integrity is maintained through precise detection and correct processing of the different DNA lesions. Oxidative clustered DNA lesions (OCDLs: two or more closely-spaced bi-stranded lesions) are a class of DNA damage hypothesized to be difficult for the cell to repair due to their close proximity. OCDL processing can potentially lead to lethal double strand breaks (DSBs) or mutations. Induction and processing of complex DNA damage (DSBs and OCDLs) has been assessed in the human breast cell lines MCF-7, MCF-10A (reduced BRCA1 expression) and HCC1937 (BRCA1 null) using an adaptation of pulsed-field gel electrophoresis with *E. coli* repair enzymes probing for different OCDLs (oxybase or abasic) and number average length analysis (NALA). γ-H2AX foci measurement has been used as an additional marker of complex DNA damage levels. Our preliminary results suggest a deficient processing of DSBs and OCDLs for MCF-7 and HCC1937 cells compared to MCF-10A. The implication of different DNA repair genes like BRCA1 is under investigation.

(PS3086) Low fluences of alpha particles do not induce SCE in cells defective in Rad51 paralogs. Hatsumi Nagasawa[1], Paul F. Wilson[2], Yuanlin Peng[1], Y-C Lio[3], Nan Liu[4], Małgorzata Z. Zdzienicka[5], Larry H. Thompson[4], David J. Chen[3], Joel S. Bedford[1], John B. Little[6]. [1]Colorado State University, Fort Collins, CO, USA, [2]Bioscience Directorate, Lawrence Livermore National Laboratory, Livermore,, CA, USA, [3]University of Texas South Western, Medical Center, Dallas, TX, USA, [4]Bioscience Directorate, Lawrence Livermore National Laboratory, Livermore, CA, USA, [5]Leiden University, Leiden, The Netherlands, [6]Harvard School of Public Health, Boston, MA, USA.

We reported previously that the homologous recombination repair (HR)- deficient Chinese hamster mutant cell line irs-3 cells (Rad51 C-/-) showed only a 50 % baseline level of sister chromatid exchanges (SCE) as compared to the wild type V79 Chinese hamster cell line. Furthermore, when irradiated with very low fluences of alpha-particle, no SCE were induced in the HR deficient line. (Rad. Res. 164: 141–147, 2005). The chicken DT 40 cells defective in any of five Rad 51 paralogs were reported to be sensitive to DNA cross-linking agents and to ionizing irradiation. (Mol. Cell. Biol. 19; 5166–5169, 1999)

In the present study, we examined four Chinese hamster cell lines defective in different Rad 51 paralogs as well as a cell line defecient in BRCA-2. Cells were seeded in normal growth medium (MEM + 10% FCS) at density of 10^5 cells on specially designed stainless steel dishes with a 1.5 μm thick Mylar base (3.8cmin diameter, 11.3cm^2surface area) coated with fibronectin to facilitate cell attachment. The culture medium was replaced with isoleucine-deficient MEM containing 5% 3X dialyzed FCS to synchronize cells in the G0/G1 phase. The culture medium was replaced with normal growth medium containing10^{-5} M BrdU immediately after α-particle irradiation. These culture dishes were returned to the incubator to allow the synchronized cells to reach the second post-irradiation mitoses. The chromosomes were prepared by the air-dry method, then stained by the FPG technique for analyzing SCE.

The frequencies of SCE measured in three non-irradiated wild type cell lines (AA8, CHO, V-79) were approximately 0.3 SCE per chromosome, whereas two Rad 51C -/- cell lines (irs-3 and CL-V4B) showed 0.161 or 0.160 SCE per chromosome, respectively, nearly the same baseline SCE frequencies obtained in other cell lines defective in xrcc-2, xrcc-3, Rad51D and BRCA 2. In wild type cells, SCE frequencies significantly higher than the baseline level were induced in 30–40% of the cells exposed to a mean dose of 1.3mGy α- particle irradiation, whereby $< 1\%$ of the nuclei were traversed by an α-particle. On the other hand, induction of SCE was minimal or absent after α-particle irradiation in all of the mutant cell lines. These results suggest that Rad 51 paralogs may contribute to DNA damage repair processes involved in the induction of SCE by very low fluences of α-Particles.

(PS3087) Persistence of radiation-induced foci after exposure of proliferating human mammary epithelial cells to sparsely and densely ionizing radiation. Torsten Groesser, Bahram Parvin, Sylvain V. Costes, Mary Helen Barcellos-Hoff, Bjorn Rydberg. Lawrence Berkeley National Lab, Berkeley, CA, USA.

The radiation risk of highly charged, high-energy (HZE) particles is highly relevant for long-term space travel such as a manned mission to Mars, because of their occurrence in cosmic background radiation and the inability to completely shield astronauts from this type of radiation. Experimental studies indicate that HZE may be a more potent carcinogen; therefore, HZE particles have a major impact when it comes to radiation risk assessment in space travel.

In our studies we hypothesized that HZE particles such as 1GeV/u Fe-ions induce more persistent DNA damage than sparsely ionizing γ-rays. To address this, we measured γ-H2AX and 53BP1 foci in proliferating human mammary epithelial cells (HMEC) after exposure to low doses of sparsely and densely ionizing radiation. Foci numbers were quantified with a computer based analysis at various time points. Preliminary results revealed the disappearance of radiation induced γ-H2AX and 53BP1 foci in HMEC have different dynamics as a function of radiation quality. While foci numbers after γ-ray irradiation returned to control level after about 12h, Fe-ion induced foci were still present at 24h. However, these persistent foci were removed during the 24 - 48h time interval after irradiation under the conditions used. The slower disappearance of foci after Fe-ion exposure could be due to the fact that HZE particles induce more complex DNA damage in the core of the ion track.

To further elucidate the mechanism of foci removal in replicating cells, we tested whether foci can pass mitosis and still be present in daughter cells. We used Cytochalasin B to positively identify binucleated cells that had divided 1 - 3h after irradiation in G2-phase. We found that the binucleated cells had the same number of foci as mononucleated cells, showing that foci, and possibly DSBs, can pass mitosis and be available for further repair in the next cell cycle. Furthermore, we have observed that at late time points gamma-H2AX foci are still present in about 50% of radiation-induced micronuclei, but this represents only a small proportion of the originally formed foci. Additional studies are underway using non-cycling HMEC cultures and HMEC that form acini when embedded in extracellular matrix.

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(PS3088) P53 inhibits *in vitro* and *in vivo* double-strand break repair in the absence of serine 15-phosphorylation. Peter Keng, Yi-Jang Lee, Dawn Mazzatti. University of Rochester, Rochester, NY, USA.

DNA Double-strand break repair (DSBR) is critical for maintaining genomic integrity and cellular survival following ionizing radiation (IR) exposure. The presence of the tumor suppressor p53 has been implicated in the suppression of DSBR following IR, due to the observations that cells expressing wild-type (wt)-p53 display increased sensitivity to IR, and sensitive tissues express low rates of DNA repair. In this study, recombinant p53 protein was used to demonstrate that p53 can bind to Rad51 and DNA-PK proteins *in vitro* in a dose dependent manner, and can disrupt DNA repair complex formation. This inhibitory effect of p53 was negated in the presence of serine15 phosphorylation. In addition, the addition of wt-p53 markedly reduced *in vitro* end-joining activity in H1299 p53-null cellular extracts, in a dose dependent manner.

To test the hypothesis that while Ser15 phosphorylated p53 retains the transcriptional activation functions of the molecule, desphosphorylation of Ser15 promotes the binding of p53 to proteins involved in both DSBR processes, the kinetics of dephosphorylation of Ser15 in H1299 cells transfected with wt-p53 was examined. The results showed that the kinetics of p53 protein binding to Rad51 and DNA-PK and disruption of DNA repair complex formation in H1299 cells expressing wild-type p53 was concomitant with the simultaneous dephosphorylation of Ser15. Our data further showed that while p53 promotes rapid, accurate repair immediately following IR treatment, during the fast component of repair, the DNA repair rate was reduced upon p53 binding to DSBR proteins. These data are consistent with the

hypothesis that the interaction of p53 with DSBR machinery may negatively influence DNA repair, thus contributing to increased radiation sensitivity of cells expressing functional p53.

(PS3089) Assessment of individual variation in DNA double-strand break repair capacity in human primary diploid fibroblasts. Paul F. Wilson, Salustra S. Urbin, Peter B. Nham, Cynthia B. Thomas, John M. Hinz, Irene M. Jones, Larry H. Thompson. Lawrence Livermore National Laboratory, Livermore, CA, USA.

DNA double-strand breaks (DSB) are considered the critical genetic lesion induced by ionizing radiation (IR) exposure. We have developed improved immunofluorescence assays to examine the induction and kinetics of DNA damage proteins that form nuclear foci at sites of DSBs. We have examined the responses of over 20 apparently normal and 10 DNA repair-deficient primary human fibroblast strains exposed to 5, 10, and 25 cGy of cesium-137 gamma irradiation. Responses are measured in G0/G1-phase cultures from 10 minutes to 24 hours post-irradiation using immunofluorescent antibodies against phosphorylated histone H2AX (gamma-H2AX, pS139) and phosphorylated ATM (pATM, pS1981). We have documented a large degree of variation (up to 20-fold) among the apparently normal cell strains in the levels of spontaneous (unirradiated) and IR-induced foci, kinetics of foci induction and repair, and residual levels of foci after 24 hours. The responses of most of the 10 DNA repair-deficient strains (derived from patients with ataxia-telangiectasia, Nijmegen Breakage syndrome, Seckel syndrome, and Fanconi anemia) fell within the range of responses of the apparently normal cell strains. Importantly, we also documented a dose-dependent decrease in repair capacity in some strains at doses of 5 and 10 cGy compared to 25 cGy, suggesting these cells may fail to register this modest additional load of IR-induced DNA damage. Future research directions include: 1) the examination of 40 additional apparently normal fibroblast strains irradiated in G0/G1; 2) the correlation of levels of residual damage foci to the induction of chromosomal aberrations and effects on long-term cell survival; 3) the processing of DNA damage induced by low IR doses in S and G2; and, 4) identification of genetic determinants of variation for cells derived from apparently normal individuals. These studies should advance development of predictors of risk of cancer from low-dose ionizing radiation exposures by quantifying inter-individual variation and the contributions of the various DNA damage-response and repair pathways. This work was performed under the auspices of the U.S. DOE by the University of California and the Lawrence Livermore National Laboratory under contract W-7405-Eng-48 and supported by the U.S. DOE Low Dose Radiation Research Program.

(PS3090) Relative biological efficiency for micro-nuclei induction after low doses of HZE Fe-ions, and the effect of polyethylene shielding. Torsten Groesser, Eugene Chun, Mary Helen Barcellos-Hoff, Bjorn Rydberg. Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

The highly charged, high-energy (HZE) particle radiation environment is a concern for space travel due to the high, but not well-characterized, relative biological efficiency (RBE) for many endpoints. We used micronuclei induction as a measure of general cytogenetic damage induced by three energies of Fe-ions and X-rays. Dose-response curves for induction of micro-nuclei (MN) was measured in Chinese hamster V79 and xrs6 (Ku80-) cells and in human mammary epithelial MCF10A cells in the dose range of 0.05 - 1 Gy. The Chinese Hamster cells were exposed to 1 GeV/u, 600 MeV/u, and 300 MeV/u Fe-ions as well as with 320 kVp X-rays as reference. Second-order polynomials were fitted to the induction curves and the initial slopes (alpha values) were used to calculate RBE. For the repair proficient V79 cells, the RBE at these low doses increased with LET. The values obtained were 3.1 (LET=151 keV/μm), 4.3 (LET = 176 keV/μm) and 5.7 (LET = 235 keV/μm), while the RBE was close to 1 for the repair deficient xrs-6 cells regardless of LET. For the MCF10A cells, the RBE determined for 1 GeV/u Fe-ions was found to be 5.4, slightly higher than the value for V79 cells. To test the effect of shielding, the 1 GeV/u Fe-ion beam was intercepted by various thickness of high-density polyethylene plastic absorbers, which resulted in massive Fe-ion fragmentation. It was found that the MN yield for V79 cells placed behind the absorbers decreased in proportion to the decrease in dose both before and after the Fe-ion Bragg peak (excluding the area around the Fe-ion Bragg peak itself), indicating that RBE did not change significantly due to shielding. At the Bragg peak, the efficiency for MN formation per unit dose was decreased, indicating an "overkill" effect by very high-LET stopping Fe-ions. The RBE values obtained are representative for overall cytogenetic damage, but are lower than values reported in the literature for complex aberrations and isochromatid breaks, which are very inefficiently induced by low LET radiation.

Supported by NASA Specialized Center of Research.

(PS3091) Changes in the distribution of human HAT1 after DNA damage. Stefan T. Tafrov. Brookhaven National Laboratory, Upton, NY, USA.

I am investigating the role of the histone acetyl transferase HAT1 in the cellular response to agents that can damage genomic DNA.

In my studies, I treated normal human keratinocytes (NHKs) in several ways:

- Exposure to 0.05mM to 0.5mM solutions of hydrogen peroxide;
- Irradiation with 0.1 cGy to 5Gy of X- or gamma - rays;
- Irradiation with 25- to 50-cGy of high atomic mass and high-energy (HZE) particles; and,
- Irradiation with 20- to 40-cGy of fast moving protons.

After treatment, the keratinocytes underwent immunostaining to reveal the distribution of the cellular Hat1 protein. In some experiments, before staining for HAT1, the cytoplasmic and nuclear membranes of the keratinocytes were permeabilized by treating with 0.5% Triton-X-100, the chromatin was digested with 80U/ml of DNase I, and extracted once with 0.1M ammonium sulfate, then with 0.65M. At each step of the protocol, a representative number of keratinocytes were stained to detect HAT1, DNA, and total histones.

Western blotting analyses were performed to identify Hat1 protein in cytoplasmic extracts, nuclear extracts, and in the insoluble nuclear matrix.

My study yielded the following results:

1. In NHKs treated with hydrogen peroxide or irradiated with HZE particles, the cytoplasmic Hat1 protein translocated to the nucleus.
2. Irradiation of NHKs with X- or gamma- rays did not trigger nuclear translocation of HAT1.
3. The bulk of HAT1 in the nucleus is attached to chromatin, but in the nuclear matrix, there are foci of Hat1 protein associated with histones that do not include DNA. Presently, it is unclear if these foci exist permanently or are created in response to treating the cells.
4. Hat1 protein can be found in the cytoplasm, the nucleoplasm, and attached to the nuclear matrix.

From my findings I conclude that exposing NHKs to certain agents causes HAT1 to translocate from the cytoplasm to the nucleus. Such transfer is not observed in keratinocytes exposed to X- or gamma rays. I hypothesize that HAT1 is translocated to the nucleus to attach to the chromatin and so protect it from potential damage. A second possibility is that in the nucleus HAT1 participates in the DNA repair through providing acetylated histone H4 molecules.

Key words: HAT1, DNA damage, hydrogen peroxide, ionizing radiation.

(PS3092) Repair of strand breaks in *E. coli* by the *Mycobacterium tuberculosis* non-homologous end-joining proteins. Douglas Wright[1], Svitlana Malyarchuk[1], Reneau Castore[1], Emily Klepper[1], Bernard Weiss[2], Aidan Doherty[3], Lynn Harrison[1]. [1]LSUHSC-Shreveport, Shreveport, LA, USA, [2]Emory University,

Atlanta, GA, USA, [3]University of Sussex, Falmer, United Kingdom.

Mycobacterium tuberculosis (Mt) expresses a Ku-like protein (Mt-Ku) and an ATP-dependent DNA ligase (Mt-Lig D) that can perform non-homologous end-joining (NHEJ). As a model system to study Mt-NHEJ, we created *E. coli* strains that express either Mt-Ku, Mt-Lig D or Mt-Ku and Mt-Lig D under the control of an arabinose-inducible promoter. This was achieved using the CRIM vector system, where the expression vectors are integrated into specific phage attachment sites in the genome. In addition to the wild-type strains, bacteria deficient in RecA, RecB, and RecJ have been generated. To determine end-joining activity, we use a plasmid that encodes carbenicillin resistance and firefly luciferase. The plasmid is linearized within the luciferase coding region and transformed into bacteria grown in the presence or absence of arabinose. Re-circularization of the plasmid results in carbenicillin resistant colonies which are analyzed for luciferase activity. Disruption of the luciferase coding region by insertions or deletions results in loss of activity. Only accurate repair results in colonies expressing luciferase activity. Transformation of the strains with the linearized DNA demonstrated that expression of both the Mt-Ku and Mt-Lig D was required for plasmid re-circularization and DNA end-joining was independent of RecA. Repair was predominantly inaccurate and resulted in the deletion of sequences ranging in size from ~20 bp to ~2 kb. Sequence analysis of the junctions revealed that the majority of the ligations occurred at regions of micro-homology (1–4 bps), eliminating one copy of the homologous sequence at the junction. This type of junction is consistent with that found in mammalian NHEJ systems. Loss of RecB did not prevent the formation of large deletions, but did increase the amount of end-joining. In order to determine if the presence of oxidative damage near the double strand break alters the efficiency of end-joining, we tested linear substrates containing 8-oxo-7,8-dihydroguanine (8-oxodG). Initial studies indicate positioning of an 8-oxodG within 4 bases of the end of one terminus did not decrease the end-joining efficiency. Future studies will examine more complex double strand breaks and determine whether RecJ is involved in the deletion of DNA containing oxidative damage prior to ligation by Mt-Lig D.

(PS3093) Characterization of radiobiological endpoints in cells from RI mice. Guanxiong Xiao[1], Hatsumi Nagasawa[1], Simon D Bouffler[2], Natalie L Degg[2], Yuanlin Peng[1], F. Andrew Ray[1], Alexander C Roby[1], Robert I Ullrich[1], Joel S Bedford[1], Michael M Weil[1]. [1]Colorado State University, Fort Collins, CO, USA, [2]Centre for Radiation Chemical and Environmental Hazards, Chilton, United Kingdom.

Recombinant inbred (RI) mouse strains have been used both for trait cosegregation studies and genetic linkage analysis. They are created by using a breeding scheme that consists of a cross between two inbred mouse strains (progenitor strains) followed by at least 20 generations of brother-sister inbreeding. Thus, RI strains are inbred (homozygous at every locus) and derive roughly half their genome from each of the two progenitor strains. The CXB RI strain set consists of 13 RI strains derived from matings of BALB/c (C) and C57BL/6 (B) mice.

The CXB progenitor strains, C57BL/6 and BALB/c, differ in their susceptibility to radiation-induced mammary tumors with BALB/c being susceptible and C57BL/6 being resistant. In part, the susceptibility difference can be explained by a polymorphism in the *Prkdc* gene which encodes the catalytic subunit of DNA-dependent protein kinase. However, other, as yet unknown, loci may be involved. The CXB RI strain set provides a useful tool to unravel the events that lead to radiation-induced mammary tumorigenesis and to understand the interrelationships of cellular radiobiological endpoints to one-another.

We have generated fibroblast strains from each of the CXB RI strains and from their progenitor strains, BALB/c and C57BL/6. Currently, we are assaying these fibroblast strains for a number of radiobiological endpoints including clonogenic survival following acute and low dose-rate exposures, cytogenetic instability and kinetics of γ-H2AX focus formation and clearance. Once these assays are completed we will determine which endpoints differ between the RI strains. Those that do differ between strains can be analyzed as genetic traits; pairwise correlations between them will be made based on the extent to which they cosegregate in the RI strains. In addition, computational methods are available that will allow multiple endpoints to be interrelated as functional networks. Ultimately, we intend to identify those cellular radiobiological endpoints that correlate with RI strain susceptibility to radiation-induce mammary tumorigenesis. In addition, we are screening the same radiobiological endpoints in heterozygous and homozygous *Atm* knock out mice.

(PS3094) Repair of dsb at a specific site of chromosome: influence of low-dose/low-dose-rate gamma-rays. Fumio Yatagai[1], Masao Suzuki[2], Noriaki Ishioka[3], Hitoshi Ohmori[1], Masamitsu Honma[4]. [1]RIKEN, Wako-shi, Saitama, Japan, [2]NIRS, Chiba-shi, Chiba, Japan, [3]JAXA, Tsukuba-shi, Ibaraki, Japan, [4]Natl. Inst. Health Sciences, Setagaya-ku, Tokyo, Japan.

It is important to study the genetic effects of low-dose/low-dose rate ionizing radiation from the aspects human health influences. A single DSB was introduced by the I-Sce I expression vector, pCMV3xnls-I-Sce I. Repair of DSB at the inserted I-SceI-site, intron 4 of the tk+ allele in human lymphoblstoid TK6 cell line TSCE5, was measured by induction of TK-deficient mutants. Similarly we also measured the revertants due to such repair in its compound heterozygote (tk-/-) cell line, TSCER2, which carried an additional point-mutation in exon 5. The former and later measurements reflect the DSB repair efficiency due to DNA end-joining (EJ) and homologous recombination (HR), respectively. As we already reported, the data suggests that EJ contributed to the repair of DSBs 100 times more frequently than HR. The pre-treatment of cells, irradiation with 30 mGy of gamma-rays at a dose rate of 1.2 mGy/hr, did not influence the EJ repair efficiency but enhanced HR repair efficiency, ~50 %. Furthermore, the I-SceI digestion, followed by much lower dose /dose-rate gamma-irradiation (8.5 mGy at 0.125 mGy/hr) during the incubation, resulted in a similar tendency of HR enhancement (~80 %). This kind of approach can be regarded as a useful simulation for elucidating the DSB repair in the low-dose region.

(PS3095) Human Rad54B associates with werner syndrome protein WRN. Yoshitaka Tomoda, Mari Katsura, Kiyoshi Miyagawa. Section of Radiation Biology, Center for Disease Biology and Integrative Medicine, Graduates School of Medicine, The University of Tokyo, Tokyo, Japan.

Homologous recombination is the main pathway for DNA double-strand brake (DSB) repair in yeast, and it has been revealed that the recombination repair pathway also plays an important role in higher eukaryotes. The Rad52 epistasis group genes are involved in homologous recombination, and they are conserved from yeast to humans. We previously cloned a human gene, Rad54B, which is homologous to yeast and human Rad54, and yeast Tid1/Rdh54. Human Rad54B (hRad54B) shares a high homology with these proteins in the central region containing the helicase motifs characteristic of the SNF2/SWI2 family of proteins, but the N-terminal region is less conserved. We found that hRad54B is a DNA-binding protein and a dsDNA-dependent ATPase like hRad54, though its rate of ATP hydrolysis is lower than that of hRad54. In addition, hRad54B associates human Rad51 (hRad51) in mammalian cells through the N-terminal domain of hRad54B, and hRad54B forms nuclear foci that co-localize with hRad51, hRad54 and BRCA1 in response to DNA damage. To clarify the role of Rad54B in homologous recombination in human cells, we generated Rad54B-deficient colon cancer cell lines by sequential gene targeting. The frequency of targeted integration in these cells was dramatically reduced compaired with that in Rad54B-expressing cells. In addition, cell survival analysis revealed that the Rad54B-deficient cells showed resistance to hydroxyurea and cladribine. We also showed that hRad54B directly interacts with the WRN protein, a member of the RecQ hericase family whose deficiency is responsible for Werner syndrome, a progeroid syndrome characterized by genomic instability. Thus, our findings provide the role of Rad54B in the maintenance of genomic stability.

(PS3096) Cohesin and the repair of radiation-induced dna double-strand breaks. Christina Bauerschmidt[1], Cecilia Arrichiello[1], Michael Woodcock[1], David L. Stevens[2], Mark A. Hill[2], Susanne Burdak-Rothkamm[1], Kai Rothkamm[1]. [1]University of Oxford and Gray Cancer Institute, Oxford, United Kingdom, [2]MRC Radiation and Genome Stability Unit, Harwell, United Kingdom.

The cohesion between sister chromatids depends on cohesin, a protein complex that contains Smc1 and Smc3. These proteins form heterodimers via a 'hinge' domain and are joined together by Scc1/Rad21, resulting in the formation of a ring. Furthermore, Rad21 binds to Scc3 which exists in two isoforms, called SA1 and SA2. Cohesin was previously reported to accumulate at sites of enzymatically-induced DNA double-strand breaks in *S. cerevisiae* and in mammalian cells at sub-nuclear regions damaged by high energy lasers.

We wanted to test whether cohesin contributes to DNA double-strand break repair and cell survival in X-irradiated human cells.

To analyse whether cohesin is recruited to sites of X-ray-induced DNA damage in human cells, immunofluorescence microscopy-based co-localisation analysis was performed in HeLa cells using antibodies for various cohesin subunits, the DNA damage markers γH2AX and 53BP1 and the S/G2 phase marker CENP-F. Recruitment of the cohesin factor Rad21 to sites of X-ray-induced DNA damage was observed in G2-phase cells, but only when high levels of DNA damage were induced in 1 μm-wide stripes across the nucleus, generated by partially shielded ultra-soft X-rays.

To determine whether cohesin contributes to the cellular response to radiation, we depleted SMC1 by RNA interference and used the colony assay to measure radiosensitivity. Increased radiosensitivity was observed for SMC1-depleted HeLa cells compared to cells transfected with non-targeting siRNA. DNA double-strand break repair kinetics in G1- and G2-phase cells were assessed by scoring 53BP1 foci in BrdU- and Cyclin A-negative versus BrdU-negative and Cyclin A-positive cells using immunofluorescence microscopy. Loss of 53BP1 foci was slower in SMC1-depleted cells than in controls in G2- but not in G1-phase cells. Micronuclei were analysed in binucleated cells after treatment with cytochalasin B. Elevated levels of micronuclei were observed in SMC1-depleted versus control cells irradiated in late S/G2.

Taken together, these results suggest that cohesin / SMC1 may contribute to cell survival by promoting the repair of radiation-induced DNA double-strand breaks in replicated chromatin. It remains to be determined whether the observed contribution of cohesin depends on homologous recombination or non-homologous end joining.

(PS3097) The role of MEF/ELF4 in potentially lethal damage repair. Chris van Bree, Nicolaas A.P. Franken, Jan Paul Medema. Academic Medical Center-University of Amsterdam, Amsterdam, The Netherlands.

INTRODUCTION: Quiescent tumor cells are better able than proliferating cells to repair the ionizing radiation-induced potentially lethal damage (PLDR). This may be relevant for radiation treatment failure (Barendsen et al., Int J Oncol, 2001, 19:247–256). The myeloid ELF1-like factor/E74-like factor 4 (MEF/ELF4) has recently been implicated in the regulation of quiescence and of radiosensitivity of hematopoietic stem cells (Lacorazza et al., Cancer Cell, 2006, 9:175–187). This study is aimed at establishing the role of MEF/ELF4 in PLDR in human tumor cell lines.

EXPERIMENTAL PROCEDURES: Exponentially growing and confluent cultures of human tumor cell lines (non small cell lung carcinoma A549 and SW-1573, colorectal carcinoma DLD-1 and glioma Gli-06) were studied for their capacity for PLDR by clonogenic assay. Cell cycle analysis was performed in fixated cells by bivariate flowcytometry of BrdU-positive (S phase) cells and DNA counterstaining. The induction of quiescence was studied in living cells by bivariate flowcytometry after simultaneous staining of RNA by Pyronin Y and of DNA with Hoechst33342. Methylation status of the MEF/ELF4 gene was studied at mRNA and protein level without or with treatment with the demethylating agent 5-azacytidine.

RESULTS: Both Gli-06 and DLD-1 cells showed significant levels of PLDR in confluent cells which increased with prolonged confluency. No PLDR was found in SW 1573 and A549 cells. All cell lines showed decreased numbers of S phase cells upon confluency, but the number of S phase cells did not correlate with the capacity for PLDR. Only Gli-06 and DLD-1 cells showed increased levels of quiescence upon confluency. In these PLDR-competent cells, methylation of the MEF/ELF4 gene lowered its expression. CONCLUSIONS: Only human tumor cell lines with PLDR show low expression of MEF/ELF4 due to gene methylation, which is correlated with the ability to induce quiescence. In order to establish the mechanisms underlying the expression of MEF/ELF4 and PLDR, an isogenic cell system will be developed.

(PS3098) Survivin-t34a and -d53a enhanced radiation-induced apoptosis through abrogation of interaction with smac/diablo. Aki Ogura, Osamu Inanami, Daisuke Iizuka, Hironobu Yasui, Mikinori Kuwabara. Laboratory of Radiation Biology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan.

Survivin, a member of IAP (inhibitor of apoptosis proteins), is highly expressed in tumor cells. This protein is phosphorylated at threonine 34 (T34) by CDC2 and suppresses apoptosis by inhibiting caspases. However, mechanisms that survivin blocks apoptosis have remained controversial. Recently, aspartic acid 53 (D53) was demonstrated to be important for direct inhibition of Smac/DIABLO, which is known as a proapoptotic protein that binds with other IAPs such as XIAP. To investigate whether survivin can become a target for radiotherapy, we have constructed conventional GFP-fused expression vectors and adenovirus-mediated expression vectors encoding a defect mutant in binding activity with Smac/DIABLO (D53A) and a phosphorylation-defect mutant (T34A).

GFP-fused expression vectors were transfected to mouse fibroblasts NIH3T3 cells and adenoviral vectors were transduced to human lung carcinoma A549 and human cervical carcinoma HeLa cells. After irradiation, the apoptosis induction was evaluated by microscopic observation and flow cytometry.

The overexpression of D53A or T34A enhanced the radiation-induced apoptosis in NIH3T3, A549 and HeLa cells. However, two phospho-mimic mutants, T34E and T34D, did not induce radiosensitization, indicating that phosphorylation-induced negative charge at T34 was important for an anti-proapoptotic property of survivin. These results suggested that alanine mutations at T34 or D53 abolished the anti-apoptotic activity of survivin and enhanced the radiation-induced apoptosis. Furthermore, co-immunoprecipitation experiments revealed that not only D53A but also T34A was not able to bind to Smac/DIABLO, which may demonstrat that phosphorylation at T34 is essential for its capability to bind with Smac/DIABLO and that survivin suppresses apoptosis through inhibition of pro-apoptotic protein Smac/DIABLO.

(PS3099) In vitro and in vivo studies of atm roles on growth kinetics, ros level, and sldr and pldr ability of human glioma cells. Chu-Chiao Wu, Chi-Shiun Chiang. National Tsing-hua University, Taiwan, Taiwan.

The loss of ATM (ataxia telangiectasia-mutated) gene leads to oxidative damage and AT disease, which is also sensitive to the cytotoxicity of radiation. This study explored the influence of ATM on intracellular ROS level and the response of glioma to radiation *in vitro* and *in vivo*. To achieve this, siRNA technique was used to suppress *atm* gene expression of a human glioma cell line, U87. ATM gene suppression by sequence specific siRNA was confirmed by RT-PCR and Western blot assays. The intracellular ROS levels were evaluated by flow cytometry using DCF-DA fluorescent probe. Colony assay was used to determine the radiation response. The ratio of proliferation cells within tumors was determined by *in vivo* BrdU labeling. The results showed that the suppression of *atm* gene level increases the intracellular ROS level and the cell cycle time from 12.25 ± 0.75 hr to 22.61 ± 0.46 hr, but no change in control siRNA (13.91 ± 0.82 hr). Cell survival assay showed that

α/β ratio was changed from 0.001 to 5.201 with primary change on α value. This indicates that ATM mainly affects the repair capacity of radiation-induced double strands breaks. This is further supported by the finding that ATM-silenced cells had reduced SLDR and PLDR ability. The reduced PLDR was further confirmed by *in vivo* assay following 4 Gy irradiation in xenograft U87 tumors. The BrdU staining showed that the central zones of ATM-silenced U87 tumors had reduced ratio of non-proliferation cells. In conclusion, this study demonstrated that ATM-regulated ROS level is associated with part of radiosensitivity of U87 cells by reducing SLDR and PLDR ability. Reduced ratio of non-proliferation cells in central zone of tumor echoed the lower PLDR ability in tumor. The implications of the results for clinical therapy and future research possibilities are discussed.

Key words: Ataxia telangiectasia-mutated; glioma; ROS formation; sublethal damage; potential lethal damage; siRNA.

(PS3100) Effect of the trifunctional antibody catumaxomab to human tumor cells (FaDu) in 3D spheroid co-cultures. Franziska Wawrsinek, Tobias Leidig, Wolfgang Mueller-Klieser. Institute of Physiology and Pathophysiology, Mainz, Germany.

There has been a significant progress in the past years in immunological cancer therapy using therapeutic antibodies. The trifunctional antibody catumaxomab has two different binding arms. One directed against CD3 of T cells, the other against EpCAM which is known as a pan-carcinoma antigen. The antibody has a third functional part and binds to $F_c\gamma I/III^+$ accessory cells (e.g. NK cells or macrophages). This unique combination allows for the hypothetic formation of a tri-cell-complex consisting of T-, cancer- and accessory cell. This strategy creates an effective therapeutic option exploiting the natural immune defense.

For mechanistic studies we employed a 3D tumor model that is well-established in our laboratory, multicellular tumor spheroids. Interactions between tumor cells of the head and neck (FaDu) and immune cells were in the focus of our interest.

Spheroids were co-cultured with peripheral blood mononuclear cells (PBMCs) and different combinations and concentrations of catumaxomab and cisplatin. The effect of combined therapy was quantified by reduction of the spheroid volume, clonogenicity and cell viability. In histological sections the infiltration of $CD45^+$ cells and the proportions of proliferating and apoptotic cells within the spheroid were assessed by immunostaining. For evaluation of the biological activity of the PBMCs, cytokines in the spheroid culture medium were analyzed by ELISA. Furthermore, the PBMCs were characterized by staining of surface antigens.

The studies on FaDu spheroids showed that catumaxomab and cisplatin cause a significant concentration-dependent reduction in volume growth of the spheroids. The viability of the tumor cells and the ability of forming colonies were reduced after treatment with cisplatin. A synergy between treatment with catumaxomab and cisplatin could be shown. This combined therapy with clinically relevant concentrations decreased colony formation to non-detectable levels. Spheroids were reduced in diameter by increased apoptosis. Obviously these apoptoses were caused by infiltrated leukocytes. Measurement of cytokines and FACS analyses suggest that these PBMCs are NK and T cells. This infiltration was strongly dependent on the used concentration of catumaxomab.

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(PS3101) AMP-activated protein kinase: a potential novel target for radiotherapy in prostate cancer. Sofie Isebaert[1], Johan Swinnen[2], Annelies Debucquoy[1], Willy Landuyt[1], William H. McBride[3], Adrian Begg[4], Karin Haustermans[1]. [1]Lab of Experimental Radiotherapy, UH Gasthuisberg, Leuven, Belgium, [2]Lab of Experimental Medicine and Endocrinology, UH Gasthuisberg, Leuven, Belgium, [3]Department of Radiation Oncology, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA, [4]Division of Experimental Therapy, The Netherlands Cancer Institue, Amsterdam, The Netherlands.

Objectives: AMP-activated protein kinase (AMPK) is a low energy checkpoint and is activated under conditions that deplete cellular ATP levels and elevate AMP levels, such as glucose deprivation and hypoxia. Once activated, AMPK switches on catabolic pathways that generate ATP (e.g. fatty acid oxidation and glycolysis) while switching off ATP-consuming processes (e.g. fatty acid synthesis and protein synthesis) and thereby influencing key enzymes involved in these processes, like acetyl-CoA-carboxylase, mTOR and p53. It has been shown that mTOR and p53 are involved in the radiation response. We therefore aimed to investigate the role of 5-aminoimidazole-4-carboxamide-1-b-4-ribofuranoside (AICAR), an AMPK activator, in the radiation response of prostate cancer.

Materials and Methods: The PTEN-null PC3 prostate cancer cell line was treated with increasing concentrations of AICAR, with ionizing radiation, or with the combination. Cell proliferation and cytotoxicity were measured by BrdU incorporation and a Sulforhodamine B assay, respectively. A colony assay was used to evaluate cell kill. Cells were irradiated at the end of drug exposure, using single radiation doses of 2–6Gy (6MV photons, Linac).

Results: Treatment of PC3 cells with AICAR decreased cell proliferation in a dose-and time-dependent manner. The IC_{50} value for proliferation after a 48h incubation was 500μM. Increased cytotoxicity was observed when PC3 cells were treated with higher concentrations of AICAR or longer incubation periods. Exposure of PC3 cells to AICAR for 48h resulted in a concentration-dependent increase in radiosensitivity, with a SF2=0.53, SF2=0.30, SF2=0.30, SF2=0.24 for concentrations of 0μM, 25μM, 125μM and 250μM, respectively. Remarkably, the radiosensitization effect was more pronounced with a radiation dose of 2 Gy in comparison with higher radiation doses. Measurements of apoptosis and Western blotting, to evaluate the effects of AICAR on key molecules involved in the radiation response, are ongoing and will be presented at the time of congress.

Conclusions: To our knowledge, this is the first report on the radiosensitizing effect of AICAR. This finding can open new perspectives for the treatment of prostate cancer and other types of cancer.

(PS3102) CI-1033, a pan-ErbB tyrosine kinase inhibitor, enhances the radiation response of human glioma cell lines. Laurine E. Wedekind[1], M. Vincent.M. Lafleur[1], T. Rianne Stoter[1], Mark Luttjeboer[2], Peter Sminia[1], Ben J. Slotman[1], Gitta K. Kuipers[1]. [1]Department of Radiation Oncology, VU University Medical Center, Amsterdam, The Netherlands, [2]Department Pediatric Oncology/Hematology, VU University Medical Center, Amsterdam, The Netherlands.

Purpose: Investigation of the potential of CI-1033 to enhance radiation-induced cell death of human glioma cells in vitro.

Materials and Methods: The human glioma cell lines D384, Gli-6 and U251 were used. ErbB (1–4) expression levels were determined by Western blotting and quantitative RT-PCR (Taqman). The effect of CI-1033 on proliferation was examined by cell growth assays and the radiosensitizing potential was assessed by clonogenic assays. Irradiation was performed in a dose-range from 0–6 Gy. For split-dose experiments, cells were irradiated with 2x2 Gy or 2x3 Gy with a time interval of 4 hours.

Results: D384 had detectable levels of all ErbB proteins, while ErbB1, 2 and 4 protein expression was present in U251 and Gli-6. CI-1033 (0–25 μM) inhibited proliferation of all cell lines in a dose- and time-dependent manner (0–96 hours), with significant cytotoxic effect at doses exceeding 10 μM. CI-1033 treatment of D384 (5 μM) and U251 (6 μM) cells 24 hours before irradiation did not result in radiosensitization, in contrast to Gli-6 cells. In these cells, incubation with 8 μM CI-1033 24 hours before irradiation resulted in a clear radiosensitizing effect. If CI-1033 incubation was prolonged to 48 hours prior to irradiation, a clear enhancement of radiation-induced cell death of D384 cells was demonstrated, while Gli-6 cells showed less radiosensitization in comparison to 24 hours CI-1033 treatment. Split-dose experiments revealed that CI-1033 markedly inhibited sublethal damage repair in D384, but not in Gli-6 cells. Incubation with CI-1033 for 24 hours after irradiation did not enhance radiation-induced cell death in all cell lines tested.

Conclusion: CI-1033 clearly enhances radiation-induced cell death of Gli-6 and D384 cells. The radiosensitizing effect of CI-

1033 on D384 cells might be explained by inhibition of sublethal DNA damage repair. The timing of CI-1033 addition seems to be important.

CI-1033 was kindly provided by Pfizer

(PS3103) Radiosensitization effects of hsp27 gene silencing in different human tumor cells. *in vivo* validation on head and neck squamous carcinoma cells xenografted tumors. Elie Hadchity[1], Marie-Thérèse Aloy[1], Patrice Jalade[2], Christian Paulin[3], André-Patrick Arrigo[4], Martin Gleave[5], Claire Rodriguez-Lafrasse[1]. [1]Department of Cellular and Molecular Radiobiology, EA3738, Lyon-Sud Medical School, Lyon, France, [2]Department of Radiation Oncology, Lyon-Sud Hospital Center,Pierre Bénite, France, [3]Cytopathology Laboratory, Lyon-Sud Hospital Center,Pierre Bénite, France, [4]Stress Cell Laboratory, UMR CNRS 5534, Lyon, France, [5]The Prostate Center, Vancouver General Hospital, University of British Columbia, Vancouver, BC, Canada.

Upregulated expression of Hsp27 protein in cancer cells leads to uncontrolled tumor growth and increased resistance to chemotherapeutic agents, suggesting that attenuation of Hsp27 gene expression may represent a new target for sensitization to radiotherapy.

We examined, *in vitro* and *in vivo*, whether downregulation of Hsp27 protein expression, by antisense or RNAi approaches, sensitizes the radio-resistant SQ20B head and neck squamous carcinoma cells to γ-irradiation. These cells express high levels of Hsp27 protein in basal conditions and do not undergo apoptosis in response to irradiation.

Stable transfection of SQ20B cells (survival fraction at 2 Gy (SF2) = 0,72) with antisense Hsp27 cDNA or Hsp27-RNAi enhanced the killing effect of a 10 Gy irradiation. This was underlined by increased clonogenic (SF2 = 0.41 and 0.39 respectively) and apoptotic cell death (355% and 386% over controls). The underlying mechanisms leading to apoptosis involved a significant decrease in glutathione basal levels associated to ROS production, a mitochondrial collapse and caspases activation. Sensitization to irradiation by Hsp27-RNAi was confirmed in other radioresistant tumor cells such as U87 glioblastoma and PC3 prostate cancer cells.

Attenuation of Hsp27 expression was also achieved in SQ20B cells by OGX-427 antisense oligonucleotide (2 × 200 nM). This treatment resulted in a 90% decrease in Hsp27 protein levels at 24 hours. If irradiated during this sensitive period, a reduced clonogenic survival was measured with a shift to 0.49 of SF2.

In vivo, nude mice bearing heterotopic SQ20B xenografts were submitted to OGX-427 intra-peritoneal injections (5 for one week then 3 injections/week at 10mg/kg during 5 weeks) associated to a locally controlled irradiation of tumors (5 × 2 Gy at second week of OGX-427). OGX-427 strongly enhanced radiation-induced cytotoxicity by increasing apoptotic cell death and reducing tumor progression (485% decrease of tumor volume compared to control mice and 350% compared to only irradiated-mice, after 6 weeks) and without significant tissue damage or toxicities.

These findings support the hypothesis that Hsp27 mediates tumor radioresistance through the inhibition of apoptosis. *In vivo* results suggest that Hsp27 gene therapy may offer a potential adjuvant to radiation-based cancer therapy.

(PS3104) MicroRNAs and Radelegans: understanding the genetic basis of the radiation response. Joanne B. Weidhaas, Imran Babar, Sunitha Nallur, Sarah Roush, Michelle Boehm, Erin Gillespie, Frank J. Slack. Yale University, New Haven, CT, USA.

Purpose/Objectives:

MicroRNAs (miRNAs) are global regulatory RNAs originally discovered in *C. elegans* and shown to have a role as human cancer loci and to regulate human oncogenes, such as *RAS*. We hypothesized that miRNAs might also play a role in a cancer cell's response to cytotoxic therapy, and that miRNA manipulation could alter this response *in vivo*.

Experimental Procedures:

We performed miRNA microarrays to determine if miRNA levels change acutely post-irradiation. We then tested the role of identified miRNA manipulation in altering the radiation response *in vitro* in the lung cancer cell line A549 using clonogenic assays. To further validate our results we applied an *in vivo* model of reproductive cell death, Radelegans. As reproductive cell death is the primary mode of death in tissue clonogens, the putative targets of cytotoxic therapy, we hypothesize that Radelegans will be most predictive of *in vivo* tumor responses to genetic manipulation.

Results:

We have found that the over 100 of 450 tested miRNAs change significantly within two hours of radiation. The *let-7* family-of-miRNAs was over-represented in a class of miRNAs that were down-regulated post-irradiation. We were able to show that *let-7* family manipulation could significantly impact the radiation response *in vitro* in mammalian cells as well as *in vivo* in Radelegans. Specifically, *let-7* loss leads to radioresistance and its overexpression leads to radiosensitivity. We have shown in Radelegans that these effects are partly regulated by the *RAS* oncogene.

Conclusions:

MiRNA manipulation may be a novel approach to modulating the tumor response to cytotoxic therapy. While miRNA research is still an emerging field, miRNAs might be especially attractive as targets for radiosensitization due to their role as global gene regulators and specific misregulation in tumor tissues.

(PS3105) Experimental study on expression property of pEgr-p16 and its anti-tumor effects induced by ionizing irradiation. Jianxiang Liu, Xu Su. NIRP, Beijing, China.

With the rapid development of "human genome project" research, gene-radiotherapy became as the new developmental direction in the tumor treatment. In this study, according to the mechanism that ionizing radiation can activate early growth response-1 (Egr-1) gene promoter,induce the expression of downstream genes and anti-tumor effect of a tumor suppressor gene p16 which was the first gene can effected to cell cycle directly and suppressed cell division, a recombinant plasmid which containing human suppressor gene p16 and radio-sensitive promoter (Egr-1) was constructed. To investigate the expression of pEgr-p16 and anti-tumor effect in HeLa and SMMC-7721 cells, which were transfected by recombined plasmids with Lipofectamine, induced by ^{60}Co γ-rays irradiation with different doses. It has been demonstrated that the expression of p16 had enhanced property induced by irradiation *in vitro*. The anti-tumor effect and mechanism of pEgr-p16 recombinant plasmid combined with ^{60}Co γ-rays irradiation had been studied. The result demonstrated that pEgr-p16 recombinant plasmid combined with γ-rays irradiation could inhibit cell growth. The mechanism may be correlated with the enhanced expression of p16, induced G_2/M arrest, decreased expression of CDK4 and CyclinD1 protein level, decrease expression of c-myc gene transcriptional level and radiation effects. These research would be provided a theoretically and experimental basis in order to enhance radiotherapeutic effects of malignant tumor.

[Key Words] p16 gene, Egr-1 promoter, pEgr-p16 recombinant plasmid, ionizing radiation, anti-tumor effect

(PS3106) High-LET radiation enhanced apoptosis but not necrosis regardless of *p53* status. Akihisa Takahashi[1], Ken Ohnishi[1], Yoshiya Furusawa[2], Takeo Ohnishi[1]. [1]Nara Medical University School of Medicine, Nara, Japan, [2]National Institute of Radiological Sciences, Chiba, Japan.

The p53 tumor suppressor protein functions as a critical component of genotoxic stress response by regulating the gene expression of effectors that control the fate of a cell following DNA damage. In fact, wild type *p53* (wt*p53*) cells are more sensitive to low-linear energy transfer (LET) radiations than mutated *p53* (m*p53*) and *p53*-null cells, because low LET radiations induce *p53*-dependent apoptosis. However, there is little information about lethal mechanism induced by high-LET radiations from different

energy particles. Therefore, we here studied the lethal pattern in response to high-LET radiations. Stable clones of a human lung cancer cell (H1299, *p53*-null) transfected with wt*p53* gene (H1299/wt*p53*), m*p53* gene (H1299/m*p53*) or the control vector *neo* gene (H1299/*neo*) were used in these studies. These cells were exposed to X-rays or high-LET radiations (13–200 KeV/μm) using different nuclei ion-beams (C-beams, 13 KeV/μm; Ne-beams, 35 KeV/μm; Si-beams, 55 KeV/μm; Ar-beams, 85 KeV/μm; Fe-beams, 200 KeV/μm) by HIMAC at the National Institute of Radiological Science. The cellular sensitivities were determined by a colony forming assay. The types of cell death (apoptosis and necrosis) were evaluated by acridine orange/ethidium bromide double staining for fluorescence microscopy. We found that (i) there was no significant difference in cellular sensitivity to high-LET radiations (> 85 KeV/μm) among these cells, although the sensitivity of H1299/wt*p53* cells to X-rays was higher than that of H1299, H1299/*neo* and H1299/m*p53* cells; (ii) The maximum of RBE against 30% survival dose were obtained at LET 200 KeV/μm of Fe-beams among these cells. The values were 3.40, 3.36 and 3.46 in H1299, H1299/*neo* and H1299/m*p53* cells, respectively. In contrast, the value was 2.29 in H1299/wt*p53* cells; (iii) X-ray-induced apoptosis was detected at higher frequencies in H1299/wt*p53* cells when compared to H1299, H1299/*neo* and H1299/m*p53* cells, although X-ray induced mainly necrosis among these cells; (iv) High-LET radiations efficiently increased apoptosis and decreased necrosis among these cells as compared with X-rays at iso-survival dose. These results suggested that high-LET radiations induce apoptosis in a *p53*-independent manner, and are useful to any types of *p53*-pacients for cancer therapy.

(PS3107) Significance of tumor heterogeneity in determining biological effectiveness of low and high LET radiation. Koichi Ando, Sachiko Koike, Akiko Uzawa, Yoshiya Furusawa, Ryoichi Hirayama, Yoshitaka Matsumoto, Masahiko Watanabe. Natl Inst Radiol Sci, Chiba, Japan.

(Introduction) A dose escalation study of non-small cell lung carcinoma in patients has shown that shape of dose-tumor control probability of carbon-ion radiotherapy is steeper than that of photon therapy, and that relative biological effectiveness (RBE) of carbon ions is larger at higher doses than at lower doses. This contradicts with the biological knowledge; RBE decreases with an increase of dose. A possible explanation is that tumors could contain heterogeneous sub-populations with different photon sensitivities. (Purpose) The purpose of present study is to clarify experimentally significance of tumor heterogeneity. (Materials and Methods) Two sarcomas of # 6107 and #9037 were transplanted into syngeneic C3H male mice. Single cell suspensions for each tumor were mixed together at various ratios just prior to transplantation. When tumors grew to reach 7 mm in diameter, leg tumors were locally irradiated with 290 MeV/n carbon ions at an LET of 74 keV/μm that were accelerated by HIMAC synchrotron in our institute. Tumor growth (TG) time was obtained by calculating days required for a tumor to reach 5 times initial volume, and Specific Tumor Growth Delay was used to obtain an isoeffect dose. RBE values were calculated by comparing isoeffect doses between carbon ions and reference Cs-137 gamma rays. (Results and Discussion) TG time of unirradiated control was similar between #6107 and #9073, i.e., 5.6 and 6.1 days. Tumors with different ratios of mixture (RM) showed various TG time such that TG times of 8.3, 3.8 and 6.1 days were for tumors with ratios of 9:1, 5:5 and 1:9 (#6107: #9073), respectively. Isoeffect doses for 5 tumors with different RM were ranging from 18 to 48 Gy for gamma rays, and from 8 to 18 Gy for carbon ions. A tumor with RM of 9:1 showed largest isoeffect dose, and was more resistant than the either parental tumors of #6107 or #9073 tumors. RBE of the tumor with RM of 9:1 was largest, and larger than that of the parental tumors with RM of 10:0 and 0:10. This means any interaction between sub-populations could modify radiosensitivity of a tumor. Possible reasons for this interaction are either hypoxic c ell fraction or G1 cell cycle phase. Factor(s) and/or signaling responsible to the interaction are unknown. (Conclusion) Tumor heterogeneity is a significant determinant for tumor radiosensitivity and RBE of high LET.

(PS3108) Normal tissue effect and growth delay of transplanted cancer in mice by synchrotron generated microplaner beam. Yoshiya Furusawa[1], Masahiro Natsuhori[2], Arane Kasuya[2], Mitsunobu Muto[2], Toshifumi Oyamada[2], Nobuhiko Ito[2], Naoto Yagi[3], Masami Torikoshi[1], Ymiko Ohno[1], Masao Suzuki[1], Akiko Uzawa[1]. [1]Natl Inst Radiol Sci, Chiba, Japan, [2]Kitasato University, Towada, Japan, [3]Jpn Synchrotron Radiat Res Inst, Hyogo, Japan.

It is reported that microplaner beams obtained from synchrotron radiations show higher radiobiological effects on tumor tissues than normal tissues. The radiobiological mechanisms are not known, but the potential of those beams for cancer therapy is discussed as a novel radiotherapy.

We have obtained a microplaner beam at beam line BL28B2 in Spring-8, JASRI (Japan Synchrotron Radiation Research Institute), Hyogo, Japan. A multi slit-block having 20 mm width in horizontally and ten 20 Ã‘Âμm slits every 200 Ã‘Âμm in 2 mm height was used. Here, we measured tumor growth delay and skin reaction of mice as a pilot study with the beam.

For tumor growth delay (TGD) experiment, 1E6 cells of NFSa fibrosarcoma were transplanted in a leg of C3H 12–15 weeks old male mice. For skin reaction scoring (SS) experiments, hair of 8–12 weeks old female mice leg were depilated by a depilatory. Those preconditioning were performed 8 days before irradiation in our home institute, and were shipped to JASRI few days before irradiation. Mice were hold on a plastic board under anesthesia, and an area of 20 mm width and 10 mm height including the tumor or the depilated skin were irradiated with the beam. Irradiated mice were transferred to Kitasato University in Aomori, Japan, and measured tumor volume and skin score every day.

The tumor volume grown to be 1000 mm3 from 200 mm3 at the day of the irradiation took 5.5 days for unirradiated group, and 10.5 days for 800 Gy irradiated groups. We could find 5 days of the TGD for the 800 Gy group. Averaged TGD increased linearly with the dose. However, in some mouse showed no increment of the tumor volume when they receive 1200 or 1600 Gy. For skin reaction, the score were increased linearly with days, and reached maxima at around 2 weeks after the irradiation. Slight moist desquamation (skin score 3.0) was found at 1600 Gy irradiated group.

(Other Contributors; Sachiko Koike, Yasushi Ohmachi, and Koichi Ando, NIRS)

(PS3109) Cellular sensitivity and *p53*-independent apoptosis on human gingival cancer cells by heavy-ion beams. Nobuhiro Yamakawa[1], Akihisa Takahashi[1], Ken Ohnishi[1], Yoshiya Furusawa[2], Takeo Ohnishi[1]. [1]Nara Medical University School of Medicine, Nara, Japan, [2]National Institute of Radiological Sciences, Chiba, Japan.

We have reported that carbon-ion (C-) beams induce efficient cellular death and apoptosis as compared with X-rays in a *p53*-independent manner. To confirm the proposal, we studied the radiation sensitivity and the induction of apoptosis after exposure to heavy-ion beams or X-rays in a human cultured gingival cancer cell line (Ca9–22) having mutated *p53* gene. The cells were exposed to X-rays or high-LET radiations using different ion-beams (C-beams, 13, 30, 70 and 100 KeV/μm; Fe-beams, 200 KeV/μm) by HIMAC at the National Institute of Radiological Science in Japan. The cellular sensitivity was determined by colony-forming assay. The induction of apoptosis was measured by Hoechst 33342 staining of apoptotic bodies. We found that high-LET radiations effectively increased the radiation sensitivity. The relative biological effectiveness (RBE) values of C-beams (13, 30, 70 and 100 KeV/μm) at D_{10} were 1.8, 2.3, 4.0 and 4.7, respectively. The RBE value was increased LET-dependently. In a similar manner, high-LET radiations effectively increased apoptosis. The RBE values of C-beams (13, 30, 70 and 100 KeV/μm) at 10% apoptosis doses were 2.9, 3.3, 6.3 and 7.5, respectively. On the other hand, the values of Fe-beams (200 KeV/μm) at D_{10} and 10% apoptosis doses were 3.5 and 5.0, respectively. The maximal RBE values for cell killing and apoptosis frequency were obtained at LET 100 KeV/μm. Our data are in agreement with the recent proposal that heavy-ion beams might be useful even to *p53*-mutated patients in cancer therapy. In addition, PARP was fragmented even at a low dose of Fe-beams as compared with X-rays. It was suggested that high-LET radiations

efficiently might induce apoptosis dependent on caspase-3 activity, not but p53. In fact, radiation-induced apoptosis was suppressed by a caspase-3 inhibitor and a caspase-9 inhibitor, but not a caspase-8 inhibitor. We will discuss the pathway of *p53*-independent apoptosis.

(PS3110) *p53*-dependent regulation of induction of angiogenic regulatory factors by ion beam irradiation *in vitro*. Masanori Hatashita[1], Keiichi Takagi[1], Kyo Kume[1], Shigekazu Fukuda[1], Sachiko Hayashi[2], Hideki Matsumoto[2]. [1]Research and Development Department, The Wakasawan Energy Research Center, Turuga, Japan, [2]Division of International Social and Health Science, University of Fukui, Fukui, Japan.

Angiogenesis is required for the growth and progression of malignancies. Recent studies have shown that the *p53* gene is one of the factors for determining cellular radiosensitivity. Previously, we have reported that the cellular radiosensitivity of wild-type (wt) *p53* cells was higher than that of *p53*-deficient or mutant (m) *p53* cells. We have also found that the radiosensitivity of wt*p53* tumors was higher than that of m*p53* tumors. These findings suggest that the induction or suppression of tumor environmental factors due to a *p53*-dependent manner may contribute to the kinetics of tumor regrowth after irradiation. However, the correlation of p53 functions and the induction of VEGF by irradiation remains unclear. In the present study, the relationship between *p53* status and induction of angiogenic regulatory factors by ion beams were examined in human cells. A-172 cells bearing wt*p53* gene were transfected with a vector carrying a neomycin-resistant gene (neo) or m*p53* gene. *p53*-deficient H1299 cells were transfected with a vector carrying wt*p53* gene or m*p53* gene. These cells were irradiated by ion beams. The induction of angiogenic regulatory factors and the accumulation of these proteins after irradiation were analyzed with RT-PCR and western blotting methods, respectively. We found that the accumulation of VEGF was induced by ion beam irradiation in the m*p53* cells, but not in the wt*p53* cells. We will discuss the contribution of *p53* gene to anti-tumor effects of ion beam irradiation.

(PS3111) Smac gene enhances the bio-effect of EJ cells induced by $^{12}C^{6+}$ ions irradiation. Zhao Baofeng, Tian Mei, Ruan Jianlei, Su Xu. National Institute for Radiological Protection, China CDC, Beijing, China.

In cancer radiotherapy, to decrease the damage of the normal cell and enhance the apoptosis effect of cancer cell has a great significance, but resistance to apoptosis of tumor cells is one of the reasons for the decreasing curative effect. Therefore, to increase radiosensitivity of tumor cells become the focus of scientists. The abnormal expression and function of regulator molecules such as IAPs in apoptosis pathways is one of reasons for tumor resistance to apoptosis induced by diverse stimulus. Second mitochondria-derived activator of caspase (Smac) is a novel proapoptotic gene which can suppress IAPs function and enhance the apoptosis effects of cancer cells induced by diverse stimulus. In this study we transferred Smac gene into human bladder cancer cell line EJ cells to observe whether smac gene can increase the bio-effect of EJ cells induced by $^{12}C^{6+}$ ions irradiation. The experiment was divided into three groups: blank control group, vector transferred group and smac gene transferred group. Western Blot and Immuno-cytochemistry results indicated that the expression of smac gene of smac gene transferred group was significantly enhanced compared with vector pcDNA3.1 transferred group. Fluorescence-activated cell sorting (FACS) results also indicated that the expression of smac gene of smac gene transferred group was notably enhanced compared with vector pcDNA3.1 transferred group, but there was no significant difference between vector pcDNA3.1 transferred group and blank control group ($P>0.05$). Following $^{12}C^{6+}$ ions treatment at 0, 2, 4 Gy, the results of colony-formation assay suggested that Smac gene transferred group showed significantly lower survival fraction than vector pcDNA3.1 transferred group ($P<0.05$). At 24 hours after $^{12}C^{6+}$ ions treatment, FACS results indicated smac gene transferred group showed apparent cell apoptosis ratios which was higher than that of vector pcDNA3.1 transferred group ($P<0.05$). These results showed Smac gene could show high expression in smac gene transferred group cells and increase the apoptosis ratio of EJ cells. Smac gene could further enhance the apoptosis effects and notably decrease the survival fraction of EJ cells when combined with $^{12}C^{6+}$ ions irradiation

(PS3112) Protein expression profiles by radiation in a rat cirrhotic model. Sookin Chung. Brain Korea 21 Project for Medical Science, Seoul, Republic of Korea.

Object

To identify the common molecular protein between liver tissue and serum by radiation in a rat model with thioacetamide (TAA)-induced liver cirrhosis.

Materials and Methods

Male Wistar rats were treated with 0.3 g/L TAA in their drinking water for 9 weeks. The cirrhotic rats were treated with 10 Gy radiation in their partial liver. The animals were then sacrificed at 3 weeks after irradiation. The development of liver cirrhosis was observed with histological study. The livers were processed for proteins extraction and the proteins were analyzed by two-dimensional electrophoresis. The proteins were identified by quadrupole time of flight (Q-TOF) mass spectrometry (MS).

Results

In proteomics analysis of liver tissue and serum, a total 60 proteins showed significant change between TAA-treated group (control) and the radiation group. When the proteins were categorized by their function, they included immune response, signal transduction, apoptosis, proliferation/differentiation and reactive oxygen species metabolism. Especially, identified common molecular proteins between liver tissue and serum by radiation were heparanase precursor and hepatocyte growth factor. The expression level of heparanase precursor increased to 2 fold in the radiation group comparing to control. In contrast, the expression level of hepatocyte growth factor decreased to 2 fold in the radiation group comparing to control.

Conclusion

Hepatic injury was enhanced by radiation in TAA-induced rat cirrhotic model. Proteomics analysis showed a list of proteins related with various functions by radiation-induced damage. Specially, the heparanase precursor and the hepatocyte growth factor were common proteins between liver tissue and serum, suggesting deterioration of liver cirrhosis by radiation.

(PS3113) Microarray Analysis of Radiation-induced Genes in PC3 and DU 145 Cells after Single (10 Gy) and Fractionated (1 Gy and 2 Gy) Dose Irradiation. Molykutty J. Aryankalayil[1], Sanjeewani T. Palayoor[1], David Cerna[1], Mike Falduto[2], Scott Magnuson[2], Norman Coleman[1]. [1]National Cancer Institute, Bethesda, MD, USA, [2]GENUS Biosystems, Inc., Northbrook, IL, USA.

We hypothesized that fractionated radiation can induce molecular targets and thereby make tumors more susceptible to molecular-targeted therapy. The purpose of this study was to investigate the gene and protein induction patterns following single and fractionated dose irradiation protocols in human prostate carcinoma cell lines. PC3 and DU145 cells were irradiated with single (10 Gy) and fractionated (2 Gy X 5 and 1 Gy X 10) dose radiation. Alterations in gene expression were analyzed at 2 hr, 6hr and 24h after irradiation by microarray analysis (GenUs BioSystems). The microarray analysis demonstrated that the fractionated dose irradiation induced more differentially expressed genes than the single dose irradiation. Of the 2 fractionation protocols 1 Gy X 10 irradiation schedule induced more differentially expressed genes compared to the 2 Gy X 5 irradiation schedule. The data also revealed significant differences in the gene expression patterns of the two hormone-independent cell lines PC3 and DU145 in response to radiation. There were many more genes differentially expressed in both 2 Gy X 5 and 1 Gy X 10 fractionated dose-treated PC3 cells than in DU145 cells. In both cell types at least 30% of the differentially expressed genes in each of the fractionated protocols

are commonly regulated in both protocols (510 in PC3 and 180 in DU145). We followed the gene expression patterns of two genes, the growth differentiation factor (GDF-15) and the activating transcription factor 3 (ATF-3), considered to be antitumorigenic and proapoptotic genes. These two genes were differentially upregulated only in the PC3 but not in the DU145 cells. Moreover, the upregulation of these two genes was observed only after fractionated irradiation and not after single dose irradiation. The changes in these two genes were confirmed at the protein level. The differential expression pattern of GDF-15 and ATF-3 supports our hypothesis that fractionated radiation can induce molecular targets. Currently we are in the process of identifying similar radiation-induced targets and to evaluate their potential for using radiation to prime cells for molecular-targeted drug therapy.

(PS3114) Biophysical calculations of cell killing probability by the MK model and the LE model for heavy-ion beams. Yuki Kase[1], Tatsuaki Kanai[1], Naruhiro Matsufuji[1], Yoshiya Furusawa[1], Thilo Elsässer[2], Michael Scholz[2]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Gesellschaft für Schwerionenforschung, Darmstadt, Germany.

In treatment planning of heavy-ion radiotherapy, it is necessary to estimate the biological effect of heavy-ion beams. Physical dose should be associated with the relative biological effectiveness (RBE) at each point. Presently, carbon ion radiotherapy has been carried out at the National Institute Radiological Sciences (NIRS) in Japan and the Gesellschaft fuer Schwerionenforschung mbH (GSI) in Germany. Both facilities take an individual approach for the calculation of the RBE value. At NIRS, the classical LQ model has been used while the local effect model (LEM) has been incorporated into the treatment planning system at GSI. The first aim of this study is to explain the RBE model of NIRS by the microdosimetric kinetic model (MKM). In addition, the clarification of similarities and differences between the MKM and the LEM was also investigated.

An amorphous track structure model was used in the MKM calculation instead of the microdosimetric stochastic energy deposition. The amorphous track structure represents the averaged radial dose distribution of many tracks as a function of distance from the passing ion trajectory. The usage of the amorphous track structure model was originally implemented in the LEM. Domain size in the MKM calculation was remained as a free parameter according to the type of cell line, because the substance of the 'domain' has not yet been understood nor observed. Then we compared the calculated results of the MKM with the LEM.

The MKM calculation could reproduce the RBE shape used at the NIRS as well as experimental results of the *in vitro* mammalian cells for mono-energetic He-, C-, Ne-ion beams with an LET ranging from approximately 18–600 keV/micron. Intercomparison of the two models revealed that both models consist of three elemental constituents: target geometry, photon survival curve and track structure, although their implementation is significantly different. In principle the MKM focuses on the stochastic energy deposition in a micron-size domain while the LEM considers the local dose of infinitesimally small regions. Considering the amorphous track structure model, the difference between the MKM and LEM is mainly caused by the different approach to calculate the biological effect for the extremely high local dose in the center of the ion track.

(PS3115) Absorbed dose calculations predict therapeutic response in sodium iodide symporter expressing tumors. Kimberly J. Krager, Andrew W. Gaut, Mark T. Madsen, Richard D. Hichwa, Michael M. Graham, Frederick E. Domann. The University of Iowa, Iowa City, IA, USA.

Head and neck squamous cell carcinoma (HNSCC) is associated with significant morbidity due to current treatment modalities. Treatments include surgery, conventional radiation therapy, chemotherapy and combined chemo-radiotherapy. The survival rates for HNSCC have remained poor for the past 30 years. A new treatment option targeting head and neck cancers is clearly needed.

Genetically targeted radiotherapy utilizes gene transfer and expression of a transgene that confer radionuclide accumulation. Sodium Iodide Symporter (NIS) has advantages over most therapeutic genes due to its ability to accumulate radionuclide that can be used for imaging or therapy depending on the nuclide chosen. Dosimetry experiments were performed using stable NIS expressing tumors to determine the dose necessary to exhibit a clinically relevant tumor response.

Athymic mice were injected with NIS stable expressing FaDu cells. When the tumors reached approximately 6 mm in diameter, the mice received varying activities of I-131 by i.p. injection. The animals were imaged over a time course using a gamma camera fitted with a pin-hole collimator to non-invasively measure the amount of radioactivity in each tumor. From the activities determined, calculations to derive estimated absorbed doses were performed. The absorbed doses were calculated based on counts detected from the tumors and the volumes of the tumors. Tumors that receiving 12–25 Gy displayed a delay in growth, whereas absorbed doses higher than 25 Gy exhibited a regression in tumor size.

Absorbed doses could be used as a prognostic indictor to predict therapeutic outcome in patients following NIS gene transfer radioiodine administration.

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(PS3116) Enhancement of DNA damage in ion beam radiotherapy through use of heavy atom doping. Jean A. Wyer, V Senthil, Karl Butterworth, Shane W. J. Scully, Colin J. Latimer, Fred Currell, David Hirst, Dolan F. Byrne, Robert J. Pollard, Mansukh B. Shah. Queens University Belfast, Belfast, United Kingdom.

Introduction:

Atoms of high Z values have very high efficiencies for X-ray absorption and this advantage can be used to provide a convenient way of enhancing the destruction of tumours while limiting the damage caused to healthy cells. In this work we investigate whether a similar enhancement can arise using fast ion beams.

Materials and Methods:

In this feasibility study a specially designed system has been constructed which consists of a vacuum irradiation chamber and a high resolution time-of-flight (TOF) mass spectrometer. Plasmid DNA samples containing controlled amounts of gold nanoparticles were prepared by freeze drying and evaporative methods on to a mica substrate and their structure was investigated using electron microscopy. They were then irradiated with 80 and 20 keV beams of H neutrals and H^+ ions. Gel electrophoresis was used to assess total DNA damage caused by varying doses of ion beam radiation. Damage profiles at the molecular level have also been studied using the TOF-MS system.

Results:

Our results show that an important feature in keV proton work is the enhanced level of double strand breaks (compared with X-rays) caused to DNA, showing that these protons are highly ionising. As the dose is increased, damage levels increase and eventually plateau and the super-coiled DNA (intact DNA) levels fall rapidly at relatively low levels of flux. This result was found to be highly sensitive to the microscopic sample structure. Our work also shows that damage levels at 80 keV are substantially larger than at 20 keV. In addition our preliminary work suggests that DNA damage occurs at lower doses when irradiated in the presence of gold.

Conclusions:

We have shown that keV ion and atom beams are highly efficient at causing damage in DNA, and furthermore it would appear that this damage can be enhanced in the presence of gold nanoparticles.

(PS3117) Specialties of animal reproduction under x-rays irradiation. Olga Petrova, Sergiy Andreychenko. Scientific Center for Radiation Medicine, Kiev, Ukraine.

Regarding the strong mutational and damaging properties of ionizing irradiation in connection with wide expansion of atomic energy consumption in the world the investigation of post - irradiation consequences on animal reproduction is to be of great importance. The present investigation has been performed with the goal to elucidate the similarities and differences in fecundation and offspring production after total body single X-irradiation either of male or female partner before crossing.

For the experiments white rats (Wistar line) were irradiated by x-rays in the dose range 0,5 −10 Gy with dose rates 0,17 and 0,34 Gy/min, respectively. The irradiated males were then crossed with intact females and vice versa. Periodically the hematological state of the blood and the contents of proteins, triglycerides as well as different enzymes, namely catalase, superoxide dismutase, glutathione peroxidase, aminotrasferases, alkaline phosphatase in the blood plasma of animals were examined. Apart from this, morphologic and physiologic characteristics of isolated spermatozoa and oocytes were studied. In addition, teratogenic analysis has been also made.

The research performed has revealed that the dose 0,5 Gy caused evident delay in fecundation and offspring production. The irradiation in dose of 10 Gy used to result in sterilization and degradation of sexual organs. However, at the doses 1,0 and 2,0 Gy the offspring production was detected without any retardation, although the dose of 2 Gy caused mass mortality in the posterity. It was noticed that body weight and dimensions were dependent on the dose value. It must be emphasized that the reproductive function has recovered slowly in the course of next few descents. Moreover, the irradiation doses that caused infertility were not crucial for the viability of male and female gametes.

(PS3118) Secondary neutron production from patients during therapy with hadrons: are there potential risks. Anwar M. Chaudhri. Klinikum Nuernberg, Nuernberg, Germany.

There is no reliable data on the production of secondary neutrons from patients during therapy with hadrons even when this is absolutely essential. By making use of the experimental neutron out put studies from thick-targets of different elements, we have been able to accurately estimate, for the first time, the fluence and energy distribution of these secondary neutrons from tissue under irradiation with different hadrons. Our results indicate that at least 4.2 neutrons , with energies greater than 5 MeV, are produced for every carbon ion of 400 MeV / u energy incident on tissue. This number reduces to 3, 1.4 and 0.3 respectively at carbon energies of 300, 200 and 100 MeV /u. The energy range of carbon ions considered here, 100 MeV/u to 400 MeV/u, corresponds to that being actually used in therapy. In the case of neon ions these figures (number of secondary neutrons / incident neon ion) are slightly higher. For irradiation with alpha particles the number of these secondary neutrons reduces to about 1 per alpha particle with incident energy of 200 MeV / nucleon. There would no doubt be even more neutrons with energies lesser than 5 MeV which so far could not be estimated due to the lack of experimental data. In the case of proton therapy the numbers of secondary neutrons from tissue are estimated to be 0.05, 0.2 and 0.4 per proton of energies 100, 200 and 300 MeV respectively.

For a physical treatment C-ion dose of 20 Gy in the Bragg Peak, the total number of secondary neutrons produced in patients are 1.6×10^{9} / cm ^{2}, 7.3×10^{8} / cm ^{2}, 2.5×10^{8} / cm ^{2} and $4{,}1 \times 10^{7}$ / cm ^{2} respectively at carbon-ions energies of 400, 300, 200 and 100 MeV / u. The corresponding figures for a physical proton dose of 20 Gy in the Bragg Peak are 2×10^{9}/cm ^{2}, 4.17×10^{8} / cm ^{2} and 4.17×10^{7} / cm ^{2} neutrons respectively at proton energies of 20, 160 and 70 MeV. The doses to various organs have also been approximately estimated in the two cases. In our opinion the large number of secondary neutrons produced from patients during therapy with hadrons, and their corresponding doses, indicate they could have real potential to cause new primary cancers and cause other harmful side-effects in patients. It is therefore strongly recommended that serious considerations should be given before deciding to treat patients with hadrons, especially children, younger people and those who still have many years to live

(PS3119) Photochemistry of the III generation photosenisitizers and Raman spectroscopy for breast cancer diagnosis. Halina Abramczyk[1], B. Brozek-Pluska[1], K. Kurczewski[1], M. Kurczewska[1], P. Ciacka[2], M. Tazbir[3], Z. Morawiec[3], P. Wozniak[4], J. Parulski[4]. [1]Laboratory of Laser Molecular Spectroscopy, Dept. of Chemistry,Technical University of Lodz, Lodz, Poland, [2]Institute of Physics, Technical University of Lodz, Lodz, Poland, [3]Kopernik Hospital in Lodz, Oncological Surgery Ward, Lodz, Poland, [4]Dr. E. Sonnenberg Hospital, Lodz, Poland.

The research presented in the lecture integrates the state-of-the-art laser technology, molecular spectroscopy and research in diagnosis of human biological tissue using laser-induced fluorescence and Raman spectroscopy. Raman scattering spectroscopy, absorption spectroscopy and femtosecond laser spectroscopy results will be presented for photochemistry of the III generation photosensitizers that are used in the photodynamic cancer therapy - sulfonated phthalocyanine derivatives and metal complexes of phthalocyanine in solutions, glassy and crystal phase and in human blood. Results obtained from Raman spectroscopy will be presented for breast tissue samples illustrating new possibilities on the implementation of the Raman spectroscopy in the medical diagnostic. We will show the empirical relations between the Raman and the emission spectra related to human breast cancer for about 60 patients.

The specific goal of the presented research is twofold:

1. Determination of mechanisms of photochemistry in selected phthalocyanine complexes with metal X (X=Zn, Mg Cu,Co) in solutions, crystal and glassy phases and in human blood. The studies will estimate photodynamic activity of the photosensitizes of the III generation in the cancer therapy

2. Application of Raman spectroscopy for medical diagnosis of the breast tissue. The research goal is to create medical tools based on Raman spectroscopy that ultimately may be used in areas such as early diagnosis of cancer and diagnostics that benefit human health.

(PS3120) Increased mercury release from dental amalgam restorations after exposure to microwave radiation emitted from mobile phones. Seyed Mohammad Javad Mortazavi, Elham Daiee. Rafsanjan University of Medical Sciences, Rafsanjan, Iran (Islamic Republic of).

Mercury is the most non-radioactive toxic element known so far. Electromagnetic fields may increase the emission of mercury from dental amalgam fillings. We have previously shown that MRI alters the level of mercury released from dental amalgam restorations.

Mercury levels in urine samples of 14 healthy university students were measured both before restoration, and 1 day, 2 days, 3 days and four days after restoration. Dental treatment was given for all 14 students (2 molars on one side, 1 class I and 1 class II restorations with identical volume and surface area of the amalgam fillings). The students had not used mobile phones before the study and had not any previous amalgam restorations. All study participants were asked not to use sea food, canned food, and hot tea/coffee from 1 week before the study to final sampling at day 4 after restoration. For each student a questionnaire was filled out and basic information regarding their possible sources of exposure to electromagnetic fields, occupation and life style recorded. The case group that was consisted of seven female students who met the inclusion criteria for our research, were exposed to microwave radiation emitted from a Nokia 3310 mobile phone (SAR=0.96 W kg-1) in talk mode for 15 min at days 1–4 after restoration. Seven female age matched students who served as controls sham exposed to microwave radiation. Urine samples were collected 1 hour after exposure. After freezing, the samples were sent to the Toxicology Laboratory of Imam Reza Hospital (Mashad, Iran) and mercury levels in samples were measured by vapor atomic absorption spectrometry.

Our study demonstrated evidence of an elevated mercury level from dental amalgam fillings after exposure to microwave radiation emitted form mobile phones. The mean concentration levels (mean ± SE) of mercury in urine samples in case group were 2.43±0.25, 2.71±0.27, 3.79±0.25, 4.8± 0.27 and 4.5±0.32 μg L-1 before the restoration, 1 day, 2 days, 3 days and 4 days after treatment,

respectively. On the other hand, the mean concentration levels in controls were 2.07±0.22, 2.34±0.30, 2.51±0.25, 2.66± 0.24 and 2.76±0.32 μg L-1 before the restoration, 1 day, 2 days, 3 days and 4 days after treatment, respectively.

These results show a significant relationship between the mercury release in urine before and after mobile phone use.

(PS3121) Individual doses incurred from 1986 to 2006 by personnel of the health care units of the Ministry of National Defence and the Ministry of Interior and Administration in Poland. Agnieszka Kowalska, Jaroslaw Jazwinski, Marek K. Janiak. Military Institute of Hygiene and Epidemiology, Dept. of Radiobiology and Radiation Protection, Warsaw, Poland.

Introduction

Individual radiological surveillance is one of the most important parts of the health protection of workers occupationally exposed to ionising radiation. Military Institute of Hygiene and Epidemiology is the surveyor of individual doses incurred by personnel of the units supervised by the Minster of National Defence (MND) and the Minister of Interior and Administration (MIA) in Poland.

The aim of the present study was to estimate the magnitude and structure of the occupational exposure to ionising radiation of medical workers at the above units.

Material and Methods

The research was conducted based on the accumulated records of occupational radiation exposure and its distribution among the MND and MIA workers in the period from 1986 to 2006. The data were collected by the Laboratory of Radiological Inspection and Individual Doses Control at the Department of Radiation Protection and Radiobiology, Military Institute of Hygiene and Epidemiology, Warsaw, Poland. The surveyed workers were divided into several groups according to profession, education, age, sex and department where individual doses were recorded and analysed. The results were demonstrated as graphs assigned to the consecutive years.

Results

Analysis of the data suggests that for a large majority of the occupationally exposed workers radiation risk was minimal: over 99% of the workers whose individual doses were recorded received less than 2 mSv per year. There were also critical groups of workers whose absorbed effective radiation doses (expressed as effective doses) ranged from 10 to 20 mSv per year. Increased, but not exceeding the established limits, doses of radiation were received by workers involved in procedures performed in the X-ray field.

Conclusion

Overall, no departure from the standing radiological protection regulations was recorded during the analysed period among the MND and MIA workers of medical units utilizing sources of ionising radiation.

(PS3122) Secondary cancers after fractionated radiotherapy: stochastic population dynamics effects. Rainer K. Sachs[1], David J. Brenner[2], Igor Shuryak[2], Hatim Fakir[3], Lynn Hlatky[4], Philip Hahnfeldt[4]. [1]UCB, Berkeley, CA, USA, [2]Columbia University Medical Center, NY, NY, USA, [3]University of Salzburg, Salzburg, Austria, [4]Caritas St. Elizabeth's Medical Center, Tufts, Boston, MA, USA.

When ionizing radiation is used in cancer therapy it can cause secondary cancers. Due to longer patient survival times these secondary cancers have recently become of increasing concern. Moreover, their study gives important insights into radiation carcinogenesis in general, since the therapy involves irradiation of humans with comparatively accurately characterized doses, dose timing, and body localization, followed by long-term monitoring. In addition to putative radiation initiation that produces pre-malignant cells, inactivation (i.e. cell killing) and subsequent cell repopulation by proliferation are important at the high doses relevant to many secondary cancer situations. A recent mathematical initiation/inactivation/proliferation (IIP) model characterized quantitatively the observed occurrence of secondary breast and lung cancers at high doses, using a deterministic cell population dynamics formalism.

To analyze the possibility that pre-malignant clones may be eradicated before repopulation can occur, we here give a probabilistic version of this IIP model. Using customized computer programs that combine Monte Carlo simulations with the explicit equations of the standard Feller-Arley time-inhomogeneous birth-death formalism, we show that repeated cycles of inactivation and repopulation, as occur in fractionated radiation therapy, can lead to situations where the distribution of pre-malignant *cells* per patient has variance much larger than the mean, even though pre-malignant *clones* are Poisson-distributed. The implication of this result is that at high doses many fewer patients would be affected, but more severely, than a deterministic model, tracking average pre-malignant cell numbers, would predict. Our results are applied to data on secondary breast cancers after adjuvant radiotherapy following breast conserving surgery and after radiotherapy for Hodgkin's disease. The stochastic IIP analysis, unlike the deterministic one, is consistent with the assumption, used in some recent applications of two-stage clonal expansion radiation carcinogenesis model, that initiated, pre-malignant cells have a growth advantage during repopulation, not just during the longer tumor latency period that follows.

(PS3123) Preliminary evidence for a dose-rate-dependent threshold for low dose suppression of low-LET radiation-induced neoplastic transformation in vitro. J. Leslie Redpath, Xiaoyan Lao, Rubena Kapadia, Eric Giedzinski, Charles Limoli, Eugene Elmore. Univ of California Irvine, Irvine, CA, USA.

Purpose: To see if adaptation against neoplastic transformation could be induced by exposure to very low dose-rate (VLDR) low-LET radiation.

Experimental Procedures: HeLa x skin fibroblast human hybrid cells were irradiated with ~30 kVp photons from an array of I-125 seeds. The initial dose-rate was 4 mGy/day. The cell cultures were split after 10 days to avoid overcrowding. Half of the culture was processed for the presence of ROS. The other half was returned to the irradiator. At 20 days the culture was again split with half being used for a transformation assay and the other half being returned to the irradiator. This procedure was continued for a 3 mo. period by which time the dose-rate had declined to 1 mGy/day (acc. dose ~250 mGy). This allowed for 4 separate assays. Transformation frequencies were compared to those of parallel unirradiated controls. At the end of 3 mo. VLDR treated cells were exposed to a high dose-rate 3 Gy challenge dose of Cs-137 gamma rays and this was compared with the effect of 3 Gy on parallel unirradiated cells.

Results: Cells exposed to VLDR radiation exhibited a reduction in neoplastic transformation frequency compared to the unirradiated controls. This reduction seemed to diminish with time indicating that the dose-rate, rather than accumulated dose, may be the more important factor in illiciting an adaptive response. This pattern was paralleled by a reduction of ROS present in the irradiated cultures compared to controls. The VLDR treated cells were less sensitive to a high challenge dose confirming the induction of an adaptive response. Since there was a suggestion of a dose-rate threshold for induction of suppression, a second experiment was started with a fresh batch of cells at an initial dose-rate of < 0.8 mGy/day. These cells were allowed to accumulate ~27 mGy over a period of 45 days. In this experiment there was no evidence of an adaptive response as indicated by a lack of suppression of transformation either in the VLDR only treated cells, or in the same cells subjected to a 3 Gy challenge dose, compared to appropriate controls.

Conclusions: VLDRs in the range 1 to 4 mGy/day induced an adaptive response against neoplastic transformation *in vitro*. This adaptive response was lost at dose-rates below ~1 mGy/day suggesting a dose-rate-dependent threshold.

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(PS3124) The biophysical model for risk extrapolation needs modification. Antone L. Brooks. Washington State University Tri-Cities, Richland, WA, USA.

The biophysical model that is currently used in risk assessment needs to be re-evaluated. It is dependent on, independent action of cells, "hit" cells being the cells at risk, a constant responsiveness or risk per unit of radiation dose and radiation induced mutation and DNA damage being the prime link between the radiation exposure and cancer risk. However, the observation that radiation changes the expression of unique genes and proteins at different radiation doses, that low doses induce change in cells oxidative status, that low doses result in differential radiation apoptosis of transformed cells, and that the normal tissue and matrix control of the expression of radiation damage all question assumptions used in the current biophysical risk models. Research on the mechanisms associated with these observations show their dependence on genetic background and link them to bystander effects, adaptive responses and genomic instability. Thus, new paradigms are need to determine the radiation risk in the low dose region where it is not possible to detect an increase in the frequency of cancer in the exposed populations relative to controls. This paper will discuss these new paradigms and demonstrate that the current radiation standards are adequately protective.

(PS3125) Lung cancer risk of Mayak workers: Modelling of carcinogenesis and the bystander effect. Peter Jacob[1], Reinhard Meckbach[1], Mikhail Sokolnikov[2], Viktor V. Khokhryakov[2], Evgeni Vasilenko[3]. [1]GSF National Research Center, Neuherberg, Germany, [2]Southern Urals Biophysics Institute, Ozyorsk, Russian Federation, [3]Mayak Production Association, Ozyorsk, Russian Federation.

Lung cancer mortality in the period 1948–2002 has been analyzed for 6293 male workers of the Mayak Production Association, for whom information on smoking, annual external doses and annual lung doses due to plutonium exposures was available. Individual likelihoods were maximized for the two stage clonal expansion (TSCE) model of carcinogenesis and for an empirical risk model. Possible detrimental and protective bystander effects on mutation and malignant transformation rates were taken into account in the TSCE model. Criteria for non-nested models were used to evaluate the quality of fit. The data were found to be not compatible with the model including a detrimental bystander effect. The model with a protective bystander effect did not improve the quality of fit of simpler model versions. In the preferred TSCE model, smoking contributed to the lung cancer deaths 57%, the interaction of smoking and radiation 27%, radiation 10%, and other causes 6%. Assessments of the relative biological effectiveness of plutonium were consistent with the ICRP recommended value of 20. At age 60, the excess relative risk (ERR) per lung dose was 0.20 (95% CI: 0.13; 0.40) Sv^{-1}, the excess absolute risk (EAR) per lung dose was 3.2 (2.0; 6.2) per 10^4 PY Sv. With increasing age attained the ERR decreased and the EAR increased. In contrast to the atomic bomb survivors, a significant elevated lung cancer risk was also found for age attained younger than 55. For cumulative lung doses below 5 Sv, the excess risk depended linearly on dose. The excess relative risk was in the TSCE model for ages attained younger than 55 significantly lower than in the empirical model. This reflects a model uncertainty of the results, which is not expressed by the standard statistical uncertainty bands.

(PS3126) Two-stage carcinogenesis modeling: acute myeloid leukemia induced by X-rays and neutrons in mice. Fieke Dekkers, Harmen Bijwaard. Laboratory for Radiation Research, RIVM, Bilthoven, The Netherlands.

We have applied a two-stage carcinogenesis model to describe radiation-induced acute myeloid leukemia (AML) in mice exposed to X-rays and neutrons. Our aim is to determine the role played by low doses of radiation in different stages of carcinogenesis and to study the effect of different radiation qualities.

In the late twentieth century, experiments were carried out at NRG (the Netherlands), HPA (UK) and ENEA (Italy) in which young CBA/H mice underwent a single acute exposure to X-rays or neutrons. Of the mice that were exposed, ca. 25% developed AML.

The two-stage carcinogenesis model that we have used to describe the data from these experiments consists of a first stage in which sensitive cells are assumed be transformed into fast multiplying intermediate cells in a (series of) mutation(s). In a second (series of) mutation(s), intermediate cells are then transformed into malignant cells, that after a certain lag time lead to fatal AML. For radiation induced AML in CBA/H mice, recent experimental research suggests two mutational events that both involve the PU.1 gene.

The model contains parameters that describe the background incidence of AML and parameters that give information on the effect of radiation on mutation rates.

Since spontaneous AML is extremely rare in CBA/H mice, values for the background parameters in the model cannot easily be determined. As a consequence, we find a family of solutions to the model instead of a unique solution. In all of these solutions, radiation only influences the first stage of leukemogenesis. This could be due to the fact that all mice used in the experiments concerned were relatively young when exposed. New experiments currently being carried out at NRG involve older mice and mice that are exposed twice. Data from these experments will become available later this year and are expected to lead to a better understanding of the effect of radiation on the second stage.

We have found neutrons to be roughly 4 times as efficient at initiating sensitive normal cells as X-rays. This last finding has important implications for future modeling of leukemia in the Japanese A-bom survivors.

(PS3127) Theoretical approaches to cancer risk estimation. Philip Hahnfeldt[1], Rainer K. Sachs[2], Lynn Hlatky[1]. [1]Center of Cancer Systems Biology, CSEMC, Tufts University School of Medicine, Boston, MA, USA, [2]Depts. of Mathematics and Physics, University of California Berkeley, Berkeley, CA, USA.

Carcinogenesis risk estimation is complicated by a number of factors, among these being the lack of a common platform to integrate and analyze the available data, and the inherently systems-biologic nature of the problem. We have investigated mechanistic approaches to radiogenic risk estimation that draw on unifying biological principles to allow input from disparate data sources. At the same time, the modeling appreciates that carcinogenesis is a multi-scale phenomenon, influenced critically by determinants not only at the molecular level, but at the cell and tissue-levels as well. A reliable theory of carcinogenesis must therefore account for these multi-scale effects. Starting from the initiation-promotion-progression paradigm, we have begun to link these scales of action. The Two-Stage Logistic model considers the molecular-level events of initiation and promotion, while incorporating in a rudimentary way the larger-scale growth-limiting role of cell-cell interactions. Likewise, to account for cell-level carcinogenesis progression as influenced by inter-tissue signaling at the next higher scale, a dynamic carrying capacity construct has been developed that quantifies the growth-limiting effect of induced vasculature on tumor growth. Recent data has revealed this to be an important factor for whether nascent tumors ever rise to become a disease threat. Ultimately, a means to quantify the overall risk from low-dose space radiations is a goal. Vital to this endeavor will be the biologic quantification of meaningful RBEs. By defining effect endpoints for high-throughput genetic and physiologic data that permit effect radiation comparisons, a major step in this direction will be achieved.

(PS3128) Verification of cancer induction model based on rat skin irradiations with different LET values. Fredric J. Burns, Krystyna Frenkel, Moon-shong Tang, Arthur Nadas, Feng Wu, Ronghe Zhang. NYU School of Medicine, Tuxedo, NY, USA.

The assessment of cancer risks in humans exposed to ionizing radiation could greatly benefit from a plausible, biologically-based extrapolation model. A modified dual-action model shows promise and has been successfully fitted to benign and malignant tumors risks in rat skin exposed to various radiation doses and LETs. As modified to apply to carcinogenesis the model is based on the assumption that 2 lesions (unspecified but probably DNA double strand breaks) interact to produce a mutation or rearrangement that establishes a probability that the affected cell will undergo neoplastic progression. The track feature of radiation provides 2 modes of lesion generation: 1) both lesions originate in the same track or 2) the lesions originate in different tracks. Based on the sum of 1- and 2-track terms $Risk(D) = CLD + BD^2$ (Eq. 1), where L is the LET, C is the 1-track coefficient and B is the 2-track coefficient. A complete reanalysis of rat skin data has shown that Eq. 1 is consistent with tumor induction in rat skin exposed to argon ions, neon ions and electron radiation. Results with the ^{56}Fe ion beam have been confirmatory, except that tumor yields were lower than expected by about 20%. Most likely this discrepancy is associated with the LET of ^{56}Fe being above the peak-efficiency LET. The quantity $D_{equal} = CL/B$ (the dose where the 2 terms in Eq. 1 contribute equally to the overall cancer risk) defines roughly the dose above which 2-track dominance would be expected. Below D_{equal} the 1-track (linear) term dominates and repair would be minimal, while above D_{equal} the reverse is true, repair is increasingly important and the 2-track term is dominant. An extreme case of 2-track dominance exists for electron radiation for which LET = 0.34 keV/μ which translates to such a low D_{equal} that the 1-track term can be neglected. Thus the tumor response to electron radiation provides a direct measure of B in Eq. 1. Results are presented indicating that both C and B are altered by dietary cancer preventive agents without compromising the basic framework of the model. In spite of the known complexities of carcinogenesis, consistency with rat skin tumor yields is strong evidence that significance of the earliest radiological event(s) is never lost.

(PS3129) Effects of oxidative metabolism on carcinogenesis *in vitro*. Hanako Yoshii, Masami Watanabe. Research Reacter Institute, Kyoto University, Osaka, Japan.

Oxygen is the essential material when we produce energy, but it also induces adverse effects. Excessive oxidative stress is thought to be involved in the critical cause of life-style related disease, aging and cancer. These oxidative stresses are principally caused as by-products of energy metabolism, and radiation also induces them. As quantity of oxygen (quantity of energy metabolism) consumed throughout the life does not depend on a creature species. However, rodent cells spontaneously overcome replicative senescence (immortalization) whereas human cells rarely do so. This suggests that ability of oxygen metabolism control in human cells is better than that of in rodent cells. In addition, it is certain that oxygen stress contributes to *in vitro* carcinogenesis and/or genetic instability. Therefore this study was carried out to clarify mechanisms of intervention of oxygen in *in vitro* carcinogenesis process and the target of the oxygen in cells being cultured under low oxygen levels.

We cultured normal human cells and rodent cells (MEF and SHE) under atmospheric (20%) and physiological (hypoxic; 2% and 0.5%) oxygen conditions, and measured cell growth, levels of intracellular reactive oxygen species (ROS) and quantity and function of mitochondria of each. As a result, by culture in hypoxic conditions, human cells extended the replicative lifespan but rodent cells immortalized.

We will present the results of level of intracellular ROS, chromosome damage and centrosome amplification of each cell, and discuss the relationship between cell transformation sensitivity and oxygen control ability.

(PS3130) A novel phenomenon "delayed division delay": evidence for delayed dna double-strand break and rejoining in the clonogenic progeny of cells surviving alpha or x irradiation. Hiroshi Sasaki. Dept. Experimental Radiology, Kyushu Univ.(retired), Fukuoka, Japan.

Lethal sectoring is the process for survival by which lethal damage remaining in irradiated cells is eliminated as offspring without reproductive integrity, and occurs through the postirradiation 1st to 4th divisions with the accompanying appearance of a clonogenic progenitor harboring no lethal damage. However, nonlethal damage remaining in the clonogenic progenitors led to an elevated incidence of delayed cell death in their progeny. From an analysis of the pedigrees which had been constructed more than 20 years ago by time-lapse observations of HeLa cells surviving α (0.45 Gy from a point source of Am-241; LET 125 keV/μm) or X irradiation (3 Gy) (20% survival dose), it became clear that the mean incidence was higher for α-particle (16.3%) than X-ray survivors (8.3%), indicating the greater potentiality for genomic instability by α particles. Evidence is available to suggest the association of misrepaired clustered DNA damage with α particles and unrepaired potentially lethal damage with X rays.

A novel phenomenon "delayed division delay (DDD)" was noticed, though occasionally (~10% per clonogenic progeny), during the postirradiation 1st ~ 3rd generations. DDD was much longer in α- (mean: ~11 h) than X-irradiated cells (~4 h), and might be triggered by delayed DNA double-strand break (DSB) and rejoining at the fragile break-misrejoining sites. It can not be excluded as one of the possibilities that delayed DNA DSBs are induced at these sites by a higher-order conformational change of chromatin fibers during mitosis. The cells undergoing DDD after exposure to α particles are characterized by the large cell-size because of a prolonged cell cycle-arrest and the presence of DNA DSBs. Such cells should be the targets for a future study on radiation-induced genomic instability.

In conclusion, the persistence of delayed DNA double-strand break and rejoining in the clonogenic progeny of survivors, if this happens also in normal human cells, may play an important role in radiation carcinogenesis.

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(PS3131) Persistent phenotypic responses of human mammary epithelial cells induced by sparsely and densely ionizing radiation. P. Kumari L. Andarawewa, Sylvain Costes, William S. Chou, Mary Helen Barcellos-Hoff. Lawrence berkeley national lab, Berkeley, CA, USA.

We have demonstrated that irradiated pre-malignant human mammary epithelial cells (HMEC), cultured in reconstituted basement membrane and TGFβ, fail to undergo tissue-specific morphogenesis, whose deregulation is strongly associated with cancer. The phenotype of irradiated HMEC cultured on traditional tissue culture plastic in the presence of TGFβ is consistent with epithelial to mesenchymal transition (EMT). EMT is frequently invoked as a non-mutational mechanism by which cancer cells acquire invasive properties. Densely ionizing radiation is more carcinogenic when compared to similar doses of sparsely ionizing radiation. In these studies, we determined whether 1 GeV/amu ^{56}Fe densely ionizing radiation exposure of finite lifespan, post-selection 184v HMEC resulted in EMT. When assayed by clonogenic survival 1 Gy ^{56}Fe exposure was approximately twice as effective as ^{137}Cs. Equitoxic doses of 1 Gy ^{56}Fe vs 2 Gy ^{137}Cs were compared for phenotype analysis. E-cadherin, which is an epithelial marker, was decreased in 184v HMEC irradiated with either densely or sparsely ionizing radiation decrease. The addition of TGFβ to irradiated 184v HMEC augmented the loss of E-cadherin and elicited spindle cell morphology. The decrease in E-cadherin was more dramatic in double treated cells exposed to sparsely ionizing radiation compared to densely ionizing radiation. Fibronectin, a mesenchymal marker associated with EMT, was significantly increased in double treated cells regardless of radiation quality. In contrast to monolayer experiments, 184v HMEC cultured in reconstituted basement membrane following densely or sparsely ionizing radiation exposure do not decrease E-cadherin. However, addition of TGFβ to irradiated 184v HMEC significantly decreased E-cadherin and increased colony size similarly following densely and sparsely ionizing radiation. Our data indicate that at equal survival there is little difference in the phenotype of irradiated HMEC as a function of radiation quality but that irradiated HMEC were more resistant to the phenotypic shift when cultured in the

context of an extracellular matrix that permits epithelial morphogenesis. *This work was supported by funds from NASA Specialized Center of Research.*

(PS3132) Determination of individuals sensitive to ionizing radiation on the base of cytogenetic examinations. Emiliya Dyomina, Natalia Ryabchenko. Institute of Experimental Pathology, Oncology and Radiobiology of NASU, Kyiv, Ukraine.

Increase of radiogenic cancer in Ukraine is connected with the elevated radiation loading on the population due to the factors of the Chernobyl catastrophe. Thus it is expedient to concentrate attention on the assessment of individual radiosensitivity (IR) of healthy contingent.

Materials and methods. Test-system of human peripheral blood lymphocytes (PBL) more than 100 healthy donors with the next metaphase analysis of chromosomal aberrations (chromatid breaks) was used. G_2-assay: test γ-irradiation of cell cultures was carried out at 1,5 Gy in the late G_2-stage.

Results. Data of cytogenetic examinations of healthy individuals made it possible to determine among them 12% with hypersensitivity to radiation (CV = 37,5%). Taking into account correlations of radiation-induced genome instability of human somatic cells with cancer predisposition it is competent to relate these individuals with the group of increased radiogenic cancer risk. The coefficient K_{IR} for the cytogenetic estimation of human IR as the attitude of individual aberration number to the cut off of the group mean value is proposed. For hypersensitive individuals K_{IR} is >1. Coefficient introduction enables not only to indicate radiosensitive individuals but also to compare their values of increased IR. Indications for the prime cytogenetic examinations (G_2-assay) of persons contacting with ionizing radiation (personnel of atomic plants, radiologists etc) with the purpose of determination of increased IR are developed.

Application of cytogenetic examinations on the basis of G_2-assay is recommended for the formation of groups with the increased cancer risk and promotes the efficiency of initial cancer prevention.

(PS3133) Investigation of hot-spots associated with elements in the breakpoint cluster regions surrounding spi.1 gene deletions on chromosome 2 in radiation-induced aml in cba mice. David G. Maranon, Michael M. Weil, Susan M. Bailey, Maria C. Muhlmann, Joel S. Bedford. Colorado State University, Fort Collins, CO 80523, USA.

Radiation-induced acute myeloid leukemias in CBA mice have been shown by several laboratories to be associated with loss of a small region of chromosome 2 containing the Spi-1 gene, which encodes the PU.1 protein, a transcription factor associated with myeloid differentiation. Bouffler and Finon and co-workers have reported that the breakpoints in chromosome 2 involved in the deletion are clustered in two regions, one proximal and one distal to the centromere and surrounding the Spi-1 gene position. The distal breakpoint cluster contains a region with a telomere-like sequence . Since telomere or telomere-like sequences have been reported to be hyper-recombinogenic, and further have been suggested as hot-spots for radiation-induced chromosome exchange breakpoint regions, we have initiated studies to follow the stability of this region on chromosome 2 using a BAC probe, RP23-373F19, containing a telomere-like sequence, and have compared its frequency of involvement in exchanges with BACS from other regions nearby or more distant from this sequence. Initial results from unirradiated mice show that rearrangements resulting in translocation and inversions of the region close to the telomere-like sequence occur much more frequently than in the regions used for comparison. We are now comparing this apparent instability with the frequencies of those events that occur after irradiation with gamma-rays and with heavy ions. The results will be compared with those obtained using C57BL /6 mice that are not susceptible to radiation induced AML.

(PS3134) Hydrogen peroxide mediates persistent radiation-induced genomic instability. Disha Dayal[1], Sean M. Martin[1], Charles L. Limoli[2], Douglas R. Spitz[1]. [1]The University of Iowa, Iowa City, IA, USA, [2]The University of California, Irvine, CA, USA.

Chronic oxidative stress is associated with genomic instability following exposure to ionizing radiation (IR). We hypothesized that radiation causes damage to oxidative metabolic processes leading to oxidative stress and genomic instability. This hypothesis is based on studies in parental hamster fibroblasts (GM10115) as well as genomically unstable (CS-9, LS-12) and stable (114, 118) clones isolated following exposure of GM10115 to 10 Gy IR. CS-9 and LS-12 cells demonstrate increased genomic instability as determined cytogenetically as well as a 2-fold increase in spontaneous mutation frequency and gene amplification relative to the 114, 118 and GM10115 cells. In addition, the unstable cells CS-9 and LS-12 demonstrate a 2- to 3- fold increase in the steady-state levels of catalase-inhibitable extracellular hydrogen peroxide. These cell lines also show a 2-fold increase in the steady-state levels of superoxide from isolated mitochondria as detected by EPR. Furthermore, catalase and glutathione peroxidase activities were found to be 2-fold elevated in the stable 114 and 118 cells, relative to wild type GM10115 and/or unstable CS-9 and LS-12 cells. These results indicate that the CS-9 and LS-12 cells have elevated steady-state levels of intracellular prooxidants and lower levels of peroxide scavenging enzymes, relative to the wild-type GM10115 and genomically stable 114 and 118 cells. Over expression of glutathione peroxidase in CS-9 cells or treatment with polyethyleneglycol-conjugated catalase, reduced the mutation frequency and gene amplification by 50% providing strong evidence for the involvement of hydroperoxides in the induction of persistent genomic instability. In addition, treatment of the stable clone 114 with catalase inhibitor 3-aminotriazole, increased mutation frequency significantly. These results support the hypothesis that IR induced chronic metabolic oxidative stress in mammalian cells mediates the propagation of genomically unstable phenotypes. Supported by DE-FG02-02ER63447, RO1CA100045.

(PS3135) Hrad9 gene expression associated with prostate cancer. Aiping Zhu[1], Xia Zhang[1], Xiangyuan Wang[1], Harshwardhan M. Thaker[2], Mahesh M. Mansukhani[2], Howard B. Lieberman[1]. [1]Columbia University College of Physician and Surgeons, Center for Radiology Research, New York, NY, USA, [2]Columbia University, College of Physician and Surgeons, Department of Pathology, New York, NY, USA.

HRAD9 was identified as a human gene homologous to yeast S. pombe rad9. Based on functions in DNA damage sensing, cell cycle checkpoints, apoptosis and genomic stability, we predict that it could influence carcinogenesis. Therefore, we examined the link between HRAD9 and prostate cancer to assess functional relationships. We found that four prostate cancer cell lines examined, PC-3, LNCaP, CWR22 and DU145, had very high HRAD9 expression compared to normal prostate cells (PrEC). Human prostate cancer tissues also had high levels of HRAD9 protein, compared to normal prostate tissue. One hundred and fifty-three prostate tumor samples with HRAD9 staining in nuclei were detected out of 339 examined, although HRAD9 expression levels and staining intensities varied from sample to sample. Only two normal prostate thin sections showed very weak HRAD9 staining out of 52 tested. The percentage of positive HRAD9 protein immunostaining is 45.1% for tumor sections and 3.8% for normal tissue controls. We also found that intensity of HRAD9 protein immunostaining depended upon tumor stage_i.e., the higher stages (III and IV) showed more intense HRAD9 staining. Further analyses indicated that the HRAD9 gene was amplified in one cancer cell line, and another one as well as two independent primary tumor tissues tested are associated with DNA hypermethylation in the intron 2 transcription silencer region of HRAD9. Furthermore, in vivo studies demonstrated that three prostate cancer cell populations (PC-3, DU145 and CWR22) induced tumors when injected subcutis into nude mice, but normal prostate cells (PrEC and RWPE-1) did not. HRAD9 siRNA was stably introduced into the cancer cell lines, and they were examined for reduction in HRAD9 protein levels. The degree with which siRNA reduced HRAD9 protein levels

varied from cell line to cell line. Importantly, there was a strong correlation between the degree of HRAD9 reduction and ability to form tumors in nude mice. The lower the levels of HRAD9 the less able were the cells to form tumors. In fact, tumors did not form 5 months post-injection of one of the cell lines into nude mice. These results indicate that HRAD9 plays an important role in prostate cancer, and at a minimum could serve as a biomarker for the disease. Further studies should indicate whether HRAD9 could serve as a novel target for therapy.

(PS3136) The "Cosmic Silence" experiment: on the potential adaptive role of environmental background radiation. Massimo Pinto[1,2], Francesca Antonelli[1,2], Fernanda Amicarelli[3], Marco Balata[4], Mauro Belli[2,5], Maria Cristina Carbone[1,3], Anna Maria Cimini[3], Laura Conti Devirgilis[3], Luca Ioannucci[4], Stefano Nisi[4], Orazio Sapora[6,5], Luigi Satta[7], Giustina Simone[2,5], Eugenio Sorrentino[2,5], Maria Antonella Tabocchini[2,5]. [1]Historical Museum of Physics and Research Centre "Enrico Fermi", Roma, Italy, [2]Istituto Superiore di Sanita' - Technology and Health Department, Roma, Italy, [3]Dip.to di Biologia di Base ed Applicata, Università dell'Aquila, L'Aquila, Italy, [4]INFN - Laboratori Nazionali del Gran Sasso, L'Aquila, Italy, [5]INFN - Gr.coll. Sanità, Sezione di Roma1, Roma, Italy, [6]Istituto Superiore di Sanita' - Environment and Prevention Department, Roma, Italy, [7]Dip.to di Energetica, Università di Roma "La Sapienza" e INFN- Laboratori Nazionali di Frascati, Roma, Italy.

To estimate the risk of exposure to low doses of ionizing radiation, the latest reports from the International Commission on Radiological Protection recommend a linear extrapolation from epidemiological data at mid-high to low radiation doses, therefore confirming the almost 50 year old Linear-no-threshold (LNT) model. However, low dose radiobiology is increasingly interested in phenomena that could imply a deviation from such model, like adaptive responses as well as either genotoxic or adaptive bystander effects, in cells which do not experience direct radiation exposure (bystanders). The environmental background radiation represents a source of chronic low dose rate exposure which may condition the response of living systems to acute exposure to genotoxic agents, also including ionizing radiation. To clarify this aspect is key to better understand the role of environmental background radiation in the development of life on Earth, and also to evaluate the risk of chronic occupational exposure. The national laboratories of Gran Sasso (LNGS) of the Italian Institute for Nuclear Physics (INFN) are located underneath the Gran Sasso mountain range in central Italy, offering a unique opportunity to continuously maintain cell cultures at extremely reduced levels of environmental background radiation.

Lead by earlier experimental evidence on S. Cerevisiae (L. Satta et. al. Mut Res 1995) and V79 Chinese hamster fibroblasts (L. Satta et. al. Radiat Environ Biophys 2002), which reported on the progressive disappearance of the adaptive response under reduced environmental background radiation conditions, Cosmic Silence aims at measuring the biological response of human lymphoblastoid cells TK6 to acute exposures to low LET ionizing radiation, in cultures that are maintained in parallel in the underground LNGS laboratories and in Rome. Any potential variations of adaptive effects under different conditions of chronic environmental background radiation are being evaluated at fixed time intervals from the establishment of the cell cultures, which are being maintained under rigorously identical conditions, with the only exception of background radiation levels. Mutations at two independent genetic loci, DNA damage, apoptosis, and levels of enzymes related to oxidative stress are being measured

(PS3137) Proteomic analysis of low dose arsenic and ionizing radiation exposure on keratinocytes. Susanne R. Berglund[1], Alison R. Santana[1], Dan Li[2], David M. Rocke[2], Zelanna Goldberg[1]. [1]UC Davis, Sacramento, CA, USA, [2]UC Davis, Davis, CA, USA.

Introduction

Low level arsenic and low dose ionizing radiation (IR) are both environmental toxicants. While there are data examining the human health effects of either toxicant individually, there are no data in the literature regarding possible interactions at these low doses. Arsenic toxicity may be affected at low dose exposure levels by concurrent ionizing radiation especially in light of their known carcinogenic actions individually at higher doses. To address this possibility and the information gap in the toxicology literature, the studies described herein were undertaken. Using a proteomics approach in a human keratinocyte model to mimic human skin exposure, we examined the interaction of IR and arsenic

Methods

Keratinocytes received either 0 or 2 μM sodium arsenite and 24 hours later were irradiated with doses of either 0, 1 or 10 cGy. Protein was isolated one or four days post irradiation. Protein was separated using two-dimensional gel electrophoresis. Gels were fixed and silver stained. ANOVA was used to identify differentially expressed proteins. Proteins were sequenced by Nevada Proteomics. Western blotting was performed on selected proteins. β-actin was used as loading control. Western blots were performed in triplicate

Results

The image analysis revealed 2002 distinct spots of which 223 were determined by ANOVA to be differentially expressed. Five proteins were differentially expressed at the day one timepoint. These include: LDH A, PDI, HSP 27, CAM 1 and cyclophilin A. At four days post exposure, 8 proteins had altered expression including annexin XI, calgranulin B, E-FABP, α-enolase, profilin, pyruvate kinase M2, cytokeratin 1, and HINT 1. Immunoblotting confirmed the changes in expression in eight of the identified proteins

Conclusion

It is known that arsenic exposure alters the expression of HSPs, annexin 1 and PDI. Ionizing radiation has been shown to alter expression of HSP 60, annexin 1, PDI and α-enolase. This study confirms overlapping responses of PDI and α -enolase in the combined exposures. These overlapping proteomic responses are consistent with the hypothesis that cellular defenses to these toxicants have at least partially overlapping response pathways, which provides a mechanistic rationale to how two sublethal exposures may combine into a lethal or carcinogenic response

(PS3138) Patched1 and DNA-repair deficiencies in radiation induced cerebellar tumors. Simonetta Pazzaglia[1], Mirella Tanori[1], Emanuela Pasquali[2], Mariateresa Mancuso[1], Simona Leonardi[1], Simonetta Rebessi[1], Vincenzo Di Majo[1], Roland Kanaar[3], Leon HF Mullenders[4], Anna Saran[1]. [1]ENEA, Ente per le Nuove Tecnologie, l'Energia e l'Ambiente, Rome, Italy, [2]INT, Istituto Nazionale Tumori, Milan, Italy, [3]EMC, Erasmus Medical Center, Rotterdam, The Netherlands, [4]LUMC, Leiden University Medical Center, Leiden, The Netherlands.

Ionizing radiation is an established risk factor for brain tumors, yet quantitative information on the long-term risk of different types of brain tumors is sparse. As a genetic risk factor, inactivation of one Patched (PTC) allele predisposes to spontaneous medulloblastoma development. To further understand the role of radiation in the etiology of CNS tumors, suitable animal models are required. Heterozygous Ptc1 mice are prone to medulloblastoma development, and exposure of newborn Ptc1+/- mice to ionizing radiation dramatically increases the frequency and shortens the latency of medulloblastoma. This shows strong analogies with the human nervous system in which increased tumor risk after high-dose radiation therapy during infancy and childhood has been reported.

Recent evidence also indicates that brain tumors, besides arising in association with Ptc1 deficiency, may be linked to defects in DNA damage repair processes, as shown by the finding that various combinations of targeted deletions in genes controlling cell cycle checkpoints, apoptosis and DNA repair result in murine medulloblastoma. This highlights the prominent role of defective response to DNA damage in cancer predisposition.

The observation that radiation-induced medulloblastomas in Ptc1+/- mice are characterized by loss of the normal remaining Ptc1 allele suggests that gene conversion may be a key event in medulloblastoma development. Recently, a prominent role of homologous recombination (HR) as a pathway for LOH and initiation of neoplastic growth in proliferating cells - especially in combination with DNA-damage - has been proposed.

To investigate the contribution of HR repair pathways to medulloblastoma development after DNA damage induced by

radiation, Ptc1 heterozygous knock-out mice have been crossed to mice with defective HR (Rad54-/-), and the double mutant progeny has been irradiated. Analysis of modifying effects of defective DNA damage response on radiogenic medulloblastoma development in Ptc1 mutant mice will be presented and discussed.

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(PS3139) Epigenetic signature of radiation exposure in the male germline. Jan Tamminga, Olga Kovalchuk. University of Lethbridge, Lethbridge, AB, Canada.

The paternal genome has been shown to be more susceptible to genotoxic stress than the maternal genome. Consequently, pre-conception paternal ionizing radiation (IR) exposure poses a serious threat to the progeny of irradiated fathers (1,2). The molecular mechanisms behind this phenomenon are unknown; however, the epigenome has been implicated in transgenerational genome instability (1,2). Notwithstanding, IR-induced epigenetic changes in the male germ cells have never been studied.

Epigenetic changes include DNA methylation, histone modification and small regulatory RNA-associated effects. Cytosine DNA methylation is the most widely studied epigenetic mechanism. It is crucially important for normal development and proper maintenance of genome stability.

DNA methylation patterns are regulated in male germ cells by two paralogous proteins, BORIS and CTCF (3). Expression of BORIS, followed by CTCF expression, takes place with the erasure and re-establishment of methylation marks, respectively.

We hypothesized that BORIS and CTCF may play important roles in the epigenetic programming in the male germline, and their dysregulation upon IR exposure may lead to the epigenetic dysregulation in the progeny.

Using a mouse model, IR exposure was assayed for its affect on BORIS and CTCF expression, and for the global DNA methylation levels in testes. Animals received the whole body X-ray exposure of 2.5Gy. In our previous studies this dose led to significant deleterious effects in the progeny (2). Sham treated animals served as controls. To dissect the IR effects on mature sperm cells or spermatogonia, the animals were sacrificed 4 or 56 days after exposure.

We found that whole body irradiation resulted in the significant loss of global DNA methylation, paralleled by a significant increase in the expression of BORIS 4 days after exposure. No statistically significant changes were noted 56 days after exposure.

Alongside with changed methylation levels we also observed altered levels of small regulatory RNAs, specifically microRNAs in the testes of exposed animals. The cellular and developmental repercussions of the observed epigenetic changes will be discussed.

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(PS3140) Differential effects of low and high dose ionizing radiation on gene networks and pathways in human epithelial cells. Sanchita Bhattacharya[1], L Ding[2], Mary Helen Barcellos-Hoff[1], AJ Wyrobek[1]. [1]Lawrence Berkeley National Lab, Berkeley, CA, USA, [2]UT South Western Medical Center, Dallas, TX, USA.

Gene expression networks induced by different radiation exposures in epithelial cells may provide insight into their sensitivity to radiation carcinogenesis. We used oligonucleotide microarrays to investigate the effects of varying x-ray doses on transcript expression for ~22,000 genes in growth-arrested, non-malignant human mammary epithelial cells (HMEC) cell line 184A1. Bioinformatics analyses identified gene networks and pathways that were differentially expressed in HMEC after low-dose (10 and 30 cGy) versus high-dose exposures (1 and 3 Gy). Genes that showed modulated transcript levels at 4 hours after low-dose exposure fell into four major gene networks ($p<10^{-20}$) and the high-dose genes fell into 7 networks ($p<10^{-15}$), each with one or more nodal genes. MYC and EGR1 nodal genes were prominent in both low- and high-dose networks, but seemed to be associated with different biological pathways in the two dose groups. The MYC and EGR1 networks were highly associated with TGF-β and Wnt/B-catenin signaling for the low-dose ($p<0.001$) but not the high-dose genes. However, p38MAPK and IL-6 signaling and cell cycle:G1/S checkpoint regulation were enriched among both low- and high-dose genes ($p<0.05$). We then compared our HMEC findings to those from a published data set for irradiated human umbilical vein epithelial cells (HUVEC; Lanza et al. J. Radiat. Res. 46:265, 2005). Comparison of radiation induced transcript expression in HMEC and HUVEC confirmed the importance of TGF-β signaling after low doses and cell cycle:G1/S checkpoint regulation after high-dose exposures in both cell types ($p<0.05$). Irradiated HMEC and HUVEC also showed several network and pathway differences that may be due to cell-type or study-design differences. Our study of HMEC and HUVEC suggests that (a) different biological pathways are recruited after low-dose exposures of epithelial cells even when nodal genes are similar, and that (b) TGF-β signaling is a consistent low-dose response in both cell types. Further studies of radiation-induced transcript and associated protein changes may help us to understand the biological pathways by which epithelial cells differ from other cell types in their radiation response and cancer risks. *Supported by NASA Specialized Center of Research.*

(PS3141) Effect of metalloporphyrin antioxidant to reduce the radiation population damage in rat retinal following proton irradiation: A pilot study. Xiao Wen Mao[1], Tsehay Mckomen[1], Nathan Lindsay[1], James Crapo[2], John Archambeau[1]. [1]Loma Linda University, Loma Linda, CA, USA, [2]National Jewish Medical and Research Center, Denver, CO, USA.

Purpose: To evaluate the histology and quantify the retinal cell population and capillaries changes following proton irradiation and an antioxidant metalloporphyrin.

Background: A major concern among clinicians for treating eye disease with radiation is that the dose tolerance of normal tissues limits the total dose that can be delivered. The search for agents that protect of cells against radiation is important in radiation therapy as a therapeutic radioprotector and also it is important for those at risk by virtue of environmental exposure.

Material and methods: 75 rats were treated with metalloporphyrin at 0.5ug/injection to one eye an hour before irradiation. Proton irradiation was given to one eye at dose of 8Gy and 28Gy. The opposite eye was used as control. Rats were sacrifice every three months.

Results: Observation up to date showed all animals developed moist reaction following radiation. By week 3, 11 out 12 rats showed healed moist reaction in AEOL10113 treated group, 0 out of 12 rats from radiation only group has any sign of healing. By week 6, all irradiated rats developed cataract.100% metalloporphyrin treated rats has 1^0 cataract while proton at 28Gy only have 75% in 3^0 and 25% in 1^0 of cataract formation. By 3 month following radiation, there was significantly loss of photoreceptor outer and inner nuclear layer in radiation only group (28Gy) compared to control and metalloporphyrin treated group.

Conclusion: Metalloporphyrin antioxidant significantly shortened the time for healing from moist reaction and reduced severity of cataract formation. These results demonstrate that metalloporphyrin antioxidant may play an important role regulating the oxidative damage induced by proton radiation.

(PS3142) Radiation-induced injury localized to the rat lung: changes in pulmonary function. Swarajit N. Ghosh[1], Marylou L. Mäder[2], John E. Moulder[2], Elizabeth R. Jacobs[1], Timothy Lowry[1], Meetha Medhora[1]. [1]Medicine, Medical College of Wisconsin, Milwaukee, WI, USA, [2]Radiation Biology, Medical College of Wisconsin, Milwaukee, WI, USA.

In the context of a terrorist attack, partial or whole body, non lethal exposure is expected at a dose equivalent of 12 Sv or below. We have developed a "terrorism relevant" animal model to characterize lung injury, free of injury to other organs. We irradiated rats (WAG/Rij/MCW) with a single, unfractionated dose

of 5 or 10 Gray to the thorax. To evaluate radiation-induced pulmonary injuries we measured respiratory rate and oxygen saturation in a non-invasive manner. Lung tissue was examined histologically and Clara cell secretory protein (CC10, a marker of lung injury) as well as total protein present in bronchioalveolar lavage fluid (BALF), were measured after sacrificing the rats. No significant evidence of radiation damage was seen in the 5 Gy cohort upto one year post irradiation and until 8 weeks in the 10 Gy group. Most (75%) of animals examined histologically and belonging to the 10 Gy cohort exhibited transient perivascular edema evident at 4 weeks. At 5 months and 1 year, 50% of the animals examined exhibited interstitial thickening and fibrosis. We observed a significant elevation in respiratory frequency in animals irradiated with a dose of 10 Gy compared to controls at 7 weeks (C:139 ±6.88, I:170.23 ±11.75, p= .047), 11 weeks (C:139.39±10.23, I:171.05±6.45, p= .026) and 12 weeks (C:150.90±4.3, I:191.20±6.33, p= .001), with no differences evident at 5 months and 1 year. Exposure to hypoxia (12% O_2, 10 min), caused a significant reduction in oxygen saturation in animals exposed to 10 Gy at 13 weeks (C: 86.1±1.61, n=11; I: 80.82±0.85, n=10; p= .004). At 5 months and 1 year, oxygen saturation values were similar in the irradiated and un-irradiated groups. A significant reduction in CC10 levels (n=6 each, p< .001) was observed at 4 weeks, while no such difference was noted at 5 months and 1 year. Total BALF protein concentration was significantly elevated at 4 weeks (n=7 each, p= .001) and at 8 weeks (n=8 each, p= .046) with no such elevation noted at 5 months and 1 year. In summary, irradiation with 10 Gy increases breathing rate between 6–12 weeks, increases total protein and decreases CC10 levels at 4 weeks in BALF with considerable recovery of these functions by 5 months. Using this model we are testing therapeutic agents to mitigate and treat these injuries.

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(PS3143) Evaluation of the radioprotective effects of genistein: survival, hematology, cytokines, and behavior. M R. Landauer[1], V Srinivasan[1], V K. Singh[1], M H. Whitnall[1], T A. Davis[2], S R. Mog[1]. [1]Armed Forces Radiobiology Research Institute, Bethesda, MD, USA, [2]Naval Medical Research Center, Silver Spring, MD, USA.

Radioprotective compounds have applications in clinical oncology, space travel, radiation site cleanup, radiological terrorism, and military scenarios. The ideal radioprotector would be non-toxic, increase survival, and would not degrade performance. Soy phytoestrogens have strong antioxidant activity and have been reported to have many beneficial health effects, including a reduction in bone loss and the incidence of some types of cancer. The most plentiful isoflavone from soybeans is genistein (4', 5, 7-trihydroxy-flavone). In the present studies, the radioprotective and behavioral effects of an acute administration of genistein were investigated in adult CD2F1 male mice. Animals were administered a single subcutaneous (SC) dose of genistein (3–400 mg/kg) either 24, 1, or 0.5 hr before or 0.5 or 1 hr after a lethal dose (9.5 Gy @ 0.6 Gy/min) of Co-60 gamma radiation. For mice treated 24 hr before irradiation, there was a significant increase in 30-day survival for animals receiving genistein doses of 25 to 400 mg/kg compared to the vehicle control group, with the optimal dose being 200 mg/kg. In contrast, the survival rates of mice given genistein at all other time points were not significantly different from those of the vehicle-treated animals. The improvement in survival was related to accelerated neutrophil and platelet recovery resulting from earlier and more pronounced multilineage hematopoietic progenitor cell recovery in the bone marrow compartment. In a separate experiment, mice were given vehicle or 200 mg/kg genistein SC 24 hr before sublethal (7 Gy) irradiation and serum cytokine levels were determined. Cytokines were found to be significantly elevated in genistein-treated mice at 4 hr (IL-6) and 4 and 24 hr (G-CSF) after irradiation. The behavioral toxicity of genistein in non-irradiated mice administered a single SC injection of vehicle or genistein (100, 200, or 400 mg/kg) was also evaluated. At these genistein doses, there were no adverse effects on locomotor activity, grip strength, testes weight, or histopathology. These results demonstrate that a single SC administration of genistein administered 24 hr before irradiation significantly increases survival in mice at doses that do not result in performance decrement.

(PS3144) Small molecule inhibitors of glycogen synthase kinase-3 beta modulate radioprotection in developing hippocampus. Dinesh Kumar Thotala, Dennis E. Hallahan, Eugenia M. Yazlovitskaya. Vanderbilt University, Nashville, TN, USA.

Neurocognitive deficits are devastating consequences in cancer patients undergoing cranial irradiation due to the damage of hippocampus. In the present study we demonstrate that small molecule inhibitors of Glycogen Synthase Kinase-3 beta (GSK-3β) protect hippocampal neurons from radiation-induced apoptosis. In pharmacodynemic studies, we utilized GSK-3β down-stream targets β-catenin and cyclin D1 to monitor GSK-3β activity. Based on the accumulation of β-catenin in mouse hippocampal neurons HT-22, the optimal concentrations of GSK-3β inhibitors SB415286, SB216763, AR-AO14418 and BIO were 25μM, 10μM, 1μM and 1μM respectively. The optimal time of treatment was 16 hours for all four inhibitors. However, when HT-22 cells were treated with GSK-3β inhibitors and 3 Gy of radiation, only pretreatment with SB415286 showed increased accumulation of both β-catenin and cyclin D1. To study effect of SB415286 on viability of irradiated HT-22 neurons, we performed clonogenic assay. Pretreatment of HT-22 with 25μM SB415286 before irradiation showed statistically significant increase in cell survival as compared to cells treated with radiation alone. To confirm the specificity of this inhibitor we overexpresed wild type (WT) or kinase inactive (KI) GSK-3β using a bicistronic vector. In clonogenic studies we observed statistically significant increase in survival of cells expressing KI-GSK-3β compared to WT-GSK-3β or vector control. To elucidate the mechanism of this increased survival we monitored for program cell death by Annexin V or DAPI staining. In both approaches, inhibition of GSK-3β either genetically with KI-GSK-3β or chemically with SB415286 resulted in a 2-fold decrease in apoptosis as compared to irradiated HT-22 cells expressing WT-GSK-3β or vector control. The protective effect of GSK-3β inhibition was also demonstrated in irradiated mice. Seven-days old C57BL6J pups were treated with SB415286 (1mg/Kg) followed by irradiation with 7 Gy. TUNEL staining revealed that SB415286 pretreatment protected hippocampal neurons from radiation-induced apoptosis. In summary, small molecule inhibitors of GSK-3β protect irradiated hippocampal neurons from apoptosis and could be developed as neuroprotectors to prevent radiation-induced damage.

(PS3145) Radioprotection of normal lung tissue by two manganese porphyrin superoxide dismutase mimics. Benjamin M. Gauter-Fleckenstein[1,2], Katharina C. Fleckenstein[1,2], Zahid N. Rabbani[1], Ines Batinic-Haberle[1], Zeljko Vujaskovic[1]. [1]Duke University Medical School, Durham, NC, USA, [2]Heidelberg University, Mannheim, Germany.

Introduction:

Chronic oxidative/nitroxidative stress is associated with development of radiation (RT) induced lung injury. MnTE-2-PyP^{5+} is a catalytic Manganese Superoxide Dismutase mimic, shown to protect lungs from RT induced injury by reducing oxidative/nitroxidative stress.

We compare MnTE-2-PyP^{5+} with newly synthesized MnTnHex-2-PyP^{5+} and hypothesize that the new compound will be more effective due to its higher lipophilic properties.

Methods:

Fisher rats were irradiated to their right hemithorax with a single dose of 28 Gy. Irradiated animals were treated with PBS, MnTE-2-PyP^{5+} (1 - 3 - 6 mg/kg), and MnTnHex-2-PyP^{5+} (0.3 - 0.6 - 1 mg/kg). Sham irradiated animals served as a control. Daily s.c. injections started 2 hours after RT and continued for two weeks. Animals were sacrificed after 16 weeks. Endpoints were: body weight, breathing frequencies, histopathology (H&E and Masson's trichrome staining), and immunohistochemistry (8-OHdG, ED-1, TGF-β, and VEGF).

Results:

After initial decrease in bodyweight after RT, there was no significant difference in bodyweight among treatment groups.

A significant radioprotective effect, as measured by breathing frequencies, was observed for animals treated with MnTE-2-PyP^{5+} (6 mg/kg) and MnTnHex-PyP^{5+} (0.3 mg/kg).

This effect was confirmed by histopathology studies.

Reduction in oxidative stress (8-OHdG) was also observed in the same treatment groups. TGF-β was significantly lower in MnTE-2-PyP^{5+} (3 and 6 mg/kg) and MnTnHex-2-PyP^{5+} (0.3 mg/kg) groups. VEGF was significantly lower only in the MnTE-2-PyP^{5+} (6 mg/kg) group. However, no significant effect was seen on macrophage activation (ED-1).

Conclusion:

MnTE-2-PyP^{5+} at dose of 6 mg/kg and MnTnHex-2-PyP^{5+} at dose of 0.3 mg/kg given daily s.c. for two weeks were able to reduce RT-induced lung injury measured by functional, histopathology, and immunohistochemistry studies. However, MnTE-2-PyP^{5+} appears to be more effective.

(PS3146) LPS pretreatment changes the activation of irradiation response pathways in small intestinal crypt cells. Fengchao Wang, Yongping Su, Yu Ning, Junping Wang, Xinze Ran. Institute of Combined Injury, State Key Laboratory of Trauma, Burns and Combined Injury,College of Preventive Medicine, Third Military Medical University, Chongqing, China.

Background and objective: Lipopolysaccharide(LPS) is though to play a radioprotective role in the mouse small intestine,which is depending on the prostaglandin E2 production from crypt microenvironment. But the intrinsic events happened in the crypt cells remain unclear; and it is difficult to explain why LPS pretreatment causes a decrease of cell apoptosis during early stages after irradiation. Our aims were to investigate the changes of activation of apoptosis and survival related stress pathways induced by irradiation after pretreated by LPS

Methods: Mice (C57BL/6, male, 18–22g) were treated with LPS (5mg/kg, ip) or vehicle for 4h and then exposed to total body irradiation (TBI) using ^{60}Co γ-rays at dose of 12 Gy. The apoptosis of crypt cells was tested by TUNEL in situ; the intestinal crypt cells were isolated from the small intestine of pretreated and control mice at 0h, 1h and 4h(n=4), and the activation of AKT, extracellular signal-regulated kinases(ERK), c-Jun N-terminal kinase(JNK), p53 and Nuclear factor -κB(NF-κB) pathways were examined by Western blots and EMSA

Results: TBI induced apoptosis in the intestinal crypt cells at 4h was markedly reduced by pretreatment with LPS. Meanwhile, at 1h after TBI, phorsphorylation of Akt and JNK was increased and the activation of ERK was modestly decreased by LPS pretreatment. The total protein levels of AKT, phosphatase and tensin homologue deleted on chromosome 10 (PTEN), ERK and JNK remained relative stable in two groups at different time points. Moreover, LPS pretreatment down-regulated p53 level at 1h after TBI. LPS pretreatment for 4h induced higher expression of inhibitor of κB α (I-κBα) in the crypt cells, but it did not prevent the activation of NF-κB pathway at 1h after TBI exposure followed

Conclusions: LPS pretreatment has effect on the activation of irradiation response pathways, which may contribute to its radioprotective role in the mouse small intestine

(PS3147) EsA protects the lung against radiation-induced peumonitis and fibrosis. Shanmin Yang, Hengshan Zhang, Wei Wang, Weimin Sun, Mei Zhang, Chaomei Liu, Yanghua Yi, Zhenyu Xiao, Paul Okunieff, Lurong Zhang. University of Rochester Medical Center, Rochester, NY, USA.

Radiation-induced peumonitis and fibrosis limit the treatment of patients with thoracic malignancy including lung, esophagus, breast cancer and lymphoma. Currently, no satisfactory regimen can effectively alleviate this pathological progression after chest radiation. Recently, we found that Esculentoside A (EsA, a saponin isolated from *Phytolacca esculenta)* has multifactorial anti-inflammatory activity and is a COX-2 inhibitor. The effect of EsA on radiation-induced peumonitis and fibrosis was studied.

The fibrosis-prone C57BL/6 mouse strain was used with thoracic irradiation of 12.5 Gy. The animals were treated with EsA (10 mg/kg) every other day for 6 months. Pathological analysis of lung was conducted at 6, 48 hr, 4, 6, 12 and 42 weeks post-irradiation.

The data showed that pneumonitis (evidenced by increased infiltration of inflammatory cells and exudates) and fibrosis (evidenced by increased fibroblast, collagen deposit, and thickened alveolar walls) were significantly reduced in mice administered EsA in comparison to the control mice. Respiratory rate and pulmonary compliance were measured 42 weeks after thoracic radiation. Mice treated with EsA had a reduced respiratory rate to a near normal level ($p<0.05$). Consistent with the respiratory rate results, lung compliance measured by plethysmograph at 42 weeks after irradiation was much improved with EsA compared to control irradiated mice. The molecular mechanism studies indicated that the EsA inhibit the production of several major cytokine and chemokines (such as IL-6, IL-1, TNFα, MCP-1, VEGF and TGFβ) both *in vitro* and in vivo as assessed by antibody array and ELISA.

Our data suggest that early inhibition of pneumonitis by EsA was associated with reduction of late fibrosis of the irradiated lung. EsA and its less toxic derivative h-EsA have great promise as mitigators of pulmonary radiation toxicity.

(PS3148) Radioprotective effects of ginsenoside rg1 on intestinal epithelial cells *in vitro* and *in vivo*. Xing Cui, Makoto Akashi. National Institute of Radiological Sciences, Chiba, Japan.

Exposure of whole body to high dose of radiation results in gastrointestinal injury. Ginsenoside Rg1 (Rg1), a steroid saponin that extracted from Panax ginseng C.A. Meyer, is known to have many pharmaceutical effects *in vitro* and *in vivo*. In the present study, we elucidated the molecular mechanisms of the radioprotective action of Rg1 in intestine *in vitro* and *in vivo*. Radiation with γ ray at a dose of 20 Gy induced apoptosis in the IEC-6 rat intestinal epithelial cells. Analyses using the Hoechst staining and TdT-mediated dUTP digoxigenin nick end labeling (TUNEL) assay showed that Rg1 suppressed radiation-induced apoptosis of these cells in a dose-dependent manner. When these cells irradiated in the presence of an inhibitor of either MAP kinase kinase (MEK, PD98059) or p38 mitogen-activated protein kinase (MAPK) (SB230580), and phosphoinositide-3 kinase (PI3K, LY294002) pathway, PD98059 or SB230580 significantly decreased the number of apoptotic cells, whereas LY294002 attenuated the anti-apoptotic effects of Rg1. Radiation activated phosphorylation of extracellular signal-regulated protein kinase (ERK), p38, and Akt proteins. Treatment with Rg1 before and after irradiation inhibited phosphorylation of ERK and p38 MAPK, but enhanced phosphorylation of Akt. Rg1 also decreased levels of Bax and, caspase 3, and increased Bcl-2 expression. Studies of immunohistochemistry found that Rg1 significantly improved recovery of crypt architecture and also reduced the number of apoptotic bodies in the crypt of irradiated mice. In conclusion, Rg1 protects and rescues intestinal injuries from irradiation-induced apoptosis *in vitro* and *in vivo* through activation of the PI3K/Akt pathways and, via inhibition of MEK/p38 MAPK pathways. These data suggest that Rg1 may play important role in the intestinal epithelium injury caused by radiation accidents or cancer therapy.

(PS3149) A gastrointestinal radioprotector, FGF-P, normalizes circulating digestive protein levels after Radiation. Mei Zhang[1], Weimin Sun[1], Louis Pena[2], Jianjun Wang[1], Shanmin Yang[1], Hengshan Zhang[1], Wei Wang[1], Chaomei Liu[1], Steven Swarts[1], Paul Okunieff[1], Lurong Zhang[1]. [1]Department of Radiation Oncology, Rochester, NY, USA, [2]Brookhaven National Laboratory, Upton, NY, USA.

The gastrointestinal (GI) tract develops the early symptoms and is the most sensitive organ to subtotal body radiation (IR). Although the crypt assay is a reliable method for quantitative measure of the extent of GI damage, the need for full dose response analysis and time consuming histological evaluation limits its use as a screen for gut radioprotectors. In addition, it cannot be used in humans. The goal of this study was to define a set of non-invasive indices that might sensitively reflect the pathphysiological GI alterations after exposure to ionizing radiation (IR), which might be used to determine the effectiveness of mitigation agents against GI damage.

BALB/c mice were irradiated with one leg shielded. Using this technique, the deaths are due to GI syndrome and not bone marrow syndrome. An IR dose of 10.5–12 Gy (LD_{80-100}) was used. Mice were treated with vehicle alone or synthetic peptide of FGF2 (FGF-P). Some mice were sacrificed 3.5 days after IR for crypt assay, and others were observed for long-term survival. As an initial screen we measured circulating levels of secretin, insulin, amylase and glucose. We found that: 1) the plasma secretin (mainly secreted by gut epithelium) decreased in an IR-dose dependent manner. FGF-P prevented the drop of secretin; 2) the plasma insulin decreased beginning on day 1, reaching a nadir on day 3.5–4 and slowly returned towards normal in surviving mice on day 9; 3) decreases in amylase were observable after IR doses as low as 2 Gy and FGF-P effectively ameliorated the decline of amylase; 4) IR also reduced plasma glucose beginning one day after IR and persisting for 9 days. This too was improved with FGF-P; 5) the proliferating crypts increased in mice treated with FGF-P; 6) FGF-P rescued mice from an otherwise lethal dose of IR. Importantly, there are positive correlation among the circulating digestive protein levels, crypt counts and survival rate. Our data suggest that the plasma level of secretin, insulin, amylase and glucose might be utilized as a non-invasive index for screening agents as gut radioprotectors. This index might also prove useful for monitoring the efficacy of treatments after radiological exposures in a clinical setting.

(PS3150) Mitigation and Treatment of Radiation-Induced Lung Damage by Genistein. Andrea Para[1], Victoria Calveley[1], Aimee Langan[1], Ivan Yeung[1], Jake Van Dyk[2], Richard P. Hill[1]. [1]University of Toronto, Toronto, ON, Canada, [2]London Regional Cancer Center, London, ON, Canada.

Introduction: The main side effects of radiotherapy in the lung are pneumonitis and fibrosis. The severity of these effects depends upon the dose, volume and region irradiated and mechanisms of the damage involve a complex interaction between many cell types. We hypothesis that inflammatory cytokines and the subsequent production of reactive oxygen species (ROS) play a key role in radiation induced lung injury. Previously, we have shown that Genistein is effective in preventing damage following single doses of radiation. The purpose of this study is to investigate protection by this agent with fractionated doses of radiation.

Methods: We irradiated mice with a dose of 9 fractions of 3.1Gy over 30 days and put them on a Genistein diet (equivalent to ~10 mg/Kg) just prior to the first fraction. The animals are maintained on the diet through the subsequent analysis period. Damage is being assessed over 28 weeks in cells obtained from the lungs by a cytokinesis block micronucleus (MN) assay and by monitoring changes in breathing rate and histology. The potential of the Genistein diet to protect tumor is also being assessed using a colony assay to determine cell survival following *in situ* irradiation.

Results: Initial results demonstrate that Genistein causes about a 50% reduction in the MN damage observed during the fractionated treatment but at this time there is no significant difference in the histology of the lungs or breathing rate changes. Further data is being accumulated as the experiment progresses out to 28 weeks as side effects develop. Using the KHT fibrosarcoma model we have demonstrated no tumor protection by Genistein treatment following a single radiation dose and fractionated irradiation. We are currently studying tumor response using H460 lung carcinoma cells.

Conclusions: The results suggest that Genistein can protect lung cells against damage caused by irradiation but that such protection does not extend to tumor cells growing in the lung.

(PS3151) Induction of manganese superoxide dismutase (SOD2) activity in normal and tumor tissues by amifostine. Jeffrey S. Murley[1], Yasushi Kataoka[1], Kenneth L. Baker[1], Mitchell C. Coleman[2], Douglas R. Spitz[2], David J. Grdina[1]. [1]The University of Chicago, Chicago, IL, USA, [2]The University of Iowa, Iowa City, IA, USA.

Amifostine is the only agent currently approved by the FDA for use in the protection of normal tissues in patients being treated with radiation. Its radioprotective effectiveness is primarily due to the ability of its active free thiol (WR1065) to scavenge radiation-induced free radicals. We recently identified an additional mechanism by which amifostine can protect cells which we termed the delayed radioprotective effect. The mechanism for this protection involves its ability to significantly elevate active SOD2 enzyme levels. Protection against cell killing is observed for cells irradiated 24 h after drug treatment, the timeframe when the maximum elevation in SOD2 levels is observed. A potential concern regarding the use of amifostine is the possibility that in addition to protecting normal tissues, treatment might also result in tumor protection. We have demonstrated under *in vitro* conditions that WR1065 can induce the delayed radioprotective effect equally in both normal and tumor cells. To characterize the effects of amifostine treatment on SOD2 activity in normal and tumor tissues, mice were injected with 400 mg/kg amifostine i.p., sacrificed 24 h or 32 h later, and six normal tissue organs or tumor removed. Increases in SOD2 activity in the lung, pancreas and spleen were observed 24 h after amifostine injection, but only the increase in the spleen was significant ($P = 0.005$). No change was observed in the heart or small intestine, while a significant decrease was observed in the liver ($P = 0.008$). SOD2 activity remained elevated in the lung, pancreas and spleen, although not significantly, 32 h after amifostine treatment, while significant decreases were observed in the liver ($P = 0.048$) and small intestine ($P = 0.008$). SOD2 activity was also measured in SA-NH tumors grown in the hind leg of each animal. Compared to the normal tissues, the SOD2 activity in the tumor was significantly less, as was expected. A significant increase in SOD2 activity in the tumors was observed 24 h after amifostine injection ($P = 0.021$), that was the largest relative increase of all the tissues sampled. SOD2 activity in the tumors returned to control levels 32 h after amifostine treatment. This work was supported by NIH/NCI grant R01 CA99005 and DOE grant DE-FG02-05ER64086 (D.J.G.), and NIH CCSG P30-CA086862 (D.R.S.).

(PS3152) Mitigation of radiation-induced skin injury by AAV2-mediated MnSOD gene therapy. Shiqing Yan, Stephen L. Brown, Andrew Kolozsvary, Svend O. Freytag, Jae Ho Kim. Henry Ford Health System, Detroit, MI, USA.

Introduction: Mitigation of radiation-induced tissue injury by a novel AAV2-mediated MnSOD gene therapy approach was tested using a skin model since reactive oxygen species (ROS) has been shown to be involved in radiation-induced normal tissue injury and manganese superoxide dismutase (MnSOD) is a ROS scavenging agent used to reduce the ROS-mediated tissue injury. **Materials and Methods:** Adeno-associated virus type 2 (AAV2) construct containing both human MnSOD and the reporter gene GFP was generated from an AAV vector pAAV-IRES-hrGFP. AAV2-MnSOD-hrGFP viruses expressing both human MnSOD and the reporter gene GFP were prepared by triple transfection of 293A cells yielding a viral titer of $2.6x10^{12}$ viral particles/ml. AAV2-mediated expression of GFP and MnSOD was characterized *in vitro* in HT1080 cells by fluorescence microscopy and western blot. AAV2-mediated GFP gene expression in C57BL/6j mice was imaged *in vivo* and *ex vivo* from tissue section after subcutaneous (sq) delivery of AAV2-MnSOD-hrGFP to the mice hind legs. Radiation-induced skin injury was studied using 30Gy to the hind leg followed immediately by sq administration of $2.6x10^{11}$ AAV2-MnSOD-hrGFP viral particles in C57Bl/6j mice (n=9) and by subcutaneous administration of the same volume of PBS in control mice (n=9) **Results:** AAV2-mediated gene expression was detectable *in vitro* using fluorescence microscopy and *in vivo* by a strong green fluorescence signal from the legs of C57BL/6j mice six days after the injection of AAV2-MnSOD-hrGFP. GFP was detectable at least up to thirty days after viral injection in tissue. AAV2-mediated MnSOD expression was demonstrated by both Western blot and MnSOD activity *in vitro* in HT1080 cells. Radiation-induced skin injury in C57BL/6j mouse group treated with AAV2-MnSOD-hrGFP reduced significantly ($p<0.05$) compared to that in PBS-treated mouse group quantified by a semi-quantitative injury score (Score range from 0 to 5). **Conclusion:**

AAV2-mediated MnSOD expression has the potential to mitigate radiation-induced skin injury presumably by decreasing ROS in tissue. **Acknowledgement:** This work was supported by a pilot grant (to SY) through U19 AI067734 (NIAID).

(PS3153) Does total body irradiation result in chronic oxidative stress in normal kidney? Marek Lenarczyk, Mukut Sharma, Brian L. Fish, Marcus A. Crosby, John E. Moulder. Mediacl College of Wisconsin, Milwaukee, WI, USA.

Radiation nephropathy is a late manifestation of renal injury in a significant number of people receiving total body irradiation (TBI) prior to bone marrow transplantation. It has been suggested that development and progression of radiation nephropathy are driven by chronic oxidative stress. Chronic oxidative stress is assessed by several indices including increased nucleic acid oxidation, lipid peroxidation and protein carbonylation. We hypothesized that TBI-induced chronic oxidative stress in the renal tissue would be detected by increased protein carbonylation.

WAG/Rij/MCW rats were administered TBI with X-rays at 19 Gy in 6 fractions over 3 days. Kidneys from irradiated and sham-treated control rats were collected at 1, 3, 5, 8, and 13 weeks post-TBI. We determined total protein by Lowry's method and protein carbonyls using 2, 4-dinitrophenylhydrazine. Protein carbonylation was expressed as nanomol carbonyls/mg protein. We also determined carbonylation in, a) rat kidney proteins at 1 hour to 56 days after TBI with single fraction 10 Gy X-rays and b) bovine serum albumin (BSA) after *in vitro* irradiation with up to 50 Gy of X-rays.

TBI with 19 Gy X-rays showed 40%, 56%, 9%, 8%, 3% increase in renal protein carbonyls *vs.* sham-treated control at 1, 3, 5, 8 and 13 weeks, respectively but the differences were statistically not significant. Single dose of 10 Gy X-rays also increased renal protein carbonylation at 1 hour to 56 days post-TBI but these changes were also not significantly different from the control group. *In vitro* irradiation of BSA with doses up to 50 Gy did not cause significant increase in protein carbonylation.

These results indicate that TBI does not result in persistent protein carbonylation in kidney that would be indicative of chronic oxidative stress. However, high variability among controls implies potential interference in the determination of carbonyl levels. Alternatively, it is possible that TBI-induced chronic oxidative stress would be detected as lipid peroxidation or DNA oxidation. Therefore, we are currently assessing 8-oxo-2-deoxyguanosine in the mitochondrial DNA of rat kidneys.

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(PS3154) Gene transfer of the multi-drug resistance 1 (MDR1) and manganese superoxide-dismutase (MnSOD) gene confers radioprotection on normal tissue cells. Frederik Wenz[1], Marlon R. Veldwijk[1], Patrick Maier[1], Katharina Fleckenstein[1], Stefanie Laufs[2], Wolfgang J. Zeller[2], Stefan Fruehauf[3], Carsten Herskind[1]. [1]Mannheim Medical Center, Mannheim, Germany, [2]German Cancer Research Center, Heidelberg, Germany, [3]Paracelsus Klinik, Osnabrück, Germany.

Radiotherapy is widely utilized in cancer treatment but is limited by the associated increase in apoptotic cell death of radiosensitive normal tissue cells like peripheral blood progenitor cells (PBPC) or microvascular cells. For minimizing this unwanted side effect, gene therapy could offer considerable potential for improving the overall efficacy of radiotherapy. The aim of our studies was to determine the radioprotective potential of overexpression of the MDR1 and MnSOD gene using lentiviral and adeno-associated virus 2 (AAV2)-based vectors.

MDR1: PBPC of 4 donors were transduced with the MDR1 virus (MOI 10) and irradiated with 0–8 Gy two days later. After 12 days in liquid culture under myeloid-specific differentiation conditions, the proportion of MDR1 expressing cells was determined. The proportion of MDR1-positive cells increased significantly with escalating radiation doses (e.g. 18–54% from 0–8 Gy). Irradiation of differentiated cells after 12 days of liquid culture also led to an increase of MDR1-pos. cells with escalating radiation doses (e.g. 12.5–17% from 0–8 Gy). Fractionated irradiation (3x2 Gy; 24h intervals) of MDR1-transduced PBPC cells resulted in an increase in MDR-pos. cells (e.g. 3%-8% from 0–3x2 Gy).

MnSOD: Three days after transduction of human primary lung fibroblast (>80% $MnSOD^+$), clonogenic survival was determined. Compared to transduction controls (TC), radioprotective effects were observed (SF-ratio MnSOD/TC>1; 1–6 Gy): MnSOD: 1.29 ± 0.08-fold ($p<0.01$; n=3). In line with these, lymphoblastoid TK6 cells overexpressing MnSOD (5-fold compared to TC) with or without catalase revealed an increase in SF over TCs (SF-ratio; 1–4 Gy); MnSOD: 1.92 ± 0.89-fold, MnSOD+Cat.: 4.03 ± 1.91-fold (preliminary data).

Our results indicate the radioprotective potential of MDR1 and MnSOD. Thus, gene therapeutic delivery of these genes increase the irradiation tolerance of normal tissue cells and thus may contribute to widening the therapeutic range in radiotherapy.

(PS3155) Absence of delayed negative sequelae in manganese superoxide dismutase-plasmid liposome intravenously treated protected survivors of total body irradiation. Joel S. Greenberger, Tracy Smith, James J. Schlesselman, Michael W. Epperly. University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA.

Organ specific localized administration of manganese superoxide dismutase plasmid/liposomes (MnSOD-PL) protects the rodent lung, esophagus, oral cavity, intestine and bladder from single fraction and fractionated ionizing irradiation by a mechanism dependent upon mitochondrial localized antioxidant effects. Intravenous injection of MnSOD-PL significantly increased survival of both male and female C57BL/6NHsd mice receiving the LD50/30 whole body irradiation dose of 9.5 Gy. To determine if systemic MnSOD-PL mediated improved survival at the expense of a deleterious delayed increase in development of cancer, life shortening, or neurodegenerative disease, 50 male and 50 female C57BL/6NHsd mice per group were injected intravenously with MnSOD-PL (100 μg plasmid DNA in 0.1 ml) and irradiated with control mice to 0, 1 or 9.5 Gy whole body irradiation twenty-four hours later. Moribund or dying mice were sacrificed, and examined for tumors, and bone marrow isolated stained with Wright Geimsa, and examined for abnormal hemogram. Small intestine was fixed in formalin, sectioned and examined for intestinal changes. Female mice pretreated with MnSOD-PL prior to 9.5 Gy (LD 50/30) had an increased survival over the first 30 days which is the time frame for death due to hematopoietic or intestinal damage with 90% survival compared to 58% for the control mice ($p=0.0059$). Between 30 and 330 days there was no significant change in survival rate between the MnSOD-PL and surviving irradiated control mice in either 9.5 Gy or 1.0 Gy irradiated groups. Male mice pretreated with MnSOD-PL and irradiated to 9.5 Gy had 75% survival after 30 days compared to 25% for the irradiated control mice ($p < 0.0001$). Between 30 and 170 days there was no significant change in survival rate between survivors in 9.5 or 1.0 Gy groups. There was no detectable difference in the incidence of deaths associated with tumor (thymoma), marrow, or intestinal damage. All mice are being followed until death. The data indicate, at this time, that systemic MnSOD-PL treatment is not detectably harmful to the increased numbers of surviving total body irradiated mice.

(PS3156) Radioprotection of protein by manganese(II). Elena K. Gaidamakova, Vera Y. Matrosova, Min Zhai, Michael J. Daly. USUHS, Bethesda, MD, USA.

Extreme resistance to gamma radiation and other forms of ionizing radiation (IR) in the bacterium *Deinococcus radiodurans* has been attributed to intracellular, non-enzymic manganese-dependent redox cycling processes that protect proteins from oxidation during irradiation. The absence of such protection has been proposed to explain why sensitive cells succumb to IR at doses that elicit little DNA damage. For *in vitro* and *in vivo* irradiation, we demonstrate a mechanistic link between Mn(II) ions and protection of proteins from oxidative modifications that introduce carbonyl

groups. Conditions that inhibited Mn accumulation or Mn redox cycling rendered *D. radiodurans* radiation sensitive and highly susceptible to protein oxidation. We present the case that extreme resistance in *D. radiodurans* and other Mn-accumulating bacteria is based on protein protection, which could open up new avenues for radioprotection in diverse settings.

(PS3157) Minicircle Plasmid delivery of the human manganese superoxide dismutase (MnSOD) transgene confers radioprotection to 32Dcl3 hematopoietic progenitor cells in vivo. Xichen Zhang[1], Michael W. Epperly[1], Mark A. Kay[2], Zhi-Ying Chen[2], Tracy Smith[1], Darcy Franicola[1], Joel S. Greenberger[1]. [1]University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA, [2]Department of Pediatrics, Stanford, CA, USA.

Manganese superoxide dismutase plasmid/liposomes (MnSOD-PL) delivered by intratracheal, intraesophageal, or intraoral routes in rodent models have been demonstrated to confer organ specific ionizing irradiation protection. In addition intravenous injection of MnSOD-PL protects mice from whole body irradiation. Currently a seven week phase I/II clinical trial is in progress in lung cancer patients consisting of twice weekly swallowed MnSOD-PL for protection of the esophagus from chemoradiotherapy damage. To prepare for a potential trial of systemic MnSOD-PL for radioprotection in humans, plasmid bacterial sequences were removed to diminish the immune response. We have now produced a new MnSOD-PL vector delivery system. The human MnSOD transgene attached to a CMV promoter and a poly A tail was inserted in the site between Spe I and Xho I into a eukaryotic expression cassette located in the p2ØC31 plasmid. The plasmid contains an endonuclease I-SceI gene which can be cleaved resulting in the formation of two minicircle plasmids. The smaller minicircle contains the eukaryotic expression cassette but no bacterial sequences while the larger minicircle plasmid contains the plasmid bacterial backbone. The minicircle MnSOD was purified and then co-transfected into 32Dcl3 murine hematopoietic progenitor cells with a plasmid containing the neo gene. To determine if the MnSOD transgene in the minicircle DNA retained radioprotective capacity, a minicircle MnSOD transfected cell line (mc-MnSOD), a clone transfected with a pRK5 plasmid containing the human MnSOD transgene (2C6), and parent 32Dcl3 cells were irradiated to doses ranging from 0 to 8 Gy, plated in methycellulose and incubated at 37°C for 7 days at which time colonies of greater than 50 cells were counted. The mc-MnSOD cells were more radioresistant than 32Dcl3 cells as demonstrated by an increased shoulder on the irradiation survival curve ($n = 4.8 + 0.2$ compared to $1.5 + 0.5$, respectively, $p = 0.0078$). In contrast, there was no significant reduction in the shoulders on the survival curve comparing mc-MnSOD and MnSOD full plasmid overexpressing 32Dcl3 subline 2C6 ($n = 4.8 + 0.2$ and $4.6 + 0.2$, respectively). Therefore minicircle DNA plasmid containing the human MnSOD transgene confers undiminished radioprotection to cells in vitro.

(PS3158) Radiation countermeasure efficacy of superoxide dismutase (SOD)/catalase (CAT) mimetic EUK-189 in mice exposed to Cobalt-60 gamma radiation. Venkataraman Srinivasan[1], Susan Doctrow[2], Vijay K. Singh[1], Mark H. Whitnall[1]. [1]Armed Forces Radiobiology Research Institute, Bethesda, MD, USA, [2]Proteome Systems Inc., Woburn, MA, USA.

Several pathological conditions are associated with an imbalance in reactive oxygen species (ROS). Superoxide and hydroxyl radicals are involved in radiation-induced tissue alterations and contribute to increased mortality. The deleterious effects of ROS are suppressed by endogenous enzymes including superoxide dismutase (SOD) and catalase (CAT). SOD suppression has been shown to increase radiosensitivity, while overexpression leads to radioresistance of tumors. Administering SOD reduced radiation pneumonitis, and administering a large PEG-SOD complex increased survival in mice exposed to gamma radiation. However, administering intact SOD may lead to biological complications. Hence, we evaluated EUK-189 (Proteome Systems), a small molecular weight salen manganese complex SOD/CAT mimetic, administered as a single injection subcutaneously (sc), as a radiation countermeasure agent in mice. Survival of C3H/HeN mice exposed to 8 Gy (0.4 Gy/min), treated with vehicle (saline) at −24 h, or EUK-189 at −24 h, −1 h, +1 h or +6 h, was 6.3, 81.0, 31.5, 81.0, and 75%, respectively. The dose reduction factor (DRF) for EUK-189 (70 mg/kg, −24 h) was 1.15, with an $LD_{50/30}$ of 7.96 Gy in the saline group, compared to 9.13 Gy for the EUK-189 group. Mice treated with 70 mg/kg EUK-189 sc, 24 h before irradiation (3 Gy), showed significantly higher platelets on day 4 ($p<0.002$), and on day 14 had higher levels of white blood cells ($p<0.02$), lymphocytes ($p<0.01$), and eosinophils ($p<0.01$), compared to vehicle-injected mice. Cytokine profiles in drug-treated (70 mg/kg, −24 h) mice were not different from those in saline-injected mice, after irradiation (8.75 Gy) or sham-irradiation. EUK-189 at 7.20×10^{-5} mM suppressed *in vitro* lipid peroxidation in liver microsomes (TBARS). However, at a lower concentration (7.20×10^{-6} mM), it enhanced lipid peroxidation. EUK-189 appears to protect gamma-irradiated mice by protecting radiosensitive hematopoietic tissues.

(PS3159) NF-kB and MnSOD mediated adaptive radioresistance in low dose-irradiated mouse skin epithelial cells. Ming Fan[1], Kazi Mokim Ahmed[1], Mitchell Coleman[2], Douglas R. Spitz[2], Jian Jian Li[1]. [1]Purdue University, West Lafayette, IN, USA, [2]University of Iowa, Iowa City, IA, USA.

Mechanisms governing inducible resistance to ionizing radiation (IR) in untransformed epithelial cells pre-exposed to low dose ionizing radiation (LDIR, ≤10 cGy) are not well understood. The present study provides evidence that pre-exposure to 10 cGy x-rays increases clonogenic survival of mouse skin JB6P+ epithelial cells subsequently exposed to 2 Gy doses of γ-rays. To elucidate the molecular pathways of LDIR-induced adaptive radioresistance, the transcription factor NF-κB and a group of NF-κB-related proteins, *i.e.,* p65, MnSOD, pERK, cyclin B1, and 14-3-3ζ were identified to be activated as early as 15 min post-LDIR. Further analysis revealed that a substantial amount of both 14-3-3ζ and cyclin B1 accumulated in the cytoplasm at 4–8 h when cell survival was enhanced. The nuclear 14-3-3ζ and cyclin B1 were reduced and increased at 4 h and 24 h, respectively, after LDIR. Using YFP-fusion gene expression vectors, interaction between 14-3-3ζ and cyclin B1 was visualized in living cells, and LDIR enhanced the nuclear translocation of the 14-3-3ζ/cyclin B1 complex. Treatment of JB6P+ cells with the NF-κB inhibitor (IMD-0354) suppressed LDIR-induced expression of MnSOD, 14-3-3ζ and cyclin B1, and diminished the adaptive radioresistance. In addition, treatment with siRNA against mouse MnSOD was shown to inhibit the development of LDIR-induced radioresistance. Together, these results demonstrate that NF-κB, MnSOD, 14-3-3ζ, and cyclin B1 contribute to LDIR-induced adaptive radioresistance in mouse skin epithelial cells

(PS3160) Internal tandem duplication of FLT3 transduces increased ROS production, increased DNA damage, and reduced end-joining fidelity: Implications for disease progression in acute myeloid leukemia. Kamal Datta[1], Kyu-Tae Kim[2], Dan Grosu[1], Annahita Sallmyr[1], Thomas A. Winters[3], Paul Shapiro[4], Donald Small[2], Feyruz V. Rassool[1]. [1]University of Maryland School of Medicine, Baltimore, MD, USA, [2]Johns Hopkins University School of Medicine, Baltimore, MD, USA, [3]National Institites of Health, Clinical Center, Bethesda, MD, USA, [4]University of Maryland School of Pharmacy, Baltimore, MD, USA.

Genomic instability is a hallmark of cancer and has been shown to be associated with disease progression. Activating mutation by internal tandem duplication (ITD) of FMS-like tyrosine kinase3 (FLT3/ITD) occurs in about 30% of myeloid malignancies with poor outcome. However, little is known about how FLT3/ITD in acute myeloid leukemia (AML) contributes to aggressive disease progression. Using a reactive oxygen species specific probe (H2DCFDA) we observed that a murine myeloid progenitor cell line (32D) stably expressing FLT3/ITD (32D/ITD) produces

increased endogenous ROS compared to its isogenic control (32D). FLT3 inhibitor, CEP-701, treated 32D/ITD cells show decreased ROS levels in a dose and time dependent manner. FLT3 mutant cells transfected with dominant negative construct of Rac1, a regulatory component of the ROS producing NADPH oxidase in our model system, showed decreased ROS. Similarly, human AML cell lines (MOLM-14 & MV-4–11) bearing FLT3/ITD showed significant decrease in ROS with CEP-701 and Rac1 inhibitor (NSC23766) treatment. Importantly, with CEP-701 treatment significant decrease in ROS was also observed in primary AML patient samples bearing FLT3/ITD mutation. Interestingly, when we did co-immunoprecipitation experiments we found that Rac1 interacts with STAT5 suggesting a non-genomic function of STAT5 in regulating Rac1 and thus ROS production in these cells. Translocation of Rac1 to gp91*phox*, a membrane bound componant of NADPH oxidase is essential for ROS production. We observed that CEP-701 treatment prevents translocation of Rac1 to gp91*phox* at the membrane thus decreasing ROS production. Blocking ROS in FLT3/ITD cells with CEP-701 also showed decrease in γH2AX foci formation indicating reduced DNA double-strand breaks. In a plasmid based *in vitro* end-joining assay the extract of human AML cells bearing FLT3/ITD showed reduced repair efficiency and increased mis-repair. In contrast inhibition of endogenous ROS production with CEP-701 showed increased repair efficiency and higher repair fidelity suggesting stabilization of the genome. In conclusion, our study suggests that the aggressiveness of AML with FLT3/ITD could be due to increased genomic instability that is driven by higher endogenous ROS, increased DNA damage and decreased end-joining fidelity.

(PS3161) CuZnSOD overexpression enhances radioresistance of human glioma cells by increasing cyclin B1 mRNA turnover and suppressing late reactive oxygen species accumulation. Prabhat C. Goswami, Zhen Gao, Ehab Sarsour, Amanda Kalen. University of Iowa, Iowa City, IA, USA.

This study investigates the hypothesis that CuZn-superoxide dismutase (CuZnSOD) overexpression confers radioresistance in human glioma cells by controlling the G_2-checkpoint pathway and cellular reactive oxygen species (ROS: superoxide and hydrogen peroxide) levels. Wild type, neo vector control, and CuZnSOD stably overexpressing U118–9 human glioma cells were irradiated (0–8 Gy) and assayed for cell survival, cellular ROS levels, and cyclin B1 expression. Flow cytometry measurements of dihydroethidine (DHE) fluorescence, which is an indicator of intracellular superoxide levels, did not show any significant change in ROS levels in wt, neo, and CuZnSOD-overexpressing cells within 1–5 h post-irradiation. However, irradiated wt and neo control cells showed a significant (40–60%) increase in DHE-fluorescence at 6–8 d post-irradiation, which was suppressed in CuZnSOD-overexpressing cells. The increase in DHE-fluorescence was followed by a decrease in cell growth and viability, and increase in percentage of cells with sub G_1 DNA content. These results were comparable to results obtained from irradiated wild type cells transiently overexpressing CuZnSOD suggesting that the results obtained from the stably overexpressing cells were not because of a clonally selected cell population. CuZnSOD overexpression enhanced radiation-induced G_2-accumulation within 24 h post-irradiation, which accompanied with an initial increase in cyclin B1 mRNA levels within 4 h post-irradiation followed by a decrease at 8–24 h post-irradiation. The decrease in cyclin B1 mRNA levels was partly due to a rapid mRNA turnover, 18–20 h in unirradiated compared to 11–12 h in irradiated cells. These results support the hypothesis that manipulations of cellular ROS levels (presumably due to radiation-induced changes in cellular metabolism) long after the initial radiation exposure could significantly affect cancer cell radiosensitivity.

(PS3162) Cross talk between cell cycle checkpoint proteins and mitochondrial antioxidant defense in irradiated cells. Ehab H. Sarsour[1], Sarita G. Menon[2], Iman M. Ahmad[3], Maher Abdalla[4], Venkatasubbaiah A. Venkatesha[1], Prabhat C. Goswami[1]. [1]The University of Iowa, Iowa City, IA, USA, [2]University at Buffalo, The State University of New York, Buffalo, NY, USA, [3]The Hashemite Uviversity, Zarqa, Jordan, [4]The Hashemite University, Zarqa, Jordan.

Research from several laboratories including our own suggests that reactive oxygen species (ROS:superoxide and hydrogen peroxide) could act as second messengers regulating multiple cellular processes including proliferation. We hypothesize that *a cross-talk between G_2-checkpoint protein cyclin B1 and mitochondrial antioxidant enzyme manganese superoxide dismutase (MnSOD) regulates cellular responses to radiation exposures*. Human normal skin fibroblasts representative of G_2-phase were irradiated with 6 Gy of ionizing radiation and harvested 4 h post-irradiation for immunostaining of cyclin B1 and MnSOD. In un-irradiated controls, cyclin B1 was found primarily in the nucleus of G_2-cells. However, following irradiation cyclin B1 was excluded from the nucleus and translocated to the mitochondrion; detected as punctate yellow staining (overlapping of green (cyclin B1) and red (MnSOD) staining). These observations were confirmed further by performing transmission electron microscopy and cell fractionation assays. Cyclin B1 was absent in mitochondria isolated from un-irradiated G_2-cells and present in irradiated G_2-cells. Bioinformatics evaluation of mitoproteome database identified multiple mitochondrial proteins including MnSOD as the potential substrates for cyclin B1/CDK1 kinase. Consistent with this prediction, mitochondria-localized cyclin B1 in irradiated cells was associated with a decrease in MnSOD protein levels and activity, which correlated with a decrease in cell viability. Radiation-induced translocation of cyclin B1 was also observed in human glioma cancer cells. We believe our observations of cyclin B1 translocation to mitochondria and decrease in MnSOD activity in irradiated cells are novel and of highest significance because cyclin B1 could be a major coordinator regulating nuclear and mitochondrial functions in response to ionizing radiation. (Supported by NIH CA111365)

(PS3163) Amplification of ATM-dependent checkpoint signals coupled with DNA double strand break repair. Keiji Suzuki[1], Motohiro Yamauchi[1], Seiji Kodama[2], Masami Watanabe[3]. [1]Division of Radiation Biology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan, [2]Osaka Prefecture University, Sakai, Japan, [3]Research Reactor Institute, Kyoto University, Sennan, Japan.

[INTRODUCTION] Ionizing radiation stimulates so called DNA damage checkpoint singalling pathway in mammalian cells, whose process is initiated by auto-phosphorylation of ATM protein at serine 1981. The aim of this study is to identify the molecular mechanism underlying the amplification of ATM-dependent DNA damage checkpoint signals. [PROCEDURES] Normal human diploid (NHD) cells, CHO and NHEJ-deficient xrs5/6 cells were used. The foci of DNA damage checkpoint and related factors were detected by immunofluorescence analysis. Digital images were captured by a CCD camera, and fluorescent intensities were examined as phosphorylation levels. [RESULTS] While it has been reported that phosphorylated ATM forms discrete foci, we found that the initial phosphorylated ATM foci grew rapidly within 1 hour after X-irradiation, especially at lower doses. Growth of the foci were also observed in the foci of phosphorylated histone H2AX, 53BP1 and NBS1. These observation provides a possibility that phosphorylated ATM foci growth plays a role in amplifying the DNA damage signals. To prove this, we examined p53 phosphorylation at serine 15, as a marker for DNA damage signal, in cells defective in non homologous end-joining (NHEJ), which is the major DNA repair pathway in the G1 cells. We used CHO cells, and xrs-5 cells that lack Ku80 involved in NHEJ. One hour after 1 Gy of X-rays, most of the initial foci grow in CHO cells, however, defective growth was observed specifically in G1 cells derived from xrs-5 cells, while cells in other cell cycle phases normally form grown foci. The cells showing defective foci growth also demonstrated weak phosphorylation signal of p53 at serine 15. [CONCLUSION] These results indicate that the growth of phosphorylated ATM foci were coupled with DNA double strand break repair, and that the foci growth is essential for the efficient transduction of DNA damage checkpoint signals.

(PS3164) Ku70/80 modulates ATM and ATR signaling pathways in response to DNA double strand breaks. Nozomi Tomimatsu[1], Candice G.T. Tahimic[1], Akihiro Otsuki[1], Sandeep Burma[2], Akiko Fukuhara[1], David J. Chen[2], Akihiro Kurimasa[1]. [1]Tottori University, Yonago, Tottori, Japan, [2]University of Texas Southwestern Medical Center, Dallas, TX, USA.

Double strand break (DSB) recognition is the first step in the DSB damage response and involves activation of ataxia telangiectasia-mutated (ATM) and phosphorylation of targets such as p53 to trigger cell cycle arrest, DNA repair, or apoptosis. It was reported that activation of ATM- and Rad3-related (ATR) kinase by DSBs also occurs in an ATM-dependent manner. On the other hand, Ku70/80 is known to participate at a later time point in the DSB response, recruiting DNA-PKcs to facilitate non-homologous end joining. Because Ku70/80 has a high affinity for brokenDNAends and is abundant in nuclei, we examined their possible involvement in other aspects of the DSB damage response, particularly in modulating the activity of ATM and other phosphatidylinositol (PI) 3-related kinases during DSB recognition.Wethus analyzed p53Ser18 phosphorylation in irradiated Ku-deficient cells and observed persistent phosphorylation in these cells relative to wild type cells. ATM or ATR inhibition revealed that this phosphorylation is mainly mediated by ATM-dependent ATR activity at 2 h post-ionizing radiation in wild type cells, whereas in Ku-deficient cells, this occurs mainly through direct ATM activity, with a secondary contribution from ATR via a novel ATM-independent mechanism. Using ATM/Ku70 double-null cell lines, which we generated, we confirmed that ATM-independent ATR activity contributed to persistent phosphorylation of p53Ser18 in Ku-deficient cells at 12 h post-ionizing radiation. In summary, we discovered a novel role for Ku70/80 in modulating ATM-dependent ATR activation during DSB damage response and demonstrated that these proteins confer a protective effect against ATM-independent ATR activation at later stages of the DSB damage response.

(PS3165) EGFR-ERK signaling through PARP coordinates DNA repair, apoptosis and proliferation. Adly Yacoub, Joseph Kelley, Timothy Wallace, Paul Dent, Michael Hagan. Virginia Commonwealth University, Richmond, VA, USA.

Purpose: Examination of poly(ADP)ribose accumulation, TGFα shedding, and binding of PARP associated proteins following ionizing radiation in the presence of active or inhibited EGFR-ERK signaling

Materials and Methods: DU145 and LNCaP prostate cell lines, EGFR-ERK dependent and independent, respectively, were examined for polyADP ribose accumulation, TGFα-shedding and DNA repair complex formation following exposure to ionizing radiation. ERK signaling was manipulated through ligand activation or specific inhibition of the EGFR, Akt, or MEK1/2, while PARP activity was reduced through the use of siRNA knockdown or the small molecular weight inhibitor PJ34. Western analysis and confocal microscopy were used to follow the kinetics and localization of poly(ADP)ribose, ERK2, TGFα, PARP, XRCC1 and γ-H2AX.

Results: Following exposure to ionizing radiation, physiologic EGF signals PARP, increasing poly(ADP)ribose to activate SP-1 and NFκB dependent transcription. As a result, TGFα is expressed and shed in a radiation-dose dependent manner. Intact PARP function is associated with up-regulation of DNA repair proteins and association of poly(ADP)ribose with the repair complex at sites of DNA damage. Activation of EGFR-ERK signaling at the time of irradiation deranges PARP activation, resulting in dramatically increased poly(ADP)ribose accumulation, but failure to localize to sites of DNA damage.

Conclusions: ERK signaling operates through PARP activation to coordinate the up-regulation of proteins involved in DNA repair as well as TGFα release. After exposure to ionizing radiation, ERK signaling enhanced formation of the DNA repair complex. EGFR-ERK signaling at the time of radiation exposure, however, increases cell-killing associated with deranged poly(ADP)ribose formation.

(PS3166) Bioinformatics of high-throughput, quantitative mass spectrometry applied to radiation-induced genome instability. John H. Miller[1], Shuangshuang Jin[2], William Morgan[3], David Springer[4]. [1]Washington State University Tri-Cities, RIchland, WA, USA, [2]Washington State University Tri-CIties, RIchland, WA, USA, [3]University of Maryland School of Medicine, Baltimore, MD, USA, [4]Pacific Northwest National Laboratory, RIchland, WA, USA.

The purpose of this study is to develop methods to extract information from genome-wide high-throughput mass spectrometry (MS) proteomics data to profile a radiation-induced genome-unstable cell line relative to parental GM10115 Chinese hamster ovary cells. When shotgun proteomics is applied to cells from higher eukaryotes, a significant fraction of the detected peptides cannot be associated with a unique protein because the protein databases for the organism contain many proteins of similar amino acid sequence. Ambiguity regarding the origin of these degenerate peptides makes their MS output data less useful for estimating the abundance of proteins in a complex mixture that has been subjected to protolytic digestion prior to MS analysis. We have devised a way to avoid this problem for cases where peptide degeneracy is within a family of proteins with related biological function. For the datasets examined thus far, we find that most of the peptide degeneracy is within families of biologically-related proteins. Consequently, our method significantly increases the number of peptides that can be used to estimate protein abundances without ambiguity. This improves the precision and reliability with which differential protein abundance can be assessed for comparison of cell lines and profiling responses of a given cell line to treatments.

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(PS3167) TNF-α-induced genomic instability in primary vascular endothelial cells. Catherine F. Gibbons[1], Mohan Natarajan[2], Sumathy Mohan[2], Munira A. Kadhim[3], Andrew J. Grosovsky[1]. [1]University of California, Riverside, Riverside, CA, USA, [2]University of Texas Health Science Center, San Antonio, TX, USA, [3]Medical Research Council, Harwell, Oxfordshire, United Kingdom.

Genomic instability has been demonstrated in the progeny of irradiated cells and unirradiated bystander cells. Bystander responses are thought to depend on the activation of cellular communication processes. In this study we examine one such mediator of cellular communication, the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α). TNF-α is known to increase in expression following ionizing radiation (IR) exposure. Upon binding to its cellular receptors, TNF-α initiates a signaling cascade mediated by reactive oxygen species (ROS) that can activate sequestered NF-κB, thus initiating a pro-inflammatory and anti-apoptotic pathway. NF-κB can in turn upregulate TNF-α expression, which when secreted can induce subsequent autocrine and paracrine stimulation of TNF-α and NF-κB. We speculate that this increase in TNF-α signaling and concomitant ROS generation could have a mechanistic role in the initiation of genomic instability and a potential involvement in producing bystander responses.

Genomic instability is induced by IR in a non-dose-dependent manner. Previous investigation by our group using primary human vascular endothelial cells has shown that both low (0.1 Gy) and high (2 Gy) doses of IR raise levels of secreted TNF-α in a non-linear manner, that both immediate genetic damage and delayed chromosomal instability can be induced at similar levels following treatment with either 0.1–10 ng/mL TNF-α or 0.1 or 2 Gy IR, and that this immediate damage was abrogated by pre-incubation with antioxidants. The current study is therefore focused on the mechanism responsible for this TNF-α-induced instability, and whether TNF-α can act as a signaling mediator of bystander-induced responses. Suppressors of TNF-α are added to either directly irradiated or bystander cell cultures exposed to low or high doses of IR, and the results are compared to cells pre-treated with antioxidants. Cellular damage is assessed by the comet assay, formation of damage-induced foci, and presence of delayed chromosomal instability. Preliminary results indicate that while TNF-α and the ROS involved in its signaling are important factors

in the initiation of genomic instability, the role of TNF-α in mediating the bystander effect is more uncertain.

(PS3168) Multiple molecular alterations in fibroblasts of a patient with radiation hypersensitivity / chromosomal fragility syndrome. Reinhard Kodym[1], Gazi Alsbeih[2], Micheal Dean Story[1]. [1]UT Southwestern Medical Center at Dallas, Dallas, TX, USA, [2]King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

A pediatric patient with a family history of severe late radiation sequelae after radiotherapy was diagnosed with medulloblastoma. A primary skin fibroblast line was created from this patient and was determined to be radiosensitive, displayed an increased basal level of chromatid-type aberrations, as well as a profound deficit in DNA DSB repair fidelity when compared to fibroblasts from normal individuals. In an effort to characterize the molecular lesion responsible for the increased radiosensitivity, functional studies and an analysis of the gene expression pattern were performed. Using this approach two molecular lesions related to cellular radiosensensitivity were identified:

Co-immunoprecipitation experiments revealed that the fibroblasts showed a significantly reduce level in the expression of Tissue Transglutaminase 2 (TGM2). As TGM2, in addition to its acyl-transferase function, acts as a G protein and has been demonstrated to show protein kinase activity we tested whether the reduced expression is responsible for increased radiosensitivity. Intracellular levels of TGM2 were altered by siRNA and by forced overexpression of TGM2. These experiments demonstrated that alteration in the TGM2 expression level can, at least partially, modulate the radiosensitive phenotype of the fibroblast line.

Microarray hybridization demonstrated that these primary skin fibroblasts overexpressed a number of secreted proteins. Among these proteins was Peroistin, which is known to bind to the integrin receptor and constitutively stimulate AKT. Examination of the AKT pathway in these fibroblasts showed increased levels of phosphorylated and therefore activated AKT. This activation could be suppressed by inhibitors of PI3 kinase and siRNA directed against Perisotin.

Taken together these finding indicate that multiple molecular alterations, like TGM2 down regulation or autocrine stimulation of AKT via Perisotin contribute to the radiosensitive phenotype of these patient derived fibroblasts.

(PS3169) Amplification of G1 checkpoint signalling by growth of IR-induced foci. Motohiro Yamauchi[1], Yasuyoshi Oka[1], Seiji Kodama[2], Masami Watanabe[3], Keiji Suzuki[1]. [1]Nagasaki University, Nagasaki, Japan, [2]Osaka Prefectural University, Osaka, Japan, [3]Kyoto University, Kyoto, Japan.

(a)

It is well established that several checkpoint-, or repair factors, such as Ser1981-phosphorylated ATM and Ser139-phosphorylated histone H2AX, form discrete nuclear foci after ionizing radiation (IR), and the foci indicate the site of DNA double-strand breaks (DSBs). Although the initially-formed foci decrease concomitantly with DSB repair, some fraction of foci remain persistently. To date, physiological role of remaining foci has not been fully understood. Thus, in the present study, we addressed the issue.

(b)

Exponentially-growing or G0-synchronized normal human diploid cells were irradiated with 1 Gy of X-rays. After fixation, immunofluorescence staining was performed using antibodies for Ser1981-phosphorylated ATM, Ser139-phosphorylated H2AX, MDC1, 53BP1, NBS1, RPA, and Ser15-phosphorylated p53. The digital images of the primary antibodies were obtained using fluorescence microscopy (Leica DM6000B, Leica, Germany). Diameter of phosphorylated ATM foci and fluorescence intensity of phosphorylated p53 were measured using Leica FW4000.

(c)

We found the remaining phosphorylated ATM foci grew time-dependently after irradiation. While the mean size of the initial foci was approximately 0.6 μm in diameter 1 h after 1 Gy of X-rays, the foci grew and the mean size reached approximately 2.0 μm 24 h after irradiation. Percentage of cells with large foci (≥1.6 μm in diameter) and the number of large foci per cell increased dose-dependently. However, foci size distribution did not change significantly between 1–8 Gy. All of the large foci of phosphorylated ATM colocalized with the foci of Ser139-phosphorylated histone H2AX, MDC1, 53BP1 and NBS1, which also grew similarly to phosphorylated ATM foci. We confirmed clear correlation between growth of a phosphorylated ATM focus and the levels of Ser15-phosphorylation of p53. Furthermore, when G0-synchronized cells were released immediately after 1 Gy of X-rays and incubated for 24 h, large foci were rarely (1.3%) observed in RPA positive S-phase cells, while the smaller foci were frequently (45.9%) found.

(d)

These results indicate that the remaining foci, grown after IR, play an essential role in amplifying signals of very few remaining damages to sufficient levels for G1 checkpoint activation.

(PS3170) Phosphorylation of c-Myc on Ser62 by CDK5 is Essential for Cyclin G1-Mediated Transcriptional Activation of Cyclin B1. Haeng Ran Seo. Korea Institute of Radiological & Medical Science, Seoul, Republic of Korea.

Abstract Cyclin G1 previously reported to overcome radiation-induced G2 arrest and increased cell death, which are mediated by transcriptional activation of cyclin B1. In the present study, we further investigated how cyclin G1 transcriptionally activates cyclin B1. Deletion mutants of cyclin B1 promoter region revealed that c-Myc binding site (E-Box) is necessary for cyclin G1-mediated transcriptional activation of cyclin B1. Cyclin G1 induced phosphorylation of c-Myc and increased stability of c-Myc, which are responsible for activation of cyclin G1-mediated cyclin B1 promoter. Furthermore, phosphorylation site by cyclin G1 overexpression was Ser 62 of c-Myc and this phosphorylation was involved in increased c-Myc stability and increased cyclin B1 promoter activity. Kinase activity of CDK5, interaction partner was also increased by cyclin G1 overexpression and in vitro kinase assay revealed that CDK5 directly phosphorylated c-Myc on Ser 62 and cyclin G1 potentiated CDK5 kinase activity. Cyclin G1 mediated increased radiosensitivity and G2/M phase arrest was attenuated by treatment of RNA interference for CDK5, which are independent of p53 function. From the data, CDK5 activation in cyclin G1 overexpressed cells involved in c-Myc phosphorylation on Ser 62, which is responsible for cyclin G1-mediated transcriptional activation of cyclin B1.

(PS3171) Importance of 5'-AMP-activated protein kinase (AMPK) for tumor development. Keith Laderoute[1], Khalid Amin[1], Joy Calaoagan[1], Merrill Knapp[1], Benoit Viollet[2]. [1]SRI International, Menlo Park, CA, USA, [2]Institut Cochin, Paris, France.

While studying the bioenergetics of hypoxic cells, we found that adenosine 5'-monophosphate-activated protein kinase (AMPK) was strongly activated by hypoxia combined with glucose deprivation, independently of HIF-1 activity. AMPK has been termed the "fuel sensor" of mammalian cells because it directly responds to the depletion of the fuel molecule ATP. During these studies we also obtained evidence that AMPK activity was activated by the same low oxygen conditions in the presence of glucose, as well as by less severe hypoxia (physiological hypoxia). These findings are directly relevant for understanding how cells adapt to ATP depletion (energy stress) within solid tumors, which naturally develop gradients of oxygen and glucose as they grow. Based on preliminary research showing that experimental tumors made from cells engineered to lack AMPK activity grew very poorly as mouse xenografts, we propose that AMPK is a novel therapeutic target. We will review these in vitro and in vivo findings and present new results concerning the importance of AMPK for solid tumor growth. Considering the critical importance of AMPK activity for the adaptation of normal cells to energy stress, we hypothesize that AMPK regulates a general mechanism to conserve total ATP in

solid tumors, and thus maintain the viability of energetically stressed tumor cells.

(PS3172) Distinct gene expression profiles following 10 Gy or iso-survival doses of radiation in human lymphoblastoid cells. Tzu-Pin Lu[1], Mong-Hsun Tsai[2], James B. Mitchell[3], Eric Y. Chuang[4]. [1]National Taiwan University Electronical Engineering, Taipei, Taiwan, [2]Institute of Biotechnology, National Taiwan University, Taipei, Taiwan, [3]Radiation Biology Branch, NIH, Bethesda, MD, USA, [4]Graduate Institute of Biomedical Electronic and Bioinformatics, National Taiwan University, Taipei, Taiwan.

Our previous study has shown that cluster analyses of gene expression profiles following 10 Gy radiation revealed distinct patterns among three closely related human lymphoblast cell lines with different p53 status. In the present study, we further investigate whether gene expression profiles following iso-survival (D_0) doses of radiation would yield similar profiles of a single 10 Gy radiation in these three cell lines. TK6 (wild-type p53), WTK1 (mutant p53), and NH32 (p53-null) cells were exposed to 81 cGy, 150 cGy, and 88 cGy, respectively. Total RNA samples were isolated at 0, 1, 3, 6, 9, 24 hr after radiation and processed for cDNA microarray analysis. Template-based clustering analysis of the gene expression over the time course was applied to select genes either up- or down-regulated by at least two-fold after radiation treatment. Based on the clustering analysis, we identified 524 genes for 10Gy and 830 genes for iso-survival. After the Venn diagram analysis, there were only 118 genes changed significantly in both sets. Furthermore, Pearson correlation coefficients and tight clustering were used to select genes with significant correlation to others. The clustered genes were further analyzed by the Ingenuity Pathway Analysis. The results showed that cell cycle related genes (including *CCNA2, CDKN1B, PTTG1, CDC2, TOP2A, and PLK1*) and carbohydrate metabolism related genes (such as *GBE1, ENO1, GAPDH, ERO1L, LDHA,* and *SUCLG*) exhibited similar expression profiles among these three cell lines following iso-survival radiation treatment, whereas these genes showed differential responses in the 10 Gy treatment. In addition, within individual cell lines, distinct expression profiles were induced following different radiation treatments. Our preliminary results may suggest that the gene expression profiles are significantly different between 10 Gy and iso-survival radiation treatments. Although our previous study indicated that p53 plays an important role in response to 10 Gy radiation treatment among these three cell lines, p53 may not be a dominant gene in the iso-survival radiation treatment.

(PS3173) Regeneration mechanisms of ontogenetic radio-adaptation in plants. Alexandr Mikhyeyev, Svitlana Sytnik, Ludmila Ovsyannikova, Alla Dyachenko, Dmytro Grodzinzky. Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine, Kyiv, Ukraine.

Experimental data were obtained that in pea seedlings modified by decapitation of main root had increased radioresistance (radioadaptation), fixed by various parameters of growth activity of lateral roots, and decreased ability to repair sublethal damages, detected by method of acute γ-irradiation dose fractionation. These facts both with enlargement of dose dependence shoulder in lateral roots of decapitated seedlings led to conclusion that main role in such mechanism of radioadaptation effect of decapitation belongs to supercellular processes such as repopulation and regeneration. Conclusion was confirmed by additional comparative investigations of cyto- and histological parameters of apical meristems of intact (control) and decapitated (experiment) lateral roots. It was shown, that decapitated seedlings had increased mitotic activity of apical meristems of lateral roots and total volume of their meristematic zone. So at the moment of application of irradiation in the test-dose decapitated variant had significantly more meristematic cells of certain size that allowed biological object to form necessary (critical) amount of elements for valid or more complete postradiation recovery.

(PS3174) Characterisation of a novel protein, FKBPL; protein interactions and implications for pathways controlling cell growth and survival. Keeva McClelland, Hayley McKeen, Andrea Valentine, David Hirst, Tracy Robson. Queen's University Belfast, Belfast, United Kingdom.

Introduction: We have previously identified a novel gene, FKBPL, which is downregulated by X-rays (Robson *et al*, 1997). Its repression can also protect cells against X-ray and UV-induced oxidative damage through increased DNA repair (Robson *et al*, 1999, 2000). FKBPL also interacts with the molecular chaperones, HSP70 and 90, which are involved in protein folding, maturation and proteosomal degradation of key oncogenic signalling proteins, steroid receptors and tyrosine kinases. Thus FKBPL could be acting as a co-chaperone in these complexes. Furthermore, FKBPL, has been implicated in the stabilization of newly synthesized p21 (Waf-1) in the presence of HSP90. Downregulation of FKBPL prevents the accumulation of p21 and cell cycle arrest following X-irradiation (Jascur *et al*, 2005). We now aim to identify other FKBPL-associated proteins

Materials and Methods: A yeast-2-hybrid system was used to identify two novel FKBPL-interacting proteins, XAP3 and USP19 with roles in ubiquitination. Interactions were confirmed using the mammalian-2-hybrid system and co-immunoprecipitation. Western blots were used to establish if XAP3 was also regulated by X-rays and to determine if overexpression of XAP3 affected FKBPL levels. Furthermore, clonogenic cell survival was used to assess effects of FKBPL or XAP3 overexpression on radiosensitivity.

Results: Mammalian-2-hybrid and co-immunoprecipitaion confirmed that XAP3 interacts with FKBPL in mammalian cells. XAP3 was regulated by X-rays in a similar manner to FKBPL; protein levels fell in L132 and DU145 cells following exposure to 0.2 Gy. Furthermore, overexpression of XAP3 led to an increase in the level of FKBPL, suggesting a role in its stabilization. Stabilization by XAP3 of the suppressor of cytokine signaling protein, SOCS6 (Bayle et al. 2006) and the HBV-X protein (Cong YS. et al 1997) has been described.

Conclusion: FKBPL may be critical to the stabilization of Hsp90 chaperone client proteins such as p21. This may be facilitated by the interacting regulatory proteins, XAP3 and USP19. The next stage is to confirm that XAP3 is directly involved in the Hsp90 co-chaperone complex. A more complete understanding of the biological activity of Hsp90 and, in particular, the co-chaperones with which it is known to interact may provide us with a new target for cancer treatment.

(PS3175) Targeting the COP9 Signalosome for Cancer Therapy. Katharine S. Richardson, Ashraful Islam, Wayne Zundel. Univ of Louisville Health Sciences Center, Louisville, KY, USA.

Emerging research has implicated the COP9 signalosome (CSN) as a significant factor in tumor initiation and progression. While its function is incompletely known, it is known to be vital for Cullin-dependent protein degradation. Several proteins degraded through CSN-dependent mechanisms are central mediators of apoptosis, cell proliferation, cell adhesion, DNA repair, and oxygen homeostasis. In addition, direct association of the CSN with known proto-oncogenes and tumor suppressors regulates their function by controlling the rate of their degradation. The CSN is comprised of 8 subunits, with CSN5 containing the catalytically active domain. While CSN5 plays a critical role in regulating key cellular processes through regulation of cullin based E3 ubiquitin ligases, CSN5 also interacts directly with known cancer associated proteins. A number of studies have examined overexpression of CSN5 in tumor models as well as clinically manifested cancers, yet the exact mechanism by which CSN5 controls various aspects of tumor development remain to be elucidated.

To evaluate the effects of CSN5 loss on tumorigenesis and potential efficacy as a drug target, we have initiated a pilot study to evaluate the effects of both genetic and pharmacologic loss of CSN5 on cullin function and tumor growth. Consequently, this proposal addresses the cellular impact of CSN5 knockdown, overexpression of a dominate negative CSN5 supplemented with overexpression of other deneddylases. Combined with pilot drug studies modeled on

the CSN5 catalytic core, this study will establish the role of CSN5 during various aspects of tumor progression and potential utility as a pharmacologic target. We propose that negative perturbation of CSN5 function will reduce cell proliferation and subsequent tumor growth due to global cullin E3 ligase dysfunction.

(PS3176) Sirt3:modulator of foxo3a activity? Mark V. Mishra, Kristi Muldoon-Jacobs, Phuongmai Nguyen, David Gius. National Cancer Institute, Bethesda, MD, USA.

Sirt3, a human homolog of the S. cerevisiae Sir2 protein known to be involved in cellular aging and in the response to DNA damage, is a NAD-dependent protein deactylase localized to the mitochondria. It has been previously shown that a nuclear human homolog of the Sir2 protein, Sirt1, deacetylates and represses the activity of the forkhead transcription factor Foxo3a to reduce forkhead-dependent apoptosis. Here we sought to determine if Sirt3 might also be involved in the regulation of Foxo3a to modulate mitochondrial functioning. This could highlight a potential role of Sirt3 in protecting from mitochondrial dysnfunction implicated in several age-related diseases, including most malignancies, diabetes, and several neurological disorders. To examine this, HCT116 cell lines stably over-expressing human wild-type Sirt3 were created and used for co-immunoprecipitation of Sirt3 and Foxo3a from mitochondrial isolates. Immunofluorescence microscopy was used to further co-localize the two proteins. Mitochondrial isolates from these HCT116 cells indicate the presence of Foxo3a in the mitochondria. Immunoprecipitation of Sirt3 from mitochondrial lysates of cells over-expressing Sirt3 resulted in co-precipitation of Foxo3a, indicating that two proteins physically interact within the mitochondria. This represents the first study to show localization of Foxo3a to the mitochondra. We propose that Sirt3 is involved in the regulation of Foxo3a function, possibly through deacetylation in the mitochondria. Further tests need to be done to determine if Sirt3-dependent Foxo3a deacetylation of mitochondrial Foxo3a affects cell survival. SIRT3 might increase longevity by shifting FOXO3a dependent responses away from cell death and towards cell survival.

(PS3177) Transcriptomic analysis of the effect of embryonic irradiation on cognitive functions. Joris Verheyde[1], Arlette Michaux[1], Ann Janssen[1], Louis de Saint-Georges[1], Luc Leyns[2], Abderrafi Benotmane[1]. [1]SCK•CEN, Mol, Belgium, [2]VUB, Brussels, Belgium.

Brain damage induced by prenatal irradiation is of major concern in radioprotection. The brain is the final result of a series of well timed consecutive or concomitant waves of cellular proliferation, migration, and differentiation. Acute irradiation during pregnancy could selectively disturb these events to result in various forms of malformation such as microcephaly, reduced cortical thickness and mental retardation. Such events were previously described in epidemiological studies of the atomic bomb survivors of Hiroshima/Nagasaki.

Using cDNA-microarrays and real-time PCR we analyzed the modulated genes upon 50 cGy X-ray irradiation in embryonic mouse brain. The main activated pathways are involved in the induction of *Trp53* dependent programmed cell death, and the intercellular signalling cascades. Although in the *Trp53* null mutant embryos, our data highlights differential expression of genes involved in cell cycle progression. Various cyclins and cyclin-dependent kinases were downregulated.

Regional analysis of the irradiated anterior brain at E15 by *in situ* hybridization *with Trp53inp1* and *Ccng1* probes, suggested that there is a specific regional dependent expression in the anterior brain. Especially *Ccng1* and the P53 downstream cell cycle regulating gene indicated that the strongest effect can be observed in the cerebral cortex.

We next investigated the differential response of two main cell populations in the cortical region. To do so, gene expression analysis was performed in 24 hour old primary neuron and astrocyte cultures extracted from an E15 brain embryo. As in irradiated astrocytes, differentiated neurons showed a P53 activation which is not coupled with an increase in the expression of *Cdkn1a*. Beside cell death, neurite outgrowth was evaluated by measuring the length of the neurites extruding from the differentiating neurons. Analysis of neurite extension resulted in a statistical significant reduction for both 50 and 100 cGy X-ray exposure.

Taken together, radiation induced cell death of astrocytes in the cerebral cortex, and reduction in neurite length in maturating neurons, may interfere with a correct patterning of the brain and could jeopardize the formation of a correct neural network, leading to cognitive deficits in the mature brain.

(PS3178) Radiation-induced stress response in human skin. Ray Warters, Sergey Zhuplatov, Sancy Leachman. University of Utah Health Sciences Center, Salt Lake City, UT, USA.

Introduction. We previously reported that exposure to ionizing radiation (IR) induces a robust stress response in the intact human skin's epidermis (Pond, Leachman and Warters *Radiation Research* 161: 739–745, 2004). More than a 13-fold increase was observed in the phosphorylation of p53 serine 15 and a 4.7-fold increase in total p53 protein per cell. Inter-individual variability in IR-induced p53 protein increase was significant in the epidermis, with the relative p53 protein levels 4 hours after 2 Gy varying between 1.2-fold and 16.5-fold relative to the untreated control. Little or no p53 protein was observed in skin dermis. Since the dermis is important to the skin's IR response, we now have performed a closer examination of the IR response of skin dermis.

Methods. Neonatal human skin was obtained from the LDS Hospital Newborn Clinic under University of Utah IRB # 8476–00. Skin was cultured *ex vivo* in F12 medium containing 5% bovine serum albumin. Whole skin was irradiated and replaced at 37°C for increasing time periods. The epidermis was peeled away from whole skin that had been heated at 60°C for 60 seconds, and the recovered epidermis or dermis dissolved into RIPA buffer by FastPrep lysis and sonication. Equal μg of protein (determined by the Bradford reaction) was separated through 4–12% gradient acrylamide gels. Western analysis was performed as previously described (Pond et al, 2004).

Results. We observed 4 times more total p53 in the epidermis of untreated neonatal skin than in the dermis. When *ex vivo* cultured intact skin was irradiated with 2 Gy, collected at 4 hours and the p53 protein in the dermis examined, there was no detectable increase in phosphorylation of p53 serine 15 (IR/control = 0.88; n = 4) and the total level of p53 protein increased 1.44 fold (n = 4) 4 hours after irradiation. Very little inter-individual variation was observed in the relative increase in p53 protein increase or p53 protein phosphorylation 4 hours after exposure to 2 Gy.

Conclusions:

Our results are consistent with an efficient recovery of cell protein from the epidermis or dermis of intact human skin. IR induces a quantitatively greater stress response in human skin's epidermis (primarily keratinocytes) than in the dermis (primarily fibroblasts). Work funded by DOE grant number DE-FG02-05ER64085.

(PS3179) Changes in the expression of Keratinocyte Growth Factor and its receptor in oral mucosa (mouse) during daily fractionated irradiation. Wolfgang Doerr[1], Astrid Fehrmann[1], Stefan Pieck[2]. [1]Radiobiology Laboratory, Dresden, Germany, [2]OncoRay, Dresden, Germany.

Early radiation reactions of oral mucosa are a frequent and severe, often dose-limiting side effect of radiotherapy for advanced head-and-neck tumours. As a response to the progressive injury, a complex re-structuring of the proliferative organisation in the epithelium, summarized as "repopulation", occurs with a delay of about 1 week after the onset of treatment. This is the basis for the increase in mucosal radiation tolerance with increasing overall

treatment time. The present study was initiated to determine changes in the expression of Keratinocyte Growth Factor (KGF) and its receptor (KGFR) during daily fractionated irradiation, which may contribute to the regulation of the repopulation processes. Moreover, these changes may be related to the mucoprotective efficacy of KGF (Palifermin) administered during irradiation.

Daily fractionated irradiation with 5 × 3 Gy/week (200 kV X-rays) was given to the snouts of the animals over a total of 2 weeks. Groups of 3 mice per day were sacrificed from day 0 to 16, and the tongues were stored in liquid nitrogen. KGF and KGFR expression was determined for days 0, 1, 2, 4, 7, 12, 14 and 18 by real time (RT-)PCR. For this, the samples from each time point were pooled after preparation of RNA from the tissues of the lower tongue surface.

Compared to un-irradiated controls, no significant or systematic change in the expression of KGF-RNA was found. In clear contrast, a significant reduction in KGFR-RNA expression to about 40% was found within the first 2 days of irradiation, followed by a slight recovery to 70% of the control over the first treatment-free weekend, and a return to low values (40%) during week 2, again followed by a recovery to ca. 70% over the second weekend. Recovery was still incomplete by day 18.

In conclusion, fractionated irradiation of oral mucosa does not affect the expression of KGF-RNA. During the first days of fractionated irradiation, the expression of KGFR at the RNA-level is significantly decreased. Subsequently, cyclic changes are found, with low values during the treatment weeks and a transient and incomplete recovery over the treatment-free weekend breaks.

The RT-PCR studies were performed in co-operation with the Dept. of Molecular Cell Physiology and Endocrinology of the Institute for Zoology of the Faculty of Sciences, TU Dresden.

(PS3180) Identification of differentially expressed genes contributing to radioresistance in lung cancer cells using microarray analysis. Guozheng Guo, Wangfeng Guo. The Fourth Military Medical University, Xi'an, China.

Radiotherapy plays a key role in the treatment of many cancers. It is difficult to determine what fraction of tumor cells is radioresistant after treatment with ionizing radiation (IR). The response of tumor cells to radiation is accompanied by complex changes in patterns of gene expression. It is highly probable that a better understanding of molecular and genetic changes can help to sensitize the radioresistant tumor cells and improve the radiocurability of tumor cells. Here we developed one oligonucleotide microarray according to the biological effect of IR to detect 143 genes's alteration expression in two different radiosensitive lung cancer cell lines. By comparing to NCI-H446, a total of 18 genes in radioresistant A549 cells showed significant fold-changes, of which 8 genes were up-regulated and 10 genes were down-regulated. Subsequantly, A549 and NCI-H446 cells were irradiated by IR. In A549 cell line, 22 (19 up-regulated and 3 down-regulated) and 26 (8 up-regulated and 18 down-regulated) differentially expressed genes were found at 6h and 24h after IR, respectively. In NCI-H446 cell line, 17 (9 up-regulated and 8 down-regulated) and 18 (6 up-regulated and 12 down-regulated) differentially expressed genes were identified at 6h and 24h after IR, respectively. To provide independent confirmation of microarray data, semi-quantitative RT-PCR was performed on a selection of genes. In present study, we identified some genes with cell proliferation and anti-apoptosis, such as MDM2, BCL-2, PKCz and PIM2 expression levels increased in A549 cells and decreased in NCI-H446 cells after IR, and other genes with DNA repair, such as XRCC5, ERCC5, ERCC1, RAD9A, ERCC4 and DNA-PK activities were higher in A549 cells than NCI-H446 cells. We employed antisense strategy to achieve partial reduction of MDM2 in A549 cells. This suppression of MDM2 resulted in increased radiosensitivity of A549 cells. Taken together, these results support the hypothesis that a few genes with DNA repair, regulation of cell cycle, cell proliferation and anti-apoptosis as candidates for radioresistance of A549 lung cancer cells. These maybe provide several gene targets to sensitize the radioresistant cells for improving the radiocurability of lung cancer.

(PS3181) Establishment of reverse genetics in Medaka. Takeshi Todo[1], Yasuhiro Kamei[2], Tomoko Ishikawa[1], Jin-hyong Kim[2]. [1]Graduate School of Medicine Osaka University, Suita, Osaka, Japan, [2]Radiation Biology Center, Kyoto University, Kyoto, Japan.

Zebrafish and Medaka have become increasingly useful as animal models through which the molecular and biochemical bases of complex human diseases can be elucidated. The most important tool in biological research is mutational analysis. Our understanding of the basic mechanism of disease has been transformed by the systematic application of mutational analysis. One of the most widely used approaches for mutational analysis is forward genetics, which is driven by the identification of mutant phenotypes. Another approach is reverse genetics. The most widely used reverse genetics method in zebrafish and Medaka is undoubtedly the use of morpholinos, but this method is not a substitute for mutations because it is a transient method and only suited for early developmental stages. Recently a general reverse genetics method was reported, which can identify mutations in genes that are known only by their sequence. The method, called TILLING (Targeting Induced Local Lesions IN Genome), includes random mutagenesis, followed by screening for induced mutations in target genes at the genomic DNA level. Furutani et al (Kondoh Differentiation Signaling Project, JST, Japan) have established a method of forward genetics in Medaka which include chemical mutagenesis and subsequent large-scale screen of mutants (Furutani et al. 2004). This successful forward genetics approach allowed us to apply the chemical mutagenesis to reverse genetics approach TILLING. Adult Cab male Medaka were mutagenized with ENU and then outcrossed with Cab female to generate F1 progeny for the library. We established 5771 ENU-mutagenized F1 male fishes. To construct a library, genomic DNA and testis samples were isolated and cryopreserved from each F1 fish. Screening for mutations was done as the collaborative work with Sakuraba and Gondo (RIKEN Genomic Science Center, Japan)(Sakuraba et al. 2005). A pilot screening revealed that the average per-base mutation frequency was 1 in 350kbp. Rev1 protein is an essential player in the production of both spontaneous and DNA damage-induced mutations. We have identified 16 mutations in Rev1 gene, which include 2 nonsense and 14 missense mutations. Summary of the screening and the phenotype of these mutants, and a possible application of this method to establish the Medaka model for human diseases will be described.

(PS3182) Develop a method to study radiation induced alternative splicing transcripts. Tzu-Hung Hsiao[1], Eric Y. Chuang[1], Konan Peck[2]. [1]Department of Electrical Engineering,National Taiwan University, Taipei, Taiwan, [2]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

In this study, we develop a method for high-throughput alternative splicing measurement by combining bioinformatics analysis, RT-PCR and capillary electrophoresis. Because of high resolution of capillary electrophoresis, this technique can not only determine splicing events (like exon junction microarray), but also measure expression of each splicing isoforms. This method is described as following. First, we analyzed all splicing events reported at the Alternative Splicing Database (ASD) from the European Bioinformatics Institute and then calculated all possible splicing transcripts. Secondly, the conserved and differential exons were compared in order to define the PCR flanking regions. Minimal sets of primers were designed to amplify all possible isoforms with different sizes which could be resolved by electrophoresis. Thirdly, RNA samples were subjected to RT-PCR and separated by capillary electrophoresis, and the eletro-phoregrams were analyzed to quantify splicing sizes and expression levels. Taking advantages of this method, we studied 300 lung cancer-specific genes derived from microarray data and the NCI CGAP. By comparing lung tumor with normal lung tissue, we found numbers of genes (*COL71A2*, *RPL13*, *VSIG2*, *GIT1*, *pRGR2*, and etc.) have quantitative changes and qualitative differences in alternative splicing transcripts. For example, the gene *COL71A2* expresses a standard form as reported at the RefSeq database in normal lung tissue, but two other isoforms were existed in lung

tumor cells. All lung cancer-specific splicing transcripts we identified may be served as biomarkers which are much more unique than those selected by gene expression profiling. Currently, we are applying this method to profile alternative splicing transcripts in lung cancer cell lines following radiation treatment.

(PS3183) Genetic sensitivity in the transcriptomic response to low dose ionizing radiation. Brynn Voy[1], Lisa Branstetter[1], Sudhir Naswa[2], Michael Langston[2], Arnold Saxton[2]. [1]Oak Ridge National Laboratory, Oak Ridge, TN, USA, [2]University of Tennessee, Knoxville, TN, USA.

The net effect of low dose ionizing radiation (IR) exposure in vivo has been difficult to elucidate due in part to its overlap with endogenous metabolic stress and to the impact of genetic background. As a first step toward defining both the adaptation to in vivo low dose irradiation and the extent to which it is modified by genetic variation, we focused on acute changes in gene expression in spleen, profiled across a panel of inbred strains of mice. Transcsriptomic profiling provided a de novo means to define both responses to IR that were robust to genetic variation and those that exhibited significant genetic susceptibility. Six common inbred strains of mice - C57BL/6J, DBA/2J, A/J, Balb/c, C57BL/6J-c (non-pigmented substrain of C57BL/6J) and C3H/HeJ - were exposed to 10 cGy of a broad-spectrum x-ray flux produced by a standard bremsstrahlung source. Spleen, chosen for its radiosensitivity, was harvested 3.5 hours after exposure, and changes in gene expression were profiled using cDNA microarrays. Mixed model ANOVA was used to identify main effects of dose as well as genes for which the response to radiation depended upon genetic background. A total of 391 genes exhibited a significant response to low dose IR (false discovery rate of 5%). Functional enrichment analysis of this gene set using DAVID (david.abcc.ncifcrf.gov) indicated that genes responsive to low dose radiation were enriched for functions related to amino acid metabolism, ubiquitin conjugating activity, tyrosine kinase activity and purine nucleotide binding. An additional 268 genes responded to low dose IR in a way that depended upon genetic background, as evidenced by a significant strain*dose interaction in the ANOVA model. Genes for which the response was strain-dependent were enriched for pathways related to immune function, such as antigen presentation and processing, and for cell adhesion molecules. Bi-cluster analysis confirmed clear strain-dependent responses to low dose IR, in particular those involving genes relevant to immune response. Collectively our data demonstrate significant genetic sensitivity in the response to low dose IR, as revealed by transcriptomic analysis of the acute response in spleen. How these differences impact the net health outcome of low dose exposure remains to be defined, and is the focus of ongoing investigation.

(PS3184) Genetic dissection of susceptibility/resistance to ionizing radiation by use of recombinant congenic strain mice. Alexander K. Vaglenov[1], Bernhard Kaltenboeck[1], William R. Brawner[1], David M. Carpenter[1], Anny Fortin[2], Henry W. Brandhorst[1], Li Yihang[1]. [1]Auburn University, Auburn, AL, USA, [2]Emerillon Therapeutics, Inc., Montreal, PQ, Canada.

The research in this study aims to identify and mapping mouse quantitative trait loci (QTL) associated with susceptibility/ resistance to X-ray exposure in recombinant congenic mouse strains (RCS) derived from A/J and C57BL/6J parental mice. This study used 9 AcB and 16 BcA strains to analyze and identify the murine quantitative trait loci (QTLs) that influence susceptibility / resistance to ionizing radiation. At 8–12 weeks of age, 404 parental A/J and C57BL/6J controls, and 390 RCS mice were used. Parental strains were irradiated in the dose range from 10 to 60 cGy to evaluate the optimal time point for phenotyping. Additionally both strains were compared at dose-rate 250 versus 10.3 cGy/min. Adaptive response, as well as diets modulations in the conditions of different dose-rate were also studied. All experiments were done with 6 MeV electron beams in Siemens Megatron 6740 Linear Accelerator. Three sets of RCS animals were sacrificed 24 h post irradiation (0, 20 and 50 cGy) and bone marrow was processed for analyze phenotype by quantifying chromosomal damages in polychromatic erythrocytes (PCE). Coded slides were scored for the incidence of micro-nucleated PCE at 1000x magnification under immersion. Approximately, 2000 PCE were scored per animal. Each group of parental or RCS (control or irradiated) contain no less than 5 mice. The optimal phenotyping time and dose are 24h after irradiation, as well as 50cGy. Linkage maps constructed with 616 informative microsatellite markers were used to identify chromosomal regions associated with susceptibility/resistance to ionizing radiation. These analyses identified first collections of chromosomal markers that best correlated with the respective outcome. Significant ($p<0.0001$) regions were identified on chromosomes 2, 4, 6, 8, 13, 14, 19. Multiple regression analysis demonstrated that a subset of seven markers, including D2Mit401, D4Mit81, D4Mit31, D6Mit340, D8Mit131, D13Mit293, D14Mit and D19Mit59, accounted for 90% of the genetic variance. Three sets of data - control, after 20, as well as 50 cGy suppose strong correlation between spontaneous and induced answers to ionizing radiation. Results from 25 RCS suggest complex genetic control of susceptibility/resistance to ionizing radiation

(PS3185) Application of assisted reproductive technologies (ARTs) for radiobiological research. Seiji Kito[1], Yuki Ohta[2], Yumiko Kaneko[1], Hiroko Yano[1], Tadahiro Shiomi[1], Naoko Shiomi[1], Shimada Yoshiya[1], Kazuo Sakai[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Science Services, Chiba, Japan.

In radiation biology, experiments using animals are inevitable for understanding radiation effects at whole body level. Especially, use of genetically modified (GM) mice in radiation biology has been explosively increased in the last two decades, and an efficient system for maintenance of these animals has become a crucial issue in many radiobiological research institutes. In the National Institute of Radiological Sciences, the Advanced Animal Research Section has been collaborating with many research groups to facilitate whole animal experiments by application of assisted reproductive technologies (ARTs). The ARTs applicable to radiation research includes (1) production of new radiosensitve GM animals, (2) short and long term cryopreservation of embryos, (3) synchronous production of large number of animals, (4) domestic and international shipping of animals by way of cryopreserved embryos, and (5) cleaning of animals infected with various pathogens. An example of practical application of ARTs is simultaneous production of large animal colony by *in vitro* fertilization-embryo transfer (IVF-ET) system. This technique fits well for reproduction of GM mice, such as *gpt*-delta transgenic mice and *aprt* (adenine phosphorribosyltransferase) heterozygous mice used frequently for investigating radiation-induces mutagenesis in various tissues. Because of infertility derived from behavioral defect in *aprt* deficient homozygous (-/-) animals, they have to be bred in the colony of the heterozygous (+/-) and wild type (+/+) parents. However, maintenance of +/- animals requires laborious maintenance of both +/- and +/+ mice followed by genotyping. By application of IVF-ET to +/+ females and only two -/- males, we successfully obtained large stock of +/- *aprt* embryos (>500). These embryos were cryopreserved and, upon request, predetermined number of animals can be supplied without genotyping, resulting in maintenance of animals less expensively, less laboriously and more efficiently than conventional methods. Although we found some limitations in ARTs for practical application, we are currently making effort to improve our ARTs further for radiation research.

(PS3186) Lab-on-a-chip-system for systems radiation biology. Stefan Thalhammer[1], Achim Wixforth[2], Wolfgang Heidenreich[1], Herwig Paretzke[1]. [1]GSF - Institute of Radiation Protection,

Neuherberg, Germany, [2]Universität og Augsburg, Department of Physics, Augsburg, Germany.

In this interdisciplinary approach the combination of quantitative experimental data with mathematical modelling will open the identification and specification of systems properties of biological responses to ionizing radiation at different hierarchical levels. This planar chip technology will provide the experimental toolbox to work on targets from a single cell to a multi-cellular level. Subsequent biochemical and irradiation experiments can be performed on the programmable chip surface.

Here we present the setup of a novel lab-on-a-chip system driven by nanofluidics and controlled by surface acoustic waves (SAW). This will combine the serial DNA-isolation-, amplification- and array-detection-process on a modified glass-platform. The fluid actuation is controlled via SAW by interdigital transducers implemented in the chemical modified chip surface. The chemical surface modification allows fluid handling in the sub-microliter range. To demonstrate the feasibility of this approach, we show results on single gene amplification in the sub-nanogram range and on chromosomal imbalances by serial combination of single chromosome isolation, total genome amplification, hapten-labelling and fluorescence in situ hybridization onto normal human metaphase spreads.

(PS4001) Discovery of Sam68 as a Biomarker of Apoptosis induced by γ-irradiation in immune system. Yang Kwang-Hee[1], Moo Hyun Choi[1], Min Young Kim[1], Seon Young Nam[1], Meeseon Jeong[1], Cha Soon Kim[1], Hee Sun Kim[1], Young-Woo Jin[1], Sungkwan An[2], Suhkneung Pyo[3], Chong Soon Kim[1]. [1]Radiation Health Research Institute, KHNP co., LTD, Seoul, Republic of Korea, [2]Functional Genoproteome Research Centre, Konkuk University, Seoul, Republic of Korea, [3]College of Pharmacy, Sungkyunkwan University, Suwon, Kyunggi-do, Republic of Korea.

The RNA-binding protein Sam68, identified as a mitotic substrate of tyrosine kinases, has been reported to participate in cell cycle, apoptosis and signaling. Especially, Sam68 has been associated with multifunctional complexes containing several transduction proteins that play a role in T cell activation. However, it has been not reported that the Sam68 proteins were cleaved in the cells undergone the apoptosis by γ-irradiation. In our preliminary study, we found that Sam68 proteins were cleaved in the apopototic immune cells by γ-irradiation. Therefore, the aim of the present study was to investigate whether Sam68 was the new apoptosis indicator of radiation. In this study, When we exposed IM-9 human B lymphoblast cell line to Cs-137 radiation, It was found that the cell death and Sam68 cleavage increased in radiation dose dependent manner. And this Sam68 cleavage formation was also observed in Sam68 proteins induced by the active forms of caspase family using in vitro translation system. To investigate which of apoptosis related-effecter proteins cleavaging Sam68 proteins, we examined the cleaved Sam68 formation by each activated caspases. We found that Sam68 proteins could be cleaved by activated caspase 3, 7, 8, 9 proteins. These results indicate that the cleavage of Sam68 may be a phenomenon indicating the loss of cell viability and a new indicator for the cell damage effects by ionizing radiation.

(PS4002) Investigation of the serum proteome to look for ionizing radiation biomarkers. Olivier Guipaud[1], Valérie Vereycken-Holler[1], Joëlle Vinh[2], Patrick Gourmelon[1], Marc Benderitter[1]. [1]IRSN, Fontenay aux Roses, France, [2]ESPCI, Paris, France.

Radiation-induced lesion outcomes of normal tissues are difficult to predict. In particular, radiotherapy or local exposure to a radioactive source by accident can trigger strong injury to the skin. The finding of prognostic biomarkers is of fundamental relevance for the prediction of lesion apparition and its evolution, and for the settlement of therapeutic strategies. In order to study radiation-induced cutaneous lesions, we developed a mouse model in which the dorsal skin was selectively exposed to ionizing radiation. Two-dimensional difference gel electrophoresis (2-D DIGE) coupled with mass spectrometry were used to investigate proteins altered in expression and/or post-translational modification in serum. Proteome changes were monitored from one day to one month post-irradiation at 40 Gy in this specific model developing reproducible clinical symptoms ranging from erythema to skin ulceration followed by wound healing. About 50 proteins (including some isoforms and likely post-translational variants), representing 20 different proteins, that exhibited significant kinetic expression changes were identified using mass spectrometry and database interrogation. Several proteins were from day one down- or up-regulated and could turn out to be good candidates to prognosticate the evolution of skin lesion. In addition, we observed shifts in isoelectric point (p*I*) of several spot trains, revealing potential post-translational modification changes which could also serve as indicators of irradiation. On the other hand, we report the first serum proteome analysis of a man strongly and locally exposed to ionizing radiation by accident. 2-D DIGE analysis of his serum compared to serums from healthy donors revealed many changes in expression level and likely in post-translational modification. Identifications of variant proteins using mass spectrometry will be presented and discussed. In conclusion, our results clearly indicate that serum protein content is dynamically modified after a local irradiation of the skin, showing that investigation of the serum proteome could be of great importance to find out diagnostic or prognostic indicators.

(PS4003) ***In vivo*** **expression of p53 and stat3 dependent genes after ionizing radiation.** Marcy B. Grace, Antonino Germana, Dadin Fu, Thomas B. Elliott, William F. Blakely, G. David Ledney. Armed Forces Radiobiology Research Institute, Bethesda, MD, USA.

Ionizing radiation (IR) injury produces temporal- and dose-dependent changes of gene expression patterns in multiple human and animal models. Several downstream genes to the transcription factor p53 are up-regulated by IR, including *CDKN1a (p21$^{Waf\text{-}1/Cip1}$), GADD45a, DDB2*, and *BAX*. Up-regulation of *CDKN1a* and *BCL-2* also occurs via the *STAT3* pathway in a mammalian stress response. Altered expression of these genes can result in growth arrest, enhanced DNA repair capacity, and/or induction of apoptosis. In preliminary studies, we irradiated human whole blood *ex vivo* to measure gene expression changes by QRT-PCR assay in samples from (i) a cohort of three healthy donors over a broad dose range (0, 0.25, 0.50, 0.75, 1, 2, 3 Gy), and (ii) a cohort of twenty healthy donors at two doses (25 cGy and 2.5 Gy). The objectives of these studies were to (1) investigate the relationship between baseline mRNA expression levels in normal individuals; (2) define expression and patterns in response to IR; and (3) determine the inter-relationship of these genes for biodosimetry applications.

By combining *ex vivo* irradiation dose-response data with temporal gene expression studies *in vivo*, we have now validated several coordinately responding genes in diverse pathways altered by IR. *In vivo* models include blood samples from irradiated rodent and *Macaca mulatta*, and radiotherapy patients undergoing total-body irradiation (TBI) prior to bone marrow transplants. Multiplex quantitative PCR assays were optimized for mouse, non-human primates, and human cDNA samples. Temporal induction of *BAX, GADD45 a, DDB2,* and *CDKN1a* transcripts, and altered ratios of *BAX*/*BCL2* mRNAs, were quantitative and dose-responsive. We also discovered $>$ 30 d persistence of several candidate biomarkers in B6D2F$_1$ female mice and Rhesus primates following sub-lethal doses of TBI. On-going studies address long term changes in gene expression and DNA repair capacity following IR. Our data indicate that these sentinel gene targets in multiplex real-time PCR assay could prove useful in mechanistic studies of novel radioprotectants, monitoring radiotherapy patients, and assessment of radiation injuries for biodosimetry when combined with other early medical parameters such as lymphocyte depletion kinetics, onset of vomiting, and protein bioassays.

(PS4004) *In vivo* murine dose-response calibration curves for early-response exposure assessment using multiple radiation-responsive blood protein biomarkers. Natalia I. Ossetrova, David J. Sandgren, William F. Blakely. AFRRI/USUHS, Bethesda, MD, USA.

The present need to rapidly identify severely irradiated individuals who require prompt medical treatment in mass-casualty incidents, as well as exposed vs. non-exposed individuals in population-monitoring radiation scenarios, prompted a murine *in vivo* dose- and time course-dependent study to evaluate the potential utility to use radiation-responsive blood protein biomarkers for exposure assessment purposes. Protein targets were measured by enzyme linked immunosorbent assay (ELISA) in male BALB/c mice (6–8 weeks old) blood plasma after whole-body ^{60}Co gamma exposure (10 cGy/min) to a broad dose range (0–7 Gy) and time-points (4–96 h).

Our research strategy involves the use of human, non-human primate, and murine models involving *ex vivo* and *in vivo* radiation exposure to identify and validate radiation-responsive protein biomarkers. Using an *ex vivo* model of human peripheral blood lymphocytes as well as an *in vivo* murine model, we earlier reported radiation-responsive changes in the expression of proteins *ras*-p21, *raf*-1, GADD45a, p53, and p21WAF1/CIP1, each with a progressive time- and radiation dose-dependent increase. These results also revealed dose-dependent correlations among this subset of protein biomarkers, demonstrating their utility to identify potentially exposed individuals during the early assessment of radiation exposure. In addition, we recently presented similar data from non-human primates exposed to whole-body 6-Gy 250-kVp x-irradiation and 6.5-Gy ^{60}Co γ-irradiation. Data analyzed with use of multivariate discriminant analysis established very successful separation of animal groups before and after irradiation.

Here we present results from on-going murine *in vivo* studies demonstrating time- and dose-dependent increases in multiple blood protein biomarkers (i.e., GADD45a, IL-6, serum amyloid A). The use of multiple protein targets was evaluated using multiple regression analysis to provide dose-response calibration curves to enhance radiation sensitivity. Results from these *ex vivo* and *in vivo* validation studies establish an initial proof-of-concept that radiation protein biomarkers could provide useful diagnostic information for radiation-exposure assessment. [AFRRI supported this research under work unit BD-10.]

(PS4005) Radiation-induced phosphorylation of p53 on ser 15 in MOLT4 cells is dose-dependent. Ales Tichy[1], Darina Zaskodova[2], Martina Rezacova[2], Jirina Vavrova[1], Zuzana Rehakova[1], Zdena Vilasova[1], Jaroslav Pejchal[1], Jan Osterreicher[1]. [1]University of Defence, Faculty of Health Sciences, Hradec Kralove, Czech Republic, [2]Charles University, Faculty of Medicine, Hradec Kralove, Czech Republic.

Objective: To improve understanding of molecular mechanisms regulating cell cycle and apoptosis induced by ionizing radiation (IR) in MOLT-4 cells (human T-lymphocyte leukemia) and to find among the molecules involved in this signaling a potential biodosimetric marker.

Upon irradiation ATM-kinase (ATM) is auto-phosphorylated (Ser^{1981}) and it activates a number of downstream targets. We detected those associated with G1 cell cycle arrest: checkpoint kinase-2 (Chk-2; Thr^{68}), p53 (Ser^{15} and Ser^{392}) and its negative regulator Mdm2 (Ser^{166}).

Methods: The cells were irradiated, lysed and the proteins were separated by SDS-PAGE and detected by Western-blotting. Phosphorylation of p53 (Ser^{15}) was evaluated by ELISA Sandwich kit.

Results: We exposed the MOLT-4 cells to cytostatic, sublethal, and lethal doses (1.5, 3, and 7.5 Gy, resp.). ATM and Chk-2 were phosphorylated from 0.5 hour post irradiation by all the tested doses. Accumulation of p53 occurred from 1 hour after the dose of 1.5 and 3 Gy and after 0.5 hour after 7.5 Gy. TP53 was phosphorylated on Ser^{15} and Ser^{392} from 2 hours after the dose of 1.5 and 3 Gy, or from 0.5 hour after 7.5 Gy, resp. Mdm2 was maximally phosphorylated concomitantly with p53, i.e. from 4 to 6 hours after the dose of 1.5 and 3 Gy, or from 2 to 4 hours after 7.5 Gy.

None of phosphorylations were dose-dependent but phosphorylation of p53 on Ser^{15}. We analyzed it also by ELISA test and found a dose-dependent response in a dose range 0.5 - 7.5 Gy 2 hours after irradiation.

Conclusion: We proved that exposure of MOLT-4 cells to IR causes rapid phosphorylation of ATM (Ser^{1981}) and Chk-2 (Thr^{68}). Accumulation of p53 is followed by its phosphorylation on Ser^{15} (attenuates interaction with its negative regulator, Mdm2) and Ser^{392} (critical for DNA binding) and apoptosis is induced subsequently. Phosphorylation of p53 is accompanied by concurrent phosphorylation of Mdm2 (Ser^{166}). Although phosphorylations of ATM, Chk-2, Mdm2 and p53 (Ser^{392}) are not dose-dependent, we demonstrated a homogeneous dose-dependent increase in phosphorylation of p53 on Ser^{15}. Our results suggest that this phosphorylation might be used as a potential biodosimetric marker of received dose of IR.

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(PS4006) Expression monitoring of six new radiation responsive genes. M. Ahmad Chaudhry. University of Vermont, Burlington, VT, USA.

The expression of many genes is modulated after exposure to ionizing radiation. Identification of specific genes may allow the determination of pathways important in radiation responses. Recently we identified a number of radiation responsive genes by employing a microarray-based strategy. In the present study we monitored the expression of six of these genes, *RGS1, CC3*, *THBS1, vWF*, *MADH7*, and a novel gene encoding a secreted protein in four human cell lines, Jurkat, TK6, HeLa and HFL1 at various time points after exposure to ionizing radiation. Our objective was to find similarities and differences in the expression of these six genes in diverse cell types. *vWF* was induced in radiation-exposed HeLa cells but its expression was downregulated in Jurkat cells. *RGS1* was downregulated in Jurkat cells but was upregulated in TK6 and HFL1 cells. The expression of *CC3* was repressed after exposure to ionizing radiation in Jurkat and HFL1 cells. On the contrary this gene was induced in TK6 and HeLa cells. The expression of *THBS1* was found to be downregulated in irradiated TK6 and HFL1 cells. A striking observation was the induction of *MADH7* in all the cell lines exposed to ionizing radiation. Taken together these results indicate a cell line specific modulation of gene expression after exposure to ionizing radiation. The ability to detect altered gene expression after ionizing radiation treatment could help to predict tumor response to radiation therapy. Additionally the identification of new radiation responsive genes could potentially be exploited to develop new biomarkers of human exposure to radiation after radiological accidents.

(PS4007) Risk assessment of radiation exposure using molecular biodosimetry. Todd F. Elliott[1], Kerry George[1], Dianne K. Hammond[2], Francis A. Cucinotta[3]. [1]Wyle Laboratories, Houston, TX, USA, [2]Enterprise Advisory Services, Inc, Houston, TX, USA, [3]NASA, Houston, TX, USA.

Current cytogenetic biodosimetry methods would be difficult to adapt to on-board spaceflight operations because they require toxic chemicals and a substantial amount of time to perform. In addition, current biodosimetry techniques applicable to acute and chronic exposures are limited to whole-body doses over about 5cGy to achieve sufficient statistical accuracy. Development of new techniques that assess radiation exposure response at the molecular level could overcome these limitations and have important implications for the advancement of biodosimetry or approaches to biomarkers. Recent technical advances include expression profiling at the transcript and protein level to assess multiple biomarkers of exposure, which may lead to the development of a radiation biomarker panel revealing possible fingerprints of individual radiation sensitivity. Many biomarkers of interest, such as cytokines and members of the DNA repair pathway, have been examined for their response to ionizing radiation. New technology, such as the Luminex system, can analyze many biomarkers

simultaneously in one sample. Results using this approach will be discussed.

(PS4008) Development of a risk assessment system of toxicants by HiCEP. Katsutoshi Suetomi, Akira Fujimori, Yoshihisa Kubota, Sentaro Takahashi. National Institute of Radiological Sciences, Chiba, Japan.

In our previous study, we have indicated a novel expression profiling method called 'high coverage expression profiling' (HiCEP) could detect an alteration in gene expression as small as 1.5-fold and covers 70% to 80% of all transcripts. As HiCEP has a great advantage over other common methods for detecting minimal alterations in gene expression as described above, our comprehensive approach using HiCEP method is thought to be a powerful strategy to develop the system of risk assessment of many chemicals.

Arsenic is one of the typical toxic metals and is also known to be carcinogen. Exposure to arsenic is supposed to increase the risk of occurrences of tumors (lung, skin, liver, bladder, and kidney). In order to investigate the efficiency of HiCEP method on developing the system of risk assessment of arsenic in the environment, we carried out HiCEP analysis of human lung embryonic fibroblasts (HFLIII) after 1 and 2 hours' exposure to 1 μM sodium arsenite. The most up-regulated gene is hemoxigenase 1 (HMOX1), which is known to be induced by arsenic. We examined the effect of arsenic on the HMOX1 expression by quantitative PCR (qPCR) after several periods of incubation time with 1–10 μM sodium arsenite. HMOX1 expression reached to the peak value after 4 hours' incubation with 1–10 μM arsenic. We also examined the effect of low level of arsenic on HMOX1 expression. HMOX1 expression reached to the peak value after 2 hours' incubation with arsenic at 0.1 and 0.3 μM, and after 4 hours' incubation with arsenic at 0.5 μM. Up-regulation of HMOX1 gene was observed in HFLIII cells treated with arsenic at 0.3 and 0.5 μM. We examined the effect of arsenic concentration on cell viability. Arsenic at less than 1 μM does hardly affect the viability of HFLIII cells.

We further explored the effect of ionizing radiation on HMOX1 expression by qPCR 0.5, 2, and 4 hours after X-irradiation at 2 and 4 Gy. At all of the period of time after X-irradiation at 2 and 4 Gy, HMOX1 expression was not up-regulated. In HiCEP analysis, HMOX1 expression after low-dose X-irradiation was not up-regulated as well.

We conclude HiCEP could be used to develop the system of risk assessment of many chemicals and HMOX1 could be a good biomarker for arsenic contamination in the environment.

(PS4009) Radiation metabolomics permits discovery of mouse urinary biomarkers for gamma radiation exposure. John B. Tyburski[1], Josef Slavik[2], Kristopher W. Krausz[1], Kathryn Doiron[3], Christian Lanz[2], Albert J. Fornace, Jr[3], Frank J. Gonzalez[1], Jeffrey R. Idle[2]. [1]Laboratory of Metabolism, Center for Cancer Research, NCI, NIH, Bethesda, MD, USA, [2]Institute of Clinical Pharmacology, University of Bern, Bern, Switzerland, [3]Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC, USA.

The purpose of the study was to develop biomarkers for gamma radiation exposure in the mouse. There are limited available data for the response of mammalian organisms to ionizing radiation exposure, in terms of changes in the complement of small molecules known as the metabolome. Such exposure biomarkers are necessary for the monitoring of populations inadvertently exposed to ionizing radiation due to nuclear accidents or acts of terrorism and warfare. We exposed C57Bl/6 mice to doses of gamma radiation from 0.1 to 11 Gy in two different laboratories and employed the emerging field of metabolomics to uncover biomarkers in various biofluids. Urinary end-products of metabolism are invariably acidic and therefore anionic. Over 2,000 24-hour mouse urines were analyzed by ultra-performance liquid chromatography coupled to electrospray time-of-flight mass spectrometry for their content of anionic species. Multivariate data analysis was applied, specifically principal components analysis, partial least-squares discriminant analysis, and batch projection to latent structures analysis, using SIMCA-P$^+$. Dose-dependent anionic biomarkers were revealed that were subjected to further validation using liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry. Furthermore, targeted metabolic profiling was employed for urinary products of protein and lipid interactions with reactive oxygen/nitrogen species. Overall, urinary biomarkers included Krebs' cycle intermediates, products of impaired lipid metabolism, DNA damage, and abnormal gut floral metabolites. Metabolomics offers a means to design high-throughput non-invasive protocols capable of detecting persons who have received non-lethal doses of ionizing radiation.

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(PS4010) A novel method for biodosimetry. Jeff W. Bacher[1], Wael Abdel Megid[1,2], Martin G. Ensenberger[1], Richard B. Halberg[2], Stephen A. Stanhope[2], Marijo G. Kent-First[3], Tomas A. Prolla[2]. [1]Promega Corporation, Madison, WI, USA, [2]University of Wisconsin, Madison, WI, USA, [3]Mississippi State University, Starkville, MS, USA.

Accurate methods for measuring the biological effects of radiation are critical for estimating an individual's health risk from radiation exposure. We investigated the feasibility of using radiation-induced mutations in repetitive DNA sequences to measure genetic damage caused by radiation exposure. Most repetitive sequences are in non-coding regions of the genome and alterations in these loci are usually not deleterious. Thus, mutations in non-coding repetitive sequences might accumulate, providing a stable molecular record of DNA damage caused by all past exposures. To test this hypothesis, we screened repetitive DNA sequences to identify the loci most sensitive to radiation-induced mutations and then investigated whether these mutations were stable *in vivo* over time and after multiple exposures. Microsatellite repeat markers were identified that exhibited a linear dose response up to 1 Gy of 1 GeV/nucleon ^{56}Fe ions and ^{137}Cs gamma rays in mouse and human cells. Short tandem repeats on the Y chromosome and mononucleotide repeats on autosomal chromosomes exhibited significant increases in mutations at $\geq$0.5 Gy of ^{56}Fe ions with frequencies averaging 4.3–10.3 $\times$ 10^{-3} mutations/locus/Gy/cell, high enough for direct observation of mutations in irradiated cells. A significant increase in radiation-induced mutations in extended mononucleotide repeats was detectible *in vivo* in mouse blood and cheek samples 10 and 26 weeks after radiation exposure and these mutations were additive over fractionated exposures. This study demonstrates the feasibility of a novel method for biodosimetry that is applicable to humans and other species. This new approach should complement existing methods of biodosimetry and might be useful for measuring radiation exposure in circumstances that are not amenable to current methods.

(PS4011) Estimating the genotoxic effects of Fe-ions: impact of cell cycle effects, apoptosis and intra-individual variability. Sylvia Ritter, Ryonfa Lee, Sylvester Sommer, Elena Nasonova. GSI, Darmstadt, Germany.

Health hazards of charged particles, in particular Fe-ions, are of major concern for the planning of manned space explorations. Among the health effects carcinogenesis is currently considered to be the main risk factor for astronauts. To estimate the cancer risk, chromosome aberration frequencies are generally measured in peripheral blood lymphocytes. Cells are cultivated for about 48h in vitro. Then, the aberration yield is determined in first cycle cells. Since our preceding studies using hamster cells and human fibroblasts have shown that heavy particles induce dramatic cell cycle delays and that these delays are related to the aberration burden of a cell, we analysed in detail the time-course of aberrations in human lymphocytes after Fe-ion exposure. Additionally, factors that might confound the aberration yield were examined. In the experiments, lymphocytes from a healthy volunteer were exposed to

either Fe-ions with differing linear energy transfer (LET) or X-rays. Lymphocytes were harvested at multiple times and aberrations were determined in 1st cycle metaphases and prematurely condensed G2-cells. Furthermore, cell cycle effects (e.g. S-phase labelling, G2-delay), apoptosis and intra-individual variations in the cytogenetic response were recorded. Our data reveal a pronounced increase in the aberration yield with sampling time after Fe-ion exposure. This increase was about threefold for LET=150 keV/μm, sevenfold for LET=400 keV/μm and tenfold for LET=3160 keV/μm. In contrast, a stable aberration yield was found after X-ray exposure. As a consequence, RBEs derived from late sampling times were significantly higher than those obtained at early sampling times. A similar picture was found for G2-phase cells. However, the increase was less pronounced than that observed in metaphase samples. Furthermore, with increasing LET the number of lymphocytes capable of entering the cell cycle decreased, while the number of cells undergoing apoptosis increased indicating a rapid removal of heavily damaged cells from the population. Finally, marked intra-individual variations in the cytogenetic response were observed. The significance of these observations for estimating health risks associated with high LET exposure will be discussed and alternative research strategies will be presented.

(PS4012) Gene expression profiles for radiation biodosimetry with a fully integrated biochip. Sunirmal Paul[1], Ralf Lenigk[2], Christine Orosco[2], Mark Richards[2], Frederic Zenhausern[2], Sally A. Amundson[1]. [1]Columbia University Medical Center, New York, NY, USA, [2]The Biodesign Institute at Arizona State University, Tempe, AZ, USA.

To fill the need for rapid, high-throughput radiation biodosimetry in the event of a large-scale radiological incident, we are developing a fully automated integrated biochip system based on gene expression. We have used Agilent whole genome microarrays to profile the gene expression response of human blood to ionizing radiation. We measured gene expression profiles of ex vivo irradiated human peripheral blood from healthy donors. This study spanned doses from 0.5 to 8 Gy, and monitored gene expression at 24 hours after irradiation. Real time PCR of CDKN1A revealed a biphasic response, with linear kinetics up to 2 Gy and further linear increases through the highest dose used. A dose-response relationship was further evident within the microarray data. Preliminary analysis yielded 109 genes that distinguish between untreated controls and four different doses of irradiation.

We have also used blood samples from patients undergoing total body irradiation (TBI) as a model to test gene expression signatures in vivo. Interestingly, in vivo CDKN1A expression after a single TBI fraction (1.5 Gy) showed induction ratios similar to the ex vivo 2 Gy samples. When pre- and post- TBI samples were tested against the preliminary ex vivo signature, the same gene set was able to discriminate control from irradiated patient samples, although differences from the healthy donors were also evident.

In order to make such gene expression signatures useful for triage, we are developing cartridges to take a blood sample and automatically perform a chemo-luminescence based gene-expression assay. The cartridges contain all necessary reagents, pumps, valves and control electronics, do not rely on molecular amplification methods such as PCR, and deliver highly consistent results (cv $<$10%). We have developed a hand-held, microprocessor-controlled prototype for sample preparation, and are modifying a commercial chemo-luminescence reader to read the microfluidic cartridges.

This biodosimetry concept was tested at the Coyote Crisis Campaign 2006, a disaster preparedness exercise in Scottsdale, Arizona. While additional studies are needed, our current findings strongly support the usefulness of gene expression signatures and our biochip approach for radiation biodosimetry.

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(PS4013) Stable amino acid end-products in proteins irradiated in the solid state: potential use as biodosimeters of radiation exposure in human populations. Steven G. Swarts, Katerina A. Naumenko, William A. Bernhard. University of Rochester, Rochester, NY, USA.

In the event of a radiological exposure incident, such as the detonation of a radiological dispersion device (RDD) in a terrorist attack or accidental exposures to sources of ionizing radiation, it is advantageous to use dose assessment methods that can be conducted rapidly on large numbers of people. Ideally, the method should use biomarkers with a dose-response that can be measured in the range of 0.5 to 10 Gy, a range which is relevant to the triage and treatment of exposure victims. The method should also be based on human specimens that can be easily obtained using minimally invasive sampling techniques.

Here we present our current efforts at exploring the use of specific radiation-induced alterations measured in the proteins making up human hair and nails as potential biodosimeters of human exposures to ionizing radiation. Specifically, our objective is to demonstrate that amino acid damage products can be reliably measured in hair and cuticle within a dose range of 0.1 to 10 Gy and that the yields of these products can provide dose estimates in increments of $<$ 1 Gy.

In our initial phase of the project, we are integrating the end-product analysis of hair and cuticle cuttings x-irradiated by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) techniques with the identification and quantification of the radiation-induced free radical signal in these human specimens. Several specific amino acid products have been identified that also possess good dose-responses above 500 Gy. However, a high background of oxidized amino acids in hair and, especially, in nails, has limited the dose range to a threshold of 100–500 Gy. To help overcome this limitation, we are devising strategies to our sample processing techniques that provide for the discriminate characterization and quantification of the radiation-induced amino acid end-products in hair and nails. These strategies and their results will be discussed.

(PS4014) Potential use of early cytokine changes as surrogate markers of low dose irradiation. Eric Hernady, Jacqueline P. Williams, Carl Johnston, Christina Reed, Jacob N. Finkelstein. University of Rochester Medical Center, Rochester, NY, USA.

Rationale: Following a radiological or nuclear terrorism event or accident, there is a need for accurate biodosimetry for triage purposes. Since lung damage may occur from both direct or inhaled irradiation, our group has begun to look at cytokine responses to low doses of radiation for use as biomarkers following both localized (whole lung) and total body irradiation (TBI), correlating changes in expression with both early and late pulmonary responses.

Method: Groups of 10 C57Bl/6 mice, 6–8 weeks of age, received either whole lung or total body external irradiation at doses of 0, 0.5, 1, 2.5, 5 or 10 Gy. Animals were sacrificed at time points between 1 hour and 1 15 months. Alterations in cytokine mRNA and protein expression were assessed in tissue (lung and liver), serum and lavage (BAL).

Results: In the lung tissue, robust, dose response changes were seen in CXCR2 mRNA expression at 1 and 6 hours post-irradiation, returning to baseline by 24 hours. These were concurrent with increases in protein levels of KC, a neutrophil chemotactic factor and a ligand for CXCR2, and interleukin (IL)-6 measured in the serum. Total cell counts (BAL) indicated that there is a rapid ($>$48 hours) dose-responsive decline in total cell numbers. In contrast, in the tissue, there was an abrupt increase in the numbers of infiltrating neutrophils over the 1 to 6 12 hour period, followed by an equally rapid decline.

Conclusion: KC (the murine equivalent to IL-8/GROα) and IL-6 may be candidates for biodosimeters. Interestingly, log transformation of the values for these two cytokines demonstrated a clear threshold for doses at or above 2 Gy. Their role in the progression of tissue response is unclear at present, nonetheless, their early change in expression may act as a surrogate for lung response. Investigations are currently underway to further examine cytokine expression at later time points and correlate these early changes with late tissue effects.

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(PS4015) Proteomic expression studies after *in vivo* irradiation. Daniela L. Stricklin, Margaretha Lundquist, Micael Granström. FOI, Swedish Defence Research Institute, Umeå, Sweden.

The expression of a wide variety of proteins may be altered after exogenous and endogenous insults. Studies of protein expression after radiation exposure afford both the opportunity to identify novel markers of exposure as well as mechanistic insight on molecular response. The aim of this study was to elucidate the broad spectrum of proteins expressed *in vivo* in mice after moderate to low dose radiation exposure. Serum was collected from control, sham, and irradiated mice. Sham and irradiated responses were examined at 6, 24, 48, and 96 hours post exposure. The serum samples were treated under different conditions and placed on three different protein chip surfaces; to capture proteins by cationic interactions, metal exchange, and via hydrophobic interactions. The chips were analyzed by a SELDI mass spectrometer and the molecular weight of proteins identified. The results of our studies indicate expression profiles included proteins are common in all treatment groups, proteins common to both sham and irradiated samples, and other proteins that are specific to only sham or only irradiated samples. Further, based on the kinetic profiles the optimal time window for measurement of different proteins is examined. This study provides insight on the wide spectrum of expression of proteins after exposure and further helps to indicate which responses are are unique to the exposure and which are stress related.

(PS4016) Differential diagnosis of responses caused by radiation or chemical exposure. Hee-Kyung Kwon, Hyung-A Kim, Hyun-Jin Yun, Ji-Eun Kim, Hye-Kyung Shin, Su-Jae Lee, Chang-Mo Kang. Korea Institute of Radiological & Medical Sciences, Seoul, Republic of Korea.

Purpose: To investigate whether the radiation exposed person from the chemical exposed person can be determined differentially with human blood lymphocytes by cytogenetical methods.

Materials and Methods: Human blood lymphocytes were analyzed to differentiate the radiation exposure or chemical exposure after radiation or chemical exposure with cytogenetical methods containing dicentric assay and micronuclei in binucleated cell assay.

Results and Conclusion: Data from both dicentric assay and micronuclei in binucleated assay were responsed to radiation exposed human lymphocytes, however, data from micronuclei in binucleated assay was only responsed to chemical exposure. These results suggest that if we employ both dicentric assay and micronuclei in binucleated cell assay, we can differentiate whether he was radiation-exposed or chemical-exposed.

(PS4017) Radio-adaptive response of cultured salmon cells exposed to ionizing radiation. Michael F. Kilemade, Jennifer A. Lennon, Douglas R. Boreham. McMaster University, Hamilton, ON, Canada.

When exposed to a variety of environmental stresses, living organisms from bacteria to humans respond promptly so that they may survive the crisis. Radio-adaptive response (RAR) refers to the phenomenon whereby biological systems irradiated with very low doses of ionizing radiation, generally between 0.10 and 0.50 Gy become refractory to the genotoxic effect of a subsequent challenge with a higher dose of radiation (>1.0 Gy).

The purpose of this study was to investigate the influence of low conditioning doses of ionizing irradiation on salmon cell survival *in vitro* prior to being challenged with subsequent higher doses and to measure DNA repair rates. RAR is well established in mammalian cells, however the kinetics in non-mammalian systems is largely unknown and ecobiodosimetry is gaining importance in the scientific arena. A RAR was examined in the Chinook salmon embryo cell line (CHSE-214). The cells were initially irradiated with a range of low conditioning doses of ^{60}Co γ (gamma) rays (0.25 - 0.75 Gy), followed by a challenge dose of 7.50 Gy at intervals of 24, 48 and 72 h. RAR in the cells was assessed using the clonogenic assay. A pilot study was set up to reveal the optimal dose for priming and challenging the CHSE-214 cells and also the most suitable incubation period between the two doses. Cell survival was determined by counting the number of colonies (viable clones) after 40 days culture. The full study revealed that cells which received the optimal priming dose of 0.50 Gy 72 h before delivering the higher challenge dose of 7.50 Gy became less sensitive to its toxic effects with an increase in cell survival of approximately 25 % over cells which received the challenge dose alone, i.e. no "priming" or conditioning dose was given.

The results represent one of the first with an *in vitro* piscine system and are consistent with other studies in a wide variety of both *in vitro* and whole-organism systems across the taxa which demonstrate a protective effect of low doses of ionizing radiation. This implies that radiation protection standards and environmental ordinances predicated on the notion that even the smallest dose of radiation carries a quantifiable risk of adverse effects to the exposed organism requires further examination.

(PS4018) Effects of low dose irradiation on the quantitative and qualitative changes of major immune parameters and on the immune surveillance in mice. Katalin Lumniczky, Tünde Szatmári, Géza Sáfrány. National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary.

Introduction: We investigated the effect of low dose ionizing radiation on the quantitative and qualitative changes of major immune parameters in healthy mice and studied how low doses influence the anti-tumor immune surveillance.

Experimental conditions: Mice were irradiated with different doses of gamma-radiation (0.01, 0.05, 0.1, 0.5 and 2 Gy) and 24 h later a moderately immunogenic mouse glioma 261 tumor was transplanted subcutaneously into the mice and tumor growth followed. To study radiation effects on various lymphocyte subsets, mice were irradiated (0.01, 0.05, 0.1, 0.5 1, 2 and 4 Gy), three or seven days later the animals were killed and lymphocytes isolated from spleen. The ratio of various lymphocyte subsets were determined by flow cytometry. The proliferative response of lymphocytes to non-specific stimuli (Concanavalin A) was also investigated.

Results: Pre-irradiation of mice with 0.01 and 2 Gy gamma radiation before tumor transplantation seriously prohibited tumor growth. Other doses were less effective. Twenty-four hours after whole body irradiation, non-specific stimuli induced lymphocyte proliferation was inhibited in a dose dependent manner; even radiation doses, as low as 0.01 Gy suppressed lymphocyte proliferation. The anti-proliferative effect persisted at least for a week. Three days after whole body irradiation, the ratio of CD4+, CD8+, CD4+CD25+ lymphocytes and NK cells increased with increasing radiation doses. This suggests that these components of the lymphocyte compartment are less sensitive to radiation as the average lymphocyte population. Nonetheless, these cells were killed by radiation in a dose dependent manner. Interestingly, these lymphocyte subsets presented hypersensitivity to radiation at low doses (10, 50 and 100 mGy). The low dose hypersensitivity was most prevalent for NK cells, where 100 mGy and 1 Gy radiation doses killed nearly the same number of NK cells (about 50% survival). The time course of these alterations was followed, and their impact on anticancer surveillance investigated.

Conclusion: The experiments suggest that even low doses of ionizing radiation might have substantial impact on various compartments of the immune system.

(PS4019) Targeted irradiation of single fibroblasts with heavy ions reveals transient cell cycle related changes but no DNA damage in bystander cells. Claudia Fournier, Philippe Barberet, Thomas Pouthier, Sylvia Ritter, Gisela Taucher-Scholz. GSI, Darmstadt, Germany.

We have shown recently that after exposure to heavy ions (LET 170 to 15000 keV/µm) bystander populations of human fibroblasts show small and transient changes with respect to cell cycle responses. We observed an overall induction of the cell cycle inhibitor CDKN1A (p21) (Western blot, immunofluorescence), also

when the transmission of signals was limited to soluble factors. This was accompanied by a transient delay in the initial G_1 cell cycle phase (determined by flow cytometry, only measured for carbon ions). Precise targeting of single cells using a heavy ion microbeam and immunofluorescence analysis revealed that cells bearing an elevated CDKN1A expression are not located in the direct neighbourhood of the hit cells or forming clusters. Furthermore, the CDKN1A induction was shown not to be related to pronounced, long-lasting effects, as analysed by assessing premature terminal differentiation, which is considered to underly fibrosis in irradiated tissue.

We now addressed the question whether the cell cycle related changes are linked to elevated levels of DNA or chromosomal damage in the bystander cells. Slightly enhanced frequencies of sister chromatid exchanges were detected for low fluencies of carbon ions. Furthermore, no pronounced bystander effect was observed with respect to the occurrence of micronuclei for carbon and uranium ion low fluence irradiation. First microbeam experiments on the formation of γH2AX foci as an indicator of DNA damage in non-targeted bystander cells showed no significant differences to control populations. The data will be discussed in contrast to reported results from literature.

In summary, the exposure of a few cells to heavy ions over a wide range of LET values revealed a small bystander effect with respect to transient, cell-cycle-related effects. The transient effects do not seem to be reflected by changes on the chromosomal level or by DNA damage in bystander cells.

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(PS4020) "Medium mediated" bystander effect induced by α-particle irradiated human fibroblasts. Francesca Antonelli[1,2], Mauro Belli[1,2], Giuseppe Esposito[1,2], Orazio Sapora[1,2], Giustina Simone[1,2], Eugenio Sorrentino[1,2], Maria Antonella Tabocchini[1,2]. [1]National Institute of Health, Rome, Italy, [2]INFN-Gr.coll. Sanità, Sezione di Roma1, Rome, Italy.

Radiation-induced bystander effects, i.e. responses detected in a wide range of unirradiated cells that are close to irradiated cells, may have important consequences for health risk assessment. Despite they have been extensively studied in the last decade, the underlying mechanisms are still largely unknown. In order to get more insight on these mechanisms, we have undertaken a study on the time scale involved in "medium mediated" bystander effects, as part of NOTE *(Non-Targeted Effects of Ionizing Radiation)* EU Project. To this purpose, the ^{241}Am alpha particle irradiator set up at the Istituto Superiore di Sanità was used. Stainless steel Petri dishes have been implemented with three independent irradiation vessels able to housing permeable membrane inserts. In the operating conditions, alpha particles impinge on the cells with LET of ~125 keV/μm, at a dose rate of ~84 mGy min^{-1}. Due to the limited range of alpha particles, only cells growing on the bottom of the Mylar based Petri dish are irradiated and bystander effects are studied in co-cultured cells placed ~1 mm apart into the permeable membrane inserts. The irradiator is located into a standard cell culture CO_2 incubator, allowing irradiation and post-irradiation incubation in the presence of irradiated cells without moving the samples. Irradiation is started and stopped trough a shutter, driven by a shaft sliding through a vacuum-tight support. After different incubation times with the shutter closed, inserts and "conditioned" medium can be immediately tested or placed in commercial Companion Plates for further incubation time(s) in the absence of irradiated cells.

Preliminary experiments were performed irradiating AG01522 human fibroblasts with 0.5 Gy and using the H2AX assay to detect DNA damage in bystander cells. The results suggest an about two fold increase in the number of cells presenting γ-H2AX foci after 1 hour incubation in the presence of irradiated cells followed by 1 hour incubation in the presence of conditioned medium only. A similar effect is observed after 2 hour incubation in the presence of irradiated cells. Parallel measurements of DAF-FM diacetate fluorescence in bystander cells suggest a widely spread presence of NO at 30 min after irradiation that decreases at longer incubation times.

This work is supported by the NOTE Project (FP6–36465)

(PS4021) Distinct neuroinflammatory responses to gamma versus HZE particle irradiation. Sean D. Hurley, Jaqueline Williams, Lee A. Trojanczyk, Michael J. Moravan, John A. Olschowka, M. Kerry O'Banion. University of Rochester Medical Center, Rochester, NY, USA.

Brain irradiation induces a modest yet significant inflammatory response. This is important because neuroinflammation is both a hallmark of brain injury and can potentiate further injury. Our previous studies with gamma-irradiation and HZE particle irradiation have shown that both forms of irradiation induce an acute neuroinflammatory response characterized by the rapid upregulation of gene transcripts for a variety of pro-inflammatory factors, including CCL2, ICAM-1, and TNF-alpha. These acute mRNA changes are accompanied, over a period of days, by morphological changes in astrocytes and microglia and by immunohistochemical changes in ICAM-1 expression. This present study sought to directly compare the dose response effects of neuroinflammation between gamma-irradiation and HZE particle irradiation. C57Bl/6 mice were exposed to a variety of doses of gamma-irradiation (0, 1, 2.5, 5, 10, 15, 25, 35 Gy, at our home institution) or 1 GeV/n ^{56}Fe HZE particle irradiation (0, 0.5, 1, 1.5, 3, 6, 9, 12, 15 Gy, at Brookhaven National Laboratory) and were sacrificed at 4 hours for gene transcript analysis or at 3 days for immunohistochemical analysis. As expected there was a greater induction of gene transcript expression for proinflammatory factors (including CCL2, ICAM-1, and TNF-alpha) at lower doses of HZE particle irradiation (0 to 6 Gy) than for gamma-irradiation. However, the dose response profiles of these proinflammatory factors for the two forms of irradiation were distinctly different. These differences in the dose response effects could be correlated with immunohistochemical differences. HZE particle irradiation primarily induced ICAM-1 expression in large vessels. In contrast, gamma particle irradiation induced ICAM-1 principally in microvessels and microglia. Our results suggest a differential neuroinflammatory response to gamma and HZE particle irradiation. This work is supported by NIH CA114587 and NASA NNJ04HD82G.

(PS4022) Delayed genomic instability in bystander cells. Burong Hu, Peter Grabham, Adayabalam Balajee, Brian Ponnaiya, Tom K. Hei, Charles R. Geard. Center for Radiological Research, Columbia University, New York, NY, USA.

Radiation-induced bystander effects pose a challenge to the assessment of radiation risk and understanding of basic mechanisms of radiation action. There is considerable evidence that exposure to ionizing radiation may induce a heritable, genomic instability that leads to a persisting increased frequency of genetic and functional changes in the progeny of irradiated cells. These may be considered to be a driving force behind carcinogenic change. Over the past decade studies on non-irradiated bystander cells have shown effects on chromosome damage for example, mutation and micronuclei.

In this study, we examine heritable genomic instability in the progeny of bystander cells. Two novel protocols were used to ensure pure populations of bystander cells and their progeny. 1) One normal human fibroblast cells population cultured in double side mylar dishes were irradiated with random short penetrability 3Gy alpha-particles. The other populations were than bystanders which could only be influenced by signal transfer through medium. 2) 20% of cells nuclei of near-confluent fibroblasts were irradiated with 30 alpha-particles each using precise Columbia University microbeam facility. This ensures that only contacting but non-hit bystander cells can survive over many cell generations. In both scenarios cells were harvested at specific time point post-irradiation. The G2 phase premature chromosome condensation's assay shows that higher level of chromosomal damage induced in bystander cell population, compared with the corresponding control, in both irradiation protocols. There are significant difference in induced chromatid breaks at the longer time point after track segment (by 5days) and microbeam (10days) irradiations. Elevated chromosomal damage can be suppressed by NS-398 (inhibitor of cyclooxygenase-2 activity) in track segment irradiation and by Octanol (inhititor of gap junctional intercellular communication) in microbeam irradiation. These results suggest that the bystander signal(s) was transferred from irradiated cells and genomic instability can be induced in bystander cell populations in both protocols. Ongoing

mFISH experiments will further indicate whether the large scale damages in chromosome of the bystander cells' progeny are induced. (supported by NIH PO1 CA49062)

(PS4023) Radiation-induced bystander responses in mouse testes. Prasad V.S.V. Neti[1], Venkat R. Narra[2], Hosea F. Huang[1], Edouard I. Azzam[1], Roger W. Howell[1]. [1]University of Medicine & Dentistry of New Jersey, NJMS, Newark, NJ, USA, [2]University of Medicine & Dentistry of New Jersey, RWJMS, New Brunswick, NJ, USA.

The present work was undertaken to assess whether ionizing radiation emitted by incorporated radionuclides (^{3}H, ^{125}I) can impart radiation-induced bystander effects in the testes of mice. Spermatogenesis in mouse testis is used as the experimental model with spermhead survival and induction of abnormal shapes in the heads of epididymal sperm serving as the biological endpoints. This *in vivo* model is extremely radiosensitive and it is rich in various forms of intercellular communication making bystander phenomena a distinct possibility.

Swiss Webster mice were anesthetized under isoflurane and injected intratesticularly with either tritiated deoxycytidine (^{3}HdC) or ^{125}I-labeled iododeoxyuridine (^{125}IUdR). These radiochemicals incorporate into the DNA of S-phase spermatogonial and preleptotene cells. The extremely short-range of the very low energy β-particles (^{3}H), and Auger electrons (^{125}I), impart radiation dose principally to the labeled cells. Immunohistochemistry studies with BrdU support this. To assess spermhead survival, the injected testes were removed 29 d post-injection, homogenized, sonicated, and the spermheads counted. Abnormalities in epididymal sperm were assayed 9 d later. Slides were smeared with sperm, dipped in nuclear emulsion, stored to accumulate decays, and developed. Spermhead abnormalities were scored blind in 1000 cells.

The ^{3}HdC dose response curve, for spermhead survival, was of a 2-component exponential nature that saturated at about 48% survival. Bolus administration of lindane, a potent gap-junction inhibitor, had no significant affect on the survival curve. Spermhead abnormalities in labeled cells increased steeply at very low testicular doses (~ 5%/cGy). The unlabeled cells receive a very small radiation dose during the early phase of testicular clearance of the radiochemical. An observed increase in abnormalities in unlabeled cells could not, based on historical studies with external x-rays, be attributed to this small dose. Therefore, the increased level of abnormalities in unlabeled cells may be attributed to bystander effects imparted by neighboring labeled cells. Additional studies with ^{125}IUdR are underway.

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(PS4024) X-ray irradiated lymphoblastoid cells caused media mediated bystander effects. Asima Chakraborty[1,2], Robert W. Redmond[2], Martin Purschke[1], Kathryn D. Held[1]. [1]Radiation Oncology, Boston, MA, USA, [2]Wellman Center for Photomedicine, Boston, MA, USA.

It is well known that radiation induces damages to the DNA either by direct ionization or by the production of reactive oxygen species, mainly OH. However, there is increasing evidence that unirradiated cells that are not exposed to the direct radiation can also exhibit a wide range of biological effects (bystander effects). Although radiation-induced bystander responses have been well documented, it is still unknown how these bystander cells communicate with the directly stressed cells and which mediators may be involved. Different reactive oxygen species and products of oxidative stress are a possibility. In order to probe the mechanisms of the radiation-induced bystander effect, human lymphoblastoid WTK1 cells were irradiated in 6 well plates using 250 kVp X-rays. A microporous insert containing naïve cells was immediately placed in the well, with the two cell populations physically separated but sharing the same media. Cellular responses including oxidative stress, apoptosis, micronuclei formation, DNA damage and γ-H2AX foci were measured in each population. Using the comet assay it has been observed that there is substantial DNA damage in bystander cells but it occurs at a later time than the damage in the irradiated cells (increase of DNA damage was five fold in directly irradiated cells @ 0 h and two fold in bystander cells @24 h). There is no significant effect on apoptosis and micronuclei formation in bystander cells before 48 h and 30 h, respectively, but apoptosis and micronuclei formation are seen at 48 h and 30 h, suggesting that different end points take different amounts of time to be expressed. The bystander effect of γ-H2AX foci formation can be triggered by a small dose (0.1 Gy) as early as 15 min, but the extent of the damage is dose dependent. Results with γ-H2AX foci also indicated that there are two waves for the bystander signaling, one that occurs immediately and lasts for 15–30 min (after the initial stress to the directly irradiated cells) and a second that occurs at 6–24 h after irradiation to the directly irradiated cells. The effects of extracellular antioxidants suggest that secondary oxidative products of the primary insult may be important mediators of bystander effects under these conditions. (Supported in part by NIH PO1 CA 095227 and NIH Training grant T32 CA009078)

(PS4025) Performance of an energy-tunable X-ray microbeam irradiation system developed at the Photon Factory. Katsumi Kobayashi[1], Noriko Usami[1], Munetoshi Maeda[1], Hiroshi Maezawa[2], Tohru Hayashi[3], Kotaro Hieda[4], Kaoru Takakura[5], Yoshiya Furusawa[6]. [1]Photon Factory, KEK, Tsukuba, Japan, [2]Tokushima University, Tokushima, Japan, [3]Hayashi-So-Ken, Tsukuba, Japan, [4]Rikkyo University, Tokyo, Japan, [5]International Christian University, Mitaka, Japan, [6]National Institute of Radiological Sciences, Chiba, Japan.

We already developed an X-ray microbeam irradiation system with monochromatic X-rays using synchrotron radiation(SR) as light source. Energy of X-rays is restricted to 5.35 keV, since the beam is deflected right angle upward using diffraction of Si(311), in order to irradiate the samples through the bottom of the dish. Presently we are usually using a microbeam cut out by a slit system, and beam shape is 5 micron square(minimum) or larger. Dose rate is about 40 R/s, which corresponds to about 10^{4} photons/s/100 micron^{2}. When we operate the system in region scan mode we can search the target and irradiate them quickly enough to obtain survival data with statistical significance. We have another, off-line, microscope, into which information of the coordinate of the cells irradiated by the irradiation system can be transferred. Recently, we have installed a confocal laser microscope into this system, so that we can get highly sensitive fluorescent image of the irradiated cells.

One of the advantages of using SR as light source is that we can choose any energy of monochromatic X-rays with practical intensity. In order to study the signal induction and repair processes which determine radiobiological responses in low dose region, from energetic viewpoint, we have constructed an energy-tunable X-ray microbeam irradiation system, by which we can irradiate with monochromatic X-ray of inner-shell absorption edges of certain elements. We have to use the horizontal beam as emitted from the electron storage ring, hence we need to overcome some difficulties such as vertical positioning and sample chamber containing wet cells. Some preliminary results will be presented.

(PS4026) Study of combined action of very low dose-rate gamma-radiation and radioactive strontium on mice *in vivo*: dose response, adaptive response, and genetic instability. Elena Niyazova, Svetlana Zaichkina, Olga Rozanova, Gella Aptikaeva, Asiya Akhmadieva, Elena Smirnova, Olga Vachrusheva. Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russian Federation.

Our previous studies of the effect of low doses of ionizing radiation (IR) showed that both acute and chronic irradiation induce the adaptive response (AR) and genetic instability in the F1 generation of mice. Adaptive response makes itself evident as a decrease in the damaging effect of high doses of radiation after

preliminary irradiation of cells with low doses (5_20 cGy). In the last few years the problem of the effect of low doses of chronic low-intensity ionizing radiation on living organisms has acquired a particular value in view of its possible combined action with different environmental factors, in particular, heavy metal ions.

The aim of this study was to investigate the dose-effect, adaptive response (AR) and genomic instability (GI) induced by combined action of chronic low-intensity IR and radioactive Sr in mouse bone marrow cells *in vivo*.

CBA/lac mice were irradiated for 40, 120, and 210 days at a dose rate of 0.17 cGy/day using a ^{137}Cs source, which corresponds to doses of 6.8, 20.4, and 35.7 cGy, respectively. Acute irradiation (1.5 Gy) was produced using a ^{60}Co source (28.2 Gy/h). $SrCl_2$ at a concentration of 70 mg/l (calculated for ions) was given to animals with drinking water throughout the irradiation period. Cytogenetic mouse bone marrow preparations were prepared by the standard method, and the frequency of polychromatophil erythrocytes with micronuclei was determined. For each mouse 3000 PCE were analyzed.

It was found that the combined action of chronic low-intensity radiation and radioactive Sr (1) does not affect the yield of cytogenetic damage over a period of 120 days, and only after a 210-day exposure, a significant increase in the damage occurs, whereas the increase in the cytogenetic damage in mice that were not treated with Sr was observed only after 360 days of irradiation; (2) induces AR after a 210-day exposure, and (3) induces genetic instability in the F1 generation obtained from males irradiated for 210 days.

Our investigation indicates that the combined action of low dose-rate γ-irradiation and ^{90}Sr, as well as high-dose-rate γ-irradiation can induce the adaptive response and genetic instability in mouse bone marrow cells *in vivo,* and the magnitude of the adaptive response is dose-dependent.

(PS4027) Tracking Genomic Instability within irradiated and bystander populations. James W. Kelly[1,2], Jeremy S. Taylor[2], Munira A. Kadhim[1]. [1]Radiation and Genome Stability Unit, Oxfordshire, United Kingdom, [2]Department of Physiology, Anatomy and Genetics. University of Oxford, Oxford, United Kingdom.

Genomic Instability (GI), defined as an increased rate in the accumulation of new genetic alterations, has been referred to as a hallmark of tumourigenesis. GI is observed in a fraction of the progeny of cells surviving direct irradiation, and also in the progeny of cells that have never been irradiated, but have communicated with irradiated cells (bystander cells). Our group has observed that both manifest similar GI (measured by chromosomal aberrations). We propose the initiation of GI in both populations is a multi-step process (with the induction of chronic oxidative stress post-irradiation playing a major role in the initiation and perpetuation mechanism of GI).

Primary human HF-19 fibroblasts were sham irradiated or exposed to 0.5 Gy α-particle (^{238}Pu; 3.2 MeV; 121.6 keV/μm) in our co-culture system, permitting communication between irradiated and bystander populations via medium-borne factors during and for defined times post-irradiation. The two populations were then separated immediately and assayed for DNA damage. In parallel, populations were continued in culture and assayed several times multiple population divisions post-irradiation for the manifestations of GI.

After 1 and 24 hours of communication post-irradiation, significant levels of DNA damage were detected in irradiated and bystander populations by the alkaline 'comet assay.' A significant increase in DNA double strand breaks (DSBs) were detected up to 24 hours in the irradiated, by 53BP1 focus formation, but only at 24 hours was there a significant increase in the bystander, suggestive of oxidative damage induced in the bystander at 1 hr. In order to confirm the direct involvement of oxidative stress in the induction of damage, the antioxidant n-acetyl-cysteine (NAC) was added to the co-culture pre/post-irradiation, and also to populations in culture several divisions post-irradiation. Co-cultures that were treated with NAC had a near five-fold decrease in DNA damage up to 24 hours post-irradiation, with work currently ongoing to establish the long term influence of these treatments on the initiation and perpetuation of GI, utilising cytogenetic analysis in addition to endpoints currently mentioned.

(PS4028) Bystander response to an X-ray microbeam using three different DNA damage response markers in epithelial cells. Eleanor A. Blakely[1], Polly Y. Chang[2], Richard I. Schwarz[1], Kathleen A. Bjornstad[1], Chris J. Rosen[1], Rajeeb Khatua[1], Christy L. Wisnewski[1], Bahram Parvin[1], Al C. Thompson[1]. [1]Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [2]SRI International, Menlo Park, CA, USA.

Ionizing radiation can break DNA in irradiated cells activating ATM, ATR and DNA-PKcs. Untargeted bystander cells adjacent to the irradiated cohort can also respond to the stress response of the irradiated cells. DNA double-strand breaks triggers a subsequent sequential cascade of signal transduction events with îł-H2AXser139, 53BP1ser25 and TP53ser15 among the various downstream repair and checkpoint proteins recruited and phophorylated at the damage sites. We have investigated the dose- and time- and spatial-dependence of these phosphorylation events in nearly confluent human mammary epithelial cells (HMEC-S1) irradiated with stripes of 12.5 keV Xray microbeam dose 100 microns wide using a highly robust set of fluorescent montages objectively evaluating the response of thousands of individual cells.

The results indicate that all three markers can detect doses down to 5 cGy in the targeted region of the dose stripe within minutes after the exposure, followed by a time-dependent decay of the response out to 20 hours after exposure. The fluorescence characteristics of bystander response in the untargeted region on either side of the dose stripe are different than the targeted signal for each marker. In addition, the spatial range of the bystander effect and the time course of the appearance are variable for the three damage response markers. Of particular interest is the progression of fluorescent signal appearing in the partially-irradiated cells at the edge of the dose stripes.

We conclude that the low-LET-induced bystander effect can be mapped using this approach to reveal novel information contributing to a quantitative understanding of the magnitude, duration and spatial range of early protein changes in specific human epithelial cell cohorts.

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(PS4029) Anti-tumor activity of murine NK cells after single or fractionated exposures to 0.1, 0.2 or 1.0 Gy X-rays. Aneta Cheda, Ewa M. Nowosielska, Jolanta Wrembel-Wargocka, Tomasz Ołdak, Marek K. Janiak. Military Institute of Hygiene and Epidemiology, Warsaw, Poland.

The reported inhibitory effects of low doses of low-LET radiation on the development of tumors may result from stimulation by such exposures of anti-cancer immune mechanisms. In view of the important role of NK cells in anti-tumor surveillance, the aim of the present study was to assess the effect of irradiations with low and higher doses of X-rays on cytotoxic reactions mediated by these cells.

In the study, murine NK cells obtained form spleens of BALB/c mice were irradiated *in vitro* with 0.1, 0.2, or 1.0 Gy X-rays or collected from animals exposed to single or fractionated X-rays so that the absorbed doses amounted to 0.1, 0.2, or 1.0 Gy. Cytotoxic activity of NK cells was estimated using the ^{51}Cr-release assay. Concanamicin A or anti-FasL antibody were used to inhibit the perforin- or Fas receptor-mediated cytolytic function of these cells. Production of IFN-γ by these cells was examined using the ELISA assay. The in vivo activity of NK cells was suppressed by injection of the anti-asialo GM_1 antibody. Apoptosis of NK cells was assessed using annexinV. The anti-mouse Pan-NK and anti-Fas ligand (FasL) antibodies were used to label NK cells.

Cytotoxic activity of the NK cells collected from mice irradiated both with single or fractionated X-rays was significantly stimulated compared to that of the counterpart cells obtained from the sham-exposed mice. This effect was totally abrogated by injection of the anti-asialo GM_1 antibody. The elevated cytotoxicity was, for the most part, mediated by perforin and FasL. NK cells obtained from the low dose-irradiated mice exhibited enhanced surface expression of FasL. Exposure of mice to all the three doses of X-rays markedly increased IFN-γ production. Furthermore, irradiation of mice or isolated NK cells with 0.1 or 0.2 Gy of X-rays did not affect the rate of apoptosis in the collected NK cells,

whereas irradiation with 1.0 Gy enhanced the number of apoptotic NK lymphocytes. In contrast, the NK-mediated cytotoxic activity and secretion of IFN-γ were not affected by the *in vitro* irradiations of these cells.

Collectively, the obtained results indicate that both single and fractionated exposures of mice to 0.1, 0.2, or 1.0 Gy X-rays stimulate the anti-tumor activities of NK cells but the effect of the latter irradiations lasts longer than after single exposures.

(PS4030) Anti-tumor activity of murine peritoneal macrophages after single or fractionated exposures to 0.1, 0.2 or 1.0 Gy X-rays. Ewa M. Nowosielska, Aneta Cheda, Jolanta Wrembel-Wargocka, Tomasz Ołdak, Marek K. Janiak. Military Institute of Hygiene and Epidemiology, Warsaw, Poland.

The reported inhibitory effect of low doses of low-LET radiation on the development of tumors may result from stimulation of anti-cancer immune mechanisms. In view of the important role of activated macrophages (Mϕ) in anti-tumor surveillance, the aim of the present study was to assess the effects of irradiations with low and higher doses of X-rays on cytotoxic reactions mediated by these cells.

In the study, murine peritoneal Mϕ obtained form BALB/c mice were irradiated *in vitro* with 0.1, 0.2, or 1.0 Gy X-rays or collected from animals exposed to single or fractionated X-rays so that the absorbed doses amounted to 0.1, 0.2, or 1.0 Gy. Cytotoxic activity of these cells was estimated in the [^{3}H]thymidine-uptake assay. Colorimetric assay with the Griess reagent was used to measure production of nitric oxide (NO). Secretion of IL-1β and TNF-α by these cells was examined using the ELISA assays. Aminoguanidine (AG) and carrageenan (CGN) were used to inhibit the macrophage-mediated function. Activity of Mϕ was also estimated after injection of mice with anti-asialo GM_1 antibody. Apoptosis of Mϕ was assessed using annexinV.

Exposure of mice to 0.1 or 0.2 Gy of both single and fractionated X-rays significantly stimulated the macrophage-mediated cytolysis of the tumor targets, the effect being associated with the enhanced production of NO in the collected effector cells. Suppression of the *in vivo* function of Mϕ by CGN significantly suppressed the Mϕ-mediated cytotoxicity and NO production. Moreover, addition of AG to the incubation medium not only shut-down the nitric oxide synthesis but also significantly suppressed the cytolytic activity of these cells. Injection of the anti-asialo GM_1 antibody did not affect the activities of Mϕ. Production of IL-1β and TNF-α markedly increased after exposures of mice to all three doses of X-rays. Furthermore, exposure of isolated Mϕ to 0.1, 0.2 or 1.0 Gy of X-rays didn't increase the rate of apoptosis in the collected cells. In contrast, cytotoxic activity of and NO synthesis by Mϕ were not affected by the *in vitro* irradiations of these cells.

Overall, the obtained results suggest that both single and fractionated exposures of mice to 0.1 or 0.2 Gy X-rays stimulate anti-tumor activities of Mϕ. This effect following fractionated irradiation occurs later and lasts longer than after a single exposure.

(PS4031) Using hybrid spheroids to assay cancer stem cell sensitivity to ionizing radiation and chemotherapeutics. Christopher S. Lange, Bozidar Djordjevic, Shy'Ann Jie, Saira Hafeez, Joshua Garren, David J. Goff, Ovadia Abulafia, Allison Wagrich, Marvin Rotman. SUNY Downstate Medical Center, Brooklyn, NY, USA.

The Cancer Stem Cell (**CSC**) hypothesis postulates that only a small fraction of a tumor (~ 1%) is responsible for its growth, and cure requires sterilization of these **CSC**s. It has been difficult to study **CSC**s directly; but we have developed a novel system of hybrid spheroids (**HS**) that allows us to isolate fresh tumor cells capable of extensive proliferation, a hallmark of stem cells. In our **HS** system, a single cancer cell, engulfed in a spheroid of fibroblasts, can grow > 10 divisions, proliferating like a stem cell, while the fibroblasts become reproductively dead and disappear.

Results:

1) Pure spheroids composed entirely of live AG1522 fibroblasts were incubated in suspension for several days. They shrank to ~ 10 - 20% of their original size within three days, after which they remained the same reduced size for > 2 weeks.

2) These fibroblasts also have a rapid and exponential loss of clonogenicity. Within 3 days after harvest, only ~ 1% of fibroblasts remain clonogenic; within four days, < 0.3% and within a week, < $1/10^6$ remain clonogenic.

3) Thus, any **HS** growth is due to cancer cell, not fibroblast, proliferation, as confirmed by morphology.

4) Cervical carcinoma cells, released from fresh tumor surgical samples by collagenase digestion, produced no colonies when 40,000 cells were plated directly into tissue culture dishes (Plating Efficiency, PE < $2.5x10^{-5}$). However, when mixed 5.5% tumor cells, 94.5% fibroblasts, to form **HS**, some cells were clonogenic and grew.

5) At least one clonogen (defined as ≥ 10 divisions) was present in 3.7 ± 0.6 % of small spheroids (88–105 μm) & 6.7 ± 1.3 % of large spheroids (105–125 μm), yielding respective PEs of 0.50±0.09% & 0.35±0.07%. The Poisson distribution correction for clonogens/spheroid is < 0.1% for small spheroids, but for large ones, a correction factor of 2.14 increases the PE to 0.76 ± 0.15%.

Conclusions:

The **HS** system provides a tumor **CSC** model for the individual patient. The PEs of 0.50 ± 0.09% to 0.76 ± 0.15% (or 1.25% to 1.9% if only 40% of the sample is tumor cells) are what would be expected for **CSC**s, well above the Courtney-Mills method PE of 0.1%. We are using the **HS** assay to determine the **CSC** survival curves for ionizing radiation and common chemotherapeutic agents. This assay could provide the basis for improving cure rates by tailoring cancer treatments to the sensitivities of each patient's **CSC**s.

(PS4032) Radiation-induced genomic instability in tandem repeat sequences is not predictive of unique sequence instability. Asao Noda, Yoshiaki Kodama, Harry M. Cullings, Nori Nakamura. Radiation Effects Research Foundation, Hiroshima, Japan.

Tandem repeat sequences, such as minisatellite sequences or partially duplicated genes, are inherently unstable, and they have been used as sensitive markers to study genetic effects of radiation exposure. However, the biological consequences of the elevated instability of such repeat sequences are not well characterized. To learn more about the characteristics of the instability at different sequences in the genome, we created mutant HT1080 cells bearing a 8.4 kb of partially duplicated allele at the *HPRT* locus by gene targeting. The cells were then tested to determine whether or not repeat-sequence instability (assessed by elevated reversion rate caused by loss of one duplicated segment) accompanied increased forward mutation rates at the restored wild-type *HPRT* allele. Following a 4 Gy X-irradiation, about 6 % of surviving cell clones showed elevated reversion rates even after many cell generations. These clones also showed general increase in the forward mutation rate, whereas the paired individual mutation rates did not correlate with each other. Furthermore, levels of intracellular reactive oxygen species (ROS) and nuclear γH2AX foci, which is a hallmark for DNA damage responses, were also generally elevated although they did not correlate with the individual reversion rates. We concluded that repeat sequence instability is not predictive of unique sequence instability, probably because the instability is generated by multiple mechanisms following radiation exposure

(PS4033) P53 mutant dependent and glutathione independent glucose regulated γ radiation response in human cancer cells. Iraimoudi S. Ayene[1], Jie Li[1], Kathleen Ward[2]. [1]Lankenau Institute for Medical Research, Wynnewood, PA, USA, [2]Saint Joseph's University, Wynnewood, PA, USA.

We have studied the impact of glucose deprivation on the response of human cancer cells to radiation since hypoxic tumors are known to have low level of glucose. The depletion of intracellular glucose as a result of glucose depletion in the medium is verified by glucose assay, and measuring the bioreduction of hydroxyethyl disulfide (HEDS), which requires glucose as the

substrate to convert HEDS into mercaptoethanol (ME). Cancer cells in glucose depleted medium showed a five fold decrease in bioreduction than that measured in glucose containing medium suggesting lack of glucose mediated intracellular metabolic activity. There was no detectable glucose in the extracellular medium as measured by glucose assay. In glucose containing medium, these human cancer cells showed different responses to radiation induced inhibition of cell proliferation. A detailed analysis of each category of cancer cells have indicated that i) p53 mutant HT29 cells are highly resistant compared to p53 wild type HCT116 and ii) p53 mutant DU145 is more resistant than wild type p53 LnCAP. However, in contrast to colon (HCT116, HT29) and prostate (DU145, LnCAP) cancer cells, breast cancer cells p53 mutant SKBR3 are more sensitive than the p53 wild type MCF7. The resistance of p53 mutant cells is enhanced when these cells are exposed to radiation in the absence of glucose. We have also measured glutathione content in these cells to determine any correlation between non-protein thiol and glucose regulated radiation response since GSH plays a major role in cellular resistance to therapy. In the presence of glucose, these cancer cells have different levels of GSH - i) HT29 cells have the same level as HCT116, ii) LNCAP has lower level of GSH than DU145, iii) MCF7 has higher content of GSH than the SKBR3 cells. The intracellular GSH levels of these cancer cells in glucose depleted medium had either decreased or remained the same, and had shown no correlation with radiation response. These results suggested that glucose regulated the response of cancer cells to γ radiation in a p53 mutant dependent pathway in some cancer cells. The additional resistance caused by p53 mutation in glucose deprived cancer cells has clinical significance since hypoxic tumors are know to have low level of glucose.This work was supported by NCI, National Institute of Health Grant CA109604 (ISA)

(PS4034) Ionizing radiation modulates HLA expression in two human melanoma cell lines. Severino Michelin[1], Diana Dubner[1], Maria del R Perez[1], Mariana Malvicini[1], Edgardo Carosella[2], Michel Bourguignon[3]. [1]Autoridad Regulatoria Nuclear, Buenos Aires, Argentina, [2]Commissariat a l'Energie Atomique, Paris, France, [3]Commissariat a L'Energie Atomique, Paris, France.

The immune system plays an important role in host anti-tumor surveillance. HLA-G is a non-classical HLA class I molecule expressed in around one third of human melanomas, providing them with a mechanism to escape the immune surveillance. The effect of ionizing radiation on the expression of HLA molecules and anti-tumor NK-cell mediated responses was evaluated in two human melanoma cell lines.

FON (HLA-G positive) and M8 (HLA-G negative) cells were exposed to 20Gy- gamma irradiation (Co60 source, single dose, dose rate of 0.8 Gy/min). Cell surface expression of molecules was analysed by flow cytometry using mouse monoclonal antibodies against human classical HLA-class I (HLA-A, -B, and -C) molecules, non classical HLA-G and -E molecules and MICA . HLA-G expression was also determined by Western blot analysis. ELISA technique was applied to measure soluble forms of HLA-G in culture supernatants. HLA-G expression increased in FON cells 3h and 6h post-irradiation (p.i.). A significant increase in the concentration of soluble forms of HLA-G were found in culture supernatants of FON cells 6h and 24h p.i. No induction of HLA-G expression was observed in M8 cells. Enhanced HLA-E expression was detected in FON cells 6h p.i., while an inverse radiation effect was observed in M8 cells. Changes in cell surface expression of MICA were not significant in FON nor M8 cells. After an initial increase (3h p.i.), the expression of classical HLA-class I molecules decreased up-to 24h p.i.. in both melanoma cell lines and returned to basal values 48h p.i. These results show a transient radiation-induced regulation of the expression of classical and non classical HLA-class I molecules in human melanoma cells. Our study also demonstrate that ionizing radiation distinctly modulates the expression of HLA-E depending on HLA-G cell status. This finding could be related to peptides derived from leader sequences of classical HLA-class I and HLA-G molecules, which bind and up-regulate the expression of HLA-E on the cell surface. ^{51}Chromium-release assays are now being carried out to evaluate the functional consequences of these changes on NK-cell mediated cytotoxicity against irradiated melanoma cells.

(PS4035) ***In vitro*** **lactate consumption in human cancer cell lines.** Kelly Kennedy, Thies Schroeder, Ashley Chi, Mark W. Dewhirst. Duke University, Durham, NC, USA.

Many solid tumors produce lactate under hypoxic conditions, which has been shown in clinical studies to be linked to increased tumor aggressiveness and poor patient prognosis and survival. Lactate is often thought of as solely the end product of glycolysis or waste to be buffered by the body or shuttled into the Cori Cycle to undergo gluconeogenesis. Many tissues within the body can utilize lactate directly as an energy source by converting it to pyruvate to be shuttled into TCA. Recently, our lab has shown that rat mammary carcinoma R3230 consume lactate under normoxic conditions; lactate was observed to be preferentially taken up when given equimolar amounts of glucose and lactate (unpublished observation). This ability to consume lactate could provide a survival advantage to tumor cells. If the better perfused cells utilize lactate as an energy source through TCA, the glucose gradient across tumor area may decrease, allowing glucose to reach the more hypoxic and glycolytic cells farther from vessels.. In order to gain a better understanding of the commonality of lactate consuming phenotype, we performed lactate consumption experiments on numerous cancer cell lines in vitro (HCT116, FaDu, MCF-7, SiHa, and MDAMB231 among others). Experiments were conducted in a normoxic environment after cells reached confluency. 20mM of lactate was added to glucose deprived media to mimic the higher concentrations found in patient biopsies. Lactate continually dropped to as low as 2 or 3mM, in some cultures (HCT116 and MCF-7). After normalizing to cell count, some cell lines, like MCF-7, consistently showed consumption rates above 0.5mM per 100,000 cells. Other cell lines did not show strong propensity to consume lactate. We are now focusing primarily on human breast cancer cell lines because these show the broad spectrum of phenotypes. MDAMB231 shows nearly 20 fold less propensity to consume lactate in comparison with MCF7, for example. Genomic analyses are planned, which we anticipate will identify a genomic signature revealing distinction between the lactate consumer verses non-consumer phenotype. Work supported by a grant from NCI40355.

(PS4036) Adaptive responses of long term radiation on tumorigenic and non-tumorignic human prostate cell lines. Danupon Nantajit, Kazi Mokim Ahmed, Ming Fan, Zhaoqing Wang, Jian Jian Li. Purdue University, West Lafayette, IN, USA.

Although accumulated data suggest that exposure of mammalian cells to different level of ionizing radiation can induce adaptive responses to radiation, the mechanism underlying such results still remains unclear. We have reported that cultured normal and tumor cells survived from long-term fractionated radiation express radioresistant phenotypes. The purpose of this research is to identify the difference in the development of adaptive radioresistance between normal and transformed prostate cell lines. The paired cell lines have been established from human neonatal prostate epithelium by transfection with *SV40* (named 267B1) or co-transfection with *SV40* and v*Ki-Ras* (named Ki). Clonogenic survival and animal xenograft experiments demonstrate that Ki cells are tumorigenic and radioresistant, while 267B1 cells are non-tumorigenic and relatively radiosensitive. A profile of clones has been established with the radiation regimen of long-term exposure with different daily doses. We are currently measuring a group of specific stress responsive proteins in the radiation-derived cell lines. We aim to determine if specific stress elements can be targeted to re-sensitize the radioresistant tumorigenic Ki but not the non-tumorigenic 267B1 cells. Data from this isogenic comparison may be informative for generating specific targets to enhance the cure rate of prostate cancer.

(PS4037) A translationally controlled angiogenic switch in breast cancer. Robert J. Schneider, Ksenia Karpisheva, Steve Braunstein, Carolina Pola, Judith Goldberg, Silvia C. Formenti. NYU School of Medicine, New York, NY, USA.

Tumors critically require the development of a vasculature to thrive, which they acquire through the process known as angiogenesis, which is activated by hypoxia (decreased oxygen). We demonstrate that in the majority of large tumors of the breast known as locally advanced breast cancers, angiogenesis is regulated by a translationally controlled switch which acts through the overexpression of translation regulatory protein 4E-BP1 and initiation factor eIF4G. 4E-BP1 is shown to orchestrate a hypoxia-activated switch that drives tumor angiogenesis and growth at the level of selective translation of mRNAs that contain internal ribosome entry sites (IRESs). These mRNAs include those encoding vascular endothelial growth factor (VEGF) which drives angiogenesis, HIF1α which orchestrates the hypoxia response, and Bcl2 which protects against apoptosis, among others. All of these mRNAs also have capped 5' ends and can translate through conventional cap-dependent mRNA translation under normoxic conditions. Model animal and cell systems demonstrate that the elevated levels of 4E-BP1 serve to trigger hypoxia inhibition of cap-dependent mRNA translation at high levels of oxygen, and in conjunction with eIF4G, to increase selective translation of cap-independent (IRES) mRNAs to promote angiogenesis, hypoxia responses and prevent cell death. Overexpression of 4E-BP1 was found to more than double tumor growth and triple angiogenesis in animal models, and may be linked to suppression of metastasis. This work adds to our understanding of the importance of translational control in breast cancer development and progression. Our findings support two molecular scenarios for translational control in breast cancer. (1) Overexpression of eIF4E and increased cap-dependent mRNA translation, associated with formation of small tumors with higher metastatic potential, achieved in part by mutational uncoupling of hypoxia inhibition translation; and (2) Overexpression of 4E-BP1 and eIF4G that stimulate cap-independent mRNA translation of select mRNAs that drive tumor angiogenesis and survival under conditions of only moderate hypoxia, associated with formation of large locally advanced breast cancers with lower metastatic potential.

(PS4038) Glioma cancer stem cells promote tumor radioresistance and angiogenesis. Jeremy N. Rich, Shideng Bao, Qiulian Wu, Roger E. McLendon, Sith Sathornsumetee, Zhizhong Li, Mark Dewhirst, Darell D. Bigner, Anita B. Hjelmeland. Duke University, Durham, NC, USA.

The mechanisms underlying tumor radioresistance remain elusive. We recently demonstrated that cancer stem cells contribute to glioma radioresistance through preferential activation of the DNA damage checkpoint response and increased DNA repair capacity (*Nature*, 2006). The fraction of tumor cells expressing CD133 (prominin-1), a marker for both neural stem cells and brain cancer stem cells, was enriched after radiation in gliomas. CD133+ glioma cells survive ionizing radiation at increased rates relative to the majority of tumor cells, which are CD133 negative. CD133+ tumor cells preferentially activate the DNA damage checkpoint in response to radiation and repair radiation-induced DNA damage more effectively than CD133- tumor cells. Furthermore, the radioresistance of CD133+ glioma stem cells can be reversed with a specific inhibitor of the Chk1/Chk2 checkpoint kinases. These results suggest that CD133+ tumor cells represent the cellular population conferring glioma radioresistance and could be the source of tumor recurrence after radiation. Targeting DNA damage checkpoint response in cancer stem cells may overcome this radioresistance and provide a novel therapeutic paradigm for malignant brain cancers.

In additional studies, we examined the potential of CD133+ glioma cells to support tumor angiogenesis (*Cancer Research*, 2006). Tumors derived from CD133+ cells were morphologically distinguishable from CD133- tumor populations by widespread tumor angiogenesis, necrosis and hemorrhage. In comparison to matched CD133- populations, CD133+ cells consistently secreted markedly elevated levels of vascular endothelial growth factor (VEGF), which were further induced by hypoxia. The pro-angiogenic effects of CD133+ glioma cells were specifically abolished by the anti-VEGF neutralizing antibody bevacizumab (*Avastin*), but bevacizumab had limited efficacy against matched CD133- populations. Together these data indicate that stem cell-like tumor cells can be a crucial source of key angiogenic factors in cancers and that targeting pro-angiogenic factors from stem cell-like tumor populations may be critical for patient therapy.

(PS4039) Radiogenomics of prostate cancer: identification of genomic markers for normal tissue radiotoxicity. Sambasivarao Damaraju, David Murray, Gino Fallone, Carol Cass, John Hanson, Matthew Parliament. Cross Cancer Institute, Edmonton, AB, Canada.

Objective: A radiogenomic approach was used to explore possible relationships between single nucleotide polymorphisms (SNPs) in candidate genes encoding DNA damage recognition/repair/response, steroid metabolism, cytokine and tissue repair proteins with respect to clinical radiation toxicity in a retrospective cohort of patients previously treated with conformal radiotherapy (3DCRT) for prostate cancer. Materials and Methods: Eighty three patients with prostate cancer who underwent 3DCRT at our institution between Sept 1996 and Dec 2000 were approached and obtained consent for blood sampling and SNP analysis using buffy coat DNA. Twenty-eight patients were documented as having experienced late toxicity for bladder or rectum (Radiation Toxicity Oncology Group, RTOG grade) with grade $\geq$2 on at least one follow-up visit. We selected 279 SNPs from 45 DNA repair genes and 30 genes from tissue repair, cytokine, apoptosis and CYP450 pathways for this study and sequenced exons and flanking introns for TGFB1, LIG4 and TP53 genes from all the 83 patients. Genotyping assays were carried out using the Pyrosequencing® technique. Results: We have completed designing assays for 185 SNPs from a total of 279 that were selected for this ongoing study. Genotyping data was subjected to Hardy-Weinberg Equilibrium (HWE) analysis and the data that deviated from HWE were eliminated from further analysis. Univariate associations with late rectal or bladder toxicity (gr. 2+) were found for several gene SNPs and those with $p < 0.2$ were entered into multivariate analysis. Significant associations with freedom from toxicity were found for *XRCC3* (A>G 5' UTR NT 4541), *BRCA2* (A>G, K1132K), *IL10* (T>C, intron 2, NT 56), *LIGIV* (T>C Asp568Asp), *CYP2D6*4* (G>A, splicing defect), mean bladder dose >60 Gray, and dose to 30% of rectal volume >75 Gray on Cox multivariate analysis.

Conclusions: This study suggests an association of genitourinary/gastrointestinal toxicity after 3DCRT for prostate cancer with select candidate gene SNPs in DNA damage recognition/repair/response-related and tissue repair. A study with larger sample size to validate these findings is warranted. Identification of SNP signatures that might predict adverse tissue toxicity in patients prior to therapy would justify radiogenomic approaches in the clinic.

(PS4040) Volume effects in the rat lung for late radiation-induced loss of lung function. Peter van Luijk[1], Hette Faber[1], Jacobus M. Schippers[2], Harm Meertens[1], Johannes A. Langendijk[1], Robert P. Coppes[1]. [1]Dept. Radiation Oncology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands, [2]Accelerator department, Paul Scherrer Institut, Villigen, Switzerland.

Purpose: In previous preclinical studies we found that early symptomatic radiation-induced loss of lung function (SRILF) can be due to two distinguishable pathologies: vascular inflammation starting at very low dose (<9 Gy) and parenchymal inflammation starting at ±16 Gy. Of these, only parenchymal inflammation progresses into late radiation fibrosis. This difference in biological background between the early response and fibrosis, may lead to a different response to partial lung irradiation. The aim of the present study is to determine whether early and late SRILF demonstrate different volume effects.

Methods: Using 150 MeV protons we performed high-precision irradiation of various sub-volumes of the lung (100%, 75%, 50% and 25%, excluding heart irradiation above 18 Gy). Breathing rate (BR) was measured bi-weekly before and up to 28 weeks after irradiation. The increase in mean BR in week 6 - 12 with respect to the mean BR in week 0 - 4 was used as a measure for

early SRILF. The increase in mean BR in week 16 - 28 with respect to the mean BR in week 0 - 4 was used as a measure for *late* SRILF.

Results: For 25% irradiated volume the tolerance dose was never reached for both end points not even up to a dose of 36 Gy. Tolerance doses of 100%, 75% and 50% lung irradiation were 12.2 (11.8–13.1, 95% confidence interval) Gy, 14.1 (13.3–14.8) Gy and 19.1 (18.3–20.0) Gy for *early* SRILF and >13.0 Gy, 16.8 (15.8–21.0) Gy and 17.3 (16.4–18.1) Gy for *late* SRILF, respectively. At 28 weeks, irradiated areas became fibrotic and vascular damage does not add to SRILF anymore.

Conclusion: The dependence of the tolerance dose of SRILF on IR volume is stronger for the early than late phase, consistent with differences in pathology. Therefore, reliable selection of high and low risk groups requires the use of different criteria for *early* and *late* SRILF. This is in contrast to current clinical practice in which often a single DVH parameter such as V13, V20 and mean lung dose is used, irrespective of the end point.

(PS4041) Impact of SNP's in risk genes on fibrosis after radiotherapy. Kerstin Borgmann[1], Inga Boeckelmann[1], Sonko Borstelmann[1], Annette Raabe[1], Oliver Zschenker[2], Ulrike Hoeller[3], Dirk Rades[4], Ekkehard Dikomey[1]. [1]Lab of Radiobiology and Experimental Radiooncology N79, Hamburg, Germany, [2]Dep. of Radiation Oncology, University of California, CA, San Francisco, CA, USA, [3]Dep. of Radiotherapy, Vivantes Medical Center, Berlin, Germany, [4]Dep. of Radiation Oncology, University Hospital Schleswig-Holstein, Luebeck, Germany.

Purpose: Individual radiosensitivity is discussed to be mediated by single nucleotide polymorphisms (SNPs) in so called risk genes. This study was performed to identify risk genes causing an increased susceptibility to radiation-induced damage, in terms of chromosomal aberration determined in vitro as well as radiation-induced fibrosis following radiotherapy.

Patients and methods: Blood samples were collected from 69 patients with breast cancer Stage I/II who had undergone breast conserving surgery and adjuvant radiotherapy applying 1.8 to 2.5 Gy per fraction with a median reference dose of 55 Gy. Fibrosis was evaluated using the LENT/SOMA score. The median follow up time was 12 years. Blood samples were analysed for SNP's in TGFβ1 (C-509T), XPD (A->C, exon 23), SOD2 (C1183T), XRCC1 (G-A, exon 10), and ATM (G557A) applying the RFLP method or MassArray technology, respectively. In 47 out of 69 blood samples chromosomal damage was determined using the metaphase technique. Results: In total 15/69 (22%) of the patients developed a fibrosis of grade 2 or 3, respectively. A combined analysis of the risk alleles TGFβ1 (CT,TT), XRCC1 (GA,AA), ATM (GA,GG), and SOD2 (CT,CC) revealed a positive correlation between the number of risk alleles and the probability to develop a grade 2/3 fibrosis, with no fibrosis grade 2/3 in patients without any risk allele but 80% of the patients carrying four risk alleles. For the analysed SNP in the XPD gene no effect on the development of fibrosis was apparent. Comparison of chromosomal damage and risk alleles in 47 out of the 69 patients showed a strong increase in the number of chromosomal damage with risk genes, particularly with the SNP in the SOD2 gene, implying a relation of these genes to individual radiosensitivity. On the other hand, a strong reduction in the number of chromosomal damage was evident in patients carrying a SNP in the XRCC1 gene, indicating the involvement of this gene in other pathways responsible for the development of fibrosis.

Conclusion: A combination of SNPs in so called risk alleles result in a higher susceptibility to late effects after irradiation in breast cancer patients. Thus, polymorphisms in specific genes might be useful to identify patients with an increased risk to develop late tissue effects after radiotherapy.

(PS4042) A little to a lot or a lot to a little: evaluation of lung response to ionizing radiation using a rat model. Vladimir A. Semenenko[1], Robert C. Molthen[2], Swarajit N. Ghosh[3], Meetha M. Medhora[3], Natalya V. Morrow[1], X. Allen Li[1]. [1]Department of Radiation Oncology, Medical College of Wisconsin, Milwaukee, WI, USA, [2]Zablocki Veterans Affairs Medical Center, Milwaukee, WI, USA, [3]Division of Pulmonary and Critical Care, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI, USA.

The lung is an organ characterized by a pronounced volume effect, i.e., the same level of tissue tolerance can be maintained by irradiating either small lung volumes to a large dose or greater lung volumes to a reduced dose. However, it is a matter of debate which irradiation scenario provides better long-term sparing of lung function in patients undergoing radiotherapy for malignancies of the thorax. We use a rat model of radiation-induced lung injury to elucidate this problem. The dorsal surface of the thorax of pathogen-free WAG/Rij/MCW rats was exposed to 300 kVp X rays. Cerrobend blocks were used to collimate the beam to irradiate either the entire lungs or a fraction of lung volume. For the latter shielding configuration, lungs were exposed through 5 mm holes in the block, allowing the beam to pass through four non-contiguous regions in the basal/apical and right/left lung. Mean lung dose, i.e., volume-weighted physical dose, was calculated from dose-volume histograms. Volumetric information was obtained from computerized tomography images of several representative animals. Irradiation times were chosen to achieve the same mean lung dose of 10, 15, or 20 Gy using both shielding configurations. Radiation-induced lung damage was evaluated as an increase in breathing frequency in irradiated versus control rats. We investigated which irradiation scenario, i.e., a lot to a little or a little to a lot, results in a greater loss of lung function, for the same mean lung dose. This project will provide additional information about whether novel radiotherapy techniques, such as intensity-modulated radiation therapy, which tend to disperse low doses of radiation over large volumes of normal tissue, are safe alternatives to the current standard of 3D conformal radiation therapy for lung cancer and other tumors in the thorax.

(PS4043) Association between polymorphisms in candidate genes and late complications to radiotherapy in Head and Neck cancer patients. Ghazi Alsbeih, Najla Al-Harbi, Khaled Al-Hadyan, Muneera Al-Buhairi, Medhat El-Sebaie, Nasser Al-Rajhi. King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia.

Most cancer patients (50–70%) receive radiotherapy (RT) during the management of their disease. However, patients vary considerably in their normal tissue response to RT even after similar treatment. Therefore, much interest in normal tissue radiosensitivity has emerged and raised the possibility of developing biomarkers or predictive assays for radiosensitivity. Recently, it has been suggested that single nucleotide polymorphisms (SNPs) in candidate genes could influence radiosensitivity and cause variations between patients. We tested this hypothesis in our local cancer patients. Fifty Head and Neck cancer patients were retrospectively recruited and fibroblast cell cultures were established from punch skin biopsies. The grade of fibrosis, a late complication to radiotherapy, was scored according to RTOG/EORTC grading system. Patients with grade 0 to 1 fibrosis were referred to as normal control (n = 25) and those with grade 2 to 3 were considered clinically radiosensitive (n = 25). The following 10 SNPs (p21 31 Ser/Arg C>A, p53 72 Arg/Pro G>C, ATM 1853 Asp/Asn G>A and 1853 Asp/Val A>T, MDM2 309 promoter T>G, MDM2 110 Ile/Val A>G, DNA Ligase IV 591 Ile/Val A>G, XRCC1 399 Arg/Gln G>A, XRCC3 241 Thr/Met C>T and TGFβ1 10 Lue/Pro T>C) that may influence protein level were genotyped in all patients by direct sequencing. Results showed significant association between XRCC1 G399A SNP and grade of fibrosis ($P = 0.05$), whereas TGFβ1 T10C and XRCC3 C241T showed borderline associations ($P = 0.07$, and $P = 0.10$, respectively). Furthermore, the number of risk alleles of these 3 SNPs showed combined effect on clinical radiosensitivity and the radiosensitive group showed significantly higher number of risk alleles ($P = 0.003$). We conclude that this case-control study confirms the association between certain SNPs and risk of radiation-induced normal tissue complications and supports the assumption that clinical radiosensitivity depends on the combined effects of polymorphic variations in several genes. This study is being expanded to include more patients and more SNPs in candidate genes that represent diverse pathways in radiation response. Supported by KFSHRC grant # 2000 031.

(PS4044) The implications of DNA damage checkpoints on acute radiation effects in normal epithelium. Ingela Turesson[1], Jan Nyman[2], Ragnhild Bernefors[2], Majlis Book[1], Ingegerd Hermansson[2], Fredrik Qvarnström[1], Martin Simonsson[1], Sunna Sigurdardottir[1], Ulf Thunberg[1], Karl-Axel Johansson[3]. [1]Uppsala University, Department of Oncology, Radiology and Clinical Immunology, Section of Oncology, Uppsala, Sweden, [2]Göteborg University, Sahlgrenska University Hospital, Department of Oncology, Gothenburg, Sweden, [3]Göteborg University, Sahlgrenska University Hospital, Department of Radiophysics, Gothenburg, Sweden.

Background: In response to ionizing radiation, cells activate DNA damage checkpoint pathways to protect the genome. This allows the cell time to repair DNA damage. The outcome of DNA damage checkpoint activation by radiation depends largely on cell type, tissue type and radiation dose. Damaged cells can be eliminated by cell death, or permanent arrest induced by cellular senescence or terminal differentiation. Alternatively, cells can survive and resume cell cycle progression upon checkpoint recovery.

Aim: To estimate the impact of DNA damage checkpoints on the keratinocyte response to fractionated radiotherapy.

Method: Skin punch biopsies were taken from prostate and breast cancer patients, before, during and after radiotherapy, from those areas of the skin receiving daily fractions of 0.05 to 2 Gy. Cell cycle progression in the basal cell layer of the epidermis was evaluated by staining for the molecular markers: Ki-67, Rb, cyclin A, cyclin B, p-Histone 3 and p21. Cell death was quantified by γH2AX staining. The numbers of stained keratinocytes in the basal cell layers were counted.

Result: The data suggest an immediate dose dependent G_0 arrest, with hypersensitivity up to 0.2 Gy that persists throughout 7 weeks of treatment. After 2 to 3 weeks, cells entered into the cell cycle, displaying a dose dependent effort of repopulation for 0.4 Gy up to 2 Gy. However, cells were trapped in G_1 and G_2 at dose dependent periods, and accordingly, entry into mitosis was suppressed. Checkpoint recovery and accelerated repopulation was only observed during the couple of weeks after completion of radiotherapy. Cell death was infrequent. p21 data indicate that permanent arrest is the major response of skin keratinocytes.

Conclusion: The acute effects observed in normal skin epithelium during radiotherapy are caused by the growth arrest of keratinocytes, induced by persistent DNA damage checkpoint activation, rather than the cell-killing of keratinocytes.

(PS4045) Heart irradiation and late radiation-induced loss of lung function. Peter van Luijk[1], Hette Faber[1], Jacobus M. Schippers[2], Johannes A. Langendijk[1], Harm Meertens[1], Robert P. Coppes[1]. [1]dept. Radiation Oncology, Universiy Medical Center Groningen, University of Groningen, Groningen, The Netherlands, [2]Accelerator department, Paul Scherrer Institut, Villigen, Switzerland, Villigen, Switzerland.

Purpose: In previous preclinical studies we demonstrated that co-irradiation of the heart with the lung enhances *early* symptomatic radiation-induced loss of lung function (SRILF). In the present study we hypothesize that this enhancement persists in the *late*, fibrotic phase.

Methods: Using 150 MeV protons we performed high-precision irradiation of two sub-volumes of the lung (50% and 25%), both including and excluding the heart. Breathing rate (BR) was measured bi-weekly before and up to 28 weeks after irradiation. The increase in mean BR in week 6 - 12 with respect to the mean BR in week 0 - 4 was used as a measure for *early* SRILF. The increase in mean BR in week 16 - 28 with respect to the mean BR in week 0 - 4 was used as a measure for *late* SRILF.

Results: Tolerance doses were 19.1 (18.3–20.0, 95% confidence interval) Gy and >36 Gy for *early* SRILF and 17.3 (16.4–18.1) Gy and >35 Gy for *late* SRILF after irradiation of 50% and 25% of the lung, respectively. If the heart was included we found tolerance doses for *early* SRILF of 17.3 (16.8–17.9) Gy and >21.6 Gy and for *late* SRILF of 15.9 (15.0–17.0) Gy and 20.6 (18.6–24.0) Gy.

Conclusion: Co-irradiation of the heart with the lung results in enhanced loss of lung function, both during the *early* (pneumonitic) phase and the *late* (fibrotic) phase.

(PS4046) p53 polymorphism at codon 72 predicts individual radiosensitivity of acute skin reactions. Ulf Thunberg[1], Jan Nyman[2], Majlis Book[1], Ingegerd Hermansson[2], Karl-Axel Johansson[3], Ingela Turesson[1]. [1]Uppsala University, Department of Oncology, Radiology and Clinical Immunology, Section of Oncology, Uppsala, Sweden, [2]Göteborg University, Sahlgrenska University Hospital, Department of Oncology, Gothenburg, Sweden, [3]Göteborg University, Sahlgrenska University Hospital, Department of Radiophysics, Gothenburg, Sweden.

Background: The tumor suppressor gene p53 is activated by stress, such as DNA damage caused by radiation, which triggers DNA repair, cell cycle arrest and induction of apoptosis. However, the effectiveness of the various functions of p53 depends on a common polymorphism at codon 72 (Arg or Pro). Cells with Arg 72 are more prone to undergo apoptosis compared to cells with Pro 72. In contrast, the Pro 72 genotype displays a higher proportion of cells in G1 phase.

Aim: To study if the p53 polymorphism at codon 72 correlate to individual radiosensitivity in terms of acute effects.

Methods: In patients who had received curative radiotherapy for prostate cancer, 3mm skin biopsies were taken regularly during the treatment period of 7 weeks from two lateral fields. The fractionation schedules were 5*0.45Gy/week and 5*1.1Gy/week. In 74 patients the reduction rate of keratinocytes in the basal cell layer of epidermis was determined and taken as endpoint for individual radiosensitivity. DNA was taken from 117 unirradiated control biopsies to determine the polymorphism at codon 72.

Results: Analysis of the p53 polymorphism at codon 72 in the prostate cancer patients showed that 65 (56%) individuals were Arg/Arg, 42 (36%) were Arg/Pro and 10 (8%) were Pro/Pro. The individual radiosensitivity was determined by the slope of the reduction rate of keratinocytes at 0.45Gy/fraction.

Patients with Arg/Arg had significantly reduced reduction rate of keratinocytes after daily fractions of 0.45Gy compared to patients with Arg/Pro or Pro/Pro ($p=0.007$). The Arg/Arg patients also had reduced reduction rate when comparing to the Arg/Pro patients only ($p=0.01$). However, there was no significant difference in keratinocyte reduction rate when Pro/Pro patients were compared to Arg/Pro or Arg/Arg patients ($p=0.9$ and $p=0.1$ respectively). The dose-modifying factor for the Pro 72 versus Arg 72 was 0.8. This was confirmed by the outcome of daily fractions of 1.1 Gy.

Conclusion: The p53 polymorphism at codon 72 explains part of the individual radiosensitivity of the skin epithelium. This will have implications for prescribing the individual dose of radiotherapy.

(PS4047) Radiation-induced pathophysiology, in particular late effects after radiotherapy, is inversely proportional to the rate of induction of radiation-induced apoptosis in T lymphocytes. Nigel E. Crompton, Joel Strehl, Natalie Kent, Catherine Carter, Elianna Bootzin, Rick Hay. Noninvasive Imaging and Radiation Biology, Van Andel Research Institute, Grand Rapids, MI, USA.

Purpose We have previously demonstrated that the severity of late effects induced by radiation exposure, typically as a consequence of radiation therapy, can be estimated flow cytometrically by quantifying radiation-induced apoptosis in T lymphocytes. Dose response curves of radiation-induced apoptosis suggest 8 Gy is the optimal dose to employ. We also investigated the time kinetics of this physiological ATM- and NBS-dependent process.

Material & Methods Pretherapy venipuncture is performed to collect 1 ml of peripheral blood in heparin tubes. The blood is diluted 1:10 in RPMI medium and irradiated. The blood is left to incubate for a fixed period of time. Thereafter, the cells are harvested into 5 ml tubes, spun down and treated with antibodies. RBCs are lysed and WBCs fixed in a formaldehyde solution, which is subsequently neutralized with HBSS. The WBCs' DNA is stained

using propidium iodide. Apoptotic yield is evaluated flow cytometrically.

Results Radiation-induced apoptosis in T lymphocytes followed a sigmoid kinetics-induction curve. As the dose increased up to a maximum of 8 Gy the sigmoid curve shifted to the left, indicating that apoptosis was induced sooner. At higher doses up to 20 Gy a slight delay was observed. After an 8 Gy exposure, maximum apoptosis (circa 90%) was reached between 72 and 96 h depending on the donor. Differences between individuals, however, were most prominent 48 h after irradiation, on the ascending region on the induction curve. Differences between individuals after 4 Gy irradiations were most prominent at 72 h, and after 2 Gy irradiations at 96 h.

Conclusion Estimation of the severity of late effects caused by radiation exposure, based on the induction of apoptosis in lymphocytes, is best evaluated in the ascending region of the sigmoid kinetics-induction curve. Maximum cumulative induction of apoptosis is observed at 8 Gy. Discrimination between individuals is greatest 48 h after 8 Gy; 72 h after 4 Gy, and 96 h after 2 Gy. The rate of induction of apoptosis varies between individuals. This rate is inversely proportional to the production of late effects.

(PS4048) Stereotactic radiosurgery (SRS) improves locomotor recovery and function after spinal cord injury. Chitti Moorthy[1], Ronald Rocchio[1], Alan Alfieri[2], Lynn Shih[1], Nagwa, Saleh[1], Richard J. Zeman[1], Xialing Wen[1], Nengtai Ouyang[1], Joseph D. Etlinger[1]. [1]New York Medical College, Westchester Medical Center, Valhalla, NY, USA, [2]Albert Einstein College of Medicine, Bronx, NY, USA.

The development of clinical stereotactic radiosurgery (SRS) has expanded the therapeutic use of radiation to focal areas, as injurious effects on non-targeted tissues inherent in conventional irradiation can be avoided. LINAC based SRS to the injured spinal cord was used to preserve adjacent and contiguous uninjured segments, reducing dose to non-spinal cord structures to further delineate optimization for locomotor recovery and spinal cord tissue sparing in the contusion spinal cord injury model.

METHODS: Diagnostic spiral CT was used to delineate the site of targeted therapy in anesthesized rats along the axis of the cord with the isocenter positioned at the contusion epicenter. SRS (Novalis, Brain Lab, 6MeV LinAc) to a 4 × 15mm spinal cord length in four sequentials fractions with beam angles 60–70degree apart to the T10 contusion site (2 hrs post weight drop injury) at doses of 2, 5, 10, 15, 20 or 25 Gy. Animals were then followed for BBB scores (functionality) and histologically for neural regeneration/damage.

RESULTS and DISCUSSION: Weight-drop contusion resulted in centrally located lesions within the spinal cord consisting of areas of gliosis and cyst formation or cavitation with sparing of a peripheral rim of tissue at the contusion epicenter 6 wks postinjury. SRS at 10Gy, but not 25Gy increased by 49% the relative and absolute cross-sectional area of spared spinal cord tissue at the contusion epicenter compared to untreated-injured spinal cords at 6 weeks postinjury. SRS significantly increased locomotor recovery (21 point BBB scale) during the 6-week observation period relative to the unirradiated-contused treatment group (final BBB scores: 9.4 vs. 7.3). Significant increases in BBB scores initiated at the 3rd week post SRS. Doses of 2–10 Gy demonstrated a linear increase in BBB scores (r^2=0.99, slope=0.21 ± 0.01 BBB score /Gy, $P<0.001$) whereas, 15–25 Gy lowered BBB scores to 6.6–8.1, respectively. The extent of locomotor recovery following SRS treatment correlated with measurements of spared spinal cord tissue at the contusion epicenter. Together these studies demonstrate the feasibility of SRS for human spinal cord-injured patients as a potential therapeutic modality.

(PS4049) Hierarchy of complex double-strand break repair: 8-oxoguanine retards DSB repair when in close proximity to the break termini. Tracey A. Dobbs, Philip Palmer, Martine E. Lomax, Peter O'Neill. MRC Radiation and Genome Stability Unit, Harwell, United Kingdom.

Complex double-strand breaks (DSBs) induced by ionising radiation, and characterised by the presence of base lesions close to the break termini, are believed to be one of the major causes of the biological effects of IR. It has been highlighted that complex DSBs pose problems to DNA repair machinery. The major repair pathway of DSBs in mammalian cells is non-homologous end-joining (NHEJ); involving recruitment of the DNA-protein kinase complex which in turn recruits the DNA ligase IV/XRCC4 complex. However, complex DSB termini may require processing prior to ligation.

In this study, synthetic oligonucleotides containing an 8-oxoguanine (8-oxoG) lesion in close proximity to the termini were used to investigate the influence of 8-oxoG on NHEJ, assess if glycosylase enzymes involved in base excision repair (BER) can remove 8-oxoG from these positions, and the interplay between the two pathways in the repair of complex DSBs.

We will discuss the impact of 8-oxoG on the ligation efficiency through *in vitro* repair assays with either T4 DNA ligase or Ligase IV/XRCC4 complex (in which oligonucleotides were ligated to each other) when a single 8-oxoG is present 2–3 bases from the 3'- oligonucleotide terminus and how the ligation becomes further reduced when an 8-oxoG is near to both 3' ends of the oligonucleotide. The orientation of 8-oxoG also has a significant effect upon ligation efficiency for when 8-oxoG is 6 bases from the 5'- terminus, ligation is reduced almost 4 fold. In addition, removal of 8-oxoG in close proximity to the oligonucleotide termini by glycosylases is severely compromised compared with the removal of 8-oxoG distant from the oligonucleotide ends; although again there is an influence from the orientation. However the inhibitory effect of a DSB on the glycosylase function can be partially recovered through an AP-endonuclease independent pathway involving human OGG1 and NEIL1.

Taken together these data demonstrate that complex DSBs are not only difficult to ligate but also to process by BER. These findings will be discussed in terms of the NHEJ repair of radiation-induced complex DSBs and their contribution to the biological effects of radiation and ultimately tumourigenesis.

(PS4050) Extremely low frequency magnetic fields enhance chemically induced formation of apurinic/apyrimidinic sites in A172 cells. Shin Koyama[1], Tomonori Sakurai[2], Takehisa Nakahara[2], Junji Miyakoshi[2]. [1]Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan, [2]Department of Radiological Technology, School of Health Sciences, Faculty of Medicine, Hirosaki University, Hirosaki, Japan.

OBJECTIVE: Extremely low frequency (ELF) magnetic fields are ubiquitous in daily life. There is increasing concern about the effects of ELF magnetic fields on human health. We previously found an increased rate of mutation following combined exposure to ELF electromagnetic fields and H_2O_2 or X-rays (Koyama *et al.* 2004, 2005). To analyze this effect, we performed experiments to detect apurinic/apyrimidinic (AP) sites in human glioblastoma A172 cells. Here, we examined whether exposure to chemical agents and ELF magnetic fields produces AP sites in DNA in A172 cells.

METHODS: Human glioblastoma A172 cells were exposed to an ELF magnetic field. A 5 mT ELF magnetic field at 60 Hz was used and the duration of ELF magnetic exposure was 2, 4, 8, 16 or 24 hr. Cells were exposed to an ELF magnetic field alone, to genotoxic agent (methyl methanesulfonate (MMS) or hydrogen peroxide (H_2O_2)) alone, or to an ELF magnetic field with genotoxic agents in the medium. After exposure to the ELF magnetic field or sham exposure, the cells were collected. Then the DNAs were extracted, and the number of AP sites was measured using a DNA damage quantification kit, based on a calibration curve prepared using standard solutions of DNA.

RESULTS: Formation of AP sites after exposure to an ELF magnetic field did not differ significantly from that with sham exposure. The number of AP sites formed in MMS-treated cells or ELF+MMS-treated cells increased in a time-dependent manner, and AP-site formation in ELF+MMS-treated cells was enhanced compared with that in cells treated with MMS alone. The numbers of AP sites formed in H_2O_2- and ELF+H_2O_2-treated cells increased

time-dependently under both conditions, but formation of AP sites in ELF+ H_2O_2-treated cells was enhanced compared with that in cells treated with H_2O_2 alone.

CONCLUSIONS: Our results suggest that exposure of cells to an ELF magnetic field alone has no effect on production of AP sites, but that the number of AP sites induced by MMS or H_2O_2 is enhanced by exposure to ELF magnetic fields.

(PS4051) Development of a true internal standard for the comet assay to minimise variability in the measures of radiation-induced DNA damage formation and repair. George Don D. Jones, Murizal Zainol, Julia Stoute, Karen Bowman, Gabriela Almeida. University of Leicester, Leicester, United Kingdom.

The Comet Assay (CA) is a highly sensitive method for assessing DNA damage formation and repair at the level of individual cells. Furthermore, due to its ability to detect a wide variety of DNA damage in cells exposed to a range of genotoxins, its ease of application, and both its low cost and low material requirement, CA is being increasingly used in human studies. However, many features of the assay, particularly of the electrophoresis stage, affect both intra-assay variability and inter-assay reproducibility.

To minimise these variables, we have designed an internal standard consisting of 'reference' cells which have had their DNA thymidine substituted with BrdU. The post-electrophoresis comets, derived from these 'reference' cells, can be readily distinguished from the 'test' cell comets present in the same gel, at the time of analysis using a fluorescently tagged *anti*-BrdU antibody together with an additional barrier filter.

In experiments to assess the impact of the internal standard on intra-assay variability and inter-assay reproducibility, the 'reference' and 'test' cells were either present in separate gels (on the same slide) or mixed together in the same gel before their co-exposure, on slides, to X-irradiation. By adjusting the individual 'reference' cell data to a determined average 'reference' cell response and then applying the derived correction factor to the corresponding individual 'test' cell data, we have obtained substantial (>2-fold) reductions in the coefficient of variation (CoV) for the measures of radiation-induced comet formation and DNA damage repair, but only when the 'reference' and 'test' cells were in the same gel; only minor reductions in CoV were noted when the 'reference' and 'test' cells were in separate gels.

These studies indicate that differences between individual gels, even when present on the same slide, significantly contribute to experimental variation in the assay. However, having both the 'reference' and 'test' cells together in the same gel provides the means of reducing variation in comet measures caused by differences in the preparation of the slides, cell/slide exposure, nucleoid electrophoresis and comet analysis. Ultimately, with further development, we anticipate that this research will deliver 'off the shelf' quality assurance materials for CA.

(PS4052) Analysis of clustered DNA damage generated by high LET radiations. Hiroshi Ide[1], Hiroaki Terato[1], Yusuke Nakaarai[1], Ryoichi Hirayama[2], Yoshiya Furusawa[2]. [1]Hiroshima University, Higashi-Hiroshima, Japan, [2]National Institute of Radiological Sciences, Chiba, Japan.

The relative biological effect (e.g., cell killing) of ionizing radiation (IR) for mammalian cells increases with an increase in linear energy transfer (LET) values up to 200 keV/um. Since high LET radiations deposit more energy on water and DNA molecules per the unit length of track than low LET radiations, it has been proposed that high LET radiations produce localized multiple damage more frequently than low LET radiations. In particular, bistranded clustered damage, which contains two or more closely opposed base lesions and/or single-strand breaks, is assumed to result in error-prone repair or double-strand breaks (DSBs), and hence leads to adverse biological effects. In the present study, we irradiated plasmid DNA and cells by IR with different LET values and quantified the yield of clustered damage to elucidate whether the yield of clustered DNA damage accounts for the biological severity associated with high LET radiations. Plasmid DNA was irradiated in Tris-EDTA by gamma-rays (0.2 keV/um), carbon ions (13 keV/um), and iron ions (200 keV/um). The fraction of clustered damage (DSB and clustered base damage (CBD)) was 2–3% of total damage and independent of the type of radiation. The ratio of the amounts of DSB and CBD was 1:1, indicating that CBD accounts for half of clustered damage. Interestingly, the yields of both isolated and clustered damage decreased in the order of gamma-rays > carbon ions > iron ions, showing an inverse correlation with LET. CHO cells (AA8) were irradiated similarly, and cell survival and clustered damage (DSBs) were analyzed. In keeping with known data, the efficacy of cell killing increased with an increase in LET. DSBs of chromosomal DNA were analyzed by static field gel electrophoresis. The yield of DSBs decreased with an increase in LET, showing a correlation similar to that observed for plasmid DNA irradiated in vitro. The inverse correlation between the yield of clustered damage and LET implies that the initial yield of clustered DNA damage does not simply account for the biological severity associated with high LET radiations. The present data suggest that additional factors, such as the processing of clustered DNA damage in cells, are likely involved in the severity of high LET radiations.

(PS4053) Role of DNA-PKcs in DSB repair following high and low dose radiation. Jennifer Anderson, Jane Harper, Peter O'Neill. Medical Research Council, Didcot, United Kingdom.

Ionising radiation induces DNA double strand breaks (DSB), which are predominantly repaired by the non-homologous end joining pathway (NHEJ). The complexity of the DSB is thought to increase with the ionisation density of the radiation. The aim of this study was to examine any dose dependence in the role of DNA-PK_{cs} (a key protein in NHEJ) in repair of DSB induced by either high- or low-LET radiation.

HF19, M059J and M059K cells were irradiated with ^{60}Co γ-rays; M059J/K cells were also irradiated with ^{56}Fe ions (1 GeV/nucleon). Immunostaining was used to detect DSB as γ-H2AX foci, a beacon of DSB, and recruitment of DNA-PK_{cs} to sites of DSB following irradiation.

DNA-PK_{cs} foci, detected following γ-irradiation of HF19 cells, showed a linear dose-response observed down to doses of 0.1 Gy, with no apparent dose threshold required for DNA-PK_{cs} recruitment. Radiation-induced DNA-PK_{cs} foci reached maximal levels ~45 mins post γ-irradiation with the majority of foci co-localising with γ-H2AX. Vanillin, an inhibitor of DNA-PK_{cs}, was able to reduce significantly the rate of DSB repair following 1 Gy γ-irradiation. However, following 0.25 Gy γ-irradiation no observable difference in DSB repair was seen between cells treated with and without vanillin, thus suggesting the repair of many DSB induced by low dose, low-LET radiation does not require DNA-PK_{cs}, in contrast to observations at higher doses.

M059J and M059K cells, which are deficient and proficient in DNA-PK activity respectively, were also used to examine the role of DNA-PK_{cs} in DSB repair. Following 1 Gy γ-irradiation similar induction of γ-H2AX foci was seen however the subsequent repair rate of DSB is slower in M059J cells than in M059K as shown previously. Using high-LET ^{56}Fe ions, the rate of repair was again slower in M059J cells compared with that in M059K, thus indicating a role for DNA-PK_{cs} in the repair of more complex breaks induced by high-LET radiation.

DNA-PK_{cs} is an important protein involved in repair of DSB induced by high- and low-LET radiation. However it appears that at lower doses of low-LET radiation DNA-PKcs is dispensable for the majority of DSB, possibly reflecting a different subset of DSB which during repair do not require DNA-PKcs. Whether they are repaired by other proteins in the NHEJ pathway, or by a different repair pathway, is an area under investigation.

(PS4054) Rbe of double-strand breaks from 211at. Kristina Claesson[1], Bo Stenerlöw[2], Lars Jacobsson[3], Kecke Elmroth[1]. [1]Dep of Oncology, Göteborg, Sweden, [2]Dep of Oncology, Radiology and

Clinical Immunology, Uppsala, Sweden, [3]Dep of Radiation Physics, Göteborg, Sweden.

The induction of double-strand breaks (DSBs) depends on several factors, for example radiation quality. High-LET radiation leads to more DSBs at the same absorbed dose than low-LET radiation and due to the clustering of ionizations by high-LET radiation, these DSBs are more difficult to repair. It has been shown that high-LET radiation induces non-randomly distributed DSBs along the DNA molecule and that the non-random component for fragments $<$ 100 kbp increases when the LET is increased. During recent years, the α-particle emitting nuclide 211At has become interesting as a potential agent for cancer treatment of small tumors and micrometastases due to its radiophysical properties, and a number of studies on its cytotoxic effect have been published. The purpose of this study was to quantify and to determine the distribution of DNA DSBs in human cells irradiated in vitro and to evaluate the RBE of the α-emitter 211At for DSB induction. Human normal fibroblasts were irradiated as intact cell monolayers with α-particles from 211At, labeled to the unspecific monoclonal antibody MX35 F(ab')2 fragment, or photons from 60Co and X-rays. The quantifications and distributions of DSB were determined by pulsed-field gel electrophoresis with fragment analysis for separation of DNA fragments in sizes 10 kbp - 5.7 Mbp. A non-random distribution of DSB after irradiation with α-particles from 211At was found, while irradiation with low-LET led to more random distributions. The RBE for DSB induction was 2.1 and 3.1 for 60Co and X-rays as reference radiation, respectively. In conclusion, irradiation with α-particles from 211At induced 2–3 times more DSB compared with 60Co and X-rays, which is significantly higher than reported earlier.

(PS4055) Rejoining of DNA double-strand breaks and clastogenic effects in higher-plant tobacco cells irradiated with gamma rays. Yuichiro Yokota[1], Seiichi Wada[2], Atsushi Tanaka[1], Issay Narumi[1]. [1]Japan Atomic Energy Agency, Takasaki, Japan, [2]Gunma Univ Grad Sch Med, Maebashi, Japan.

There is an interesting but still-unsolved issue in radiation biology: a part of higher plants show high radiation tolerance in spite of their large genomes. We challenge to solve this issue using single cells prepared from tobacco BY-2 cell line (*Nicotiana tabacum* L., 2n = 4X = 48, 12.3 Gbp DNA per cell nucleus) as model cells. Compared radiation tolerance and initial yield of DNA double-strand breaks of single tobacco cells to mammalian cells, we have found that the former were about 10 times more radiation tolerant (Int. J. Radiat. Biol. 79, 681–685, 2003) and suffered less DNA double-strand breaks (DSBs) per bp but could tolerate more DSBs per cell than the latter (Radiat. Res. 163, 520–525, 2005). In this study, we investigated the rejoining ability of DSBs in tobacco cells by pulsed-field gel electrophoresis (PFGE) assay and clastogenic effects by micronucleus (MN) assay. In the PFGE assay, an equal level of DSB rejoining ability was observed between tobacco cells and Chinese hamster ovary CHO-K1 cells. Tobacco cells may have a larger chance to rejoin DSBs than CHO-K1 cells do since the doubling time of their cells is approximately 28 h and 18 h respectively at normal culture conditions. To draw a conclusion, cell cycle arrest time should be analyzed. We monitored MN formation in tobacco cells and found that the rate (cells having at least one MN per all observed cells) reached the maximum at 2 day-post-irradiation (DPI). The MN formation rate at 2 DPI increased with gamma ray dose and was 40% at LD_{50} (27.4 Gy). Our MN assay did not use cytochalasin B, an inhibitor of cytokinesis, since plant cells have different mechanisms of cytokinesis (i.e. division by cell plate). Thus, the MN formation rate is certainly underestimated both by undivided cells and second round cell division. The cytochalasin B generally induces 30% or less MN formation rates (cells having one or more MNs per binucleated cells) in mammalian cells irradiated with LD_{50} of gamma rays. In summary, tobacco cells are permissive to clastogenic effects of ionizing radiation, and this appears to contribute the radiation tolerance. In the congress, we will refer to the genomic instability observed in the progeny of irradiated tobacco cells.

(PS4056) Induction of strand breaks, and base lesions in dry plasmid DNA films induced by 270 - 560 eV ultrasoft X-rays. Kentaro Fujii, Akinari Yokoya, Naoya Shikazono. Japan Atomic Energy Agency, Ibaraki, Japan.

K-shell photoabsorption of DNA constituent atoms is predicted to induce the critical DNA damage because the atoms can be selectively ionized by the *K*-shell photoabsorption, and ejected secondary low-energy electron tracks cause local multiple damage sites to DNA molecules. To investigate the *K*-shell photoabsorption effect (photoelectric effect and subsequent secondary electron effect) on DNA damage, dry plasmid DNA (pUC18) films were irradiated with synchrotron monochromatic ultrasoft X-rays. Four photon energies, 270, 380, 435, and 560eV, a value below the carbon *K*-edge, below and above the nitrogen *K*-edge, and above the oxygen *K*-edge, respectively, were chosen for the irradiation experiments. Irradiated plasmid DNA was analyzed by agarose gel electrophoresis and the yields of strand breaks were determined by measuring the band intensities of the separated closed circular, open circular and linear forms of the plasmid DNA. The yields of base lesions were determined by the post-irradiation-treatment of the DNA with enzymatic probes (Fpg and Endo III) which convert base lesions into detectable strand breaks. The obtained yield of strand breaks by irradiation of 560eV photon energy was 2.5 times larger than those obtained by other energies (270, 380 and 435eV). On the other hand, the yields of base lesions at 435eV (above the nitrogen *K*-edge) were larger than that of 380eV (below the nitrogen *K*-edge). The role of *K*-shell photoabsorption effect of carbon, nitrogen and oxygen will be discussed in terms of their efficiency to induce strand breaks, base lesions, and clustered damage sites.

(PS4057) A novel methodology for characterizing strand-break termini and damaged bases in plasmid DNA exposed to ionizing radiations. Ken Akamatsu[1], Seiichi Wada[2], Yasuhiko Kobayashi[2]. [1]Japan Atomic Energy Agency, Ibaraki, Japan, [2]Japan Atomic Energy Agency, Gunma, Japan.

We have developed a *de novo* methodology to characterize radiation damage in DNA. An enzyme system consisting of the 3'→5' exonuclease snake venom phosphodiesterase (SVPD) and calf intestine alkaline phosphatase (CIAP) was used to examine the 3' termini of strand break sites. In this study, we hypothesized that the strand-break termini can be divided into two categories: CIAP-independent SVPD sites and CIAP-dependent SVPD sites. The former consists of strand-break termini that can directly be recognized and digested by SVPD without CIAP pretreatment, whereas the latter includes the termini that cannot be digested by SVPD without CIAP pretreatment. In addition, the apparent radiation-chemical yield (*G*-value) can be estimated using the level of intact 2'-deoxynucleotides produced during a 15-min incubation with SVPD. The *G*-value for total strand breaks in fully dried DNA irradiated with Co-60 γ-rays was estimated to be 0.1 μmol/J. Moreover, the *G*-values of CIAP-dependent and CIAP-independent SVPD sites were estimated to be 0.078 and 0.024 μmol/J, respectively. These values suggest that 3'-phosphate termini are more likely to be produced than 3' termini without phosphate. Furthermore, piperidine-treated irradiated plasmid DNA was also treated with the same enzyme system to examine the piperidine -labile sites. As a result of the treatment, the *G*-value of the CIAP-dependent SVPD sites increased to 0.16 μmol/J, whereas no significant increase was seen in the *G*-value of the CIAP-independent SVPD sites. This observation implies that most piperidine -labile damaged bases can be eliminated to form AP sites, which are completely removed by piperidine treatment to form 3' phosphate termini, and that prompt CIAP-independent SVPD sites are piperidine -resistant.

(PS4058) DNA double-stranded breaks indicate general cell stress. Jennifer S. Dickey, Olga A. Sedelnikova, Mykyta V. Sokolov, William M. Bonner. NIH, Bethesda, MD, USA.

Cell stress induces genomic instability. Stress can be caused either from the extracellular environment or from internal cellular processes. One example of stress initiated in the environment is the bystander effect. Cells that are in close proximity to cells that are directly damaged by agents such as radiation display effects similar to but distinct from those in the irradiated cells. DNA double-stranded breaks (DSBs) are a direct consequence of cell irradiation, and they have also been found in bystander cell populations. However, the role of DSBs in propagation of the bystander effect as well as in other forms of cellular stress has not been examined directly. In order to investigate the bystander response to DSB induction, we used a system in which DSBs could be induced with UVA light. Cells were labeled with BrdU and Hoescht dye and half the cells were irradiated with UVA to induce DSB formation while the other half were shielded from the UVA rays. DSB induction was measured for both irradiated and bystander cells using the gamma-H2AX focus formation assay. Results indicate that DSBs formed in cells irradiated with UVA led to the formation of DSBs in unexposed bystander cells. Increased levels of damage led to an increased bystander response though the intensity of this response varied by cell type. Tumor cells behaved distinctly from primary cells and fibroblasts were less sensitive than epithelial cells. Also, filtered media from irradiated cells generated a bystander response in undisturbed control cells. In addition to examining the role of DSB propagation in the bystander effect, we were interested in the role DSBs play in other forms of cell stress. We found that DSBs were generated in response to many forms of cell stress including UVC exposure to neighboring cells, wound formation by scratching, alterations in media pH, and exposure to irritants like SDS. Additionally, normal primary fibroblasts showed an increase in levels of DNA DSBs after exposure to media conditioned on various tumor cell lines. These results indicate that the bystander effect might be a general response to cellular stress and this effect might be an important consideration not just for radiation therapy but also for other cellular assaults and general cell stress.

(PS4059) DNA strand breaks, DNA-protein cross-links and apoptosis in mice exposed to low dose-rate gamma-radiation. Andreyan N. Osipov. N.I. Vavilov Institute of General Genetics Russian Academy of Sciences, Moscow, Russian Federation.

The aim of the present work was to study of DNA strand breaks, DNA-protein cross-links and apoptosis induction in spleen cells of CBA male mice continuously (40–365 days) exposed to very low dose-rate γ-radiation at a dose rate of 0.17 cGy/day. The comet assay study on spleen cells showed that very low dose-rate irradiation resulted in statistically significant increase in DNA strand breaks level, starting from a dose of 20 cGy. Further prolongation of exposure time and, hence, increase of a total dose did not, however, lead to further increase in the extent of DNA strand breaks level. A dose-response curve for DNA single-strand breaks is good fitted by a polynomial regression $y= 0.6209+0.0313*x-0.0004*x^2$, where y is the average comet index, x is a dose in cGy. The DNA-protein cross-links level was statistically increased only on day 40 (6.8 cGy). At the days 120, 270, and 365 of the chronic irradiation (20, 45, and 61 cGy, respectively), approximately two-fold increase over a control level in the apoptotic cell fraction was observed. As expected, a correlation ($r=0.86$; $P<0.05$) between an overall level of DNA strand breaks and percentage of apoptotic cells was noticed. Overall increase in the DNA strand breaks and DNA-protein cross-links levels in mouse spleen cells as a result of chronic low dose-rate gamma-radiation exposure can be associated with the chromatin rearrangement accompanied by gene overexpression, increase in ROS production rate, and DNA repair activation, processes known to be triggered after low doses of ionizing radiation.

(PS4060) Understanding interstrand cross-link formation in bromodeoxyuridine substituted DNA. Marie-Eve Dextraze[1], Sylvain Cecchini[2], Sonia Girouard[1], Richard J. Wagner[1], Darel J. Hunting[1]. [1]University of Sherbrooke, Sherbrooke, PQ, Canada, [2]National Heart Lung and Blood Institute, Bethesda, MD, USA.

Interstrand cross-links impede critical cellular processes such as transcription and replication and are considered to be one of the most toxic types of DNA damage. Both natural products (psoralens) as well as several agents used in chemotherapy (such as carboplatin and Mitomycin C) induce interstrand cross-links. However, these agents remain largely nonspecific with respect to the localization and specificity of the cross-link. Recently, we reported the formation of interstrand cross-links by γ-rays that were specific for mismatched nucleotides within bromodeoxyuridine (BrdU) substituted DNA. In order to reach a better understanding of the cross-linking mechanism, we investigated the formation of interstrand cross-links as a function of DNA sequence in BrdU substituted DNA. Here, we report that the efficiency of cross-link formation is highly dependent on the nature of the mismatched bases, both in the brominated and non-brominated strand, with cytosine giving the highest crosslinking efficiency and guanine the lowest. Furthermore, several distinctive cross-link structures were observed with specific DNA sequences, clearly indicating that the cross-linking process is highly dependent on the nature of the surrounding bases. In addition, irradiation of BrdU-substituted DNA also induces the formation of strand breaks in both the substituted and unsubstituted strand of mismatched DNA, thus providing us with evidence of the possible pathways leading to cross-link formation. Given its ability to be naturally incorporated into cellular DNA *in vitro* and *in vivo* without undue cellular toxicity, BrdU has great potential as a conformation and sequence dependent cross-linking agent.

(PS4061) Double strand break repair in human lymphocytes irradiated with ionising radiation and incubated in microgravity. Maddalena Mognato[1,2], Roberto Cherubini[3], Lucia Celotti[1,2]. [1]Department of Biology, Padova, Italy, [2]University of Padova, Padova, Italy, [3]National Laboratories of I.N.F.N., Legnaro-Padova, Italy.

a) Space missions are characterised by the exposure to both ionising radiation and a significant reduction of gravitational force. The aim of the study is to assess whether cellular radiosensitivity is modified under conditions of microgravity, thus evaluating the risk occurring during space flights.

b) We investigated whether modeled microgravity (MMG) interferes with DNA repair processes, by analysing the induction and repair of double strand breaks (DSBs), the most severe and mutagenic lesion induced by ionising radiation. DSB can be quantified by in situ monitoring the kinetics of foci formation and disappearance of g-H2AX and other proteins that accumulate at sites of DSBs. The rate of loss foci strongly correlates with the rate of DSB repair. Peripheral blood lymphocytes (PBL) were irradiated with 1Gy of low energy protons (LET=31 keV/mm) or g-rays (5Gy) and incubated in normal gravity (1g) and in modeled microgravity (MMG, i.e. 10–2g). At 30', 2h, 6h and 24h from irradiation PBL were recovered, stained for immunofluorescence and analyzed by confocal laser scanning microscopy. We evaluated the phosphorylation of the DNA damage sensor protein ATM (Ataxia Telengiectasia Mutated), the first to be activated in the presence of DSB, together with NBS1 (Nijmegen Breakage Syndrome 1) and 53BP1 (53 binding protein 1) proteins, both involved in the early steps of DSB repair.

c) Clustered and larger g-H2AX foci along a defined linear path were evident in cells irradiated with protons compared with those irradiated with g-rays, where the foci were randomly distributed. Our results show that the percentage of g-H2AX foci positive cells is significantly higher in MMG at 6h and 24h from irradiation with both protons and g-rays, in comparison with 1g condition. Also the mean number of g-H2AX foci per nucleus is significantly higher in PBL incubated in MMG. At the same time points, 53BP1 and NBS1 foci persisted in a significant high fraction of PBL incubated in MMG. Instead, the co-localisation of these proteins with g-H2AX foci doesn't seem to be affected by MMG. ATM activation also seems to be affected by MMG.

d) The persistence of unrepaired DSB in MMG may contribute to the significant increase of HPRT mutant frequency previously measured as well as to the significant increase of apoptosis.

(PS4062) Cancer cells modulate DNA DSB/Repair in non-transformed cells. Afshin Beheshti[1,2], Heiko Enderling[1,2], Matthew Perkins[1,2], Aaron Burg[1,2], Katarina Luptakova[1,2], Amir Abdollahi[1,2], Philip Hahnfeldt[1,2], Lynn Hlatky[1,2]. [1]Tufts University School of Medical, Boston, MA, USA, [2]Caritas St. Elizabeth's Medical Center, Boston, MA, USA.

γ-H2AX (phosphorylated H2AX from the core histone H2AX) and 53BP1 (p53 binding protein 1) co-localize at sites of DNA damage with a number of proteins involved in DNA damage repair and signaling, thus facilitating DSB repair and rejoining. γ-H2AX and 53BP1 play a critical role in suppressing oncogenic translocations. Although induction of γ-H2AX foci and 53BP1 foci are frequently utilized as assays to investigate DSB/repair following irradiation, minimal attention has been given to the generation of spontaneous DSB/repair foci in cancer cells and cells of the tumor microenvironment. We investigated the constitutive level of DNA damage/repair, as indicated by standard γ-H2AX and 53BP1 foci assays, in cells of the tumor and adjoining stroma *in vivo*. *In vitro* studies were also done on a panel of murine tumor cells (glioma and lung carcinoma) and human tumor cells (lung carcinoma), in both direct and indirect co-cultures with primary nontransformed cells (murine lung fibroblast, human dermal fibroblast). *In vivo* studies on a panel of tumor models, including human tumor xenografts (liposarcoma), murine tumors (Lewis lung carcinoma), and the spontaneous K-ras LA2 lung tumor model were also performed. DSB repair foci in both *in vitro* and *in vivo* tumor stromal populations were quantified. *In vivo* spatial distributions were compared to other measured tumor features (e.g. vascularization, oxygenation, etc). An increase in DSBs occurred in nontransformed cells in the presence of tumor cells *in vitro*. Conversely, preliminary data suggest that stromal fibroblasts may induce a reduction of DSB foci in the tumor cells. In the *in vivo* tumor models, γ-H2AX and coupled 53BP1 expressions were detected in several cell types (e.g. endothelial, fibroblasts, adipocytes etc.) in the adjacent tumor microenvironment. At distances away from the tumor, these same cell types, showed null or minimal levels of γ-H2AX and 53BP1. Our studies demonstrate, both *in vivo* and *in vitro* that tumor cells can induce DSB/repair foci in adjacent nontransformed cells. This suggests that tumor cells may transmit signals into the microenvironment that result in DNA damage to neighboring nontransformed cells. We termed this effect the "constitutive-break bystander effect."

(PS4063) UV-C radiation induces single strand breaks in DNA by inducing conformational relaxation of the helix and affects the restriction profile of DNA. Chaitali Bhattacharjee. NEHU, Shillong, India.

Ultraviolet (UV) radiation is known to be a mutagen. Of the various components of the UV radiation, UV-C is of the shortest wavelength (200–290 nm) and therefore, known to be most lethal. The lethality of UV-C radiation is attributed primarily to its ability to induce cyclobutane pyrimidine dimers (CPD), in addition to the induction of varying amounts of pyrimidine-pyrimidone photoproducts ((6–4)PP) and their Dewar isomers, other dimeric nucleotides and adenine dehydrodimers. In addition, UV-C radiation is also known to induce single stranded breaks (SSB), though in small numbers. These SSB, though small in number, can prove to be highly mutagenic if they are left unrepaired or are misrepaired. The mechanism of the formation of SSB by UV-C is not known. It is, however, known that the energy absorbed by the DNA when it is irradiated with UV-C, is not enough for the direct induction of strand breaks. Nonetheless, SSB are induced in small numbers upon UV-C exposure. This suggests that induction of SSB must be a secondary effect of the changes that the DNA undergoes as a result of irradiation with UV-C. This investigation was undertaken using repair proficient and repair deficient *E. coli* to understand the mechanism that underlies the induction of SSB on DNA as a result of assault by UV-C radiation. In addition, the effect of the presence or absence of *recF* gene on the repair of the damages induced on the DNA was studied. Our studies indicated that UV-C induced conformational relaxation in DNA caused significant torsional stress on DNA helix. The torsional stress caused break in DNA strand in form of one or more SSB. Since UV-C affects mainly pyrimidine nucleotides (NT), it is expected that NT sequences rich in such NT would be modified by UV-C. To test this, we analyzed restriction profiles of DNA before and after UV-C exposure using restriction endonucleases whose restriction sites were either rich or poor in pyrimidine NT. Our studies indicate that in addition to the expected effects on pyrimidine-rich sites (e.g., AT-rich), other NT sequences were also getting modified by UV-C radiation.

(PS4064) A novel technique using DNA denaturation to analyze clustered DNA damage sites induced by densely ionizing radiation. Akinari Yokoya[1], Naoya Shikazono[1], Takeshi Ushigome[2,1], Ayumi Urushibara[1], Kentaro Fujii[1]. [1]Japan Atomic Energy Agency, Ibaraki, Japan, [2]Ibaraki University, Ibaraki, Japan.

One of the goals of our study is to clarify the nature of DNA damage in relation to the energy deposition pattern of radiation, in particular of densely ionizing radiation such as ions and of low energy secondary electrons constituting radiation track-end. Many studies on damaged DNA to date have focused on yields of single- and double-strand breaks in closed-circular plasmid DNA. Oxidative base lesions and cluster damages composed of them have also analyzed using base excision repair enzymes, which convert a base lesion to a detectable strand breaks. The enzymatic assay, however, does have some limitations when revealing clustered DNA damage sites. For instance, clustered damage sites induced by high LET radiation are predicted to be more complex, in other words contain more SSBs or base lesions within the cluster. Monte Carlo simulation studies have shown that the proportion of SSB which are complex, i.e. one or more base lesions close to SSB terminus, is significant. These complex SSBs, detected as enzymatically revealed additional SSBs, would be underestimated since this latter SSB will not cause additional conformational changes of the closed-circular plasmid DNA if it is on the same strand as the prompt SSB or on the opposite strand but separated sufficiently (>6 bp) from the prompt SSB so as not to induce a DSB.

In order to observe these enzymatically invisible clustered damages, we developed a novel technique using DNA denaturation by which irradiated DNA is analyzed as single strand DNA. The prompt or enzymatically revealed additional SSBs which arise in both strands of pUC18 plasmid DNA, but do not induce a DSB, are measured as molecular size distribution of single strand DNA using gel electrophoresis. To avoid induction of heat labile sites by high temperature used for a general denaturation treatment, we have determined much lower denaturation temperature (39 degrees centigrade) using formamide (50% v/v), which prevented the induction of additional damages. We have also examined gel staining giving quantitative signal intensity of the single strand DNA fragments distributed from 20 to 2000 nucleotides. Obtained results will give us new knowledge of damage clustering patterns in irradiated DNA.

(PS4065) Formation of DNA repair protein foci at clustered damage sites in high-LET irradiated cells. Bo Stenerlow[1], Irina Radulescu[2], Kristina Viktorsson[2], Martin Simonsson[3], Fredrik Qvarnstrom[3], Karin H. Karlsson[1], Rolf Lewensohn[2]. [1]Biomedical Radiation Sciences, Uppsala, Sweden, [2]Department of Oncology/Pathology, Stockholm, Sweden, [3]Department of Oncology, Uppsala, Sweden.

We here used local irradiation, in the form of high linear energy transfer (LET) ions, to monitor the spatial dynamics of DNA damage and repair processes in human fibroblast cells. Cells were irradiated with low doses of accelerated nitrogen ions (LET=80–320 eV/nm) and foci of repair-related proteins were detected by immunofluorescence analysis. Activation of ATM (Ataxia telangiectasia mutated) protein kinase, *i.e.* autophosphorylation (Ser1981) and dissociation of dimeric ATM, is one of the earliest responses to ionizing radiation. In cells irradiated with ions under a low angle there were few larger p-ATM foci that clearly correlated with γ-H2AX foci within the tracks, and smaller p-ATM foci forming a punctuate/diffuse staining dispersed throughout the whole nucleoplasm, more than 10 micrometers away from visible γ-H2AX foci. Foci of γ-H2AX, MRE11 and 53BP1 rapidly co-localized along the particle tracks and track morphology varied with LET and repair

time. However, the mean distance between two adjacent γ-H2AX foci was about 1.7 micrometer, and was constant irrespective of LET (80–160 eV/nm) and repair time (5 min - 1h). This was in strong contrast to the number of DSBs present in each track, indicating that a single γ-H2AX focus might contain clusters of several DSBs within 1–2 Mbp of chromatin. In summary, our data provides new insights on the spatial dynamics of DNA damage signaling and repair on a subnuclear level along a high-LET particle tracks.

(PS4066) Analysis of DNA damage spectra induced by irradiations with the same HZE ion and different energies. Deborah J. Keszenman, Betsy M. Sutherland. Brookhaven National Laboratory, Upton, NY, USA.

Bistranded clustered DNA damages–defined as two or more strand breaks, abasic sites and oxidized bases within a few helical turns–are induced by ionizing radiations. This type of DNA damages constitutes potentially lethal and or mutagenic lesions since they are difficult to repair. Previous studies have shown that the damage spectrum, that is the relative levels of double strand breaks compared to abasic clusters and oxypurines clusters, varies with the DNA microenvironment. The yield of damage clusters is lower when DNA is irradiated in high radioquenching solutions. Also, different spectra of clustered damages are produced by photons and by charged particles including high energy protons. The absolute yield of damages decreased with increasing linear energy transfer (LET) of the radiation. However, the species of HZE ions used also increased in atomic number, Z. To study whether the determining factors of this dependence are LET, Z or both, we have investigated the damage spectra induced by irradiation of megabase pair genomic DNA with ionizing radiation species of same Z but different energies, which varies the LET. DNA samples were irradiated in quenching and non-quenching conditions with ion beams of protons at 1 GeV, 500 MeV and 200 MeV, iron at 1 GeV/nucleon, 600 MeV/nucleon and 300 MeV/nucleon and, silicon at 600 MeV/nucleon and 300 MeV/nucleon. The frequencies of different types of clustered DNA damage- double strand breaks, oxypurine clusters and abasic clusters- were determined using gel electrophoresis, electronic imaging and number average length analysis. Our results show that the variation of the yields of damages induced by irradiation with particles of the same Z and different LET was less than expected. The determination of the different damage spectra for each HZE ion is in progress.

(PS4067) Visualization of the damage induction and the accumulation of RAD51 in the cells irradiated with synchrotron X-ray microbeam. Noriko Usami[1], Kiyomi Eguchi-Kasai[2], Masahiko Mori[2], Katsumi Kobayashi[1]. [1]Phoron Factory, KEK, Tsukuba, Japan, [2]Natl. Inst. Radiol. Sci., Chiba, Japan.

An X-ray microbeam irradiation system using synchrotron radiation has been developed at the Photon Factory, KEK, Japan, and is now being fully operated for various biological experiments. The energy of microbeam X-rays is 5.35 keV which is suitable for experiments using mammalian cells, because the X-rays can penetrate the thickness of cells and the range of secondary electrons is within few micrometers. The synchrotron radiation has extremely low divergence, so it is easy to get X-ray beam with micrometer-dimension. The high-precision slit installed in the system is the simplest way to get a microbeam without focusing. The minimum beamsize by the slit is 5-micrometer-square, and the size of the microbeam can be changed quickly and arbitrarily. This specification might be ideal to investigate localization of the damage induction and subsequent cellular responses in cells irradiated with subcellular-sized beam.

To visualize the DNA damage induction, the microbeam-irradiated cells were immuno-stained by phosphorylated histone H2AX (gamma-H2AX) antibody. Most DNA double strand breaks were observed in localized area in cell nuclei, the size of which was almost the same as the beam size. Dose dependence of gamma-H2AX induction was also clearly observed.

GFP-tagged protein enables us to visualize cellular responses in living cells. We constructed Chinese hamster ovary (CHO) cells having GFP-tagged RAD51, one of the protein related homologous recombination. Within 1 hour after irradiation, significant number of foci of GFP-RAD51 could be observed at the microbeam-irradiated site in the majority of the irradiated cells, and remained at least 5 hours after irradiation. The foci were appeared in the restricted area in the cell nuclei, which was corresponded to the damage induced area visualized with gamma-H2AX. In some of the cells, the foci could not appeared at all even after 12 hours, and the percentage of the population was almost same as the estimated population of G1-phase cells at the irradiated time.

(PS4068) Iodine-125 radioprobing of intramolecular quadruplex conformation of human telomeric dna: effects of flanking sequences, ionic conditions and quadruplex-specific drugs. Timur I. Gaynutdinov, Ronald D. Neumann, Igor G. Panyutin. Department of Nuclear Medicine, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD, USA.

A repeated, non-coding, DNA sequence $d(TTAGGG)_n$ is present in the telomeric ends of all human chromosomes. These repeats can adopt multiple inter- and intra-molecular non-B-DNA conformations that may play an important role in biological processes. NMR spectroscopy and X-ray crystallography have solved three intra-molecular structures of the telomeric oligonucleotides: antiparallel, parallel and a "mixed-type" hybrid structure containing both parallel and anti-parallel strands. It was found that in a potassium-containing solution stable NMR-resolvable conformation can only be formed if certain sequences are placed upstream of the $GGG(TTAGGG)_3$ core. We applied ^{125}I -radioprobing to assess the conformation of the human telomeric DNA fragments with different flanking sequences in the presence of different ions (sodium or potassium) and in complex with the quadruplex-specific drugs: (i) cationic porphyrin TMPyP4 and (ii) telomestatin. These compounds have been proposed as anticancer agents due to their potential to inhibit telomerase. The probability of DNA breaks caused by decay of ^{125}I is inversely related to the distance between the radionuclide and the sugar unit of the DNA backbone; hence, the conformation of the DNA backbone can be deduced from the distribution of breaks. Obtained data indicate that intramolecular quadruplex conformation of telomeric repeats strongly depends on the presence of flanking sequences adjacent to the core and on the type of counterion present, i.e. Na^+ or K^+. Based on our analysis of the breaks distribution we propose a novel conformation of telomeric quadruplex, which is characterized by one lateral edgewise loop and two double-chain reversal loops configuration and a mixed parallel/antiparallel backbone structure. Telomestatin favors an antiparallel conformation of telomeric oligonucleotides regardless of the type of the counterion. Dramatic changes in the quadruplex conformation were observed upon binding TMPyP4. In the presence of some flanking sequences and K^+ it promotes formation of a parallel-stranded conformation.

(PS4069) Cultured endothelial human cells prematurely enter senescence as a non-cancer effect of high- and low-LET irradiation. Lorenzo Manti[1], Marco Durante[1], Cecilia Arrichiello[2], Thilo Elsasser[3], Giancarlo Gialanella[1], Mariagabriella Pugliese[1], Sylvia Ritter[3], Paola Scampoli[1], Gianfranco Grossi[1]. [1]University of Naples-INFN Naples Section, Naples, Italy, [2]University of Naples, Naples, Italy, [3]Gesellschaft fur Schwerionenforschung (GSI), Darmstadt, Germany.

It has been shown that sub-lethal doses of ionizing radiation can cause cultured cells to senesce prematurely. The underlying mechanisms are unclear. From a cellular viewpoint, such a response may represent a stress-induced avoidance of transformation but it might entail destabilisation of the cell's genome and thus enhance its neoplastic potential. Either fate depends on whether cells are driven into senescence by a (onco)gene-controlled pathway or by telomere attrition, respectively. It is therefore of interest to study the onset of senescence and its relationship with telomere length reduction and genomic instability following exposure to radiation of

different qualities. We used human umbilical vein endothelial cells (HUVECs) as *in vitro* system because of their well-defined pattern of spontaneous ageing. Passage 2 HUVECs were given 0.5 Gy and 2 Gy using the therapeutic 270 MeV/u ^{12}C ion beam at GSI (Darmstadt, Germany) at the positions of the plateau (LET = 13 keV/μm) or the spread-out Bragg peak (SOBP, LET = 100 keV/μm) along the beam ionization path. X-ray irradiation was also performed as low-LET reference. At regular post-irradiation time intervals, cells were either harvested for histochemical and cytogenetic analysis or re-plated. As early as the first passage post-irradiation, premature senescence was revealed by positivity for β-galactosidase activity and occurred in a clear dose- and LET-dependent fashion. At later times, the senescent fraction remained more elevated in the irradiated samples compared to sham-irradiated cells but followed a rather complex time pattern. These data will be related to measurement of telomere length by interphase quantitative (IQ) FISH. Karyotyping, assessment of persistent delayed growth impairment and de novo arising micronuclei will be used to monitor genome stability.

(PS4070) Hyper diploid lymphocytes due to aging in a woman living in the high level natural radiation area in Ramsar, Iran. Masako Minamihisamatsu[1], Akira Furukawa[1], Mojtaba Saghirzadeh[2], Reiko Kanda[1], Tsutomu Sugahara[3], Isamu Hayata[1]. [1]NIRS, Chiba city, Japan, [2]Univ. of Tabiat Modarres, Tehran, Iran (Islamic Republic of), [3]Health Reserch Foundation, Kyoto, Japan.

Chromosome aberrations to be induced by radiation usually take the form of structural aberrations. In our study one woman in a high background radiation area in Ramsar, Iran, had cells with additional chromosomes in peripheral lymphocytes. She was a 62-year-old housewife without any genetic abnormality. She married at the age of 20 years old, and started to live in the house where thereafter she was exposed to the high level of natural radiation. The exposed dose was measured 3 times using a pocket dose meter (Aloka PDM-101) put on her body for 24 hours. The measured doses were 80.4 μSv, 96.6 μSv and 136.9 μSv, respectively. The accumulated exposed dose after her marriage was estimated to be 1.64 Sv that was calculated with the average measured values of 104.6 μSv/day. The frequency of dicentrics plus centric rings (markers of radiation exposure) in 2038 cells analyzed was 12, which is about 6 times higher than normal frequency. Thus the effect of radiation is clearly shown in the lymphocytes. There were totally 18 hyper diploid cells: one cell had 7 additional C group chromosomes; one cell had two additional C; 13 cells had one additional C; one cell had one additional A; one cell had one additional D; and one cell had one additional E. Most of the extra chromosomes showed acentric like morphology of the premature centromere separation. FISH chromosome painting performed in her different chromosome slides revealed that the origin of the additional C was X chromosome. There were cells with 2 X-bodies indicating that they were inactive X. It seems these unusual X chromosomes had ability to replicate. This replication was associated with non-disjunction leading to aneuploid cells. The lymphocytes with additional X chromosome(s) were found in an age matched woman in control also who had cells with additional C in the similar frequency. The finding of 7 additional C in a lymphocyte in the normal blood is extremely unusual. It is assumed that the induction of the aneuploid cells might be the result of aging rather than the effect of radiation. The aneuploid cells in the lymphocyte will be discussed in terms of aging and radiation.

(PS4071) Telomere dysfunction and dna repair deficiency: markers of sensitivity to mutagens and carcinogens? Jennifer Newman[1], Birendranath Banerjee[1], Lakshmidevi Balakrishnan[1], Manikandan Jayapal[1], Aik Kia Khaw[1], Anuradha Poonepalli[1], Rabindra N. Bhattacharjee[2], Rajamanickam Baskar[1], Han-Woong Lee[3], Alirio Melendez[1], M. Prakash Hande[1]. [1]National University of Singapore, Singapore, Singapore, [2]Osaka University, Osaka, Japan, [3]Yonsei University, Seoul, Republic of Korea.

Genetic susceptibility to environmental disease is believed to play an important role in determining individual differences in the development of cancer. Genetic alterations in critical regulatory pathways (for e.g. control of genomic stability, including DNA repair mechanisms, cell-cycle checkpoints, apoptosis and telomere length and control of micro-environmental factors and others) may predispose cells to carcinogenesis . We have studied the role of DNA repair factors in eliciting the effect of damage induced by a carcinogen, arsenic. Arsenic is an environmental contaminant and a potent human carcinogen, which induces oxidative stress on cells via the generation of ROS. In an earlier study, we have demonstrated that cells lacking key DNA repair factor, PARP-1, displayed enhanced sensitivity to arsenite. In this study, we used telomerase deficient (mTERC$^{-/-}$) mouse embryonic fibroblasts with long and short telomeres to investigate the extent of oxidative damage by comparing the differences in telomere loss and chromosome aberrations at 24 and 48 hours of exposure to arsenite. Peptide Nuclei Acid Fluorescence in situ hybridisation analysis revealed that increasing doses of arsenite augmented the chromosome aberrations, which contributes to genomic instability and lead to possibly apoptotic cell death and cell cycle arrest. Elevated DNA damage detected by alkaline comet assay points to an impaired repair ability of arsenite induced DNA lesions in mTERC$^{-/-}$ mouse cells with short telomeres. By using micro-array analysis, we have identified few genes which are differentially expressed upon arsenite treatment and we are elucidating the signal transduction pathways involved in arsenic-induced oxidative damage. The genes involved in telomere length regulation and maintenance (such as ATM, Adprt1, NBS1, TRF1 and 2 etc) are differentially expressed in mTER-/- cells with short telomeres. Taken together, data from the present study and our ongoing studies on different DNA repair deficient mammalian cells imply that short dysfunctional telomeres impair the repair of oxidative damage caused by arsenite and point to the increased sensitivity of telomerase-deficient mouse cells to carcinogens and mutagens.

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(PS4072) Radiation quality effect on telomere elongation of irradiated mammalian cells. Francesco Berardinelli[1], Antonella Sgura[1], Antonio Antoccia[1], Giacomo Cuttone[2], Roberto Cherubini[3], Silvia Gerardi[3], Caterina Tanzarella[1]. [1]University of Rome, Roma, Italy, [2]INFN-Laboratori Nazionali del Sud (LNS), Catania, Italy, [3]INFN-Laboratori Nazionali di Legnaro (LNL), Legnaro, Padova, Italy.

Telomeres are specialized nucleoprotein complexes that serve as protective caps of linear eukaryotic chromosomes. It has been reported that radiation-induced DNA lesions, may promote the loss of functional telomeres and, consequently, chromosome fusions and chromosome instability. So far the effect of radiation quality on telomere length variation and activation of telomerase has not been investigated.

In this respect, we tested whether and how protons of different energies were able to affect telomere structure. Human primary fibroblasts (HFFF2), (MEFs) and lymphoblastoid cell cultures were irradiated with 4 Gy of 3 MeV of protons (7 MV Van de Graaff CN, INFN-LNL, Italy) and with 4 Gy of 62 MeV of protons (CATANA facility, INFN-LNS, Italy). Experiments with X-rays (250 kVp, 6 mA), as reference radiation, were also carried out.

Cells were fixed after either 24 hrs or 15 days from irradiation. Before harvesting, chromosomes were allowed to condense by incubating cells for 30 min in the presence of Calyculin-A, an agent able to induce premature chromosome condensation. To evaluate radiation-induced alterations of telomere length, Q-FISH staining was performed on condensed chromosomes and interphase nuclei with fluorescent PNA telomeric probe and telomere size was analysed with TFL-TELO software.

Our preliminary results obtained in HFFF2 human fibroblasts treated with either Low-LET 62 MeV protons or X-rays indicated that telomeres were not modified in their length as evaluated 24 hrs after exposure, whereas a significant increase in telomere length was detected in cells harvested at later times. Contrastingly, 3 MeV protons (28,5 keV/μm) induced a significant increase of telomere length at short as well as at long harvesting time from treatment. However, telomerase activity as measured by TRAP assay, showed no modulation of activity as result of irradiation with either High- or Low-LET radiation.

These results seem to indicate that lesions characterized by different complexity, as those expected after low-energy protons and those induced by sparsely ionizing radiation are able to modulate telomere elongation, though at different time. The mechanism responsible for such elongation remains to be clarified.

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(PS4073) Association of radiation-induced senescence of articular chondrocytes with plakoglobin accumulation. Eun-Hee Hong, Ji-Yeon Park, Su-Jae Lee, Sang-Gu Hwang. Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea.

Radiotherapy may be as effective for the management of primary cancer as conventional chemotherapy, but little is known about the influence of the radiation on cartilage. In chondrocyte monolayer cultures, radiation caused a dose dependent decrease in cell proliferation without cell death. Cell morphology was bigger and a population had more diverse morphotypes than control cells after radiation. These data show that radiation induces an increase in the senescence of primary cultured articular chondrocytes. Reactive oxygen species (ROS) production was increased dose and time dependently when chondrocytes were exposed to radiation. Pretreatment chondrocytes with ROS inhibitors NDGA or GSH reduced the level of ROS production after radiation, whereas no effect was observed in cell proliferation and morphology. These results suggest that radiation-induced senescence of chondrocytes was independent on release of ROS. We found unexpectedly that cell-cycle responses do not occur in irradiated chondrocytes although eukaryotic cells irradiated with high doses of radiation exhibit cell-cycle responses referred to as G1/S, intraS, and G2/M checkpoints. Instead, cells at all stages of the cell cycle carry lesions into S phase and delay cell-cycle progression. We did Western blotting to determine the expression of cell adhesion molecules N-cadherin, p120, α-catenin, β-catenin, and plakoglobin in chondrocytes. A highly significant correlation was observed between the increased expression of plakoglobin and increased senenscense. These data suggest that the S-phase DNA damage checkpoint pathway and plakoglobin accumulation are an important physiological role in senescence of chondrocytes response to radiation. [KRF-2005–041-C00344; National Nuclear Technology Program of MoST]

(PS4074) Base damage near double-strand break ends affects rejoining efficiency and the chronology of repair events for these lesions. Shubhadeep Purkayastha, Kamal Datta, Ronald D. Neumann, Thomas A. Winters. National Institutes of Health, Bethesda, MD, USA.

Radiation-induced DNA double strand breaks (DSB) are critical cytotoxic lesions that have been implicated as potentially lethal events for a cell. Previously, we have shown that highly cytotoxic ^{125}I decay-induced DSBs constitute extremely complex lesions, with variable overhang end-morphology and clustering of damage (base damage and AP sites) immediately upstream (within 8–10 bp) from the DSB end. These DSB ends also possess 3′-phosphate (3′-P) and 3′-OH ends but no 3′-phosphoglycolate (3′-PG).

The present work investigates the effect such structures have on DSB repair kinetics by employing defined duplex oligonucleotides as end-joining substrates in *in vitro* end joining assays. We have found that HeLa cell extract mediated end joining is partially inhibited by blocked 3′-ends (3′-P or 3′-PG), and by 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG) positioned 1 nucleotide upstream (−1n) from a ligateable 3′-OH DSB end. Furthermore, when 8-oxo-dG was replaced by an AP site at −1n, end joining was completely inhibited, as was end joining of an oligonucleotide with an A-C mismatch at the same position or an 8-oxo-dG mismatched with a T. These results suggest that structures likely to cause destabilization of hydrogen bonding and/or base stacking between the DSB-end terminal base pair might interfere with end joining. To examine this relationship further, we have employed oligonucleotide substrates with 8-oxo-dG placed progressively further from the DSB terminus and assessed end joining efficiency. In addition, the role of nucleotide position vis-a-vis the DSB end has also been examined for its effect on the chronologic sequence of the repair events. We have observed that 8-oxo-dG at −1n with respect to the DSB end is not removed or repaired by HeLa extracts prior to end joining.

This result suggests that excision of the damaged base is not a prerequisite to, or integrated with the end joining reaction, and that repair of damaged bases proximal to the DSB end may occur subsequent to end joining. To investigate if this effect is related to position, we assessed end-joining products of the 8-oxo-dG substrates described above for the continued presence of 8-oxo-dG.

(PS4075) A proteolytic fragment of Cyclin E enhances apoptosis through inhibition of DNA repair by interacting with Ku70 and preventing the recruitment of XRCC4, Ligase IV, and XLF. Dragos C. Plesca[1,2], Suparna Mazumder[1], Alex Almasan[1,3]. [1]Cleveland Clinic, Cleveland, OH, USA, [2]Kent State University, Kent, OH, USA, [3]Dept. of Radiation Oncology, Cleveland Clinic, Cleveland, OH, USA.

Cyclin E/Cdk2 is a critical regulator of cell cycle progression from G1 to S phase in mammalian cells that is deregulated in many types of neoplasms. We found that genotoxic stress leads to a dramatic decrease in CycE levels in hematopoietic tumor cells, coinciding with the timely appearance of its proteolytic fragment, p18CycE. Overexpression of p18CycE in a variety of cell types induces apoptosis. We have identified Ku70, a critical component of non-homologous end joining DNA repair (NHEJ) as a new interacting partner of p18CycE (Mol Cell Biol: PMID: 17325036). Non-toxic levels of p18CycE sensitize cells to etoposide and ionizing radiation. Neutral comet assays used to determine residual double-strand breaks indicative of an ineffective NHEJ, showed significant difference in both tail length and tail moment in the presence of p18CycE following irradiation. Moreover, a plasmid reactivation assay indicated that p18CycE reduced the colony formation known to be associated with NHEJ activity to ~10% as compared to ~60% for cell lysates containing wild-type CycE. Gel electrophoretic analyses indicated an impairment of end-ligation dependent on the expression levels of p18CycE. DNA pull-down assays showed that the assembly of Ku70-Ku80 and DNA-PKcs is not affected by the presence of p18CycE. However, the recruitment of XRCC4, Ligase IV, and the recently identified accessory factor XLF was greatly impaired. These data indicate a profound effect of p18CycE on cellular survival and NHEJ that is dependent on its interaction with Ku70 and probably caused by interference with the recruitment of the XRCC4-Ligase IV heterocomplex to the sites of double-strand breaks. Mapping the p18CycE interaction domain of Ku70 using a series of deletion mutants of Ku70, revealed that their interface resides at the N-terminus of Ku70, which is different from the Bax binding site located at the C-terminus. Recently, we have shown that binding of p18CycE to Ku70 results in release of Bax leading to amplification of the apoptotic signal. Interaction of Ku70 with p18CycE and Bax may be unique in linking cell cycle control, DNA repair and activation of apoptosis following genotoxic stress as well as provide mechanistic insights into the choice between cell death and cell survival depending on the cell type and nature of the genotoxic challenge.

(PS4076) The human RAD51AP1/PIR51 protein is required for homologous recombination and genomic stability. Claudia Wiese, Torsten Groesser, David W. Collins, Bjorn Rydberg, David Schild. Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

Homologous recombination serves to repair DNA double-strand breaks (DSBs) and stalled replication forks, and the RAD51 recombinase is the key protein required for this DNA repair pathway. RAD51AP1 (RAD51 Associated Protein 1; aka PIR51, Protein interacting with RAD51) is a novel RAD51-interacting protein whose function had remained elusive but, as shown in this study, is necessary for homologous recombinational DNA repair (HRR). We have investigated the consequences of RAD51AP1-

depletion in human cells using RNA interference. We report that knockdown of RAD51AP1 confers increased cellular sensitivity to the DNA crosslinking agent mitomycin C (MMC), and also sensitizes human cells to camptothecin and UV-C radiation and S-phase cells to X-rays. Moreover, RAD51AP1 suppression impairs homology-mediated repair of a site-directed DSB, and leads to elevated levels of chromatid breaks both spontaneously and after exposure to MMC or X-rays. However, suppression of RAD51AP1 has no effect on FANCD2 mono-ubiquitination, and, somewhat surprisingly, does not impair X-ray-induced RAD51 foci formation. In this last aspect, RAD51AP1 is similar to RAD54, which also is dispensable for RAD51 foci formation, and dissimilar to most genes required for HRR. We present a model in which RAD51AP1 functions downstream of the recombinational mediators, and that incorporates the limited sequence conservation between the RAD51-interacting C-terminal domain of RAD51AP1 and the RAD51-interacting N-terminal part of RAD54. Overall, our findings provide evidence that RAD51AP1 is a new gene functioning in RAD51-mediated HRR and critical for maintaining genomic integrity.

(PS4077) The role of homologous recombinational repair (HRR) in determining radiosensitivity throughout the mammalian cell cycle. Paul F. Wilson, John M. Hinz, Salustra S. Urbin, Peter B. Nham, Larry H. Thompson. Lawrence Livermore National Laboratory, Livermore, CA, USA.

DNA double-strand break repair by "error-free" homologous recombinational repair (HRR) has been postulated to underlie the increased radioresistance and reduced mutability observed in mammalian cells irradiated during S-phase, as compared to other phases of the cell cycle. To further test this hypothesis and to determine whether HRR capacity contributes to the recovery of cells irradiated in the G1 phase, we examined the role of HRR in promoting cell survival and the repair of chromosomal aberrations (CA) throughout the cell cycle following low LET ionizing radiation exposure in wild-type (AA8), HRR-deficient Rad51D-knockout (51D1), and Rad51D-complemented 51D1 (51D1.3) Chinese hamster ovary (CHO) cells. Asynchronous cultures of cells were irradiated with 150 and 300 cGy doses of cesium-137 gamma rays and synchronized by centrifugal elutriation. The relative survival of cells from each synchronized fraction spanning early G1 to late G2/M (determined by thirty minute BUdR pulse-labeling and flow cytometry for 18–20 fractions total) was measured by single-cell colony formation ability. Wild-type CHO AA8 and Rad51D-compleneted 51D1.3 cells were relatively more radioresistant in S-phase as compared to G1 and G2, while the HRR-deficient 51D1 cells peaked in radioresistance in G1 (with survival levels similar to AA8 and 51D1.3) while becoming progressively more radiosensitive throughout S and G2. Metaphase spreads were collected following colcemid treatment for the first, every subsequent third, and final elutriation fractions at the first post-irradiation mitosis and scored for CA. Irradiation of the HRR-deficient 51D1 cells in S and G2 resulted in an ~3-fold increase in chromatid-type aberrations and the generation of complex chromosome exchanges. Complex exchanges were detected in the irradiated Rad51D-complemented 51D1.3 cells only in the final elutriation fraction (late G2/M), suggesting NHEJ-mediated DSB repair occurs either more rapidly than HRR or preferentially in cells irradiated near the G2/M transition point. This work was performed under the auspices of the U.S. DOE by the University of California and the Lawrence Livermore National Laboratory under contract W-7405-Eng-48 and supported by grants CA89405 and CA112566 from the National Cancer Institute, U.S. DHHS.

(PS4078) Molecular basis of radioresistance in glioblastomas: proficient repair of dna double-strand breaks in astrocytes expressing egfrviii. Bipasha Mukherjee, Cristel Vanessa Camacho, Robert Bachoo, Sandeep Burma. UTSouthwestern Medical Center, Dallas, TX, USA.

Glioblastomas are brain tumors arising from the glial cells of the brain (astrocytes). These are the most radioresistant tumors and are refractory to radiation therapy. The basis for radioresistance of these highly aggressive tumours is not well understood. About 50% of glioblastomas have dysregulated epidermal growth factor receptor (EGFR) and about half of those express a mutant, constitutively active receptor subtype, EGFRvIII. We have generated mouse astrocytes expressing EGFRvIII in different genetic backgrounds comprising of the signature glioblastoma mutations (p53, Rb, Ink, Arf, PTEN,) and have examined their contribution to radioresistance. We find that, no matter what the oncogenic background may be, expression of EGFRvIII in astrocytes invariably renders them significantly radioresistant while a kinase dead version of the receptor has no such effect. This radioresitance can be extended to orthotopic tumors in mice - gliomas arising from astrocytes expressing EGFRvIII are significantly resistant to radiotherapy. The most deleterious damage inflicted by ionizing radiation is DNA double strand breaks (DSBs). We find that astrocytes expressing EGFRvIII can repair radiation-induced DSBs more efficiently compared to the parental lines. A few recent reports indicate that EGFR signaling impinges on DSB repair via the repair enzyme DNA-PK. To examine this possibility in gliomas, we expressed EGFRvIII and the kinase dead version in the human U87 glioblastoma line. This already radioresistant line can be rendered even more resistant by EGFRvIII. Interestingly, expression of the mutant receptor results in hyperactivation of DNA-PK (assayed by the phosphorylation status of its catalytic subunit) upon irradiation. In support of this observation, we also show that specific inhibitors of DNA-PK negate the proficient repair seen in EGFRvIII-expressing astrocytes and renders them sensitive to radiation. Our results, thus far, indicate that expression of EGFRvIII results in hyperactivation of DNA-PK and proficient DSB repair providing us with a basis for radioresistance and a target for therapy of gliomas. The mechanistic aspects of the putative link between EGFRvIII and DNA repair in astrocytes are currently being investigated.

(PS4079) Modulation of the dna damage response to hze particles by shielding. Bipasha Mukherjee, Jack Miller, Sandeep Burma. UT Southwestern Medical Center, Dallas, TX, USA.

High atomic number and high energy (HZE) particles are the most ionizing component of galactic cosmic rays and pose a significant cancer risk to astronauts. The properties of these particles are altered during passage through spacecraft shielding and the human body, and this can either exacerbate or ameliorate their effects. It is therefore essential to accurately quantify the biological effects of HZE particles both in order to estimate risks to astronauts as well as to design better shielding materials. Moreover, these particles are also important from a biomedical standpoint as they are being pioneered for use in cancer therapy.The most deleterious effect of ionizing radiation is induction of DNA double-strand breaks (DSBs). We have used two DSB-responsive proteins - gammaH2AX and 53BP1 - to generate, for the first time, 3D reconstructions of DNA damage inflicted by HZE particles. Moreover, we show that the biological imaging we employ can be used to visualize the effects of shielding materials on these particles and to quantify if the net effect of shielding is beneficial or not. We find that human cells are unable to repair a significant portion of DSBs inflicted by iron (Fe) ions. Aluminum, representing the hull of the ISS, affords scant protection against these particles while lead, a poor shield, actually exacerbates the effects of these ions. In sharp contrast, polyethylene, a favored shield, results in DNA damage that is repaired more efficiently. This is presumably due to the fragmentation of Fe ions into smaller ions and protons that inflict DNA damage that is more amenable to repair. We show that human cells are indeed able to efficiently repair DSBs induced by chlorine ions (representing a smaller fragmentation product of Fe) and protons (representing the smallest fragmentation product) providing a basis for the efficacy of polyethylene shielding.

(PS4080) Does non-homologous end joining (NHEJ) prevent repair of clustered DNA damages from converting to double strand break (DSB)? Svitlana G. Malyarchuk, Lynn Harrison.

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Multiply damaged sites (MDSs) are introduced by ionizing radiation and certain chemotherapeutic drugs and can consist of base damage, abasic (AP) sites or single strand breaks (SSBs) closely situated on opposite DNA strands. *In vitro* studies indicate that attempts to repair MDSs by base excision repair enzymes can result in the formation of potentially lethal double strand breaks (DSBs). Previous studies in our laboratory showed that attempted repair of two closely opposed uracils in bacteria resulted in DSB formation whereas the same MDS did not form a DSB in Hela cells. Since *E. coli* cannot perform non-homologous end joining (NHEJ), one possible explanation is that NHEJ in mammalian cells prevent the formation of DSBs following incomplete repair of closely opposed base damages. We have established an assay to determine whether synthetic MDSs are converted to DSBs in mouse embryonic fibroblast (MEF) cell lines. Double-stranded oligonucleotides containing no damage, a single damage or the MDS were ligated into the Firefly luciferase coding region of a non-replicating mammalian vector (pCMV3'luc). pCMV3'luc constructs were introduced into MEFs by nucleofection with pRL-CMV that expresses R*enilla* luciferase and is used to normalize for transfection efficiency. Cell-free extracts were prepared after 6 hours and the ratio of Firefly:*Renilla* luciferase was calculated and compared to the undamaged sequence. A MDS containing two opposing uracils situated 5 bp apart did not significantly change luciferase activity compared to the undamaged sequence in MEF cell lines deficient in Ku70, Ku80 or DNA-PKcs. Addition of a PARP inhibitor to $Ku80^{-/-}$ cells also did not change the activity. This indicates that even in the absence of NHEJ, the uracils were not converted to a DSB. Interestingly, initial studies using two tetrahydrofurans situated 5 bp apart showed a significant reduction in luciferase activity in wild type and $Ku80^{-/-}$ cells (6.7% ± 1.9% and 3.8%±1%, respectively) which indicates DSB formation. Future studies using cells over-expressing uracil DNA glycosylase (UDG) will help to determine whether the UDG expression level is limiting the conversion of uracils to AP sites in mammalian cells and so preventing DSB formation. Other types of MDSs will be tested to determine whether they can be converted to DSBs in cells.

(PS4081) Molecular dynamics (MD) simulation of Ku heterodimer with double strand DNA molecule. Hirofumi Fujimoto[1], Miroslav Pinak[2], Juraj Kotulic Bunta[2], Toshiyuki Nemoto[3], Naoko Takada[1], Hideaki Maekawa[4], Kozo Tsuchida[1]. [1]National Institute of Infectious Diseases, Tokyo, Japan, [2]Japan Atomic Energy Agency, Ibaraki, Japan, [3]Research Organization for Information Science and Technology, Ibaraki, Japan, [4]University of the Ryukyus, Okinawa, Japan.

OBJECTIVES: Double strand breaks (DSBs) on DNA molecule arising by exposure to ionizing radiation or various chemical agents are considered one of the most serious damage to living organisms. The Ku, a heterodimer consisting of Ku70 and Ku80, is an abundant DNA-binding protein that initially recognizes and binds with high affinity to the end of double strand DNA and facilitate the onset of subsequent process of non-homologous end-joining (NHEJ) that is a principal pathway for the repair of DSBs.

X-ray crystallographic study reported that the Ku heterodimer has a ring structure that can encircle DNA. The next question is how the Ku heterodimer recognizes and pauses at the DNA end. In this study, approaches based on molecular dynamics (MD) simulations have been applied to examine conformational changes of the Ku heterodimer and DNA.

METHODS: In order to study the time-dependent structural evolution of the Ku complex, several models were designed, in which DNA molecule in the reported data of the crystallographic structure was replaced with 3 DNA molecules that had a same 14 mer sequence with the original one and extended 3 base pairs to either or both sides. Then, MD simulations were performed for these models during 2 nanoseconds (ns).

RESULTS: Ku heterodimer seemed not to move on DNA molecules in each model during 2 ns of simulated time. When a force enough to pull DNA from the complex was added using the targeted molecular dynamics technique, double-helix structures of DNA molecules were found to collapse completely. These results suggest that Ku heterodimer in this study has no ability to slide along DNA molecules. Structural analysis showed that one of 3 models was more stable than others, suggesting that Ku heterodimer binds to the DNA end in the proper direction.

CONCLUSIONS: It is concluded that Ku heterodimer comes from outside DNA, binds to the DNA end in the appropriate orientation, and then stays there. Our first goal is to construct *in silico* a proper model of the complex of Ku heterodimer and DNA molecules, which may provide a new solution for the experimental study.

(PS4082) Response to the challenging dose of X-rays in lymphocytes of prostate cancer patients and healthy donors. Antonina Cabulska-Wasilewska[1], Zygmunt Dobrowolski[2], Zofia Rudek[3], Mateusz Krzysiek[3], Nazym Balegenowa[4], Zbigniew Drag[5], Agnieszka Panek[3], Stanislaw Krasnowolski[3], Waclaw Lipczynski[2], Barbara Dobrowolska[2]. [1]Deaprtment of Radiation and Environmental Biology, Institute of Nuclear Physics PAN, Epidemiology and Preventive Medicine Collegium Medicum Jagiellonian University, Kraków, Poland, [2]Department and Clinic of Urology CM UJ, Kraków, Poland, [3]Deaprtment of Radiation and Environmental Biology, Institute of Nuclear Physics PAN, Kraków, Poland, [4]Institute of Nuclear Physics PAN (the MSC foundation' fellow from Kazakh National University, Almaty, Kazakhstan), Kraków, Poland, [5]Epidemiology and Preventive Medicine, Collegium Medicum, Jagiellonian University, Kraków, Poland.

An individual's genetic constitution and lifestyle, e.g., diet and levels of physical activity, can affect the body's response to various exogenous agents including therapeutic treatment. There is a strong need to combine studies on variability in a cellular response to the challenging dose with predisposition of the patient to diseases development and healing. In this study a variation in responses to challenging dose of X rays in lymphocytes from healthy donors and prostate cancer patients were compared on the molecular and mitotic levels. Blood was collected from healthy, prostate cancer and benign prostate stage (BPS) patients. Among cancer patients 33% never smoke 46.7% were former smokers. Immediately after collecting the blood a challenging irradiation was performed followed by culturing procedures for classic cytogenetics and FISH techniques with a probe for the whole chromosome 1. In DNA damage investigations, isolated and cryopreserved lymphocytes were thawed and their viability examined. To evaluate individual susceptibility, defrosted lymphocytes were exposed to 4 Gy dose of X-rays and the extent of DNA damage was studied right after irradiation with the alkaline version of the single cell gel electrophoresis (SCGE) assay. To asses variability in the DNA repair competency the residual (unrepaired) DNA damage was detected again after one hour of incubation, during which irradiated cells had the condition allowing to complete the DNA repair process. No significant difference between susceptibilities to the challenging dose between investigated groups was observed. However, repair efficiency of the DNA damage induced by the challenging treatment was significantly lower in lymphocytes of prostate cancer patients than that of benign prostate stage (BPS) and healthy donors ($p<.05$). Results show also a stronger variation in capacity to repair of the X-ray induced DNA damage between cancer patients then that observed between BPS donors.. That findings correlate with results from cytogenetic studies. Significantly higher amount of cytogenetic damage was detected in irradiated lymphocytes of prostate cancer than in BPS patients. Preliminary results from FISH techniques again confirmed those findings. Our results, clearly suggest a possible predictive value of the repair competence assays applied.

(PS4083) Deletion of histone modifying enzymes Bre1 and Dot1 causes sensitivity to ionizing radiation. Kelly E. McCann[1], Tatiana Spicakova[1], Marsha Williamson[2], John C. Game[2], J. Martin Brown[1]. [1]Stanford University, Stanford, CA, USA, [2]Lawrence Berkeley Laboratory, Berkeley, CA, USA.

Efficient DNA double-strand break repair is essential for maintaining the stability of the genome. Humans with defects in

DSB repair are sensitive to DNA damaging agents and are predisposed to developing cancers, as exemplified by such diseases as Nijmegan Breakage Syndrome, Ataxia Telangiectasia, and breast cancer susceptibility in women with mutations in the BRCA1 and BRCA2 genes.

Repair of DNA damage requires activation of cell cycle checkpoint controls, recruitment of repair proteins to DNA lesions, and transcriptional activation of relevant genes. As shown by our data, deletion of genes for proteins involved in histone modification produces sensitivity to ionizing radiation in *Saccharomyces cerevisiae*, suggesting a role for chromatin modification enzymes in the repair process as well. We are specifically interested in the roles of Bre1p, a ubiquitin ligase responsible for ubiquitination of H2B on lysine 123 (H2B-K123) and Dot1p, a methylase specific for lysine 79 on histone H3 (H3-K79).

We have confirmed that *bre1*Δ and *dot1*Δ are sensitive to IR and have placed *bre1*Δ and *dot1*Δ into the Rad6 post replication repair and Rad51 homologous recombination repair pathways via epistasis analysis. We have shown that *bre1*Δ and *dot1*Δ strains have a G1 checkpoint defect, but an intact G2 checkpoint. Because a G1 checkpoint defect alone is insufficient to cause the radiation sensitivity we observe, we have focused on determining other causes for IR sensitivity in *bre1*Δ and *dot1*Δ mutants. We hypothesize that Bre1p and Dot1p are necessary for repair of the DNA break itself rather than as accessory proteins in the cell cycle checkpoint response. By monitoring phosphorylation of H2A over time, we have determined that the repair of DSB after IR in *bre1*Δ and *dot1*Δ mutants is defective as compared to wild-type yeast.

Our current studies are focused on quantifying the frequency and repair of DSB after IR in bre1 and dot1 null mutants by analyzing the chromosomes by pulsed field gel electrophoresis (PFGE), analyzing the genome-wide expression patterns in WT, *bre1*, *dot1*, and *bre1 dot1* deletion mutants to determine if Bre1p and Dot1p have roles in upregulation of DNA repair genes, examining the relationship between Bre1p and Dot1p by exploring their potential interactions, and searching for protein candidates for binding to methylated H3 K79.

(PS4084) Ionizing radiation induces microhomology-mediated non-homologous end joining in yeast and mammalian cells. Zorica Scuric, Cecilia Y. Chan, Kurt Hafer, Robert H. Schiestl. Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

DNA double-strand breaks (DSB) can be repaired by non-homologous-end joining (NHEJ) without extended sequence homology. Some NHEJ events, called microhomology-mediated non-homologous-end joining (MMEJ), occur between several basepairs (bps) of homology; about 50% of these events result in large deletions causing human genetic diseases. Ionizing radiation (IR) induced genome rearrangements often show such microhomologies at their junctions, implying that a radiation-induced DNA DSB is preferentially repaired by MMEJ. We investigated the effect of IR on MMEJ using an end-joining plasmid assay in yeast and mammalian cells. The cells were exposed to IR before the transfection of linearized plasmid so radiation exposure is physically separated from plasmid introduction. This enables us to analyze the effect of radiation on rejoining of the un-irradiated plasmid substrate. In the yeast assay, we exposed RSY12 cells to 50 Gy, and after one hour of incubation transformed with linearized YEp*lac*195 plasmid. After 4 days, Ura^+ transformants were selected and rejoined plasmids were rescued in *E. coli* and junctions were sequenced. In un-irradiated cells, 34% of clones had 2–5 bps of microhomology while 68% of clones from irradiated cells displayed 2–6 bp of microhomology at ($p<0.0005$) at the junctions. In particular, 57% events from irradiated cells showed $\geq$4 bp of microhomology whereas only 14% of events from unirradiated cells utilized $\geq$4 bp ($p<0.00005$). The events from irradiated cells are also significantly different from random occurrence ($p<0.00001$). A similar assay was performed in the Chinese hamster ovary CHO-K1 cell line where cells were first exposed to 3 Gy of radiation and then transfected with linearized pCMS-end plasmids. Among recovered clones, 54% of them from irradiated cells utilized $\geq$4 bp of microhomology compared to 0% of clones from non-irradiated cells ($p<0.00001$). The frequency of microhomology usage from $\geq$4 bps in irradiated cells is significantly different from the random occurrence ($p<0.005$). Here we report that exposure of yeast and mammalian cells to IR before substrate transfection demonstrate enhanced MMEJ events. These results indicate that DNA DSBs caused by IR induce a MMEJ pathway that might be involved in the generation of large deletions and rearrangements if it happens genome-wide.

(PS4085) Ionizing radiation and restriction enzymes induce microhomology-mediated illegitimate recombination in trans in Saccharomyces cerevisiae. Cecilia Y. Chan, Markus Kiechle, Palaniyandi Manivasakam, Robert H. Schiestl. Univ of California, Los Angeles, Los Angeles, CA, USA.

Ionizing radiation causes DNA double-strand breaks and genome rearrangements that can lead to cancer. DNA double strand breaks can be repaired by illegitimate recombination without extended sequence homology. Some illegitimate recombination events, also called microhomology-mediated recombination, occur between several basepairs of homology in the genome; about 50% of these events result in large deletions causing human genetic diseases. Ionizing radiation-induced genome rearrangements often show such microhomologies at their junctions, implying that a portion of radiation-induced DNA double-strand breaks are preferentially repaired by such recombination events. However, events at undamaged sites have so far not been documented. Here we report that both ionizing radiation and restriction enzymes induce microhomology-mediated integration of the DNA substrate in trans at sites that are not damaged. Irradiated yeast cells displayed 82% microhomology-mediated nonhomologous integration, compared to 27% in unirradiated cells. Restriction enzymes enhanced integration events at random non-restriction sites via microhomology-mediated recombination. Generation of a single I-SceI-mediated double-strand break also led to microhomology-mediated integration randomly throughout the genome. These results indicate that DNA double-strand breaks caused by ionizing radiation or restriction enzymes induce a genome-wide microhomology-mediated illegitimate recombination pathway that facilitates integration in trans at non-targeted sites and might be involved in the generation of large deletions and rearrangements. We propose that this inducible recombination pathway could be a potential mechanism of evolution and carcinogenesis.

(PS4086) Heterogeneity in the response of the Fanconi Anemia pathway to genotoxic stress. Lisa A. Kachnic[1], Chen-Mei Luo[1], Li Li[1], Martin Purschke[1], Kerstin Borgmann[2], Kathryn D. Held[1], Simon N. Powell[3], Henning Willers[1]. [1]Massachusetts General Hospital, Boston, MA, USA, [2]University of Hamburg, Hamburg, Germany, [3]Washington University, St. Louis, MO, USA.

Fanconi Anemia (FA) is a rare heterogeneous chromosomal instability and cancer predisposition syndrome characterized by multiple abnormalities including a profound hypersensitivity to DNA crosslinking agents, such as mitomycin C (MMC). In response to replication fork-blocking interstand crosslinks (ICLs), a multiprotein nuclear core complex of eight FA proteins is required for mono-ubiquitination of the central downstream effector FANCD2, which then relocates into chromatin and co-localizes with other DNA damage proteins such as Rad51. Accumulating evidence suggests that the biochemical pathway formed by the FA proteins is not a linear pathway, but rather represents a complex network responding to diverse genotoxic stresses. To this end, we analyzed the functions of the central effector protein FANCD2 and the nuclear core complex protein FANCA in the response to MMC-induced ICLs and oxidative DNA damage caused by hydrogen peroxide (H2O2). We utilized well-characterized isogenic pairs of fibroblasts derived from patients with FA group D2 (PD20) or group A (PD220) and their retrovirally complemented counterparts expressing the respective wild-type protein. We found that FANCD2-deficient cells demonstrate decreased clonogenic survival and increased apoptosis in response to H2O2 compared to cells complemented with wild-type FANCD2. Interestingly, mono-ubiquitination of FANCD2 by the nuclear core complex and the formation of FANCD2 and Rad51 subnuclear foci are not required for cellular resistance to oxidative damage. Furthermore, FANCD2-

deficient PD20 cells treated with H2O2 do not demonstrate the hallmark chromosomal breakage phenotype seen with crosslinkers. Thus, FANCD2 may protect against oxidative damage through a mechanism that is distinct from the response to ICLs. In support of this hypothesis, we discovered that FANCA-deficient PD220 cells, which retain resistance to H2O2, demonstrate a profound hypersensitivity to MMC, i.e., 1,000- to 10,000-fold compared to wild-type cells, while FANCD2-deficient cells are only about 10-fold more sensitive. We conclude that FANCA is more important for crosslinker resistance than FANCD2, while the opposite is true for the cellular response to H2O2. In part supported by Susan G. Komen for the Cure (to LAK) and NIH P01 CA095227 (to KDH).

(PS4087) Vitamin D antagonizes radiation-induced expression of Rad51 in head and neck squamous cell carcinoma. Christopher A. Bradley, Shey-Jen Shih, Andrew T. Vaughan, Danny J. Enepekides, Gregory Farwell, Joanna S. Albala. UC Davis School of Medicine, Sacramento, CA, USA.

Squamous cell neoplasms of the head and neck occur as the result of an accumulation of adverse genetic events that ultimately lead to changes in the function of cellular proteins. These changes result in dedifferentiation of the epithelium, proliferation, invasion, and finally, tumor metastasis. Our objectives are to examine how the active form of vitamin D, 1-α, 25-dihydroxyvitamin D_3 (VD_3), modulates the radiation response in head and neck cancer. The molecular mechanisms through which VD_3 mediates these effects remain unknown. Rad51 is a key protein involved in the repair of DNA double-strand breaks induced by ionizing radiation and overexpression of Rad51 has been shown to increase cellular resistance to radiation and chemotherapy in several cancer types. High levels of endogenous Rad51 protein in head and neck squamous cell carcinoma have been correlated to poor prognosis and resistance to treatment. Preliminary work in our lab has shown that VD_3 down-regulates Rad51 protein expression in squamous cell carcinoma *in vitro*. Furthermore, VD_3 antagonized radiation-induced induction of Rad51. Pretreatment of SCC25 cells with VD_3 twenty-four hours prior to irradiation reduced the formation of H2AX foci and decreased sublethal DNA damage repair. Combined treatment of VD_3 and radiation showed an increase in caspase-3 cleavage, a measure of increased apoptosis. This suggests that application of VD_3 may decrease the ability of head and neck cancer cells to survive radiation by impairing the DNA damage response ultimately leading to apoptosis. This has obvious therapeutic implications as vitamin D-mediated inhibition of Rad51 expression could modulate the response of head and neck cancers to treatment.

(PS4088) Werner Syndrome Protein is Phosphorylated by DNA-PK and regulates DNA double-strand break repair. Asaithamby Aroumougame[1], Steven M. Yannone[2], David J. Chen[1]. [1]University of Texas Southwestern Medical Center, Dallas, TX, USA, [2]Lawerence Berkeley NAtional Laboratory, Berkeley, CA, USA.

Werner syndrome (WS) is a rare genetic disorder characterized by premature aging, genomic instability and an elevated risk of cancer. A number of studies indicate that Werner Syndrome protein (WRN), protein responsible for WS, is involved in DNA repair, replication and telomere maintenance. Earlier studies have shown that DNA-dependent protein kinase catalytic subunit (DNA-PKcs) phosphorylates WRN and also regulates its enzymatic activities. In this study, we show that WRN is phosphorylated on Serine 319 (S319) by DNA-PK in response to ionizing radiation (IR) in human cells. Similar to WS cells and as reported previously, WRN S319A mutant cells also showed a reduced ability of non-homologous end-joining (NHEJ) by an IsceI reporter assay system. Interestingly, live cell imaging analysis reveals that phosphorylation on S319 has no effect on WRN's recruitment but affects its kinetics of retention and fluorescent recovery after photobleaching (FRAP) on accumulated WRN shows that WRN phosphorylation alters its dynamics of exchange rate at the sites DNA double strand breaks (DSBs) suggesting that WRN phosphorylation may affect its stability at the sites of DSBs. While WS cells bears no significant sensitivity to low LET IR, interestingly, measuring cellular sensitivity by survival assay and γH2AX foci formation in response to high LET and high energy Fe particles reveals that WS and S319A cells show reduced cell survival and slower γH2AX repair kinetics as compared to low LET IR. Thus, our studies strongly suggest that WRN phosphorylation may be involved in the repair of IR-induced complex DNA DSBs via NHEJ pathway.

(PS4089) Mice lacking DNA polymerase POLQ have increased radiation-induced micronuclei in vivo and radiosensitization of marrow stromal cells in vitro. Julie P. Goff[1], Michael W. Epperly[1], Donna Shields[1], Tracy Smith[1], Mineaki Seki[2], John Wittschieben[2], Richard D. Wood[2], Stephen Dertinger[3], Dorothea Torous[3], Joel S. Greenberger[1]. [1]Department of Radiation Oncology, Pittsburgh, PA, USA, [2]Department of Pharmacology, Pittsburgh, PA, USA, [3]Litron Laboratories, Rochester, NY, USA.

The DNA polymerase POLQ (pol theta) can bypass AP sites and thymine glycol (Seki *et a.l*, EMBO J 2004, 23:4484), and may have a back-up role in base excision repair (Yoshimura 2006, 24:115). To explore the role of POLQ in hematopoiesis and in repair of DNA damage following irradiation, we established long-term bone marrow cultures and marrow stromal cell lines from the *PolQ*+/+ and *PolQ*-/- mice derived by Shima *et al*. (Genetics 2003, 163:1031). Cobblestone islands in cultures from *PolQ*-/- mice increased from weeks 10 through 23, but with no increase in number of nonadherent cells or colony forming potential compared to *PolQ*+/+ cultures. *PolQ*-/- bone marrow stromal cell lines had a saturation density about two-fold lower than *PolQ*+/+ lines. *PolQ*+/+ cells had a doubling time of 18 hr compared to 24–30 hr for *PolQ*-/- clones. Plating efficiencies of *PolQ*+/+ and *PolQ*-/- cells were similar. *PolQ*+/+ cells and three independent clones of *PolQ*-/- cells were exposed to 0 - 8 Gy of gamma radiation, and colony forming ability was measured. *PolQ*-/- cells were more radiosensitive with D_o values of 1.27 ± 0.16 Gy, 0.98 ± 0.10 Gy ($p = 0.032$), and 1.08 ± 0.01 Gy ($p = 0.01$) for three clones, compared to a D_o of 1.38 ± 0.06 Gy for *PolQ*+/+ cells. Mice exposed to 7 Gy gamma radiation were monitored for restoration of white blood cell, red blood cell, and platelet counts for 34 days. No differences in the responses of *PolQ*+/+, +/-, or -/- mice were apparent. Micronucleated reticulocytes were measured in peripheral blood of *PolQ*+/+, +/- or -/- mice after irradiation with 0.75 Gy. Before irradiation, *PolQ*-/- mice had a higher fraction of micronucleated reticulocytes (0.91 ± 0.05%) compared to 0.41 ± 0.02% and 0.32 ± 0.03% for *PolQ*+/+ and +/- mice respectively ($p < 0.0001$). Forty hr after irradiation, micronucleated reticulocytes in *PolQ*-/- mice increased to 8.53 ± 1.48%, compared to 2.35 ± 0.12% and 2.55 ± 0.17% in *PolQ*+/+ and *PolQ*+/- mice ($p = 0.0032$ and 0.0039, respectively), confirming the observations of Shima *et al*. The increased frequencies of spontaneous and radiation-induced micronuclei in peripheral blood red cells indicate that POLQ has a role in maintaining genomic integrity. We further find that bone marrow cell lines derived from these mice are more sensitive to ionizing irradiation, suggesting a role for POLQ in tolerance of ionizing radiation-induced DNA damage.

(PS4090) Differential expression of DNA repair genes following irradiation of human fibroblast and endothelial cells. Swati Girdhani[1], Amir Abdollahi[1], Philip Hahnfeldt[1], Sharon Kunder[1], Christian Schwager[2], Ute Wirkner[2], Peter Huber[2], Lynn Hlatky[1]. [1]Center of Cancer Systems Biology, Caritas St. Elizabeth's Medical Center, Tufts University School of Medicine, Boston, MA, USA, [2]Department of Radiation Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany.

Variation in the expression of DNA repair genes following gamma irradiation was examined using pan-genomic arrays. The differential expression of 14 categories of human DNA repair genes (e.g. base excision repair (BER), homologous recombination (HR), etc) was investigated as a function of cell type and of dose. Two representative non-transformed tissues, human lung microvascular endothelial cells and human dermal fibroblasts were studied. DNA repair represents a function required of all cells, irrespective of

tissue type. Yet, it was determined that, contrary to being consistently expressed across cell type in response to gamma-irradiation, the expression of important DNA repair genes varied as a function of cell type. This was the case for a significant number of genes involved in major repair processes, like HR, with genes being up-regulated in one tissue were down-regulated in another. For example NBS1 as found to be more expressed in the endothelial cells rather then the fibroblast. The Fanconi anemia family of genes, involved in repair of DNA cross-links was also found to be differentially regulated according to the cell type. Strikingly, the differential regulation of particular repair genes as a function of cell type was greater than their differential regulation as a function of dose (2 vs. 10 Gy). Repair gene regulation may be part of a larger regulatory phenomenon, as revealed by our hierarchical cluster analysis, that cell type is more important than dose with regard to overall radiation gene transcription. Further experiments are under progress to confirm the regulation of these selected genes by quantitative RT-PCR, Western blotting and immunofluorescence studies.

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(PS4091) Homologous recombination is the principal pathway for the repair of DNA damage induced by tirapazamine. Sophia B. Chernikova[1], James W. Evans[1], Lisa A. Kachnic[2,3], Judith P. Banath[4], Olivier Sordet[5], Yvette M. Delahoussaye[1], Alejandro Treszezamsky[2], Brian Chon[2], Zhihui Feng[2], Yves Pommier[5], Peggy L. Olive[4], Simon N. Powell[2], J. Martin Brown[1]. [1]Stanford University, Stanford, CA, USA, [2]Harvard University School of Medicine, Boston, MA, USA, [3]Boston University Medical Center, Boston, MA, USA, [4]British Columbia Cancer Research Centre, Vancouver, BC, Canada, [5]National Cancer Institute, Bethesda, MD, USA.

Tirapazamine (TPZ) is a promising hypoxia-selective cytotoxin that has demonstrated significant activity in advanced clinical trials in combination with radiotherapy and cisplatin. The current study aimed to advance our understanding of TPZ-induced lesions and the pathways involved in their repair. TPZ has been shown to induce base damage, single-strand breaks (SSBs), and as we have found earlier, double-strand breaks (DSBs) that at least partially result from poisoning topoisomerase II by TPZ under hypoxic conditions. This study demonstrates that homologous recombination (HR) plays a critical role in repair of TPZ-induced damage, as cells defective in XRCC2, XRCC3, BRCA1 and BRCA2, all defective in HR, are particularly sensitive to TPZ. On the other hand, we show that the non-homologous end-joining pathway, which in mammalian cells is the predominant pathway to deal with "frank" DSBs, is not involved in the repair of TPZ induced DSBs. Consistent with HR involvement in the repair of TPZ damage, we observed significantly elevated sister chromatid exchanges after treatment with TPZ. In addition, we found that cells deficient in the nucleotide excision repair (NER) factors XPF and ERCC1, but not other NER factors, XPD, XPG, and XPC, are hypersensitive to TPZ treatment. Repair of base damage and SSBs plays an important role in resistance to TPZ as cells deficient in base excision repair (BER) factors XRCC1 (EM9 cell line) and Polβ are hypersensitive to TPZ treatment. Furthermore, we show that after treatment with TPZ γ-H2AX is synergistically induced in EM9 cell line in S-phase, suggesting that unrepaired SSBs collapse replication forks, triggering recombinational repair. We have previously shown that TPZ induced stable DNA-topoisomerase II complexes, and in this study we found that TPZ induces stable DNA-protein crosslinks with topoisomerase I. These observations lead us to an overall model of TPZ damage in which DNA strand breaks, base damage and DNA-protein crosslinks (including the topoisomerase I and topoisomerase II cleavable complexes) produce stalling and collapse of replication forks, the resolution of which results in DSB intermediates, requiring HR and XPF/ERCC1 for their repair.

(PS4092) Processing of low dose γ-radiation induced DNA strand breaks in eukaryotic cell lines *in vitro*: Insight from *pGFP* transfected SCID and +/+ cells. Rajeshwar N. Sharan. North-Eastern Hill University, Shillong, India.

The net outcome of radiation induced changes in DNA and their processing by inherent repair systems decide the criticality of the irradiation on well-being and survival of an organism. This study was undertaken to understand the processing of these changes or damages at molecular level in an eukaryotic system. Earlier studies using a plasmid DNA construct, *pMTa4*, transformed into *E. coli* have revealed vulnerability of GC-rich nucleotide sequences to γ-irradiation generating pre-mutagenic lesions in a non-random way and critical roles of *RecF-RecA* proteins, especially *RecA* protein, in high fidelity rejoining of strand breaks. In this study, a reporter plasmid construct, *pGFP* (harboring green fluorescent protein gene), was transfected into repair proficient or deficient human cell lines (+/+ & SCID) before γ-irradiating them to a doses of 0.5, 1 and 2 Gy. The plasmid was recovered from the sham-irradiated and γ-irradiated cell lines under repair permissive (R^+) and non-permissive (R^-) conditions and analyzed for induction and repair of single strand breaks (SSB) and double strand breaks (DSB).

The efficiency of transfection was, in general, higher for radiohypersensitive SCID cells (clonogenic survival $\leq 1\%$ at 2 Gy) than its radioresistant +/+ (wild) counterpart (clonogenic survival $\cong$ 80% at 2 Gy). γ-irradiation up to a dose of 2 Gy did not affect the difference in transfection efficiency. While +/+ cells showed a near-dose dependent increase in induction of SSB and DSB and near-complete repair under R^+ condition, SCID cells failed to do so. The slope of curve for induction of strand breaks was biphasic; higher slope at lower dose. No cell cycle arrest was noticed up to 1 cell cycle after irradiation. Differences in damage fixation in SCID and +/+ cell lines seem critical to processing repair of the induced damage. The presentation shall focus on implication of these findings on genome instability.

(PS4093) Homing of transplanted stem cells in irradiated tissues. Mohi Rezvani[1], Marc Cranfield[1], Steve Ray[1], Uday Tirlapur[2]. [1]Systems Biology Laboratory, Abingdon, United Kingdom, [2]Department of Engineering Science, University of Oxford, Oxford, United Kingdom.

Radiation damage to the normal tissues is associated with depletion of clonogenic stem cells. Therefore, a stem cell-based therapy to regenerate the lost tissue by transplanted stem cells can be a beneficial modality for the treatment of radiation-induced lesions. Homing of transplanted cells in target organ is an important issue in stem cell transplantation. We have used Mesenchymal stem cells (MSCs), from bone marrow of rats expressing green fluorescent protein (GFP)were used to study the homing of transplanted cells in irradiated brain in rat.

Brains of 8 week old Sprague-Dawley rats were irradiated with 22 Gy single dose of X-ray. MSCs were transplanted *iv* 4 months after X-ray irradiation. Subsequently, at specific time points after transplantation the brain and other tissues were removed and frozen at −70 °C. Thick cryo-sections of unfixed tissue, counter-stained with propidium iodide (PI), were studied by a multiphoton laser scanning microscope.

It appeared that the transplanted green MSCs do not directly migrate into the irradiated brain but that they initially get incorporated into the bone marrow (as early as 4 hours after transplantation). Subsequently, at later times they migrate and reach the damaged brain; perhaps in response to long-distance molecular signals originating from radiation damaged brain tissue. In unirradiated control animals while only a few GFP-expressing cells were found to be incorporated into the bone marrow, none were observed in the brain tissue even after several weeks following transplantation.

This was followed by the study of the ability of stem cell therapy in modification of CNS damage in a rat myelopathy model and it was observed that the incidence of radiation myelopathy was significantly reduced (67% vs 100% in controls) by exogenous MSC cell transplantation.

It was concluded that exogenous MSC cells, transplanted systemically, home to radiation-damaged CNS tissue and significantly reduce the incidence of radiationmyelopathy. However, the number of exogenous cells observed in homing studies appeared to be too low to justify the functional changes observed. This might

suggest that the exogenous cells initiate a process of recovery/regrowth of the endogenous stem cells that lead to an improved functional response.

(PS4094) Bmi1 polycomb gene has a novel radioresistance function in nasopharyngeal carcinoma. Nehad M. Alajez, Wei Shi, Angela BY Hui, Fei-Fei Liu. Ontario Cancer Institute, Toronto, ON, Canada.

Introduction: There is compelling evidence in the literature demonstrating the existence of cancer stem cells (CSC) in different types of tumours. BMI1 PcG gene was shown to play a critical role in self-renewal of normal and malignant stem cells. Over-expression of BMI1 has been associated with poor prognosis in many cancer types; however, the exact mechanism by which BMI1 confers biological aggressiveness remains elusive. In this report we investigate the ramifications of BMI1 knockdown in C666–1 nasopharyngeal carcinoma (NPC) cell line, which expresses several stem-cell related genes (e.g. CD133, CD44 and BMI1).

Methods: C666–1 cells were transfected with BMI1 or control siRNAs and on day 3, cells were exposed to 6 Gy ionizing radiation (RT). qRT-PCR was used to asses mRNA level on day 3 post siRNA treatment. Cell viability was measured by MTS on Days 4 and 6. Apoptosis was measured on days 4 and 6.

Results: qRT-PCR showed overexpression (2–17 fold) of BMI1 in 64% (11/17) of primary NPC specimens. We observed lower viability in the BMI1 siRNA-treated C666–1 cells compared to control cells (82.4%, and 100.0% respectively). More interestingly, we observed significantly lower viability when exposing BMI1 siRNA-treated cells to RT (6 GY) compared to control cells (32.0%, and 64.9% respectively). The reduction in cell viability was accompanied with a marked increase in apoptosis (8-fold increase for BMI1), suggesting a possible anti-apoptotic function of BMI1 in NPC. Interestingly, this function appears to be independent of the inactivation of the CDKN2A locus, as we did not observe any changes in $p16^{INK4A}$ or $p14^{ARF}$ transcript levels in BMI1 siRNA-treated cells. We are currently using apoptosis PCR array to identify potential down-stream targets that have been dysregulated in BMI1 siRNA-treated cells. The therapeutic potential of targeting BMI1 in combination with radiotherapy is currently being evaluated *in vivo*.

Conclusion: Our data are the first report indicating that BMI1 is conferring radioresistance in NPC. Since BMI1 is also expressed in cancer stem cell (CSC), our data suggest the postulate that NP CSC could survive RT, which in turn contribute to the relapses observed in NPC patients. Hence, developing small molecule inhibitors targeting BMI1 could sensitize NPC to RT, thereby improving curability of this disease.

(PS4095) Prx1 interacts with androgen receptor and enhances its trans-activation by hypoxia/reoxygenation. Soo-Yeon Park[1], Xiaofei Yu[1], Clement Ip[2], James L. Mohler[3], Paul N. Bogner[4], and Young-Mee Park[1]. Department of [1]Cell Stress Biology, [2]Cancer Chemoprevention, [3]Urologic Oncology and [4]Pathology, Roswell Park Cancer Institute, Buffalo, NY 14263, U.S.A.

Abstract

Although hypoxia is accepted as an important microenvironmental factor influencing tumor progression and treatment response, it is regarded usually as a static global phenomenon. Consequently, less attention is given to the impact of dynamic changes in tumor oxygenation in regulating the behavior of cancer cells. The androgen receptor (AR) signaling plays a critical role in prostate cancer. We reported previously that hypoxia/reoxygenation, an *in vitro* condition used to mimic an unstable oxygenation climate in a tumor, stimulates AR activation. In the present study, we demonstrated that Prx1, a member of the peroxiredoxin protein family, acts as a key mediator in this process. We found that the aggressive LN3, C4–2, and C4-2B prostate cancer cell lines derived from LNCaP possess constitutively elevated Prx1 compared to parental cells, and display greater AR activation in response to hypoxia/reoxygenation. Although the cell survival enhancing property of Prx1 has traditionally been attributed to its antioxidant activity, the ROS-scavenging activity of Prx1 was not essential for AR stimulation, since Prx1 itself was oxidized and inactivated by hypoxia/reoxygenation. Increased AR trans-activation was observed when wild type Prx1 or mutant Prx1 (C52S) lacking antioxidant activity was introduced into LNCaP cells. Reciprocal immunoprecipitation, chromatin immunoprecipitation, and *in vitro* pull down assays corroborated that Prx1 interacts with AR and enhances its trans-activation. We also show that Prx1 is capable of sensitizing a ligand-stimulated AR. Based on the above information, we suggest that disrupting the interaction between Prx1 and AR may serve as a fruitful new target in the management of prostate cancer.

(PS4096) Human Prx1 and Prx2 are not duplicative proteins: The unique presence of Cys^{83} in Prx1 plays a critical role in providing structural and functional differences between Prx1 and Prx2. WeonSup Lee[1], Kyoung-Soo Choi[1], Jonah Riddell[1], Yun-Jeong Kim[1], Clement Ip[2], Debashis Ghosh[3,4], Jong-Hoon Park[5], and Young-Mee Park[1]. Department of [1]Cell Stress Biology, [2]Cancer Prevention, and [3]Pharmaology & Therapeutics, Roswell Park Cancer Institute, Buffalo, NY 14263; [4]Department of Structural Biology, Hauptman-Woodward Medical Research Institute, Buffalo, NY 14203; [5]Department of Biological Sciences, Sookmyung Women's University, Seoul, Korea

Abstract Human Prx1 and Prx2 are more than 90% homologous in their amino acid sequences. Prx1 and Prx2 are elevated in various cancers, and are shown to influence diverse cellular processes. Although their growth regulatory role has traditionally been attributed to the peroxidase activity, the physiological significance of this function is unclear since the proteins are highly susceptible to inactivation by H2O2. A chaperone activity appears to emerge when their peroxidase activity is lost. Structural studies suggest that they may form a homodimer or doughnut-shaped homodecamer. Little information is available, however, whether human Prx1 and Prx2 are duplicative in structure and function. We noted that Prx1 contains a cysteine (Cys83) at the putative dimer-dimer interface, which is absent in Prx2. We studied the role of Cys83 in regulating the peroxidase and chaperone activities of Prx1, because the redox status of Cys83 might influence the oligomeric structure, and consequently the functions of Prx1. We show that Prx1 is more efficient as a molecular chaperone, while Prx2 is better suited as a peroxidase enzyme. Substituting Cys83 with Ser83 (Prx1C83S) results in dramatic changes in the structural and functional characteristics of Prx1 in a direction similar to those of Prx2. Intact protein analysis by mass spectrometry revealed that while Prx1 is present predominantly as a decamer, both Prx2 and Prx1C83S are primarily in a dimeric form. These findings are consistent with the hypothesis that human Prx1 and Prx2 possess unique functions and regulatory mechanisms, and that Cys83 bestows a distinctive identity to Prx1.

(PS4097) Characterization of spatio-temporal fluctuations in vascular pO_2 in three rat tumor lines. Laura Isabel Cardenas-Navia[1], Daniel Mace[1], Rachel Ann Richardson[1], Siqing Shan[1], David F. Wilson[2], Mark W. Dewhirst[1]. [1]Duke University, Durham, NC, USA, [2]University of Pennsylvania, Philadelphia, PA, USA.

Many studies have shown the limitation of traditional therapies such as radio- and chemotherapy on hypoxic cells in solid tumors. More recently, many investigators have measured the presence of so-called "intermittent" or "acute" hypoxia in murine tumors, human xenografts, and spontaneous canine tumors. However, none of these studies have been able to directly assess the spatio-temporal prevalence of pO_2 fluctuations. This study contains the first data directly measuring fluctuations in tumor vascular pO_2 over time in three rat tumor lines, using Fourier analysis to characterize the spatial as well as temporal differences. In this study, phosphorescence lifetime imaging is used to measure fluctuations in vascular pO_2 in rat fibrosarcomas (FSA), 9L gliomas, and R3230 mammary adenocarcinomas grown in dorsal window chambers (n=6 for each tumor type). Tumors were imaged every 2.5–5 min over 60–90 min intervals. Fourier analysis performed on tumor vascular pO_2 maps showed that the dominant period of fluctuations was usually 40 min, although R3230 also showed up to

15% of the map with fluctuations between 16–20 min. Despite this common period of fluctuating pO_2, R3230 and FSA tumors showed distinctly different spatial patterns compared with 9L. Both R3230 and FSA showed clear coordination of pO_2 fluctuations while 9L fluctuations were spatially diffuse. The most significant result of this study is that pO_2 is constantly fluctuating at every point within a tumor. Previous characterization of hypoxia as perfusion-limited or diffusion-limited are simply the extreme cases of O_2 delivery dominant or O_2 metabolism dominant areas in tumors; most tumor tissue does not fall into either category and the local pO_2 is heavily influenced by both. Additionally, despite a common characteristic of low frequency tumor pO_2 fluctuations, tumor type has a clear impact on the spatial characteristics of the pO_2 fluctuations. This suggests that despite a common mechanism controlling the temporal pO_2 fluctuations, other factors clearly influence the spatial characteristics of pO_2.

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(PS4098) Inhibitory effects on tumor growth and suppressive effects on hypoxia of a ribonucleoside anticancer drug, tas106 in x-irradiated tumor. Hironobu Yasui[1], Osamu Inanami[1], Taketoshi Asanuma[1], Daisuke Iizuka[1], Akira Matsuda[2], Mikinori Kuwabara[1]. [1]Laboratory of Radiation Biology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan, [2]Laboratory of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan.

The purpose of this research was to examine the *in vivo* antitumor efficacy of X irradiation combined with administration of a ribonucleoside anticancer drug, 1-(3-C-ethynyl-β-D-*ribo*-pentofuranosyl)cytosine (TAS106, ECyd). To investigate the mechanism of antitumor efficacy of TAS106, we also examined the effects of TAS106 on irradiated-tumor cells in hypoxic condition both *in vitro* and *in vivo*.

Colon26 murine rectum adenocarcinoma cells and MKN45 human gastric adenocarcinoma cells were inoculated into the footpad in BALB/c mice and SCID mice, respectively. They were treated with low doses of X irradiation (2 Gy) and TAS106 (0.1 mg/kg and 0.5mg/kg). The tumor growth was monitored by measuring the tumor volume. Histological analyses for proliferative and apoptotic cells in the tumors were performed using Ki-67 immunohistochemical- and TUNEL-staining. The expression of survivin, a key molecule related to tumor survival, was assessed using quantitative PCR and immunohistochemical analysis. In the experiments of hypoxia, MKN45 cells were exposed to hypoxic condition (oxygen concentration = 3 mmHg) by passing 100% N_2 gas continuously in a gas-exchangeable chamber. Hypoxic regions in MKN45-transplanted tumors were stained using anti-pimonidazole immunohistochemistry.

When X irradiation and TAS106 treatment were combined, significant inhibition of tumor growth was observed in both types of tumors compared to mice treated with X irradiation or TAS106 alone. Parallel to this phenomenon, the suppression of survivin expression and appearance of Ki-67-negative and apoptotic cells were observed. Furthermore, 5 μM TAS106 inhibited the hypoxia-induced accumulation of HIF-1α to the level of normoxic condition *in vitro*. In MKN45 xenograft, a significant reduction in tumor hypoxia was observed in mice treated with a low dose of X-rays and TAS106.

In conclusion, X irradiation and TAS106 effectively suppress tumor growth in mice. The inhibition of survivin expression by TAS106 is thought to mainly contribute to the suppression of the tumor growth. Additionally, TAS106 enhances radiation-induced inhibition of tumor growth through the inhibition of hypoxia-induced expression of survival factors.

(PS4099) Tumor pO_2 of orthotopic gliomas and their response to irradiation and hyperoxygenation: how this information could be potentially used to individualize and optimize radiotherapy. Nadeem Khan[1], Hongbin Li[1], Huagang Hou[1], Jean P. Lariviere[1], Shi Y. Lu[1], Eugene Demidenko[1], David J. Gladstone[2], Julia A. O'Hara[1], Harold M. Swartz[1]. [1]Dartmouth Medical School, Hanover, NH, USA, [2]Dartmouth - Hitchcock Medical Center, Lebanon, NH, USA.

We have investigated tissue pO_2 of two orthotopic brain tumor models (9L and C6) and their response to irradiation and hyperoxygenation using the unique capabilities of *in vivo* EPR (Electron Paramagnetic Resonance) oximetry. Our aim is to use this information to enhance therapeutic efficacy by determining the effects of various fractionation schemes on tumor pO_2 and to facilitate tumor irradiation at the time of optimal tumor oxygenation.

High Spatial Resolution Multi-Site EPR oximetry was used to make simultaneous repeated non-invasive pO_2 measurements at two sites in the tumor and at one site in the contralateral brain. Briefly, the tumors were established in the left hemisphere of rats by injecting cells (10 μl of 40K cell suspension) through two burr holes, using a stereotaxic technique (AP-3.0, DV-3.2, L1.5 and L3.5). Oxygen sensitive lithium phathalocyanine (LiPc) crystals were implanted one week after cell injection into the same burr holes and in the contralateral brain (AP-3.0, DV-2.5, L1.5) as a control.

The pO_2 measurements were done for five consecutive days. The 9L tumors at its orthotopic position were not as hypoxic as when they were grown subcutaneously. Orthotopic C6 tumors were hypoxic. Both tumors responded to carbogen breathing. However, the increase in tumor pO_2 was different on different days and a decreased effect of hyperoxygenation was observed with an increase in the tumor size. This was likely due to increased intracranial pressure and compromised tumor vasculature with increase in tumor volume. A single dose of 9.3 Gy irradiation resulted in a modest increase in intracranial tumor pO_2 on day 2, but not in the non-irradiated tumor nor in the contralateral brain. The response to irradiation by each tumor was different, with a maximum increase in tumor pO_2 that varied from day 1 to day 3. These data strongly suggest the need to individualize oxygen dependent therapy based on the response of each tumor.

The ability of EPR oximetry to make repeated tumor pO_2 measurements could be used to individualize therapy based on the response of individual tumors, and this could greatly enhance the therapeutic efficacy. Recently, we have initiated a clinical study to measure and follow pO_2 of peripheral tumors in humans.

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(PS4100) Modulation of peripheral tumor hypoxia by topical vasodilator (benzyl nicotinate): an EPR oximetry study. Huagang Hou[1], Zrinka Abramovic[2], Marjeta Šentjurc[2], Jean P. Lariviere[1], Hongbin Li[1], Shiyi Lu[1], Eugene Demidenko[1], David J. Gladstone[3], Harold M. Swartz[1], Nadeem Khan[1]. [1]Dartmouth Medical School, Hanover, NH, USA, [2]Jožef Stefan Institute, Ljubljana, Slovenia, [3]Dartmouth - Hitchcock Medical Center, Lebanon, NH, USA.

Tumor hypoxia is a known factor in radioresistance. One important limiting step in the optimization of strategies which have been developed to modulate tumor hypoxia and/or to alter the type of treatment to enhance the efficacy of radiotherapy is the lack of non-invasive techniques that can measure and follow tumor pO_2 as desired. We have used EPR oximetry to non-invasively and repeatedly measure tissue pO_2 of peripheral tumors and sought to reduce tumor hypoxia by topical application of vasodilator benzyl nicotinate (BN), and investigated the effect of BN formulation on tumor pO_2 and tumor growth inhibition when tumors were irradiated at times of increased tumor oxygenation.

Oxygen sensitive lithium phthalocyanine (LiPc) crystals were implanted in the subcutaneous RIF-1 tumors at a distance of 4 mm and a depth of 2 mm from the tumor surface. After 30 minutes of baseline pO_2 measurements, BN formulations (2.5% or 8% in microemulsion) were topically applied on the tumor surface and the pO_2 measurement was continued for another 60 minutes. The tumors were irradiated (4Gy) at the time of maximum increase in tumor pO_2 and the experiment was repeated for five consecutive days.

The magnitude of the tumor pO_2 increase from baseline and the time to reach the maximum pO_2 after BN application varied during 5 days of the experiment. A maximal increase in tumor pO_2

was observed at 20 - 30 minutes after application of 2.5% BN and the maximum tumor pO_2 varied from 16 - 24 mm Hg on day 1 to day 4. Topical application of 8% BN also resulted in a significant increase in tumor pO_2. Tumors irradiated at the time of maximum increase in tumor pO_2 after BN application resulted in a significant growth inhibition from day 3 to day 5 of the experiment as compared to control groups.

This approach provides dynamic information on the changes in tumor pO_2 and the effectiveness of BN in enhancing tumor oxygenation over 5 consecutive days. Such information could be exploited to enhance the efficacy of radiotherapy of hypoxic peripheral tumors by using appropriate doses of BN. EPR oximetry, which now is being used clinically, could be used to repeatedly monitor these pO_2 changes and irradiate tumors at times of optimal tumor oxygenation to increase radiotherapeutic efficacy.

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(PS4101) Hypoxic and acidic tumor environment markedly alters the radiation-induced gene expression and radiosensitivity of tumor cells. Yeon Hee Kook[1], Hyewon Youn[1], Eun Taex Oh[1], Kyung Hee Park[1], Chang Won Song[2], Eun Kyung Choi[3], Byung Uk Lim[1], Heon Joo Park[1]. [1]Inha University, Incheon, Republic of Korea, [2]University of Minnesota Medical School, Minneapolis, MN, USA, [3]University of Ulsan, Seoul, Republic of Korea.

Introduction: While the effect of hypoxia on the cellular radiosensitivity has been well documented, relatively little known about the effect of acidosis on cellular radiosensitivity. Our goal is to shed light on the effect of combination of hypoxic and acidic environments on radiosensitivity of tumor cells.

Material and Methods: The gene activation in tumor cells exposed to hypoxia and acidosis (pH 6.6) alone or combined was determined with western blot. The cell proliferation rate was assessed based on the increase in cell numbers and flow cytometry method. The radiosensitivity of tumor cells in different environment was determined with clonogenic assay and induction of apoptosis.

Results: The radiation-induced apoptosis and clonogenic cell death decreased in hypoxic and acidic condition. The G2 arrest caused by a 4 Gy irradiation lasted longer in hypoxic and acidic environment than that in normal. We also have investigated radiation-induced changes in various molecular signals elicited in hypoxic and acidic environment. The acidic environment, particularly in combination with hypoxia, significant enhanced the radiation-induced p53 expression. In the cells irradiated with 4 Gy, the phosphorylation of p53 and chk2 markedly increased, particularly 48–72 h after the irradiation, in hypoxic environment or acidic environment. The radiation-induced phosphorylation of p53 and chk2 was more pronounced when the environment was both hypoxic and acidic. The elevation of ATM, which is intimately related to DNA repair, in hypoxic and acidic environment was far greater and longer than that in hypoxic and acidic environment alone. Importantly, we found that acidic environment also activates hypoxia-inducible transcriptional factor-1 (HIF-1). The cell proliferation was almost completely ceased when cells were remained in hypoxic and acidic environment.

Conclusion: The delay in cell cycle progression in acidic and hypoxic environment appeared, at least in part, account for the increase in radioresistance in hypoxia and acidic environment. The expression and phosphorylation of various genes which affect proliferation and radiosensitivity of tumor cells are markedly altered by environmental acidity and oxygenation status.

(PS4102) Hypoxic induction of neurotensin in lung carcinoma cells: its involvement in a resistance to γ-radiation and anticancer drug. Tae Lim Kim[1], Jee Sun Oh[1], Kug Chan Kim[1], Il Lae Jung[1], Eun Wie Cho[2], Sang Ki Paik[3], In Gyu Kim[1]. [1]Korea Atomic Energy Research Institute, Daejon, Republic of Korea, [2]Korea Research Institute Biotechnology and Bioscience, Daejon, Republic of Korea, [3]Chungnam National University, Daejon, Republic of Korea.

Purpose: The hypoxic status of various solid tumors has been related to an increased resistance to radiotherapy and chemotherapy treatments in a variety of tumor types with a more malignant phenotype. The aim of this study is to investigate a new important hypoxia inducible factor that makes cells resistant to a γ-radiation, anticancer drugs and toxic chemicals.

Experimental procedure: A549 cells were subjected to hypoxia (0% and 1%) by placing them in a humidified airtight chamber and neurotensin expression was analyzed by real time PCR and western blotting. Neurotensin-overexpressed A549 cells were constructed and these cells were plated in a T25 flask ($1X10^6$ cells/flask). 24–48h after irradiation with a single exposure to a dose of 20 Gy (^{60}Co γ-ray source; dose rate, 1Gy/min), cell viability was analyzed by flow cytometry.

Summary: Little is known regarding a hypoxic regulation of the autocrine peptide growth factor, neurotensin, which is widely distributed through neural and endocrine tissues, and its direct involvement to a resistance or tolerance in cancer cells. Here we showed that neurotensin was significantly induced by hypoxia in a lung carcinoma A549 cell. Cells with a neurotensin overexpression became resistant or tolerant to cell death caused by a variety of DNA damaging agents such as a γ-radiation, anti-cancer drug and toxic chemical. Neurotensin was not involved in the HIF-1α induction or stabilization. We confirmed that the application of siRNA for neurotensin sensitized cancer cells to these environmental stresses. Radio- and chemo-protective effects by a neurotensin induction in the hypoxic A549 cells may be achieved by a cellular cAMP upregulation, showing that addition of dibutyryl cyclic AMP, cAMP analogue, had a significant protective effect on stresses-induced cell death.

Conclusion: This study not only provides the first demonstration of a severe hypoxic regulation of neurotensin but also suggests the participation of novel mechanisms regarding resistance to a γ-radiation and anticancer drugs caused by hypoxia.

(PS4103) Radiosensitization by the combination of SR-2508 and paclitaxel in hypoxic human tumor cells in vitro. Cheng Jin[1], Ling Bai[2], Guozhen Guo[1]. [1]Department of Radiation Medicine, Fourth Military Medical University, Xi'an, China, [2]Department of Clinical Laboratories, Xi'an Gaoxin Hospital, Xi'an, China.

The two radiosensitizers SR-2508 (etanidazole) and paclitaxel (taxol) have different dose-limiting toxicities in humans. Combination of the two radiosensitizers may increase radiosensitization without increasing toxicity. This study was carried out to determine the synergistic radiosensitizing effect of the combination of SR-2508 and paclitaxel in two hypoxic human tumor cell lines: a breast carcinoma (MCF-7) and a carcinoma cervicis (HeLa). The 3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay was used to determine the number of surviving cells. Cell cycle was evaluated by flow cytometry. Cell viability was measured by the ability of single cells to form colonies in vitro. Our data demonstrated that the radiosensitization produced by the two radiosensitizers was additive in hypoxic HeLa cells while held in the G_1 phase of the cell cycle. On the other hand, there was no synergistic radiosensitizing effect in hypoxic MCF-7 cells by combination of the two drugs. Our results suggested that the synergistic radiosensitizing effect of SR-2508 and paclitaxel may be tumor-dependent and that breast cancer may not be a good candidate. This study may provide a new combination of radiosensitizers in radiotherapy for cervical carcinoma.

(PS4104) Antizyme suppression leads to induction of HIF-1α protein and increment of cellular redox potential in lung carcinoma cells: its involvement in resistant to γ-radiation. Tae Lim Kim[1], Jin Sik Kim[1], Sang Gi Paik[2], Hai Won Chung[3], In Gyu Kim[1]. [1]Department of Radiation Biology, Environment Radiation Research Group, Korea Atomic Energy Research, Daejeon, Republic of Korea, [2]Department of Biology, College of Natural

Sciences, Chungnam National University, Yusong, Daejeon 305–764, Korea, Daejeon, Republic of Korea, [3]School of Public Health and Institute of Health and Environmental Sciences, Seoul National University, Seoul 110–460, Korea, Seoul, Republic of Korea.

Purpose: Intracellular polyamine content has been related to an increased resistance to radiotherapy and chemotherapy treatments in a variety of tumor types. The aim of this study is to investigate the role of antizyme (AZ) in a γ-radiation-induced cell death.

Experimental procedure: Human lung carcinoma A549 cells were cultured in RPMI-1640 media supplemented with 10% FBS and penicillin/streptomycin in a humidified 5% CO_2 atmosphere at 37°C. AZ-suppressed cell lines were constructed with siRNA and AZ expression was evaluated by western blotting. The cultured cells were irradiated with a single exposure to a dose of 20 Gy (^{60}Co γ-ray source; dose rate, 1Gy/ min).

Summary: Mammalian AZ has been found to promote an ubiqutin-independent degradation of ornithine decarboxylase. In these studies, we showed that AZ suppression affected the cell death in human A549 cells, but made cells resistant to DNA damaging agents including γ-radiation. RNA interference for AZ caused A549 cells to increase hypoxia inducible factor-1α (HIF-1α) and cellular redox potential (GSH/GSSG ratio) and thus to diminish the intracellular ROS important for signaling cellular physiology. Cell death and cell proliferation inhibition caused by AZ suppression resulted from highly decreased cellular ROS, showing that exogenous H_2O_2 supplement partly rescued cell death. Moreover, the resistance to γ-radiation may result from HIF-1α induction and cellular redox potential. Increment of redox potential or scavenging intracellular ROS, by AZ suppression was caused by overexpression of cytoplasmic $NADP^+$-dependent isocitrate dehydrogenase (ICDH). We also showed that ICDH overexpression is involved in HIF-1α induction. Therefore cotransfection of ICDH-siRNA with AZ-siRNA partially increased intracellular ROS and decreased HIF-1α induction. Correspondingly, inhibitor blocking reduction pathway of oxidized GSH also increased intracellular ROS and thus recovered cell death and HIF-1α accumulation.

Conclusion: These results suggest that AZ suppression induced partial cancer cell death due to excessive ROS scavenging caused by a high increment of redox potential, but at the same time, a increment of redox potential and HIF-1α induction provides cells with radio- and chemo-resistance and allows tumor cells to adapt to environmental stresses.

(PS4105) Lifespan of Rat-1 fibroblasts overexpressing dominant negative Ku70 under hypoxic conditions. Muneyasu Urano, Yun-Fong Huang, Fuqiu He, Clifton Ling, Gloria Li. Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

Ku70, a component of a protein complex, is known to bind to DNA double-strand breaks, leading to DNA damage repair. Ku70-deficient cells are unable to repair the DNA double-strand breaks and show increased radiation sensitivity. Based on studies of Ku70 and Ku70/Ku80 heterodimers, dominant negative fragment involving a N-terminal deletion (DNKu70) was generated and plasmids containing this fragment were stably trasfected into Rat-1 fibroblasts. Our previous study on the cell survival following fractionated radiation doses showed that Rat-1 cells proliferated under 30 hours-hypoxia, while DNKu70 cells showed negligible proliferation and cell death. This suggested a potential role of Ku70 in the cell proliferation and raised a question whether hypoxia could lead to more substantial death of cells overexpressing DNKu70 than parental Rat-1 cells; thus we studied the lifespan of Rat-1 cells overexpressing DNKu70 kept under hypoxic conditions.

Rat-1 cells and DNKu70 cells were cultured in DMEM medium supplemented with 10% fetal calf serum and antibiotics. Cell survivals were obtained by colony-formation assay. To begin with, 20,000–30,000 cells were plated in each glass flask. Three days later, medium were replaced with HEPES-containing medium, nitrogen gas containing 0 or 0.1% oxygen was flashed into these flasks for 15 min and cells were incubated in Hypoxic WorkStation for 0–96 hours. Every 24 hours, cells were trypsinized, counted, plated in 60 mm dishes and incubated for ~2 weeks in a 5%CO2 incubator for colony formation.

Surviving fractions of Rat-1 and DNKu70 cells kept in 0% oxygen for 24 hours were ~0.85 and ~0.65%, respectively, and those of Rat-1 and DNKu70 cells kept for 96 hours were ~0.4 and ~0.1%, respectively. Rat-1 cells kept in 0.1% oxygen proliferated by a factor of ~3.5 during the first 72 hours and 100% cells survived. DNKu70 cells kept in 0.1% oxygen showed slight proliferation during the first 48 hours but surviving fractions were similar to those kept in 0% oxygen.

These results show that overexpression of DNKu70 lead to a quick loss of reproductive capability, suggesting a potential involvement of Ku70 in cell survival under low oxygen conditions. Further studies are needed to explore the cause of this quick cell death.

(PS4106) Using hypoxic hypoglycemia to selectively starve hypoxic cancer cells: influence of hypoxia and glucose availability on glucose consumption and cell survival. Thies Schroeder, John P. Kirkpatrick, Mark W. Dewhirst. Duke University Medical Center, Durham, NC, USA.

Hypoxia and high glucose turnover are hallmarks of solid tumors. We previously showed that the tissue gradient of glucose in experimental solid tumors follows hypoxia, leading to hypoglycemic conditions in perinecrotic, hypoxic regions (Schroeder, 2005). Studies of human head-and-neck cancers indicate that such gradients exist in these tumors and that severe hypoglycemia is common in hypoxic regions. This may render hypoxic tumor cells particularly vulnerable to further glucose deprivation.To investigate how systemic glucose deprivation can selectively starve hypoxic cancer cells we first quantified oxygen-dependent glucose consumption in several human tumor cell lines in vitro, using a controlled-atmosphere incubator. In FaDu (head and neck cancer), MCF-7 and MDAMB-231 (metastatic breast ca), PC-3 (prostate ca), SiHa (uterine cervix ca) and WiDr (colorectal ca), glucose consumption rose significantly when pO2 fell below 10 mmHg. In the same cells, glucose deprivation for 12 h led to far greater cell kill under hypoxia (2 mmHg pO2, reduction of viable cells by 18 to 91%) than at ambient pO2 (159 mmHg, −6 to +37%). We then determined glucose kinetics under normoxia and anoxia for PC-3, SiHa and WiDr cells, at varying glucose concentrations. For modeling, we assumed first-order kinetics with $d[Glc]/dt = -k_1[Glc]$. Under normoxia/anoxia, k_1 ($hr^{-1}10^6cells^{-1}$) were 0.0068/0.024 for PC-3, 0.0061/0.0074 for SiHa, and 0.011/0.014 for WiDr. Interestingly, glucose consumption in all cell lines fell with reduced glucose availability and increased pO2. In turn, more complex mass transport models are constructed using oxygen/glucose-dependent kinetics (Kirkpatrick, 2003) to quantitatively model [Glc]. Our data suggest that hypoxic tumor cells have the highest glucose demands yet have the lowest available glucose concentrations. This provides strong rationale for systemic reduction of blood glucose to selectively starve and kill cancer cells, improving the efficacy of radiotherapy and most chemotherapies. Moderate glucose reduction is well tolerated in humans, and clinical tools are available to reduce blood glucose, including drugs, diet and exercise. We are investigating the efficacy of systemic blood glucose reduction in tumor-bearing mice to selectively reduce the hypoxic tumor cell fraction.

(PS4107) Short-term effects of a 15Gy - 79keV synchrotron tomographic irradiation on healthy mice brain microvasculature. Clement Ricard[1], Jean-Claude Vial[2], Sonia Teypaz[1], Jerome Gastaldo[1], Manuel Fernandez[1], Francois Esteve[1], Christoph Segebarth[1], Boudewijn van der Sanden[1]. [1]Joseph Fourier Univ, Grenoble Institute of Neuroscience, Grenoble, France, [2]CNRS, Grenoble, France.

A new kind of chemo-radiotherapy has been developed at the European Synchrotron Radiation Facility (ESRF) in Grenoble. This method called PhotoActivation Therapy (PAT) consists in a tomographic irradiation of the tumor volume one day after the

intratumoral injection of high Z-compounds, such as cis-platinum (PAT-plat). In preclinical studies, the irradiation energy (79keV) has been tuned just above the Pt K-edge of the platinum with a 15Gy dose for a maximal cure of rats bearing F98 gliomas.

For future clinical trials, it is important to assure that tomographic irradiations of healthy brain adjacent to the tumor do not induce any vascular damage. Therefore, the aim of the present study was to measure changes in the blood brain barrier (BBB) permeability and vessel density after a 15Gy - 79keV synchrotron tomographic irradiation using intravital multiphoton microscopy and quantitative immunohistochemistry.

Healthy nude mice were irradiated in a 1,75mm^3 volume in the left parietal cortex using the ESRF light source. The permeability of the blood brain barrier (BBB) was assessed at different time delays after the irradiation using multiphoton microscopy. This imaging method allows the observation of the brain vasculature *in vivo* up to 600µm below the dura with a micrometric spatial resolution. BBB permeability was observed after the intravenous injection of two fluorescent vital dyes with different molecular weights: FITC-dextran (4 kDa) and RhodamineB-dextran (70 kDa). Their distribution in the vascular compartment and in the surrounding tissue was imaged in the irradiated area and in control mice. After the intravital studies, brains were removed and vascular parameters were assessed with an immunostaining of collagen IV and analyzed with home made software.

Here, it is reported that a 15Gy - 79keV synchrotron irradiation induces no BBB leakage for the 4kDa and 70kDa fluorescent molecules from day 1 to day 30 after the irradiation. Moreover, vascular parameters, such as: the density and vessel diameters were not modified in the irradiated area vs motor cortex in control animals.The clinical transfer of the PAT seems feasible, since the structure and properties of the vasculature in healthy brain were not damaged or modified by the irradiation. We are currently studying the effects of the PAT-Plat on tumor vasculature.

(PS4108) Combining antiangiogenic therapy with radiotherapy enhances tumor response without functionally normalizing the tumor vasculature. Bruce M. Fenton, Scott F. Paoni. University of Rochester Medical Center, Rochester, NY, USA.

Two hypotheses have become almost dogma regarding the effects of antiangiogenic strategies on vessel morphology and function. The first is that antiangiogenics selectively prune inefficient vasculature, resulting in a transiently normalized vascular configuration. The second is that pericyte-coated vessels are more resistant to therapy, thus producing a net increase in vessel maturity in the surviving vessels. While these results may be true in response to specific VEGF inhibiting strategies, less is known regarding the pathophysiological effects of combining antiangiogenics with conventional therapy. Utilizing DU145 human prostate tumor xenografts, a receptor tyrosine kinase inhibitor of VEGFRs (AG-013736, kindly provided by Drs. Dana Hu-Lowe and David Shalinsky of Pfizer Global Research, La Jolla, CA) was administered in combination with fractionated radiotherapy (RT). Automated image processing of immunohistochemically stained images was used to quantify total/perfused blood vessel spacing, overall tumor hypoxia, and pericyte/collagen coverage. Although tumor volumes rapidly increased by >50% over two weeks of fractionated RT, tumors grew only 12% over three weeks of combination therapy. At the same time, vascular density declined proportionately for both total and perfused vessels, and overall tumor hypoxia increased. Coverage of alpha-sma+ pericytes increased, but was overshadowed by a more striking increase in both PDGFR-beta+ and alpha-sma+ pericyte-vessel dissociation, coupled with the appearance of empty type IV collagen-coated basement membrane sleeves. These findings argue against a treatment-induced functional normalization of the tumor vasculature. Instead results suggest that the combination produces a loosening of pericyte coverage, an ablation of the vasculature, and ultimately a reduction in functionality. Despite the decrease in tumor oxygenation, combination therapy with AG-013736 plus radiation remained effective and tumor progression was minimal over the entire three weeks of therapy.

(PS4109) Importance of scheduling of anti-VEGFR2 antibody DC101 combined with fractionated irradiation (FXRT) in the treatment of human head and neck carcinoma xenografts. Oliver Riesterer[1], David Valdecanas[1], Kathy Mason[1], Walter Hittelman[2], Daniel Hicklin[3], Luka Milas[1], Kian Ang[1]. [1]Department of Experimental Radiation Oncology, The University of Texas M D Anderson Cancer Center, Houston, TX, USA, [2]Department of Experimental Therapeutics, The University of Texas M D Anderson Cancer Center, Houston, TX, USA, [3]ImClone Systems, Inc., New York, NY, USA.

Introduction: There exists considerable evidence showing that targeting the vascular endothelial growth factor receptor (VEGFR) enhances the antitumor effect of ionizing radiation. However, the question of how to optimally combine both treatment modalities is far from being resolved.

Materials and Methods: In vivo experiments were performed using xenografts derived from human FaDu head and neck squamous cell carcinoma cells injected into the hind leg of nude mice. Tumors were exposed to FXRT, 2.5 Gy given daily for 6 consecutive days, starting when tumors were approximately 7.8 mm (SD ± 0.38 mm). The anti-VEGFR2 antibody DC101, at a dose of 1mg per mouse, was administered i.p. two times with a 3-day interval between two injections before (neoadjuvant), during (concurrent) or after (adjuvant) FXRT. Neoadjuvant administration started 4 days before FXRT, concomitant administration started 6 h before the first radiation dose, and adjuvant administration started 1 day after FXRT was completed. Tumor growth delay [(days from treatment initiation (6mm) to 12 mm] was the treatment endpoint.

Results: Both DC101 and FXRT delayed tumor growth when given as single treatments, but in combination they exerted a supra-additive effect in all treatment schedules tested. This DC101-induced enhancement of tumor radioresponse depended on the sequencing of DC101 and FXRT. The enhancement factors (EF) were 0.2 for neoadjuvant, 1.8 for concurrent and 2.3 for adjuvant application of DC101. An additional group was included where DC101 was administered before, during and after completion of FXRT, and this treatment schedule resulted in EF of 2.2.

Conclusion: These results show that DC101 increased the antitumor efficacy of FXRT the degree of which depended on scheduling of the two agents. The strong efficacy observed after adjuvant application of DC101 suggests that the so called tumor bed effect was substantially affected, and mechanistic experiments testing this hypothesis are in progress.

(PS4110) Systemic overexpression of angiopoietin-2 promotes tumor microvessel regression, inhibits angiogenesis and tumor growth. Yiting Cao[1], Pierre Sonveaux[2], Shanling Liu[3], Yulin Zhao[1], Jing Mi[1], Bryan M. Clary[1], Chuan-Yuan Li[4], Christopher D. Kontos[1], Mark W. Dewhirst[1]. [1]Duke University Medical Center, Durham, NC, USA, [2]Unit of Pharmacology & Therapeutics, Université Catholique de Louvain (UCL, Brussels, Belgium, [3]West China Second University Hospital, Sichuan University, Chengdu, China, [4]University of Colorado Health Science Center, Aurora, CO, USA.

Local angiopoietin-2 (Ang-2) upregulation shows either proangiogenic or antiangiogenic activities depending on the level of vascular endothelial growth factor (VEGF). However, the effects of systemic overexpression of Ang-2 on tumor vasculature and growth are unclear. Because new blood vessels are more sensitive to Ang-2 for regression and neovasculature comprises a significant fraction of tumor vasculature, we hypothesize that systemic overexpression of Ang-2 may cause tumor vessel regression and inhibit tumor growth. We use adenoviral vectors encoding mouse Ang-2 or soluble VEGF receptor-1 (sVEGFR-1) to systemically overexpress Ang-2 or neutralize VEGF, respectively. Mouse dorsal skin-fold window chamber model and flank xenografts of HCT116 human colon cancer show that systemic Ang-2 overexpression leads to tumor vessel regression. Ang-2, sVEGFR-1 and Ang-2+sVEGFR-1 treatments significantly decrease tumor vascular density while improve vascular perfusion in survived tumor vessels. However, the treatments do not change tumor APT levels. Single-dose Ang-2 treatment transiently exacerbates tumor hypoxia. Confocal microscopy reveals that the mechanism of Ang-2-induced tumor vessel regression is the impaired association between tumor

pericytes and endothelial cells rather than the loss of tumor pericyte. In addition, systemic Ang-2 treatment significantly inhibits tumor angiogenesis and growth even without concomitant inhibition of VEGF. Multiple-dose adenoviral treatments of Ang-2, sVEGFR-1, or the combined Ang-2+sVEGFR-1 show similar antiangiogenic and pro-apoptotic effects. In contrast, none of the sustained treatments affect tumor cell proliferation or hypoxia. These findings, for the first time, demonstrate that systemic overexpression of Ang-2 has pleiotropic effects on tumor vessel stability, angiogenesis, hypoxia, tumor vessel perfusion, apoptosis, proliferation and bioenergetics. Therefore, this study lays a firm foundation for the development of a novel therapeutic strategy based on Ang-2-induced destabilization of tumor vasculature, which may have a significant impact on current antitumor antiangiogenic therapies.

(PS4111) Inhibition of cytosolic phospholipase A2 ($cPLA_2$) leads to decreased function in irradiated vascular endothelium. Amanda G. Linkous, Kyle C. Cuneo, Andrej Lyshchik, Dennis E. Hallahan, Eugenia M. Yazlovitskaya. Vanderbilt University, Nashville, TN, USA.

Clinically relevant low doses of ionizing radiation (2–3 Gy) stimulate pro-survival signaling in tumor vasculature resulting in enhanced endothelial viability and an overall decrease in therapeutic index. Recently, we have demonstrated that the viability of irradiated endothelial cells is regulated by radiation-induced activation of cytosolic phospholipase A2 ($cPLA_2$). Our present study establishes a regulatory role for $cPLA_2$ in the function of irradiated vascular endothelium. Inhibition of $cPLA_2$ resulted in decreased migration and attenuated tubule formation in irradiated HUVEC. In order to determine the effects of $cPLA_2$ inhibition *in vivo*, we used a heterotopic tumor model of C57BL6 mice with Lewis Lung Carcinoma in the hind limb. When tumors were > 2 mm, the mice received intraperitoneal injections of 70% EtOH or the $cPLA_2$ inhibitor $AACOCF_3$ (10 mg/kg) prior to tumor irradiation with 3 Gy. Mice were treated once per day for 5 consecutive days and, 24 hours after the final treatment, the tumor vascularity was analyzed by Power Doppler sonography and vessel counts of hematoxylin-stained tumor sections. After Power Doppler sonography, Vascular Index was calculated as the ratio of color coded pixels representing blood flow to the total pixel volume of the tumor. The average Vascular Indices were 12% and 15% for untreated and irradiated tumors, respectively. Treatment with $AACOCF_3$ resulted in a 1.6-fold decrease in tumor Vascular Index as compared to untreated mice (7.5% vs. 12%). This decrease was further potentiated in mice that received a combined treatment of $AACOCF_3$ and radiation (2-fold, 6% vs. 12%). A similar trend in tumor vascularity was observed when histological tumor sections from treated mice were examined for blood vessel formation. In comparison to vessel number in untreated mice (10%), microscopic analysis revealed a significant decrease in vessel number in mice treated with $AACOCF_3$ followed by radiation (2%). Treatment with the $cPLA_2$ inhibitor MAFP produced comparable results. Taken together, these results indicate a key regulatory role for $cPLA_2$ in the functional response of irradiated endothelium and suggest that $cPLA_2$ inhibition may increase the efficacy of radiation therapy. In summary, these findings identify $cPLA_2$ as a potential molecular target for tumor sensitization to radiotherapy.

(PS4112) The bone marrow derived myelomonocytic cells restore vasculogenesis in irradiated tumor bed by secreting matrix metalloproteinase-9. G-One Ahn, J. Martin Brown. Stanford University, Stanford, CA, USA.

A significant number of cancer patients treated with radiotherapy develop tumor recurrence within irradiated field. We determined whether the bone marrow (BM)-derived cells contribute to the tumor recurrence after irradiation (IR) using the mouse model of tumors grown in irradiated bed. Resembling recurrent primary tumors, this model allows tumor vessels to form by vasculogenesis because angiogenesis of the local sprouting is blocked by IR. We found by transplanting the BM cells from Tie2lacZ transgenic mice that the contribution of endothelial progenitor cells to this vasculogenic process is minimal. However, there were CD11b myelomonocytic cells secreting matrix metalloproteinase-9 (MMP-9) in those tumors. These cells conferred radioresistance when grown in irradiated bed of MMP-9 knock out mice. These radioresistant tumors also showed immature tumor vessels when examined for vessel maturity. Depletion of the CD11b myelomonocytic cells secreting MMP-9 by bisphosphonate significantly sensitized these tumors to IR. Overall this study demonstrates that the BM-derived myelomonocytic cells secreting MMP-9 are an important target for adjunct therapy to maximize the response of tumors to radiotherapy.

(PS4113) TNF-alpha-related apoptosis-inducing ligand (TRAIL) enhances radiation-induced cell killing in human carcinoma *in vitro* and *in vivo*. Momoko Takahashi, Osamu Inanami, Mikinori Kuwabara. Hokkaido University, Sapporo, Japan.

Various anti-tumor drugs and sensitizing compounds are used to enhance efficacy for the radio-therapy in tumor therapy. Recently, we showed that ionizing radiation induced the expression of death receptor, DR5, on the cell surface in tumor cell lines and death receptor of TNF-alpha-related apoptosis-inducing ligand, TRAIL, enhanced the apoptotic pathway. In present study, to obtain further mechanism of apoptosis induced by the combination of TRAIL and X irradiation, involvement of caspase family was examined in human lung carcinoma A549 cell line. As a result, we showed that the activations of caspase-8 and caspase-3 were associated with enhancement of the radiation-induced apoptosis by TRAIL treatment. These data suggested that radiosensitization by TRAIL was due to the enhancement of caspase-8/-3 cascade-dependent apoptosis through the high expression of DR5. Furthermore, this enhancement of radiation-induced apoptosis by the TRAIL was observed in A549 cells irradiated under the hypoxic condition to induce radio- and chemo-resistance in tumor cells. Next, we tested the effect of combination treatment of TRAIL and X irradiation in human gastric carcinoma MKN45 xenograft model. As a result, the growth curve obtained from the group which was treated with TRAIL and X irradiation was significantly slower than that with TRAIL or X irradiation alone, suggesting that the combination of TRAIL and X irradiation induced to delay tumor growth. These data suggest that TRAIL would be a candidate for a radiosensitizer compound in radiotherapy of solid tumor.

(PS4114) Radiosensitization of multicellular tumor spheroids by 2-deoxy-D-glucose is stimulated by a combination of TNFα and glucodeprivation induced oxidative stress. Bilikere S. Dwarakanath, Divya Khaitan, Sudhir Chandna. Institute of Nuclear Medicine &, Delhi, India.

Radiosensitization by the glycolytic inhibitor, 2-deoxy-D-glucose (2-DG) in multicellular spheroids (MTS) of a human glioma cell line (BMG-1) has been found to be 2.5 folds higher than in the monolayer cultures (MLC), which correlated with enhanced glycolysis. This was accompanied by a profound induction of apoptosis in MTS as compared to cytogenetic damage linked mitotic death, being the major death pathway in MLC. Since alterations in endogenous and inducible oxidative stress in MTS due to changes in physiologic (oxygen, TNF levels etc) and metabolic (glycolysis) status, could elicit differential death responses, we investigated the role of oxidative stress in the enhanced radiosensitization of MTS by 2-DG. Oxidative stress (ROS by DCFH-DA), TNFα levels (immunoflow cytometry), GSH and GSSG levels (biochemical assay), DNA damage (comet assay), micronuclei (microscopy), induction of apoptosis (microscopy and flow cytometry) and clonogenic survival (macrocolony) were studied.

Presence of 2-DG (5mM; equimolar with glucose) for 4 h following irradiation of MTS resulted in an increase in the number of comets with large amount of DNA damage suggestive of

apoptosis, which was accompanied by the induction of ROS that persisted for several hours. Addition of 1 mM pyruvate for the last 30 min during exposure to 2-DG in MTS completely abolished the ROS induction by 2-DG+radiation and enhanced clonogenic survival. N-Acetyl-cysteine, on the other hand, had a marginal effect, thereby suggesting the strong pyruvate dependent antioxidant defense in spheroids. Addition of 10 ng/ml TNFα (which enhances GSSG/GSH ratio) in MLC (that matched with the endogenous levels in MTS) enhanced the ROS level and also their susceptibility to radiation ($SF_{5Gy} = 0.11$) as compared to spheroids ($SF_{5Gy} = 0.43$), suggesting that enhanced pyruvate in spheroids confers resistance. The radiosensitization of TNFα-treated MLC by 2-DG was also comparable to MTS. These results suggest that the enhanced radiosensitization by 2-DG in MTS is due to a synergy between TNFα mediated endogenous oxidative stress and glucode-privation induced oxidative stress linked to pyruvate depletion resulting in enhanced interphase death in the form of apoptosis.

(PS4115) Combining radiation therapy with interstitial radiation-inducible TNF-α expression for local regional cancer treatment. Mira Jung, Alexandre Dimtchev, Arron Foxworth, Anatoly Dritschilo. Georgetown University Medical Center, Washington, DC, USA.

Brachytherapy is used in the treatment of human cancers, including cancers of the cervix, breast, prostate and head and neck. The primary advantage of brachytherapy lies in the spatial conformation of the radiation deposition, and the isotopes may be delivered within millimeter sized metallic sources. We have shown that similar techniques (using hollow metallic cylinders) may be used to deliver gene therapy vectors capable of expressing the radiation sensitizing cytokine, TNF-α, within a restricted volume of tissue. We report here, radiation sensitization of cancer cells using a radiation-inducible TNF-α expressing vector, based on the IEX-1 promoter (pIEX-TNF-α). TNF-α, determined by ELISA assays increased between 5 to 10 fold, 48 hours following exposure to radiation, and radiation sensitization was comparable to that observed in cells in which TNF-α was constitutively expressed under control by the CMV promoter (pCMV-TNF-α). The efficiency of induced TNF-α radiation sensitization was also observed in a PC-3 xenograft tumor model. IEX-1 driven TNF-α expression and radiation exposure resulted in enhanced regression of tumor volumes as compared to radiation alone or TNF-α alone. These data support the enhanced effect on tumor regression by treatment with radiation inducible TNF-α expression in combination with radiation exposure. The IEX-1 promoter provides a useful construct for temporal activation of radiation sensitizing gene expression.

(PS4116) Differential effect of low and high dose X-rays on mutation induction by *N*-ethyl-*N*-nitrosourea in thymocytes of B6C3F1 *gpt*-delta mice. Kazumi Yamauchi[1], Shizuko Kakinuma[1], Satomi Sudo[1], Seiji Kito[1], Yuki Oota[1], Takehiko Nohmi[2], Ken-ichi Masumura[2], Mayumi Nishimura[1], Yoshiya Shimada[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]National Institute of Health Sciences, Tokyo, Japan.

Carcinogenesis in human is considered as a result of the combined exposure to numerous environment factors. Little is known, however, about the mechanism of combined effect. Since thymic lymphoma has been studied as a good model of combined exposure, we attempted to about determine the influence of combined exposure to ionizing radiation and *N*-ethyl-*N*-nitrosourea (ENU) on mutation induction in thymus using B6C3F1 *gpt*-delta transgenic mice.

Four to 5 weeks of age Mice were exposed weekly to whole body X irradiation at 0.2 or 1 Gy for 4 consective weeks, ENU at 200 ppm in drinking water or X-irradiation followed by ENU. Thereafter, genome DNA was prepared from thymus and examined for *gpt*-delta mutation assay.

The results obtained were as follows; (1) ENU alone increased mutant frequency by 10-folds compared to untreated control, especially increased in G:C to A:T transition at non CpG sequence and A:T to T:A transversion mutation, and over 80% of mutans were clonally expanded, (2) unexpectedly, X-irradiation alone, either 0.2 Gy or 1 Gy, reduced mutant frequency, (3) combined exposure to 0.2 Gy with ENU dramatically suppressed mutation induction, specifically G to A transition, compared to ENU alone, (4) 1 Gy exposure, by contrast, enhanced mutant frequency by about 30-folds and appeared to accelerate clonal expansion of mutated cells.

In conclusion, low dose (0.2 Gy) radiation suppressed clonal expansion and mutation induction by ENU, while high dose (1 Gy) enhanced and accelerated clonal expansion of mutated thymocytes, indicating that the mode of combined effect is dose dependent.

(PS4117) Leukemogenesis and early loss of *PU.1* on chromosome 2 in CBA/CaJ and C57BL/6 mice after irradiation with HZE iron ions. Yuanlin Peng, Christy L. Warner, Xianan Liu, Paula C. Genik, Matthew A. Callan, F. Andrew Ray, Michael M. Weil, Robert L. Ullrich, Joel S. Bedford. Colorado State University, Fort Collins, CO, USA.

Loss of one allele of the *PU.1* gene (*Sfpi1*) is necessary but not sufficient for development of acute myeloid leukemia in mice. The spontaneous incidence of AML in CBA/CaJ mice is negligible (<1%), but these mice are susceptible to radiation induced AML. C57BL/6 mice do not develop AML either spontaneously or after irradiation. Susceptible (CBA/CaJ) or resistant (C57BL/6) mice were irradiated with 0, 0.1, 0.2, 0.4, and 1.0 Gy doses of 1GeV/amu iron ions at the NRSL at Brookhaven National Laboratory, and 24 hours, 30 days or 1 year later we harvested bone marrow to determine the frequency of loss of a segment of chromosome 2 containing the *PU.1* gene. For comparison, we have also irradiated both strains with gamma-rays and harvested bone marrow cells at similar time points.

Without irradiation 1% of mitotic bone marrow cells from both mouse strains showed loss of one allele of *PU.1*. At the 24 hour sampling time the dose-response for loss of *PU.1* was not significantly different from linear for both strains, and the slope of the dose-response was about 5% per Gy for CBA/CaJ and 3% per Gy for C57BL/6 mice. This difference is consistent with an increased sensitivity of CBA/CaJ mice to AML induction by radiation. Similar differences have been observed by others for gamma-ray exposures, but doses approximately 3-fold greater were required for the same levels of *PU.1* loss that we observed for the HZE Fe ions. We are currently cross-validating the gamma-ray data in our laboratory. The RBE of the 1 GeV/amu Fe ions relative to gamma-rays for AML induction will soon be available for comparison with the RBE for early *PU.1* loss. The scoring for *PU.1* loss at 30 days and 1 year after irradiation are nearly complete and suggest that in CBA mice the number of *PU.1* deleted cells post-irradiation never returns to baseline.

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(PS4118) Radiation increases the outgrowth of p16INK4a(-) human mammary epithelial cells in serum-free cultures. Rituparna Mukhopadhyay, Alexey Bazarov, William C. Hines, Mary Helen Barcellos-Hoff, Paul Yaswen. Lawrence Berkeley National Lab, Berkeley, CA, USA.

While to date, many studies of ionizing radiation have focused on its effects on primary DNA structure, comparatively few have focused on its effects on epigenetic phenomena such as gene methylation. Locus specific hypermethylation has been demonstrated to play a role in the abrogation of expression of certain tumor suppressor and DNA repair genes. Such epigenetic phenomena have been increasingly implicated in carcinogenesis in human cells and tissues, and in some cases, may represent predominant mechanisms of oncogenesis. Epigenetic silencing of the cyclin-dependent kinase inhibitor $p16^{INK4a}$ (p16) in human cancers, for example, is often

associated with methylation of the p16 promoter by unknown mechanism(s). We are using cultured human mammary epithelial cells (HMEC) to evaluate the potential of ionizing radiation to cause persistent pre-malignancy-associated epigenetic changes. In serum-free growth medium, HMEC from histologically normal breast tissue growth arrest after 5–20 population doublings, exhibiting senescent morphologies and elevated expression of p16. Such HMEC cultures spontaneously yield at low frequencies ($1x10^{-5}$ to $1x10^{-8}$) variant p16(-) cell populations that are capable of long term growth (50–100 total population doublings), and which are susceptible to genomic instability associated with telomere dysfunction. Using this manipulable cell culture model, we sought to determine whether radiation would alter p16 expression, p16 promoter methylation, and/or long term growth potential in HMEC. Surprisingly, in replicate experiments with HMEC from three individuals, we determined that 2 Gy X-irradiation causes increases in the appearance of p16(-) cells with long term growth potential. Flow cytometry confirmed that the differences in BrdU incorporation in the treated and untreated populations were statistically significant ($P = 0.0016$). Based on these data, we hypothesize that radiation, possibly acting through the generation of reactive oxygen species, combines with endogenous oxidative damage to cause epigenetic changes in p16 expression. We are currently exploring the mechanistic underpinnings of this phenomenon.

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(PS4119) Identification of radiation specific gene signatures in rat mammary tumors. Hae-June Lee. Korea institute of Radiological and Medical Sciences, Seoul, Republic of Korea.

The aim of this work was to identify a set of genes which involved in radiation-induced mammary tumors. Thus, mammary tumors were produced by treatment of rats with either radiation or dimethylbenz(a)anthracene (DMBA). Pathological analysis showed that radiation-induced mammary tumors showed various stages of pathological phenotypes such as fibroadenoma, papilloma, and carcinoma. However, in the case of DMBA-induced tumors, all of them were carcinoma. Much more TUNEL and PCNA positive cells were more in radiation-induced tumors than in the DMBA induced tumors, while DNA damage responses including p53 accumulation and histone H2AX phosphorylation were higher in DMBA induced tumors. Mutation analysis of p53 gene using carcinoma tissues, revealed that more frequent p53 mutations (100%) of exon 6 and 7 in DMBA-induced tumors was observed, when compare to those of IR-induced tumors (40%). cDNA microarray analysis, using radiation- or carcinogen-induced tumor tissues which showed similar pathological grade (carcinoma, grade I), revealed that 32 genes were specific for radiation-induced tumors and 11 genes were specific for DMBA induced tumors. Ten genes were induced in both radiation and DMBA induced tumors. Real time RT-PCR analysis indicated that stanniocalcin, interferon regulatory factor, interleukin 18 binding protein, and chloride channel calcium activated 3 were expressed in both DMBA and radiation induced tumors, and arachidonate 5-lipoxygenase activating protein 1 (ALOX5AP) and cathepsin S were expressed only in radiation induced tumors. Soft agar growth assay was carried out to identify the cancer features of IR-specific genes. Expression of mRNA for ALOX5AP and cathepsin S was increased by radiation in MCF10A cells, and the cells stably transfected with ALOX5AP and cathepsin S showed morphological changes compared to control cells, such as obvious pseudopods. These data suggest that ALOX5AP and cathepsin S may be associated with cellular transformation induced by radiation, and that they may possible be a radiation specific gene signature in radiation-induced mammary tumors.

(PS4120) ROS levels and mutations in atrophic thymuses after γ-irradiation. Ryo Kominami[1], Hiroyuki Ohi[1], Masaki Maruyama[1], Kenya Kamimura[1], Yukio Mishima[1], Ohtsura Niwa[2]. [1]Molecular Genetics, Niigata University Graduate School of Medical and Dental Sciences, and Transdisciplinary Research, Niigata University, Niigata, Japan, [2]Radiation Biology Center, Kyoto University, Kyoto, Japan.

Ionizing radiation induces a variety of tumors in humans and mice probably through generation of ROS (reactive oxygen species). Mouse thymic lymphomas are one of the classic models of radiation-induced malignancies, and several biological studies have suggested that irradiation results in atrophic thymus that founds 'prelymphoma' cells. However, further analysis of the prelymphoma and their precursor cells has not been performed. Thus, we examined effects of γ-ray on ROS levels in atrophic thymus at 5 and 7 days after γ-ray and also clonal growth and DNA alterations at 30 and 100 days after. We used congenic mice of two different *Mtf-1* genotypes that showed distinct susceptibility to thymic lymphomas. *Mtf-1* encodes the metal responsive transcription factor involved in the radiation-induced signaling pathway that regulates the ROS levels. Here we show that susceptible mice tended to retain proliferating large thymocytes with higher ROS levels more than resistant mice did. Such tendency was not found in a different type of susceptible mice lacking one allele of *Bcl11b* tumor suppressor gene. The high retention of the large thymocytes in atrophic thymus is probably a reflection of prolonged compensatory regeneration response and the response may augment the development of prelymphoma cells. On the other hand, examination of DNA rearrangement at TCRβ locus in atrophic thymuses revealed clonal expansion of thymocytes in some of them even 30 days after irradiation. We also detected DNA changes in the five genes, *Ikaros*, *Bcl11b*/*Rit1*/*CTIP2*, *Myc*, *Notch1* and *PTEN*, the changes occurring at *Bcl11b* and *Myc* earlier than at *Ikaros*, *Pten* and *Notch1*. The stage difference in accumulation of DNA changes suggests the order of mutations, which can be conformed to the sequential model proposed for the colorectal cancer development, and an implication of their contribution to lymphomagenesis.

(PS4121) Distinct structural abnormalities of chromosomes 11 and 12 associated with loss of heterozygosity in X-ray-induced mouse thymic lymphomas. Akifumi Nakata, Mitsuaki A. Yoshida, Miho Akiyama, Shizuko Kakinuma, Toshihiko Sado, Mayumi Nishimura, Yoshiya Shimada. National Institute of Radiological Sciences, Chiba, Japan.

Mouse thymic lymphomas (TLs) are one of the classical models for spontaneously developed, and radiation- or chemical carcinogen-induced malignancy and are a model for mechanistic study of human acute lymphoblastic leukemia. In the early studies of chromosome abnormality, trisomy of chromosome 15 was identified frequently in TLs and seemed to be the specific change associated with the development of this malignancy. Recent studies have demonstrated that radiation-induced TLs show frequent loss of heterozygosity (LOH) on chromosomes 4 (*p15*/*p16*), 11 (*Ikaros*), 12 (*Bcl11b*), 19 (*Pten*) and X. Since cytogenetic analysis of murine thymic lymphoma has not been studied for these two decades, we think it worthy to re-evaluate the genetic changes in TL using both classical cytogenetic and molecular cytogenetic method focusing on the frequent LOH region. In this study, C57BL/6 (B6), C3H and F1 (B6 x C3H) mice were used. TLs were induced by whole-body irradiation with 4- to 5-week-old mice exposing to X-ray (1.2 or 1.6 Gy/exposure) once a week for four consecutive weeks using a PANTAK X-ray generator at 0.7 Gy/min (200 kVp, 20 mA, with filters of 0.5 mm Cu and 0.5 mm Al). The mice were observed for 3 months or until moribund and were sacrificed under ether anesthesia to remove the enlarged thymus. Chromosomally abnormal cells were present in 25 TLs examined (25/26, 96%). The most frequent abnormality was trisomy or partial trisomy of chromosome 15 (16/26, 62%), being consistent with previous studies. Structural abnormalities of chromosome 11 with interstitial deletion of the proximal region and chromosome 12 translocations with deletion of the distal region were newly identified in 7 (27%) and 12 (46%) cases, respectively. Monosomy of chromosome X was also found in 4 cases (15%). No apparent aberrations were detected in chromosomes 4 and 19. Only three cases had a reciprocal translocation, t(X;17), t(3;X) and t(1;12). These results indicate that a distinct mechanism contributes to LOH of each tumor suppressor gene in different chromosomal locations.

(PS4122) Influence of genetic background on hair-cycle dependent basal cell carcinoma tumorigenesis in irradiated *Ptc1*$^{+/-}$ mice. Mariateresa Mancuso[1], Simona Leonardi[1], Mirella Tanori[1], Emanuela Pasquali[1,2], Simonetta Rebessi[1], Vincenzo Di Majo[1], Simonetta Pazzaglia[1], Anna Saran[1]. [1]ENEA-Casaccia Research Center, Rome, Italy, [2]Istituto Nazionale Tumori, Milan, Italy.

Ionizing radiation is a well-known genotoxic agent that increases cancer risk both in humans and in mice and is well known to cause skin cancer, especially basal cell carcinoma (BCC). We have previously shown that BCC incidence in irradiated *Ptc1*$^{+/-}$ mice can be affected by growth/quiescence of the hair follicles during exposure. Qualitative differences in BCC tumorigenesis were also observed based on age/hair-growth phase at irradiation, and a BCC subtype with features of myoepithelial differentiation was detected in mice irradiated at postnatal day 3 (P3), in addition to typical BCCs. This previously undetected BCC variant, characterized by anti-CK14 and -smooth muscle actin (SMA) reactivity, exhibits a striking morphological and histochemical similarity with the follicular outer root sheath (ORS) of anagen skin, suggestive of a derivation from ORS progenitors. We tested whether the interaction of the highly penetrant *Ptc1* mutation with the skin tumor susceptible background of Car-S mice could facilitate formation of either BCC subtypes. To this aim, F1 crosses of CD1*Ptc1*$^{+/-}$ mice with Car-S mice (F1S) were irradiated with 3 Gy X rays at P3, P35 or P60, and monitored for BCC development. Although P3-irradiated F1S mice were susceptible to BCC induction, all tumors observed were typical BCCs, based on morphological criteria and on lack of SMA reactivity. No SMA-expressing myoepithelial BCCs were detected in F1S irradiated mice, although the skin of P3 F1S*Ptc1*$^{+/-}$ mice was in early anagen phase, characterized by massive proliferation of ORS cells. The *Snail* family of transcription factors has previously been implicated in the differentiation of epithelial cells into mesenchymal cells. To investigate the observed genetic background-related difference in BCC tumorigenesis, the induction of *Snail* is being investigated in irradiated normal skin of CD1*Ptc1*$^{+/-}$ and F1S*Ptc1*$^{+/-}$ mice. As aberrant expression of *Snail* may promote tumorigenesis through increased resistance to apoptosis, *Snail* expression and apoptotic response will be compared in the skin of mice of the two different backgrounds at 3 and 6 h post-irradiation. The balance between *Snail* and E-cadherin expression will also be examined in the irradiated normal skin of the two lines and in BCCs.

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(PS4123) Sex- and tissue- specific microRNAome changes upon irradiation in a mouse model. Yaroslav Ilnytskyy, Olga Kovalchuk. University of Lethbridge, Lethbridge, AB, Canada.

Ionizing radiation (IR)-induced cancers were reported to occur at different frequencies in males and females. IR-induced thymic lymphoma in mice and IR-induced leukemia and lymphoma in humans have been reported to occur with a higher incidence in males. The underlying mechanisms responsible for these sex differences have yet to be established. Our laboratory has found significant differences in the IR-induced gene expression patterns in spleen and thymus of male and female, with myriads of pathways affected in a sex-specific manner. Our findings have also revealed the significant sex differences in IR-induced apoptosis as well as changes in the levels of a cellular proliferation and the significant sex specificity of the IR-induced epigenetic changes (1–4). In search for the fundamental mechanisms of the sex differences in IR responses, we now looked into the changes in the levels of small regulatory RNAs (specifically microRNAs) in the exposed tissues of male and female mice. Small molecules are important components of the epigenetic machinery and potent expression regulators. Yet, up to now the effect of genotoxic stressors in general and IR in particular on the microRNA levels is still unknown. Here, 45 day old male and female mice were subjected to the whole body X ray irradiation (0.5Gy) and sacrificed 6 or 96 hours after exposure. Spleen and thymus tissues were sampled upon irradiation. MicroRNAome profiling was conducted using LC Sciences microRNA microarray platform. We found intriguing sex- and organ-specific microRNAome signatures of the radiation exposure. The initial analysis revealed that 22 and 8 microRNAs were uniquely changed in male and female mouse thymus tissue, respectively. In murine spleen, 36 and 13 microRNAs were differentially regulated in male and female animals. Amongst those, 4 microRNAs were changed in both sexes. These were miR-290, miR-346 and miR-468, putatively targeting Gadd45a, Rad9, Rad1 proteins important for radiation responses. The forth microRNA was miR-34, confirmed to target Notch 1. The microRNA expression changes were confirmed by qRT-PCR and Northern blotting. Consistently with our previous observations, the changes in male tissue were more profound than in female tissue. The observed microRNAome changes may be used as sex- and organ-specific biomarkers of IR exposure.

(PS4124) Combined effects of radiation and estrogen on the epigenetic processes in rat mammary gland. Kristy Kutanzi, Igor Koturbash, Rocio Rodriguez-Juarez, Olga Kovalchuk. University of Lethbridge, Lethbridge, AB, Canada.

Ionizing radiation (IR) is generally accepted as a breast carcinogen. Average IR exposure doses linked to breast cancer development range widely between 0.02 and 20 Gy. Estrogen is another well-known breast carcinogen with both initiating and promoting properties. Women with elevated estrogen levels are considered to be a high-risk group for breast cancer development and would likely be exposed to diagnostic IR procedures on a more frequent basis. Similarly, many women with estrogen-induced breast cancer undergo IR treatment and are exposed to relatively high X-ray doses to the healthy breast. *In vitro* application of both IR and estrogen led to the malignant transformation of normal breast epithelial cells. We have recently shown that estrogen as well as IR exposures applied separately result in profound epigenetic dysregulation in the rat mammary gland.

Do those carcinogens act synergistically to promote deleterious epigenetic alterations *in vivo*?

We have analyzed the nature and roles of epigenetic changes in the estrogen and IR-induced breast carcinogenesis using a rat model. The six-week-old female rats were randomly assigned to one of the following treatment groups: (i) sham treated controls; (ii) estrogen treated group; (iii) IR treated group; (iv) IR + estrogen treated group. Animals were sacrificed at 4 weeks, 12 weeks, 18 weeks after the irradiation.

We have found that IR-induced global DNA hypomethylation was observed 4, 12 and 18 weeks after IR exposure. Combined IR and estrogen exposure had cumulative effects on DNA hypomethylation. We also noted a loss of trimethylation of histone H4K20 and H3K9.

Furthermore, we have found that estrogen, IR and combined exposure resulted in the significant alterations in the levels of microRNAs as compared to the age matched controls. Amongst microRNAs with altered expression were the known oncogenic microRNAs - miR-17–5p, miR-106b, miR-20a, miR23, miR-181. Experimentally confirmed targets for those miRNAs are E2F1 and AIB1, RB1, TGFBR2, Notch1 and HES1, and Tcl1 oncogene, respectively. We also noted altered expression of miR-199b which targets laminin gamma2 gene, an important component of extracellular matrix. The expression changes of the aforementioned miRNAs were confirmed by qRT-PCR. The cellular repercussions of the observed changes will be discussed.

(PS4125) The irradiated stroma increases tumors arising from non-irradiated, p53 null mammary epithelium. David H. Nguyen[1], Hellen A. Oketch-Rabah[1], Daniel Medina[2], Mary Helen Barcellos-Hoff[1]. [1]Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [2]Baylor College of Medicine, Houston, TX, USA.

Current paradigms for cancer initiation and progression generally focus on epithelial cell mutations, although it is increasingly clear that the development of cancer is highly intertwined with microenvironmental factors. We have shown that ionizing radiation (IR) induces persistent phenotypic changes in the mouse mammary stroma that are in part mediated by activation of transforming growth factor β1 (TGFβ). We developed a model of

stromal-epithelial mammary chimeras to determine whether the irradiated host affects mammary tumor development. This model takes advantage of the fact that the mammary epithelium can be removed from prepubertal mammary glands and the parenchyma-free stroma can then serve as the recipient of transplanted mammary epithelial cells or tissue. To examine whether host irradiation affects mammary cancer progression and its dose dependence, we studied transplanted *TP53* null mouse mammary epithelial cells to Balb/c wildtype mice. Approximately 60% of Balb/c *TP53* null mammary transplants develop carcinomas at approximately a year of age (Medina et al. Faseb J, 16: 881–883, 2002). The advantage of using *TP53* null mammary epithelia is that this model has been shown to recapitulate many features of human breast cancer. Inquinal glands of wildtype Balb/c hosts were cleared of epithelium at 3 weeks of age and the mice irradiated whole body with 0.1, 0.5, and 1 Gy ^{60}Co γ-radiation at 12 weeks of age and transplanted three days with syngenic p53 null mammary tissue from 10–12 week old donors. Tumor frequency of hosts irradiated with up to 1 Gy increased from 60% to 93–100%. Median latency was tended to be decreased by host irradiation. To assess the contribution of TGFβ, Balb/c *Tgfβ1* heterozygote hosts were subjected to a similar protocol and transplanted with Balb/c *Tgfβ1* wildtype, *TP53* null mammary tissue. Compared to wildtype hosts, *Tgfβ1* heterozygote hosts showed similar tumor frequency at 1 year of age, but median latency was significantly increased (342 *vs* 264 days) and the effect of irradiation was absent. This model provides a novel means of investigating how host irradiation effects mediated through the microenvironment, or genotype, affect carcinogenesis. *Supported by the Bay Area NIEHS Breast Cancer and the Environment Center.*

(PS4126) Study on radiation-induced adaptive response in fetal mice. Bing Wang, Kaoru Tanaka, Yi Shang, Guillaume Vares, Yasuko Morimoto, Tetsuo Nakajima, Mitsuru Nenoi, Isamu Hayata. National Institute of Radiological Sciences, Chiba, Japan.

A radiation-induced adaptive response, which was judged as reduction of both fetal death and digital malformation, was demonstrated in fetal mice by priming low-dose radiation on E11 prior to a high-dose irradiation on E12. Suppression of the digital defects was correlated to the inhibition of radiation-induced apoptosis. The priming dose was mouse strain-related. Both Trp53 alleles were essential for induction of the adaptive response. Furthermore, existence of the dose-rate effect was verified for delivery of the priming dose. There was a high postnatal mortality in the offspring that survived from prenatal death. Postnatal retardation, alteration in adult behavior, and life span shortening were observed in the survivals. In addition, a significantly increased radiosensitivity to the killing effect was also observed in the postnatal survivals. These results indicate that radiation-induced adaptive response in fetal mice is due to a complex interplay between dose, dose rate and animal factors such as the strain, developmental stage, and gene background. Though the priming dose could rescue some mice from high dose irradiation, the survivals are not healthy as alterations in development, behavior and radiosensitivity were observed. This work was supported in part by the Budget for New Nuclear Crossover Research from the Ministry of Education, Culture, Sports, Sciences and Technology, Japan.

(PS4127) Adaptive response in embryogenesis: comparative microarray analysis of gene expressions in mouse fetuses. Guillaume Vares, Bing Wang, Yi Shang, Harumi Ohyama, Kaoru Tanaka, Tetsuo Nakajima, Mitsuru Nenoi, Isamu Hayata. National Institute of Radiological Sciences (NIRS), Chiba, Japan.

Exposure of sublethal doses of ionizing radiation can induce protective mechanisms against a subsequent higher dose irradiation. We previously demonstrated the existence of this phenomenon, called radiation-induced adaptive response (AR), in mice during late organogenesis. For better understanding of molecular mechanisms underlying AR in this model, we performed a global analysis of transcriptome regulations (14000 different genes) in cells collected from whole mouse fetuses, 24 hours after *in utero* exposure to priming irradiation. Several combinations of radiation dose and dose-rate were applied to induce or not an AR. While many genes were not modulated at all after radiation exposure and 166 genes were deregulated for all conditions, we identified a panel of 861 genes (so called "*AR* genes") showing significantly different expression ratios under AR-inducing and non AR-inducing conditions. A sub-list of 119 *AR* genes ("*ARS* genes") with higher discriminative characteristics was also created. We applied a functional classification algorithm which clustered *AR* genes in a limited number of functionally related groups. Our results emphasized the role of signal transduction mechanisms in the induction of AR. We also observed that a significant proportion of *AR* genes were p53-related. Furthermore, many *AR* genes were important for fetal development, like growth factors or genes involved in signaling cascades. Other AR-related studies suggested that priming irradiation could trigger the induction of DNA repair mechanisms. Even though no evidence of specific DNA repair modulation at the transcriptional level was observed in adapted fetuses, we studied H2AX phosphorylation kinetics following challenging irradiation in fetal liver cells and observed that H2AX phosphorylation peak appeared earlier in adapted than in non adapted cells (while disappearance rates were similar), which could indeed suggest a possible modulation of DNA repair induced by priming irradiation. However, no modulation of radiation-induced cell death by AR was described in fetal liver cells. Taken together, our results present the first evidence of molecular mechanisms modulation by AR *in utero*, which may have potentially important consequences for further developmental processes, and could even influence tumorigenesis.

(PS4128) Simvastatin ameliorates radiation enteropathy development after localized, fractionated irradiation by a protein C-independent mechanism. Junru Wang[1], Marjan Boerma[1], Qiang Fu[1], Louis M. Fink[2], Martin Hauer-Jensen[1]. [1]Arkansas Cancer Research Center, Little Rock, AR, USA, [2]Nevada Cancer Institute, Las Vegas, NV, USA.

Background: Microvascular injury plays a key role in both early (inflammatory) and delayed (fibrotic) radiation responses. Statins, in addition to their lipid-lowering effects, have many vasculoprotective properties, notably enhancement of the thrombomodulin (TM)-protein C system, which may counteract the effects of radiation on vascular endothelium and normal tissues. This study examined whether the administration of simvastatin reduces acute and chronic intestinal radiation injury, and whether the effect of simvastatin depends on generation of activated protein C.

Methods: Male rats underwent fractionated X-irradiation (5 Gy x 9) of a 4-cm loop of small bowel. The animals were fed either regular chow or chow containing simvastatin from 2 weeks before irradiation until termination of the experiment. Groups of rats were euthanized at 2 weeks and 26 weeks for assessment of early and delayed radiation injury, respectively, by quantitative histology, morphometry, immunohistochemistry, and quantitative RT-PCR. Dependency on protein C activation was examined in $TM^{Pro/-}$ mutant mice, in which the ability to activate protein C is deficient.

Results: Administration of simvastatin was associated with lower radiation injury scores ($p<0.0001$), preservation of mucosal surface area ($p=0.0009$), less thickening of the intestinal wall and subserosa ($p=0.008$ and $p=0.004$), less neutrophil infiltration ($p=0.04$), and less accumulation of collagen I ($p=0.003$). The effect of simvastatin was consistently more pronounced for delayed than for early radiation responses. Surprisingly, and in contrast to findings after total body irradiation, simvastatin reduced intestinal radiation injury in $TM^{Pro/-}$ mutant mice, indicating that the enteroprotective effect of simvastatin after localized irradiation is unrelated to protein C activation.

Conclusions: Simvastatin significantly ameliorates the intestinal radiation response. In contrast to the situation after total body irradiation, the radioprotective effect of simvastatin after localized small bowel irradiation does not appear to be related to protein C activation. Statins should undergo clinical testing as a strategy to minimize the acute and long term adverse effects of radiation on the intestine and other normal tissues.

(PS4129) Radiation-induced changes in vasoconstriction of rat pulmonary arteries to angiotensin II are mitigated by captopril. Rong Zhang, Ying Gao, Swarajit Ghosh, John Moulder, Brian Fish, Elizabeth Jacobs, Meetha Medhora. Medical College of Wisconsin, Milwaukee, WI, USA.

Vascular injuries in the lung caused by irradiation have not been characterized in humans and animal models. We are developing an injury model consisting of a single dose of radiation (5 or 10 Gray) to the thorax of rats (strain Wistar, WAG/Rij/MCW), which will allow us to define spatial and temporal pulmonary derangements in vascular reactivity. These doses are relevant to exposure that may be sustained and survived in case of a bioterrorist attack. We have examined changes in vasoreactivity of isolated pulmonary arteries (PAs) to increasing concentrations of angiotensin II (Ang II, 10^{-10} to10^{-7} M) at different times post irradiation (PI). The PA rings were mounted on a force transducer in an organ bath. Our data demonstrated no radiation-induced changes in reactivity to Ang II in PAs with doses of 5 Gy up to 5 months PI. However, after 4 weeks following irradiation (10 Gy) there was a significant loss of constriction to Ang II (10^{-8} M & 10^{-7} M) which was even more marked after 8 weeks PI. Interestingly, there were no changes in reactivity of PAs after 5 months PI and the vasoreactivity of PAs from radiated rats was diminished over that of controls again after 12 months PI. Currently, we are using captopril (an FDA-approved Angiotensin Converting Enzyme (ACE) inhibitor) to determine mitigating doses and schedules on irradiation-induced injury in the animal model. The attenuated constriction to Ang II after 8 weeks of radiation (10 Gy) was not observed in animals treated with captopril (500 mg/L) immediately after radiation. Our results indicate that irradiation (10 Gy) results in attenuation of vasoreactivity between 4–8 weeks after exposure, temporary recovery after 5 months and loss of vasoreactivity again after 12 months PI. With captopril there is recovery of the loss of constriction to Ang II after 8 weeks following irradiation (10 Gy). Supported by AI 067734 (NAIAD).

(PS4130) Evaluation of carnosine as a radiation countermeasure agent. Theodor A. Zainal, Venkataraman Srinivasan, Mark H. Whitnall. Armed Forces Radiation Research Institute, Bethesda, MD, USA.

As a result of intensifying terrorist activity, dissemination of nuclear materials, and nuclear proliferation, there is an imperative need to develop radiation countermeasures designed to protect military personnel and emergency responders, as well as the general population, from ionizing radiation-induced injury. The objective of the present study was to evaluate the efficacy of the clinically relevant histidine-containing dipeptide carnosine (β-alanyl-L-histidine) as a radiation countermeasure agent. Due to its radioprotectant and immunostimulatory activity, long term stability, low toxicity, practical oral administration route, preexisting safety data, and representation of different mechanisms, carnosine is a viable candidate. Thirty-day survival studies at the radiation $LD_{90/30}$ in 12-week-old, male CD2F1 mice were used to evaluate countermeasure efficacy. Mice were exposed bilaterally to gamma-radiation (8.75 Gy, 0.6 Gy/min) from a cobalt-60 source. Subcutaneous administration of carnosine (2300 mg/kg) 24 hours prior to whole body irradiation resulted in 88% survival compared with 25% for the saline vehicle control animals. Carnosine is an effective countermeasure agent against ionizing radiation-induced injury in mice.

(PS4131) Exploring mechanisms for the efficacy of ACE inhibitors and AII blockers in radiation nephropathy. John E. Moulder, Brian L. Fish, Amy A. Irving, Marylou Mader, Eric P. Cohen. Medical College of Wisconsin, Milwaukee, WI, USA.

We have previously shown that angiotensin converting enzyme (ACE) inhibitors and angiotensin *II* (A*II*) type-1 (AT1) receptor antagonists can be used to mitigate and treat experimental radiation nephropathy. We have also shown that ACE inhibitors and AT1 blockers are effective in the treatment of clinical radiation nephropathy. The most obvious explanation for the efficacy of these agents is that radiation causes upregulation of the renin-angiotensin system (RAS). To date, however, we have found no evidence for radiation-induced upregulation of the RAS at the level of renin, A*II* or A*II* receptors. We are now evaluating possible mechanisms that do not directly involve the RAS:

- ACE inhibition leads to increased levels of the hematopoietic cytokine AcSDKP, and AcSDKP may be a renal anti-fibrotic;
- Aldosterone promotes nephrosclerosis in other models of renal injury, and suppression of the RAS also suppresses aldosterone;
- ACE inhibition (and possibly AT1 blockade) leads to increased levels of bradykinin; and in other models of renal injury, a drop in A*II* and a rise in bradykinin act synergistically;
- A*II* is a pro-oxidant and chronic oxidative stress may be involved in the pathogenesis of radiation nephropathy;
- A*II* metabolites such as Ang(1–7) may have anti-fibrotic and anti-proliferative activity.

Post-irradiation infusion of excess AcSDKP had no effect on the development of radiation nephropathy, largely eliminating a role for AcSDKP. A preliminary experiment found that chronic post-irradiation treatment with an aldosterone receptor blocker (spironolactone) had no effect on the development of radiation nephropathy, which cast doubts on a role for aldosterone. A role for bradykinin is being assessed in bradykinin-deficient (Brown Norway Katholiek) rats, and studies of a role for chronic oxidative stress are presented in a companion poster.

To date, we have no unambiguous explanation for how ACE inhibitors and A*II* blockers work in the mitigation and treatment of radiation nephropathy.

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(PS4132) Ramipril mitigates radiation-induced impairment of dentate gyrus neurogenesis. Kenneth A. Jenrow, Jianguo Liu, Andrew Kolozsvary, Stephen L. Brown, Jae Ho Kim. Henry Ford Hospital, Detroit, MI, USA.

Strategies to reduce cognitive impairment following whole brain irradiation (WBI) are being investigated. WBI at doses as low as 5 Gy are sufficient to disrupt granule cell neurogenesis within the rat dentate gyrus, and this disruption has been associated with an impairment of cognitive function. Anti-inflammatory agents have previously been shown to partially preserve/restore granule cell neurogenesis following WBI. The angiotensin converting enzyme (ACE) inhibitor, Ramipril, also has anti-inflammatory effects and has been shown to mitigate radiation-induced demyelination of the optic tract. These studies were designed to measure the effects of Ramipril on neurogenesis in the rat dentate gyrus following WBI. Groups of male Fischer 344 rats received WBI doses of 0 (control), 5, 10, 15, and 20 Gy, using a Cs-137 irradiator. Drug therapy was initiated within 24 hours of irradiation and continued until sacrifice. Twenty four hours prior to sacrifice, BrdU injections (50 mg/kg, i.p.) were administered once every two hours over a 6 hour interval to label mitotically active (proliferating) cells. Separate cohorts were sacrificed at 2, 4, 8, or 12 weeks post irradiation. BrdU positive neurons within the dentate granule cell layer were counted from three adjacent immunohistochemically-stained sections within the dorsal hippocampus. Consistent with previous reports, the disruption of neurogenesis within the dentate gyrus was proportional to radiation dose between 5 and 15 Gy, and was maximally disrupted at 15 and 20 Gy. Ramipril administration (1.5 mg/kg/day, oral) significantly preserved/restored granule cell neurogenesis and partially preserved the microvascular niche in which neurogenesis occurs, evidenced by the persistence of granule cell progenitor clusters near microvessels within the subgranular proliferative zone. Granule cell neurogenesis diminished over time in both our treated and untreated groups, however, the mitigating effects of Ramipril were still evident up to eight weeks post-irradiation. Activation of ACE may contribute to impaired neurogenesis in the brain following WBI. Administering ACE inhibitors post-irradiation may represent a novel approach to mitigating undesirable radiation effects, perhaps including impaired cognition.

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(PS4133) Ramipril mitigates whole brain radiation injury observed by contrast enhanced MRI. Stephen L. Brown, James R. Ewing, Sanath Kumar, Swayamprava Panda, Kenneth A. Jenrow, Joseph D. Fenstermacher, Tavarekere N. Nagaraja, Andrew Kolozsvary, Kelly Ann Keenan, Jae Ho Kim. Henry Ford Health System, Detroit, MI, USA.

Contrast enhanced magnetic resonance imaging (CEMRI) has the potential to monitor subtle changes in brain anatomy following brain irradiation. We hypothesized and tested that chronic effects of whole brain radiation exposure can be non-invasively evaluated by CEMRI. Twenty Fischer 344 rats received either 20Gy whole brain irradiation alone (n=8), 20Gy whole brain irradiation followed by ramipril given in drinking water at a dose of 1.5mg/kg/day (n=8), or no treatment (n=4), and imaged using Gd-based CEMRI (T1, T2, diffusion weighted). MRI was performed multiple times in the groups of rats up to 6 months post-irradiation. Some selected rats were administered fluorescence tracers (Evans blue which binds to plasma albumin and FITC-labeled dextran of 2000 kDa molecular weight), sacrificed and analyzed for signs of vascular leakage. No change in gross brain anatomy was observed in any group up to approximately 12 weeks. Starting at 12 weeks post-irradiation, the brains of rats receiving 20Gy whole brain irradiation began showing contrast enhancement on MRIs. The region of enhancement appeared to be localized to the ventricles and with some possible involvement of periventricular space. Such regions with Gd enhancement increased in both intensity and volume as a function of post-irradiation time. Interestingly, the rest of the brain parenchyma showed no obvious injury either in terms of CEMRI or Evans blue fluorescence. By 6 months, the ventricles of irradiated rats were grossly enlarged and exhibited marked Gd accumulation. In contrast, ventricles of ramipril treated rats showed significantly less contrast-enhancement (both magnitude and volume) than rats receiving radiation alone. Quantitative analysis of the rate of change of ventricular Gd enhancement is ongoing with the intent that a clinically useful tool to assess brain injury following whole brain exposure can be developed. This work is supported by U19 AI067734 (NIAID).

(PS4134) Defined doses of the radioprotectors amifostine and phosphonol protect against chromosomal inversion in pKZ1 spleen. Antony M. Hooker[1], David J. Grdina[2], Madhava Bhat[3], Pamela J. Sykes[1]. [1]Department of Haematology and Genetic Pathology, Flinders University and Medical Centre, Bedford Park, Australia, [2]Department of Radiation and Cellular Oncology, University of Chicago, Chicago, IL, USA, [3]Adelaide Radiotherapy Centre, Adelaide, Australia.

The greatest perceived risk by the general population following exposure to radiation is that of persistent genetic damage and the development of cancer. Chemical radioprotectors, may provide protection against the long-term effects of radiation exposure and carcinogenic risk. The pKZ1 transgenic mouse recombination assay is a sensitive assay for studying chromosomal inversion responses after low dose radiation exposure. When an inversion occurs within the transgene in the pKZ1 assay, the *lacZ* gene product (β-gal) can be detected in tissue sections using histochemistry. The aim of this project was to study the effect of the radioprotectors and free radical scavengers, amifostine and Phosphonol™ on the pKZ1 inversion frequency. Three - 4 month old pKZ1 mice were injected intraperitoneally with radioprotectors either alone or 3 hrs post 250 mGy X-irradiation and their spleen analysed for inversions.

For both amifostine and phosphonol (100 mg/kg) the inversion frequency was significantly reduced to below the endogenous inversion frequency. However at 1 and 10 mg/kg, the inversion frequency increased with decreasing concentrations of amifostine. This was likely due to the Fenton reaction in which free radicals are produced at low concentrations of thiols such as amifostine and phosphonol. Conflicting results were observed at the higher dose of amifostine and phosphonol (400 mg/kg) with amifostine reducing the inversion frequency to below the endogenous frequency whilst phosphonol, significantly increased the inversion frequency. It is unclear at this stage why two radioprotectors with similar modes of action produced different inversion effects. The inversion frequency was significantly reduced to below the endogenous inversion frequency in animals receiving amifostine (100 or 400 mg/kg) 3 hrs post-irradiation in a similar manner to amifostine alone.

Our results demonstrate the importance of determining the appropriate dose of radioprotectors in radiation protection. This research was funded by the Low Dose Radiation Research Program, Biological and Environmental Research (BER), U.S. Department of Energy, grant # DE-FG02-05ER64104 and by the Flinders Medical Centre Foundation.

(PS4135) ACE inhibition immediately following irradiation may increase GI morbidity and mortality. Mary F. Otterson, Shawn Leming, Jennifer Callison, John E. Moulder, Parvaneh Rafiee. Medical College of Wisconsin, Milwaukee, WI, USA.

ACE (Angiotensin converting enzyme) inhibitors have shown promise in the treatment of late radiation nephropathy and pneumonitis. We explored the effect of the ACE inhibitor enalaprilat (0.625 mg iv 2x/day) on the early gastrointestinal (GI) effects of radiation. Methods: Nine mixed breed dogs were instrumented with strain gauge transducers to record circular muscle contractions. Following recovery form surgery, control recordings were made. Animals were irradiated using parallel opposed lateral fields with a single dose of 6 Gy. Five animals received placebo and 4 received enalaprilat. Results: The frequency of vomiting and the contractions associated with vomiting (retrograde giant contractions, RGCs) were increased with radiation and this was not altered by enalaprilat. In the absence of enalaprilat, the contractions associated with diarrhea and abdominal cramping (giant migrating contractions, GMCs) peaked on post rad Day 2 and 3 ($p<0.05$, ANOVA, repeated measures). This effect was muted with enalaprilat. Enalaprilat and radiation produced bloody diarrhea and bloody emesis. All dogs receiving enalaprilat drooled excessively. Two of 4 dogs receiving enalaprilat were sacrificed during the first week after radiation; 1 of 4 was sacrificed during the second week. No animals in the control radiation group experienced bloody diarrhea or bloody emesis. None were sacrificed. Conclusions: Enalaprilat administered immediately after 6Gy increased the GI symptoms and lethality experienced by dogs, resulting in the death of 3 our of 4 experimental animals. The cause of death does not appear to be related to GI contractile changes. While ACE inhibitors show great promise for radiation nephropathy and pneumonitis, their use should be delayed until after the acute phase of radiation injury. The absorptive, secretary and crypt turnover of the GI tract are being investigated.

This work was supported by a cooperative agreement with NIAID (AI067734).

(PS4136) Amifostine modulates lethal and non-lethal toxicities induced in mice by gamma-ray and neutron exposure. Tatjana Paunesku[1], David Paunesku[1], Andrew Wahl[1], Yasushi Kataoka[2], David Grdina[2], Gayle E. Woloschak[1]. [1]Northwestern University, Chicago, IL, USA, [2]University of Chicago, Chicago, IL, USA.

We used statistical analysis to study effects of amifostine [S-2-(3-aminopropylamino) ethylphosphorothioic acid (WR2721)] and WR151327 [S-3-(3-methylaminopropylamino) propylphosphorothioic acid] radioprotectors on types and incidence of lethal and non-lethal toxicities induced in mice treated by gamma-ray or fission neutrons. We used for this study a pathology database that describee and cross-references type and source or radiation, dose, type and presence/absence of radioprotector treatment and tissue/animal morphology and pathology for 4000 mice. Radiation doses were 206 or 417 cGy gamma-rays or 10 or 40 cGy fission neutrons. We found that non-lethal toxicities modulated by amifostine that overlap between gamma-ray and neutron irradiations are Harderian gland non-tumor toxicity and vascular tumors. On the other hand, lethal toxicities modulated by amifostine that overlap between gamma-ray and neutron irradiations were development of liver tumors and Harderian gland tumors. Unique to gamma-ray non-lethal toxicities modulated by amifostine were acute infections, hemorrhages, lung and adrenal gland tumors, as well as development of secondary tumors. Unique to gamma-ray lethal toxicities

modulated by amifostine were development of cysts, chronic renal disease, hemorrhage, pneumonitis, non-thymic lymphoma, lung and kidney tumors. Unique to neutron non-lethal toxicities modulated by amifostine were development of bloody ascites, non-tumor toxicity to testis and epididymis as well as uterus, and tumors of Harderian gland, kidney and liver. Unique to neutron induced lethal toxicities was development of acute infections. Radioprotectors used bind electrostatically to the minor groove of DNA, stabilize damaged sites in DNA, facilitate their correct repair, and delay cell cycle progression thus decreasing mutation rate. These data suggest that toxicities not usually associated with increased mutation rate also benefit from use of radioprotectors.

(PS4137) Amifostine metabolite WR-1065 mitigates high and low LET radiation-induced genomic instability. Janet E. Baulch[1], Jaroslaw Dziegielewski[1], Jeffrey S. Murley[2], David J. Grdina[2], William F. Morgan[1]. [1]University of Maryland, Baltimore, MD, USA, [2]University of Chicago, Chicago, IL, USA.

In this study we used WR-1065 to determine how protection from direct effects of radiation exposure influences the subsequent genetic stability of surviving cells. WR-1065 is the active form of amifostine (WR-2721), one of the few radio-protectors currently in clinical use. Amifostine is also the only drug approved by the FDA as a protecting agent in the case of a radiation accident. Compounds that can protect cells and tissues from radiation injury are important for clinical use, in the event of an accidental or terrorist-generated radiation event, and for astronauts traveling in space. However, one of the major concerns regarding the use of radio-protective agents is that they may protect cells initially, but predispose surviving cells to increased genomic instability later. Effects of radiation-induced genomic instability include chromosomal rearrangements, micronuclei, gene amplification, gene mutations, and/or cell death. We evaluated the efficacy of WR-1065 in reducing either high or low linear energy transfer (LET) radiation-induced genomic instability (^{56}Fe ions and x-rays, respectively). WR-1065 protected cells from immediate radiation-induced effects (e.g. cell death) following either high or low LET radiation exposures. In addition, the drug decreased genetic instability in the progeny of irradiated cells, as measured by hyperrecombination/deletion events (green fluorescent protein assay), micronucleus induction, hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutagenesis and chromosomal aberrations. These observations indicate that WR-1065 may be utilized as an effective radio-protective drug without increasing genomic instability or, possibly, the risk of delayed carcinogenesis.

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(PS4138) Epithelial mesenchymal transition (emt) in radiation (rt) induced pulmonary fibrosis. Isabel Jackson[1], Vasily Yakovlev[2], Ross Mikkelsen[2], Mitchell S. Anscher[2], Zeljko Vujaskovic[1]. [1]Duke University Medical Center, Durham, NC, USA, [2]Medical College of Virginia, Richmond, VA, USA.

Objective: To investigate EMT in irradiated lungs harvested from animals receiving single or fractionated RT with or without administration of anti-TGFβ antibody (1D11, Genzyme Corp.). We hypothesize RT-induced changes in cellular nitroxidative stress initiate events leading to activation of β-catenin via a p38 MAPK dependent pathway. To our knowledge, direct evidence for EMT has not previously been associated with RT-induced pulmonary injury.

Materials and Methods: Single dose of 28 Gy or fractionated (5 × 8 Gy) were delivered to the right lung of Fischer 344 rats using a 150 kV orthovoltage machine. Animals were sacrificed 1d, 3d, 6w, 10w, 14w, 20w or 6 months post radiation. The right lung was harvested and fixed for histology or snap frozen in liquid N_2 for molecular analysis. Animals receiving fractionated RT were randomized into groups to receive saline control or anti-TGFβ antibody (1D11).

Results: Immunohistochemistry (IHC) revealed significant collagen deposition and fibrosis, which could be attenuated with TGFβ antibody administration. However, the limited effectiveness of TGFβ/Smad inhibition suggests alternative pathophysiological mechanisms. In the single dose RT study, β-catenin mediated EMT, determined by α-SMA and cyclin D1 expression was significantly upregulated post-RT and increased throughout the observation period (20 weeks). β-catenin mediated EMT was increased following fractionated radiation which was unaffected by TGFβ/Smad inhibition. Protein levels of iNOS, and CTGF were elevated significantly (1 wk, 6 wks post-RT). In addition, western blot analysis demonstrated nitroxidative stress induced posttranslational modification and activation of p38 MAPK, an upstream activator of β-catenin. We are continuing to evaluate protein expression levels for Wnt, GSK3β, p-GSK3β, Lef1 and Lef1/ β-catenin interaction.

Conclusion: Herein, we have for the first time demonstrated EMT to be a potential mechanism leading to RT-induced lung fibrosis in a TGFβ independent manner. Targeting both pathways could be a more effective therapeutic strategy to ameliorate RT-induced lung injury.

(PS4139) CBLB600s: a family of novel compounds with radioprotective and hematopoetic stem cells stimulating activity, acting via activation of TLR2 receptor complexes. Frederic Bone, Eugenia Strom, Jason Young, Yevgeniy Kononov, Andrei Gudkov, Elena Feinstein, Alexander Shakhov. Cleveland Biolabs, Cleveland, OH, USA.

CBL is developing radiation countermeasures based on natural and artificial activators of Toll-like receptors. Lipoproteins from different bacteria stimulate cell surface TLR2-containing complexes. Synthetic analogues of the natural Mycoplasma lipopeptides were synthesized and proved to be equally effective. Both synthetic and natural bacterial lipopeptides were previously shown to activate B-cells, monocytes, neutrophils, and platelets and to act as potent immunoadjuvants *in vivo* and *in vitro*. We have investigated the properties of one the well-known synthetic lipopeptides, R,R-Pam_2Cys-SKKKK, designated CBLB601, as a radiation countermeasure in mouse model system. This drug was the first in the CBLB600 series of radioprotectors developed by optimization of the peptide portion of lipopeptides. CBLB600 drugs show outstanding radioprotective efficacy rescuing 100% of mice from mortality elicited by 10 Gy total body irradiation ($LD_{100/30}$) when injected 24 hrs prior to irradiation. DMF_{30} for CBLB600s obtained in optimal administration conditions is ~1.6. Significant radioprotection was also observed when the drugs were administered at any time between 48 hrs and 30 minutes prior to irradiation. At irradiation dose of $LD_{90/30}$, CBLB600s administered between 15 minutes and 9 hrs after irradiation also mitigate radiation injury rescuing up to 70% of mice. Radioprotection by CBLB600s is TLR2-dependent since the drugs are ineffective in TLR2 KO mice. Mice protected by CBLB600s survived for at least 9 months after lethal irradiation showing no signs of hematopoietic failure. This result was indicative for effective rescue of long-term repopulating hematopoietic stem cells (HSC) by CBLB600s and prompted us to test the influence of the drugs on HSC. Our data indicate that *in vivo* administration of CBLB600s to mice and monkeys not only results in increased amount of almost all HSC populations in bone marrow 24 h after injection but also in a robust accumulation of HSC in blood peaking at 72 hours after the drug administration. HSC stimulating and mobilization activity of CBLB600s may be attributed to increased production of G-CSF as detected in our assays and/or with direct stimulation of HSC via triggering TLR2 receptor (Nagai et al, 2006, *Immunity* **24**, 801–12)

(PS4140) Radioprotection of murine hematopoietic and human bone marrow cells by Ex-Rad, ON 01210.Na, a novel radiation protectant. Stephen C. Cosenza[1], A Kang[1], M Bonagura[1], M V. Reddy[1], M Maniar[2], A A. Alfieri[3], S Ghosh[4], K S. Kumar[4], E P. Reddy[1]. [1]Fels Institute for Cancer Research and Molecular Biology, Philadelphia, PA, USA, [2]Pharmaceutical Discovery & Development, Onconova Therapeutics, Princeton, NJ, USA, [3]Radiation Oncology, Albert Einstein College of

Medicine, Bronx, NY, USA, [4]Armed Forces Radiobiology Research Institute, USUHS, Bethesda, MD, USA.

Background: Radiological terrorism is an increasing threat to National Defense and Homeland Security. Available countermeasures, particularly those addressing free-radical scavenging, are limiting due to narrow time of administration prior to radiation exposure, severe toxicities, and difficulties of mass administration. Onconova Therapeutics is developing a small molecule synthetic compound Ex-RAD (ON 01210.Na). Radiation survival studies in mice have demonstrated that Ex-RAD protects mice from lethal exposure to radiation by protecting both the gastrointestinal and the hematopoietic systems by a mechanism involving enhancement of DNA repair and delay in apoptosis.

Purpose: Radioprotection by ON 01210.Na of murine hematopoietic cells *in vivo* and human bone marrow cells *in vitro* was studied.

Methods: C3H/HEJ mice were injected subcutaneously with various doses and schedules of ON 01210.Na prior to exposure of sublethal levels of radiation. Bone marrow cells were isolated on various days and the number of colony forming units (CFUs) was determined. Human bone marrow cells isolated from volunteers were treated with increasing concentrations of ON 01210.Na followed by exposure to radiation. The cells were plated and total number of CFUs was determined.

Results and Conclusions: When ExRad was injected either 24 hrs and 15 min before or only 15 min before irradiation, significant recoveries of both the colony forming units and the peripheral white blood cells of irradiated mice were noticed. The results also showed that the schedule and dose of ON 01210.Na that resulted in 100% survival correlated very closely with the recovery of the hematopoietic system. In addition to the *in vivo* model, we also investigated the ability of ON 01210.Na to protect normal human bone marrow cells using an *in vitro* model. Initial toxicity studies showed that ON 01210.Na treatment was not cytotoxic with exposures to high concentrations for 24 hrs. Radiation protection assays showed that ON 01210.Na protected human bone marrow cells in a dose dependent manner. This protection compared very favorably to protection studies using amifostine. These studies show that ON 01210.Na is safe and effective in the radioprotection of murine and human bone marrow cells.

(PS4141) Radioprotective effect of zinc yeast is P53 dependent manner to human lymphoblastoid cells. Yoshihiro Fujii[1], Takamitsu A. Kato[1], Akira Fujimori[1], Nobuo Kubota[2], Ryuichi Okayasu[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Ibaraki Prefectual University, Ibaraki, Japan.

It was shown that the administration of zinc-containing yeast (zinc-yeast) to the abdominal cavity significantly increased the survival of mice irradiated with 7.5 Gy X-rays ($LD_{50/30}$ assay result). In order to elucidate the molecular mechanism of this radioprotection effect, we examined TK6 (p53 wild type) and WTK1 (p53 mutant) lymphoblast cell lines exposed to 0.1mg/ml zinc-yeast. In TK6 cells, the cell growth was impaired after administration of zinc-yeast and the cells seem to accumulate at S/G2 phase in the cell cycle. On the other hand, in WTK1 cells, no effect on cell growth was observed. Furthermore, we measured the occurrence of apoptosis in X-irradiated cells (10 Gy) treated with zinc-yeast post-irradiation. In TK6 cells, a significant reduction in apoptosis was observed with the combined treatment, while there was no clear difference observed in WTK1 cells under the same treatment. These data indicate that the radio-protective effect induced by zinc-yeast might be affected by the p53 status of irradiated cells

(PS4142) Modulation of radiation induced haematological alterations in swiss albino mice by brassica compestris (var sarason) seed extract. Anil K. Soni, Manish Kumar, Shalini Shukla, Punar Dutt Meena, Madhu Kumar, Ashok Kumar. University of Rajasthan, Jaipur, India.

Introduction - The present study reports the protective effect of ethenolic Brassica compestris seed extract on radiation induced haematological and biochemical changes in Swiss albino mice. Brassica compestris seed extract was given orally for seven consecutive days prior to radiation exposure.

Materials and Methods - Adult male Swiss albino mice (8-weeks old) were obtained from the animal facility (JNU, New Delhi). The animals were provided with standard mice feed (Hindustan Lever Ltd., India) and tap water ad libitum. The colony was maintained at room temperature of 25 ± 2 0C and the light: dark exposure of 12 hr: 12 hr. The animals were whole body exposed to gamma radiation by a Co60 source (dose rate = 0.98 Gy/min), at a distance of 101.98 cm from the source at Department of Radiotherapy, SMS Medical College & Hospital, Jaipur, India.

Results-The dose reduction factor (DRF=1.59) for Brassica compestris seed extract was calculated from LD50/30 values. The haematological parameters were assessed at different intervals of post-irradiation from day 1 to 30. The average hemoglobin (Hb) level, total erythrocyte count (TEC) and total leucocyte count (TLC) in experimental group were significantly elevated as compared to the control group of animals. Also, Brassica compestris seed extract treatment significantly elevated reduced glutathione (GSH) level in liver against radiation-induced depletion. Treatment with Brassica seed extract caused a significant decrease in malondialdehyde (MDA) formation in the liver, suggesting its role in protection against radiation induced membrane and cellular damage.

Conclusion - The results of the present study suggest that Brassica compestris seed extract modulate the radiation induced haematological and biochemical alterations in Swiss albino mice.

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(PS4143) Biomimetic lanthanide & actinide decorporation agents: preclinical development. Patricia W. Durbin[1], Eleanor A. Blakely[1], David K. Shuh[1], Polly Y. Chang[2,1], Kenneth N. Raymond[3,1]. [1]Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [2]SRI International, Menlo Park, CA, USA, [3]University of California, Berkeley, CA, USA.

Therapy for radionuclide contamination of a large population by a dirty bomb or other event will require a cocktail of decorporation agents because of the variety of possible radionuclides and their chemical/biological properties. Decorporation is the only way to reduce the health effects of incorporated radionuclides. Fission product lanthanides and the actinides are among the most intractable of these elements to decorporate. Diethylenetriaminepentaacetic acid (DTPA) is the standard for actinide/lanthanide decorporation therapy since its development and use by the U.S. Atomic Energy Commission in the 1950′s, but it is limited in efficacy. A new family of sequestering agents has been developed using a biomimetic design based on the similar biochemical transport properties of plutonium(IV) and iron(III) and the siderophores, natural iron chelators of bacteria. These chelators are more selective and have higher affinity for plutonium(IV) and a number of other actinide metal ions. Toxicity and efficacy studies using a mouse model have been published, and limited tests have been done in rats and baboons. The results established that several of the new agents are much more effective than DTPA and, unlike DTPA, are orally active. This goal of this project is to take two lead compounds 3,4,3-LI-1,2-HOPO (an octadentate ligand) and 5-LIO-Me-3,2-HOPO (a tetradentate ligand) toward clinical use by scaling up the synthesis, establishing preparation methods suitable for good manufacturing practice (GMP), carrying out limited efficacy and toxicity studies for combinations of the two chelators in a mouse model, completing toxicity studies in human cell lines, and establishing preclinical safety in a rat model of the candidate ligands under good laboratory practice (GLP) guidelines. The objective of this research is to bring forth these two new decorporation agents in tandem and successfully accelerate their development to a pre-IND stage where only primate studies remain prior to a full IND application. Accomplished by an effective partnerships of Lawrence Berkeley National Laboratory (LBNL) that has expertise in ligand design, synthesis, and laboratory testing, with SRI International which possesses expertise in GLP testing and bringing pharmaceutical products to market. This work is supported by NIH #AI074065–01

(PS4144) Cytokine expression after 5-androstenediol administration and gamma-irradiation in mouse hematopoietic tissues in vivo. Vijay K. Singh, Marcy B. Grace, Kenneth O. Jacobsen, Cheng-Min Chang, Vaishali I. Parekh, Cynthia E. Inal, Randi L. Shafran, Alexander D. Whitnall, Tzu-Cheng Kao, William E. Jackson, Mark H. Whitnall. AFRRI, Bethesda, MD, USA.

The development of an effective pharmacological countermeasure is needed to reduce the morbidity and mortality in military and civilian populations associated with possible exposure to ionizing radiation. Previous studies in mice have shown that a single subcutaneous injection of the natural steroid androst-5-ene-3β,17β-diol (5-androstenediol, 5-AED), 24–48 h prior to a lethal dose of whole body ^{60}Co gamma-radiation, enhanced survival. 5-AED also induced proliferation of granulocyte-macrophage progenitors in bone marrow, elevations in numbers of circulating granulocytes, monocytes, natural killer cells, platelets, and red blood cells, and stimulation of innate immune cell function. The purpose of this study was to obtain evidence relevant to our hypothesis that 5-AED-induced cytokines may be responsible for these hematological and immune effects in normal and irradiated animals. We quantified mRNAs of cytokines in mouse spleen and bone marrow by quantitative real time PCR (QRT-PCR), and cytokine protein levels in serum by multiplex Luminex. We also studied the pharmacokinetics of 5-AED after sc administration and compared it with buccal delivery. 5-AED administration was associated with modulation of various cytokine mRNAs in conjunction with irradiation in bone marrow and spleen. Protein levels of granulocyte-colony stimulating factor were significantly elevated in 5-AED-treated mice 4 h after sham irradiation or irradiation as compared to vehicle control. Macrophage inflammatory protein-1γ (MIP-1γ) was significantly elevated 4 h after irradiation in 5-AED-treated mice compared to vehicle control. Plasma 5-AED peaked 2 h after sc injection (30 mg/kg), and remained significantly above control after 4 days, but not 8 days. The time course of plasma 5-AED after buccal delivery (60 mg/kg) was similar, but levels were significantly lower compared to sc delivery. Plasma 5-AED 24 h after administration was not significantly different between sc and buccal delivery. Since survival is enhanced when 5-AED is administered 24 h before irradiation by the sc but not the buccal route, the results suggest that radioprotection is not dependent on the 5-AED concentration at the time of irradiation, but rather on a cascade of cytokine-mediated effects triggered during the first few hours after administration.

(PS4145) Metastasis dissemination is mediated by CXCR4 receptor in HPV/E6+ cells and can be therapeutically controlled by combination of Cidofovir with irradiation. Amine Abdessamad, Bourhis J and Vozenin MC. Institut Gustave Roussy, Villejuif, France.

Because metastasis is the main cause of cancer death, new therapies that prevent metastatic dissemination are desirable. Here we show that the invasive phenotype of HPV/E6+ cells is mediated by the membrane-associated receptor CXCR4 and downstream activation of Rho/ROCK. Inhibition of CXCR4 signaling by a blocking antibody, the anti-viral agent Cidofovir (CDF)/ ionizing radiation (IR), or a ROCK inhibitor abolishes metastatic dissemination and could constitute an efficient anti-metastatic therapy for HPV/E6+ carcinoma.

Two HPV+ cell lines were used in this study: the human cervix carcinoma cell line, HeLa and the murin E6-transfected cell line, TC-1. Cells were irradiated (1Gy-9Gy) and exposed to CDF (10 μg/mL). In vivo metastasis assay was set up by injecting TC-1 cells intravenously to C57BL/6 mice. Follow up of pulmonary metastasis development was performed by histopathological analysis 2 weeks after injection. Before injection TC-1 cells were either irradiated, treated with CDF or irradiated and treated with CDF in vitro.

The first result of the present study was that ionizing radiation potently induced CXCR4 expression. In addition, this induction was associated with enhancement of the pro-invasive activity of HPV+ cells as assessed both in vitro using the matrigel assay and in vivo using the TC-1 model. Lastly, the CXCR4 blocking antibody (12G5) abrogated cell invasion into the matrigel, thus supporting the idea that radiation-induced CXCR4 expression stimulates HPV+ cell invasion. The direct involvement of the viral oncoprotein E6 and E7 in the radiation-induced CXCR4 up-regulation is currently under investigation.

The second result was that the association of CDF with IR, already known to induce E6 inhibition and subsequent p53 restoration, also control radiation-induced CXCR4 expression. In addition, as observed with the CXCR4 blocking antibody, CDF + IR abrogated cell invasion into the matrigel, thus suggesting that the anti-invasive action of the combination is at least partly mediated through CXCR4 inhibition. Studies are currently performed in the TC-1 lung metastasis model in order the confirm the relevance of the present findings in vivo

(PS4146) U87 glioblastoma cells are radiosensitized by double transfection with EGFR and PTEN. Phyllis R. Wachsberger[1], Rochelle Halko[1], Lindsay Uribe[1], Paul Mischel[2], Adam P. Dicker[1]. [1]Thomas Jefferson University, Philadelphia, PA, USA, [2]UCLA Medical Center, Los Angeles, CA, USA.

Introduction: Deletions of the PTEN tumor suppressor gene and/or overexpression or mutation of the EGF receptor (EGFR) occur in 30–40% of human glioblastoma (GBM) and lead to activation of PI3K/Akt and MAPK signaling pathways. Both MAPK and PKB/Akt signaling downstream can increase available pool of HIF-1α protein which has been reported to increase or decrease radiation-induced apoptosis, depending on tumor model. The objective of this study was to determine how signaling through EGFR and PTEN, either singly or doubly transfected into isogenic p53-positive U87 GBM cell lines may interact to determine relative radiosensitivities. An additional objective was to assess the effect of induced expression of EGFR and PTEN on HIF-1 α expression and radioresistance

Methods: Isogenic U87GBM (ATCC) cell lines were stably transfected with wildtype (wt) PTEN, wtEGFR, mutant EGFRv111 or a combination of wtPTEN and wt EGFR or EGFRv111. Relative radiosensitivities were determined by clonogenic cell survival and an apoptosis assay using a caspase inhbitor marker (CaspACE FITC-VAD-FMK in situ marker (Promega). HIF-1α, phosphorylated EGFR, Akt and Mapk protein levels were detected by western blots

Results: U87wtEGFR/wtPTEN exhibited the highest radiation sensitivity relative to the other transfected lines. The greatest difference in radiosensitivity was seen between U87PTEN (D10=10Gy) and U87EGFR/wtPTEN (D10 = 6.5 Gy). Blockade of EGFR by Iressa reverted the radioresponse of U87EGFR/wtPTEN to that of the more radioresistant U87PTEN phenotype. Apoptosis induction was highest (>80% of total cell population) in U87EGFR/wtPTEN compared to other transfectants 48 hr following 6 Gy. Under serum-free conditions, pAkt and pMapk protein levels were increased in U87wtEGFR compared to U87wtEGFR/wtPTEN 30 minutes following 6 Gy irradiation and addition of EGF. Hif-1 alpha protein levels were highest in U87PTEN 24h following 6 Gy and lowest in U87wtEGFR/wtPTEN

Conclusions: Survival in U87 GBM cells induced to overexpress EGFR is dependent upon EGFR-activated PI3K/Akt and Mapk signaling. When EGFR and PTEN are co-expressed, signaling through the network is diminished, leading to increased radiosensitivity and apoptosis

(PS4147) Spatio-temporal responses to different UV wavelengths in human skin organ culture. Eiichiro Mori[1], Akihisa Takahashi[1], Ken Ohnishi[1], Yoshiya Furusawa[2], Takeo Ohnishi[1]. [1]Nara Medical University School of Medicine, Nara, Japan, [2]National Institute of Radiological Science, Chiba, Japan.

Apoptosis plays an important role in eliminating damaged cells from populations when such cells have been subjected to damage from UV irradiation. The apoptotic process is regulated by the activation of pro-apoptotic genes and the down-regulation of anti-apoptotic genes. To learn more about the mechanisms of gene expression during apoptotic cell death in human skin organ culture, skin samples obtained during surgery were exposed to a series of UV doses at different wavelengths. UV irradiation was performed at the Okazaki Large Spectrograph (OLS) in the National Institute of

Basic Biology (NIBB) in Japan. First, apoptosis after UV irradiation at 280–365 nm was measured by the presence of chromatin condensation. This is considered present when the nucleus is less than 50% of the average relative size of the nucleus in untreated cell populations. At all wavelengths used in this study, the exposure-response curve for the apoptotic index increased in a dose-dependent manner. An action spectrum for the induction of apoptosis was obtained from the inverse values of D_{25} (exposures producing a 25% apoptosis induction) at each wavelength, normalized to that at 300 nm. The peak was observed at 300 nm in the spectra of D_{25}, and the sensitivity for apoptosis induction decreased along with increases in the wavelengths in the region at 300–320 nm, but no additional decrease was observed in the UVA region (>320 nm). The effectiveness of apoptosis induction at 300 nm was 1,000 times higher than at 320 nm. Furthermore, UV-induced expression level of genes related to apoptosis was immunohistochemically measured. At 3 hours after UV irradiation, p53 was phosphorylated at Ser 15 and Ser 46, and accumulated in the cell nuclei. Hsp90 levels were reduced at 3 hours after exposure, and a further reduction in its expression level was observed at 9 hours after UV irradiation; the same behavior was also observed for Hsp60 levels. Accumulations of Bax, active Caspase-3 and cleaved PARP were detected in apoptotic cells 24 hours post-exposure, along with a reduction of Bcl-2 levels. These results suggest that p53 and Bax are activated to suppress HSPs and Bcl-2 leading to efficient apoptosis in the presence of irreversible damage, while less extensive damage promotes cell cycle arrest and DNA repair by p53, coupled with HSPs and Bcl-2 accumulation.

(PS4148) PPARs reduction by γ-irradiation as a mechanism to inflammatory and immune process in rat colon. Christine Linard, Marc Benderitter. IRSN, Fontenay aux Roses, France.

Radiation-induced intestine injuries, including inflammation remain a limiting factor for pelvic radiotherapy effectiveness and patient quality of life during and after the radiotherapy. Previouly, we have shown that the irradiation-induced sustained local cytokine production influences the Th2 immune polarization-mediated pathologic development. Peroxisome proliferation-activated receptor (PPARs) agonists are now emerging as therapeutic drugs for various inflammatory diseases, characterized by impaired expression of PPARs. In addition, PPARs agonists have been known to affect immune responses, by regulating the Th1/Th2 balance. Using abdominal γ-irradiation (10Gy), the purpose of this study was to investigate the profile of PPARs expression in rat colonic mucosa. We tested the possibility to modulate pharmacologically both the inflammation and the Th polarization in the colon by using the anti-inflammatory properties of 5-aminosalicylic acid (5-ASA), activator of PPARs. Rats received a single abdominal dose of 10 Gy γ-irradiation. 5-ASA (250mg/kg/day) was administered orally once daily for 6 days, starting 7 days before irradiation. Analyses were performed in mucosa 3 and 7 days post-irradiation.

Irradiation induced a drastic repression (mRNA and protein levels) of PPARα, γ and the heterodimers RXRα 3 days post-irradiation. 5-ASA treatment limited both the PPARγ and RXRα repression at 3 days post-irradiation and contributed to over-expressed PPARα 7 days post-irradiation. By promoting the PPARs expression and translocation into nucleus, 5-ASA interfered with the NF-κB pathway by both reducing irradiation-induced NF-κB p65 translocation and increasing the IκB inhibitor protein expression (mRNA and protein). The consequence is that 5-ASA prevents irradiation-induced inflammatory process as well as TNF-α, MCP-1 and iNOS expression than macrophage infiltration at 3 and 7 days post-irradiation. In addition, 5-ASA modulated STAT1 pathway restoring the Th1 cytokine IFN-γ expression impaired at 7 days post-irradiation. Collectively, these results raise the possibility that PPARs agonist might be effective in the prevention of inflammatory process and immune response during and after pelvic radiotherapy.

(PS4149) Analysis of crosstalk between low-dose-radiation-induced signaling and insulin signaling in human breast cancer cells. Tetsuo Nakajima, Mitsuru Nenoi. National Institute of Radiological Sciences, Chiba, Japan.

Various cellular regulatory factors like growth hormones are considered to mediate cell proliferation and senescence in cells exposed to radiation, leading to cellular dysfunction and carcinogenesis. We have previously reported that, in the presence of insulin, MCF7 cells (human breast cancer cells) appear to be sensitive to low-dose irradiation (0.25 Gy) in MTS assays, which is based on measuring the total viable cell number. If how insulin modifies radiation-induced cell survival signaling is clarified in the cells, it is thought to be a unique model, to demonstrate signal cascades induced by low-dose irradiation. In this study, insulin-mediated sensitization of MCF7 cells was characterized by measuring cell survival using the MTS assay and colony-forming assays. Five hours after seeding, it was observed that insulin reduced cell survival in MTS assay. Using colony-forming assays, however, no change in the cell survival was observed. These results suggest that insulin induces growth inhibition in the cells after low-dose irradiation. In addition, it was observed that insulin also sensitizes to 0.25 Gy irradiation, 24 h after seeding, when cells have been fully attached to dishes and spread. This result implies that cell-cell contact and cell spreading have no effect on the insulin mediation. Since phosphatidylinositol 3 (PI3)-kinase-related cascades are known to participate in the insulin-related cascades, effects of a PI3 kinase inhibitor on the insulin mediation were evaluated. LY294002, one of PI3-kinase inhibitors sensitized to irradiation with 0.25 Gy in the absence of insulin, similarly to the insulin mediation. These results suggest that PI3-kinase works to maintain cell growth after low-dose-irradiation, and the function is possibly inhibited in the presence of insulin. To confirm the PI3-kinase participation and explore involvement of other cascades such as protein kinase C signaling in it, results of experiments using siRNA and microarray assays will be also discussed.

(PS4150) Possible intervention strategies to reduce the initiation and progression of radiation-induced atherosclerosis. Saske Hoving[1], Sylvia Heeneman[2], Hans te Poele[1], Jeffrey Pol[2], Nicola Russell[1], Marion Gijbels[2], Mat Daemen[2], Fiona Anne Stewart[1]. [1]Netherlands Cancer Institute, Amsterdam, The Netherlands, [2]Cardiovascular Research Institute Maastricht (CARIM), Maastricht, The Netherlands.

Earlier diagnosis and better treatment options have lead to improvements in cancer specific survival for most tumor types, but unfortunately this also results in an increased number of patients at risk for developing treatment related side effects. Several clinical trials have shown an increase in localized atherosclerosis after radiotherapy for Hodgkin's disease, breast and head and neck cancer and there is good evidence to identify radiation as an independent risk factor in vascular disease.

We previously demonstrated that local irradiation (1×8–14 Gy or 20×2 Gy) to the neck of mice lacking functional ApoE ($ApoE^{-/-}$) accelerated development of an inflammatory, thrombotic plaque phenotype. The aim of this study is to further investigate the mechanism underlying the development and progression of radiation-induced atherosclerosis and to explore the protective effect of the antiplatelet drugs aspirin (ASA) and novel nitric oxide releasing aspirin (NO-ASA), which reverse endothelial cell dysfunction and inhibit vasoconstriction, in addition to their anti-inflammatory and antithrombotic properties.

$ApoE^{-/-}$ mice were given single doses of 14 Gy or sham treatment (0 Gy) to the neck and the carotid arteries were harvested at 1, 4 and 30 weeks after irradiation. ASA and NO-ASA were given in the chow at doses known to produce antiplatelet or anti-inflammatory effects, from 1 week before irradiation until termination of the experiment. Specific immunohistochemical stainings are being performed to investigate expression levels of thrombotic and inflammatory markers of endothelial cell damage (e.g. thrombomodulin, ICAM-1, VCAM-1, P-selectin and KLF2) at early times after irradiation ± drugs. This damage will be correlated with the subsequent development of atherosclerotic lesions (plaque number, size and phenotype).

Carotid artery preparations are currently being evaluated and results should provide more insight into the mechanism of radiation-induced atherosclerosis and help in the design of effective

intervention strategies to prevent the development of atherosclerotic changes in patients following radiation therapy.

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(PS4151) Characterization of the role for p38 MAP kinase in the in vivo radiation-induced inflammatory response. Henghong Li[1], Hukjin Cha[1], Dmitry V. Bulavin[2], Albert Fornace[1]. [1]Georgetown University, Washington, DC, USA, [2]University of Singapore, Singapore, Singapore.

Ionizing radiation can induce an inflammatory response by activation of NFkB and consequent upregulation of TNF and cytokines. We and others have observed gene induction of pro-inflammatory cytokines, chemoattractants, adhesion molecules and matrix metalloproteinases in irradiated cultured cells. We also noticed moderately increased p38 MAP kinase activity. p38 plays a central role in the signaling pathway that regulates inflammation. While p38 is not induced rapidly in some cell types by ionizing radiation, we hypothesized that it may be critical for the radiation-induced inflammatory response in vivo since it is activated in some cell types including lymphoid cells. While the major isoform, p38α, is required for viability in mice, we have constructed a knock-in dominant-negative mutant of p38 (p38KI) that is viable but shows marked attenuation of p38 activation. By expression profiling in spleen and thymus from irradiated mice, expression profiling showed marked changes in radiation-induced gene expression in p38KI compared to wt. There was less induction of certain p53 downstream genes such as Cyclin G, p21 (Cdkn1a), Bax and Apaf1. p38KI also exhibited a reduction or no response for some inflammation-associated factors such as VCAM1 and MMPs, and transcripts for some enzyme involved in leukotrienes biosynthesis such as arachidonate 5-lipoxygenase. However microarray data from TK6 treated with SB239063, a specific p38 inhibitor, showed little difference compared to the untreated irradiated control, which suggests that p38 has no effect in IR induced gene expression regulation in the tissue culture system. These initial studies indicate that p38 signaling might have a critical role in the in vivo response to radiation, and offer potential targets in the treatment of radiation injury.

(PS4152) Molecular crosstalk between PIKK and MAPK in cellular response to ionizing radiation. Yanrong Su, Jarah A. Meador, Adayabalam S. Balajee. Columbia University, New York, NY, USA.

Exposure of cells to ionizing radiation (IR) activates a myriad of intra-cellular signaling pathways. Among them, the notable ones are mediated by phosphatidylinositol-3 kinase like kinases (PIKK) and mitogen activated protein kinases (MAPK). Although IR activates both of these pathways, the intrinsic molecular link between them in cellular radiation response is not clearly established. In this study, we have investigated the molecular link between PIKK and MAPK pathways in human cell lines after exposure to low and high doses of γ-rays radiation. To determine the molecular cross talk between PIKK and MAPK pathways, phosphorylation of MAPK family members was monitored by proteomic array based approach as a function of radiation dose in both PIKK proficient and deficient {ataxia telangiectasia mutated (ATM), ATM and Rad3 related (ATR) and DNA dependent protein kinase (DNA-PK)} human cell lines. The phospho-proteomic approach revealed distinct differences in the activation of MAPK members between PIKK proficient and deficient human cells. Most notably Hsp27, which is a downstream target of p38 MAP kinase, showed a radiation dose dependent increase in phosphorylation, which was detected even at a very low dose of γ-rays radiation (0.1Gy) in PIKK proficient primary human fibroblast cells (MRC5). In contrast, induction of phosphorylated Hsp27 was impaired in cells deficient in ATM, ATR and DNA-PK. Also, differences in the kinetic induction of phosphorylated forms of p38 MAP kinase (α,β,γ and δ) were observed between PIKK proficient and deficient cells. The results of this study suggest that PIKK modulates the activation of MAPK after IR and that the disruption of molecular link between MAPK and PIKK may predispose the cells to radiosensitivity.

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(PS4153) Identification and characterization of factors involved in delayed effects of radiation. David L. Springer[1], Jonathan S. Peters[1], Cheryl L. Baird[1], Donald S. Daly[1], Ronald J. Moore[1], Jin Shuangshuang[1], William F. Morgan[2], John H. Miller[3]. [1]Pacific Northwest National Lab., Richland, WA, USA, [2]University of Maryland, Baltimore, MD, USA, [3]Washington State University Tricities, Richland, WA, USA.

Evidence from *in vitro* tissue culture studies, *in vivo* animal models, and radiotherapy patients, indicated that a variety of biological effects, including genomic instability, occur outside the radiation field and to the progeny of irradiated cells. It has also been shown that medium conditioned by the growth of transformed GM10115 cells, made genomically unstable by radiation exposure is toxic to the parental cell lines. We hypothesized that the transformed cells produce one or more factors, most likely signaling proteins that are responsible for these adverse effects. To characterize the differences between stable and unstable CHO cells we are using global proteomic and bioinformatics approaches to determine the nature of the factor(s). Toward this end we have developed methods to identify up- and down-regulated proteins from whole cell preparation using mass spectrometry based proteomics data. For this we use ion current values along with restricted maximum likelihood statistical estimates. Our results indicate that the abundances of several proteins are altered in unstable cells relative to the stable parental cells. Current efforts are focused on bioinformatics approaches that help us identify the pathways and networks associated with the altered proteins and presumable involved in the genomic instability and related effects.

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(PS4154) Impact of partial marrow sparing on plasma inflammatory molecules after total body irradiation. Paul Okunieff, Weimin Sun, Shanmin Yang, Hengshan Zhang, Wei Wang, Chaomei Liu, Mei Zhang, Steven Swarts, Bruce Fenton, Lurong Zhang, Paul Okunieff. University of Rochester Medical Center, Rochester, NY, USA.

Total body irradiation (TBI) causes combined trauma involving multiple organs. Bone marrow (BM) and the gastrointestinal (GI) tract are the two most sensitive organs. Fortunately even very partial shielding of the BM can result in greatly improved tolerance in the case of sub-TBI. A major goal in biomarker research is to identify patterns of circulating protein expression that predict lethality. It is reasonable to expect that partial marrow shielding in an exposure event would greatly alter the levels of many circulating inflammatory molecules (IM). In this study, we explored the difference of IMs in mice exposed to TBI and sub-TBI.

Male BABL/c mice (7 weeks old) were exposed to 7 Gy of TBI (lethal) or sub-TBI (well tolerated) (5 mice/group). Two days later, the mice were sacrificed and the plasma was collected and subjected to membrane array in which 96 inflammatory molecules were detected with 96 paired specific antibodies using the FluoChem SP system, and then analyzed with GenePix Pro 6.0 software. Each sample was assayed in duplicate. The mean dot density of each sample was compared with that of six reference dots that were run simultaneously with the samples in the same membrane and had a consistent density in all membranes. The relative density ratio was compared between: 1) the normal mice (not receiving IR) and IR mice (either TBI or sub-TBI); and 2) the TBI and sub-TBI. The 96 IMs were classified into 7 categories. Significance was calculated by t-test and a $p<0.05$.

The data suggest that **1)** two days following 7 Gy TBI, the severe depletion of BM resulted a suppressed host IMs response, and 100% death in under 10 days (combined GI and BM death); **2)** the partial protection of bone marrow lead to 100% long term survival and a more pronounced IM response; **3)** there was some

similarity of the IM response to 7 Gy between the TBI and sub-TBI, indicating some common mechanisms of response; **4)** the IMs in chemokines category had the greatest number of molecules significantly changed, followed by the growth factors in both TBI and sub-TBI models; and **5)** about half of IMs remain unchanged, suggesting that host is using some mechanism to maintain the stability. The results suggest that the IM response of the host to a radiation event may be greatly altered by the protection of even a very small compartment of bone marrow.v

(PS4155) Biomarkers of radiation exposure: *ex vivo, in vitro* and *in vivo* studies. Karen Thomas[1], Paul Babyn[1], Diana Wilkinson[2], Wendy Doda[1], Hillary Boulay[2], Louise Prud'homme-Lalonde[2], Sylvie Lachapelle[2], Sami Qutob[3], Stacey Gibson[4], Louise Lemyre[4]. [1]The Hospital for Sick Children, Toronto, ON, Canada, [2]Defence R&D Canada, Ottawa, ON, Canada, [3]Consumer and Clinical Radiation Protection Bureau, Ottawa, ON, Canada, [4]University of Ottawa, Ottawa, ON, Canada.

In response to radiation, gene expression is expected to differentially affect the levels of systemic proteins secreted in the plasma and saliva of irradiated individuals. We hypothesized that the changes in concentrations of these secreted proteins may identify radiation responsive biomarkers for rapid screening of potentially irradiated individuals. However, preliminary studies on *ex vivo* irradiated blood samples did not identify any potential biomarkers of exposure. As a result, we concluded that a more complex system was required and developed a multi-cell culture system as a surrogate for *in vivo* studies to simulate systemic responses to radiation. At the same time, preliminary *in vivo* studies have been conducted on three different population cohorts; psychologically stressed individuals, radiologists exposed to very low doses of radiation, and paediatric patients exposed to radiation for the purpose of medical imaging. In all cases pre- and post-exposure samples were monitored for concentration changes in blood plasma and saliva proteins as well as $CD4^+$ and $CD8^+$ T-cell total numbers and cell ratios. With these preliminary *ex vivo*, *in vitro* and *in vivo* studies we hope to be able to narrow the panel of potential markers for rapid screening and identification of irradiated individuals. (Funded by CRTI Project #0027RD)

(PS4156) Role of TNF-alpha in radiation-induced bystander effects. Vladimir N. Ivanov, Hongning Zhou, and Tom K. Hei. Center for Radiological Research, College of Physicians and Surgeons, Columbia University, New York, NY10032

Although radiation-induced bystander effects have been demonstrated with a variety of biological endpoints in many rodent and human cell lines, the precise mechanism of this phenomenon is not known. There is evidence that cyclooxygenase-2 (COX-2) signaling pathway mediates the bystander effects in non-irradiated human lung fibroblasts co-cultured with alpha particle irradiated cells. Since TNF-α in concert with IL1-β is a powerful inducer of COX-2 expression via the NF-κB pathway, we focused our attention on this combination of the cytokines as possible mediators of signaling transmission from irradiated to bystander cells. Monoclonal inhibitory antibody against TNF-α substantially suppressed COX-2 expression in bystander cells via inhibition of the NF-κB pathway. Bay 11–7082, a pharmacological inhibitor of NF-κB activation, demonstrated a similar effect in suppressing COX-2 activities. Furthermore, such inhibition of NF-κB in bystander cells made these cells very sensitive to apoptotic signaling induced by TNF-α. Suppression of the direct cell-cell interactions by gap junction inhibitor octanol also blocked TNF-dependent bystander effects, indicating that membrane form of TNF-α might be involved in mediating bystander signaling. TNF-α expression is known to be induced in many types of cells following stress conditions. TNF-α controls expression of numerous NF-κB-dependent genes, which regulate both survival and damaging functions in the cells, including ROS/RNS production. Role of this cytokine in radiation-induced bystander effects suggest a possible protective function of anti-TNF mAb in inhibition of TNF-mediated inflammatory reaction and apoptosis.

(PS4157) Proton radiation induced fibrosis: effects of protein kinase C on integrin expression. Pinal R. Pandya[1,2], Virginia GC Serra[1,3], Leticia S. Ortloff[2], Lora M. Green[1,4]. [1]Loma Linda University, Loma Linda, CA, USA, [2]Radiobiology Program-Radiation Medicine Loma Linda University, Loma Linda, CA, USA, [3]Radiobiology Program-Radiation Medicine Loma Linda University, Loma Linda, CA, USA, [4]JL Pettis Memorial Veterans Medical Center, Loma Linda, CA, USA.

The sequences of events in fibrosis are similar to those in wound healing however; the normal termination and resolution stages do not take place. The initial cellular response following ionizing radiation involves accumulation of the ECM (extra cellular matrix) including collagen, fibronectin and the interaction of many growth factors (cytokines) with their receptors. There are a number of unanswered questions regarding many aspects of radiation-induced fibrosis, including the initial triggers and physical changes that initiate the process. We have confirmed that elevated pKC and other cellular changes following radiation are similar to those found at sites of inflammation. This information leads us to the following hypothesis. We hypothesize that radiation-induced fibrosis is in part a result of altered signal transduction which directly modulates integrin expression and may indirectly effect ECM elaboration. Our objective was to determine whether the increase in pKC post radiation, leads to an alteration in integrin expression, which may contribute to fibrosis. To test our goal, we used thyroid tissue from Lewis Rats.

The animals were proton irradiated in the neck region with therapeutic dose of 40Gy delivered either as 5Gy per day (8 days) and 10Gy per day (4 days) fractions. The animals were sacrificed 1 week and 11 weeks post radiation and thyroid tissue was extracted. We performed H&E staining to document changes in the thyroid tissue pre and post irradiation. To visualize ECM accumulation, especially collagen, we performed trichrome staining. Immunohistochemistry was performed to quantify the levels of several isoforms of pKC, integrins, and ECM proteins fibronectin, collagen I, II, III and IV and laminin.

We measured an accumulation of ECM in the thyroid tissue, which was coincident with a loss of tissue organization and follicularization. There were increased levels of several pKC isoforms post irradiation, which coincided with modulation of integrin expression. Levels of fibronectin, laminin and collagen proteins were also altered. In vitro modulation of thyroid cultures supported the direct role of pKC in these altered properties. Collectively the results document that pKC contributes to alteration of key players in thyroid tissue structures following radiation-induced fibrotic changes.

(PS4158) Radiation-induced sialyltransferase involves in radioresistance. Minyoung Lee, Hae-June Lee, Yun-Sil Lee. Laboratory of Radiation effect, Korea Institute of Radiological and Medical Sciences, Seoul, 139–706, Korea

Recently, we demonstrated that the radiation-induced gene alterations in C57BL6 mice were organ specific and highly related to apoptosis. Among the tissue-specific genes, β-galactoside α-2, 6-sialyltransferase 1 (ST6 Gal I) was one of the candidates for radiation detection markers in spleen and intestine which are the radiosensitive organs. ST6 Gal I is a member of glycosyltransferase that catalyzes the transfer of sialic acid from CMP-sialic acid to lactosaminic termini of glycoproteins. The encoded protein which is localized in Golgi, can be proteolytically processed to a soluble form, is involved in the generation of cell-surface carbohydrate determinates. Here, we present evidences that ionizing radiation significantly induced the ST6 Gal I expression in spleen and in cell lines (U937, Daudi and Raw264.7). We also attempted to characterize the sialylated proteins, especially integrin β1, CD71 (Transferrin receptor), and CD45 (Protein tyrosine phosphatase), as well as their roles in radiation-induced apoptosis using ST6 Gal I-expressing clones of SW480, human colon cancer cell line. ST6 Gal I transfectants was more resistant to radiation exposure than vector-control cells. Furthermore, integrin β1, which is implicated for cell survival, was increased by overexpression of ST6 Gal I or exposure to ionizing radiation. These data suggested that ST6 Gal I is induced by radiation, which further implies its intrinsic involvement in defense mechanisms after radiation.

(PS4159) The chemopreventive agent Curcumin, is a potent radiosensitizer of human cervical tumor cells by a mechanism that involves increased ROS production and overactivation of the MAPK pathway. Prashanthi Javvadi. University of Pennsylvania, Philadelphia, PA, USA.

Cervical cancer is the second most common malignancy among women and is highly radioresistant. For locally advanced disease, radiation combined with chemotherapy is the standard of care. Curcumin, is a natural chemopreventive agent which in phase I clinical trials showed minimal toxicities. We therefore investigated if curcumin radiosensitizes cervical tumor cells. Using two cervical carcinoma cell lines, HeLa and SiHa, we found that pretreatment with curcumin resulted in a time- and dose-dependent radiosensitization with Dose Enhancing Ratios ranging from 1.4 to 1.6. In contrast, pretreatment of normal human fibroblast did not radiosensitize but in fact protected these cells from radiation-induced death.

To identify the molecular targets involved, we investigated the effects of curcumin on IR-induced NF-κB and AKT activation, which are known to be involved in radioresistance. Curcumin pretreatment did not significantly inhibit NF-κB activity and unlike the PI-3 kinase inhibitor LY294002, it did not inhibit the basal or IR-induced AKTphosphorylation on ser473, suggesting that radiosensitization by curcumin is not caused by inhibition of NF-κB or AKT activity. However, pretreatment with curcumin did cause a normally transient IR-induced ERK1/2 phosphorylation to extend for up to 6 hours after radiation. Since overactivation of ERK1/2 can result in increased apoptosis, these results suggest that this event may contribute to curcumin-dependent radiosensitization.

Curcumin is a polyphenolic compound which can have both anti- and pro-oxidant activities, depending on the cellular redox status. Pretreatment with the free radical scavenger N-acetyl cysteine (NAC) prior to IR, nearly abolished curcumin-induced radiosensitization, indicating that curcumin radiosensitizes tumor cells at least a part through an increase in cellular oxidative stress. Interestingly, NAC also abolished the prolonged phosphorylation of ERK1/2, further suggesting a link between ROS production, ERK activation and radiosensitization.

We are expanding these studies to *in vivo* xenograft models of cervical carcinoma. Positive results from these *in vitro* and *in vivo* studies will set the stage for clinical trials using curcumin for better radiotherapeutic management for patients with advanced cervical cancer.

(PS4160) Molecular switches of the cytogenetic radioadaptive response in human cells. Francesco Marchetti[1], Sanchita Bhattacharya[1], Matthew A. Coleman[2], Andrew J. Wyrobek[1]. [1]Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [2]Lawrence Livermore National Laboratory, Livermore, CA, USA.

Preexposure to low doses of ionizing radiation (<0.1 Gy; priming dose) can confer protection against the damaging effects of subsequent exposure to a high dose of radiation (>2 Gy; challenging dose), a phenomenon known as radioadaptation. Previously, we showed that human lymphoblastoid cell lines differed in their ability to mount the radioadaptive response and identified a set of genes whose expression levels was associated with radioadaptation (Coleman et al, 2005, Rad Res 164:369–387). Subsequent bioinformatics analyses provided further mechanistic insight into the networks and biochemical pathways that control the radioadaptive response. Of the 301 genes that were modulated in response to radiation exposure, 138 genes were expressed similarly in three cell lines independently of the radioadaptive outcome (priming dose genes). Another 163 genes had similar expression in the two cell lines that adapted and different expression in the cell line that did not adapt and were therefore predictive of the radioadaptive outcome (adaptation genes). The adaptation genes were assigned to 7 networks (p-value range: 10^{-67} to 10^{-7}) and the top network contained the MYC gene as the central node. The affected genes in the MYC-network are associated with various signaling pathways (ERK/MAPK, p38 MAPK, PDGF, IL-6 and EGF). Pathway analyses found little functional overlap between the priming dose and adaptation genes. Priming dose genes were primarily associated with ribosome functions while adaptation genes were mainly associated with signaling pathways. Our analyses suggest that the cytogenetic radioadaptive response is controlled by a molecular switch in which TP53 function is required during the time interval between the priming and challenging doses and down regulation of MYC-associated functions is an essential component after the challenging dose. Further studies are needed to study the protein components and mechanistic properties of these radioadaptive response switches. *Supported by the DOE Low Dose Program.*

(PS4161) Transcriptional analysis of the effect of ionising radiation at the gastrula stage. Abderrafi Benotmane, Arlette Michaux, Ann Janssen, Jasmine Buset, Mieke Neefs, Paul Jacquet. SCK•CEN, Mol, Belgium.

Early postimplantation development in mammals is associated with a dramatic increase in the proliferation rate of undifferentiated stem cells that form the primary embryonic layers, ectoderm, endoderm and mesoderm, and with the start of differentiation of the embryo. Interestingly, several studies reported the upregulation of *ATM* and *p53* in both the embryonic and extraembryonic region within 1 h post-irradiation in "wild-type" embryos, but only in the embryonic region did this upregulation lead to the induction of apoptosis. What are the differences between the embryonic and extraembryonic lineages that lead to a difference in the ability to undergo apoptosis in response to DNA damage is not known. There are a number of genes that have distinct expression patterns in the embryonic versus extraembryonic regions during the pre-gastrula period, however, whether any of these genes are responsible remains to be determined.

Gene expression analysis have been performed in our laboratory to analyse the differential expression induced 2h after 0.5Gy whole body irradiation between the embryonic and extraembryonic parts of the gastrula. A high number of modulated genes are down-regulated in the embryonic part, while the same genes are up-regulated in the extraembryonic part. This may explain the high sensitivity of the embryo at this stage and the role of the extraembryonic part in its protection. Down-regulation of several function at this stage might involve drastic consequences on the survival and further development.

(PS4162) Transcriptional regulation of endothelial cell thrombomodulin by statins. Qiang Fu[1], Junru Wang[1], Marjan Boerma[1], Xiaohua Qiu[1], Louis M. Fink[2], Martin Hauer-Jensen[1]. [1]Arkansas Cancer Research Center, Little Rock, AR, USA, [2]Nevada Cancer Institute, Las Vegas, NV, USA.

Background: Normal tissue radiation injury is associated with deficient levels of thrombomodulin (TM), an important anticoagulant and anti-inflammatory endothelial glycoprotein. Statins upregulate TM and ameliorate normal tissue radiation toxicity. We investigated the mechanism by which statins regulate endothelial TM.

Methods: Human endothelial cells were studied *in vitro*. Relevant transcription factor binding sites in the TM promoter were identified by deletion/mutation analysis of reporter constructs. Subsequently, detailed analyses were performed using immunoprecipitation assay, western blotting, and chromatin immunoprecipitation assay in combination with specific signaling pathway modulators and/or siRNA knockdown.

Results: Atorvastatin dissociated heat shock factor 1 (HSF1) from heat shock protein 90 (HSP90), an effect that was mimicked by NO-donors and blocked by an NO scavenger. Subsequent nuclear translocation of HSF1 and binding of HSF1 to specific heat shock elements (HSEs) in the TM promoter were essential and sufficient to increase TM expression. Inhibition of HSF1 by KNK437, in addition to inhibiting upregulation of TM, also inhibited upregulation of tissue plasminogen activator (tPA), but did not influence statin-induced downregulation of thrombomospondin (TSP), plasminogen activator inhibitor 1 (PAI-1), or connective tissue growth factor (CTGF). Knockdown of the HSF1 chaperone, 14-3-3β, by siRNA and inhibition of HSF1 phosphorylation by the MEK-inhibitor, PD98059, further enhanced the effect

of statin on TM and tPA, but did not affect downregulation of TSP, PAI-1, or CTGF.

Conclusion: Statins upregulate endothelial TM by a mechanism that involves NO-dependent dissociation of HSF1 from HSP90, nuclear translocation of HSF1, and activation of specific HSEs in the TM promoter. Knockdown of the HSF1 chaperone, 14-3-3β, or inhibition of nuclear export of HSF1 further enhances the effect of statins on TM. Analogous HSF1-dependent mechanisms appear to apply to genes that are upregulated by statins, but not to genes that are downregulated by statins. These findings have broad implications and point toward opportunities for individual regulation of pleiotropic statin effects.

(PS4163) Involvement multiple factors in regulation of the *CDKN1A* gene promoter in response to ionizing radiation. Mitsuru Nenoi[1], Kazuhiro Daino[2], Tetsuo Nakajima[1], Keiko Taki[1], Ayana Kakimoto[3]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]CEA, Fontenay-aux-Roses, France, [3]Tokyo University of Science, Noda, Japan.

TP53 is a tumor suppressor protein functioning as a transcription factor to regulate plenty of genes involved in physiological responses to DNA damages, such as apoptosis, cell cycle arrest, DNA repair, etc. When induction of various TP53-target genes in various organs of mouse following whole body exposure to ionizing radiation (IR) was examined, it was shown that, in a same organ, induction varies from gene to gene, and, for a same gene, induction varies from organ to organ. Selective regulation of TP53-target genes may be the basis for the multi-functional property of TP53. In this regards, it is noteworthy that some transcription factors, such as Sp1, GKLF, Ets1, and IRF-1, have been reported to play a role in TP53-dependent transcriptional activation. It may be hypothesized that variable transcription factors and cofactors participate in regulation of TP53-target genes depending on organs, their physiological conditions, radiation doses, dose-rates and so on. We here comprehensively investigated the factors involved in regulation of a TP53-target gene *CDKN1A*(*p21*) after irradiation. We first carried out an electrophoretic mobility shift assay to analyze DNA-protein interaction in the upstream regions of the *CDKN1A* gene using 115 probes covering the region of approximately 2.9 kb in length. A drastic change of the DNA-protein interaction was observed at −2193bp/-2185bp which is closely located to the TP53 recognition site at −2261bp. Interestingly alteration of the DNA-protein interaction at −1368bp/-1333bp and −1268bp/-1233bp was observed only after irradiation with IR in the dose range of 0.2–2 Gy. Next, we carried out a reporter gene analysis using variously deleted *CDKN1A* gene promoter linked to the luciferase-encoding sequence. It was found that deletion of the region −1398bp/-1119bp drastically diminished the induction of the *CDKN1A* gene promoter after IR in the dose range of 0.2–2 Gy. As this region contains the loci where alteration of the DNA-protein interaction occurred after IR in the dose range of 0.2–2 Gy, it was suggested that some transcription factors, inducible after IR of this dose range, bind DNA at the loci −1368bp/-1333bp and −1268bp/-1233bp to regulate the *CDKN1A* gene promoter in cooperation with TP53.

(PS4164) Role of the WW binding motif of the EGR-1 in its binding and transactivation function. Anna Reeves[1], Marius Sudol[1], Mark Bedford[2], Mohammed Momin Shareef[1], Mansoor M. Ahmed[1]. [1]Weis Research Center, Danville, PA, USA, [2]M.D. Anderson Cancer Center, Smithville, TX, USA.

Ionizing radiation (IR) exposure is associated with activation of certain immediate-early genes including EGR-1. The EGR-1 is composed of two modules: the DNA-binding domain and the NH_2-terminal transactivation domain. EGR-1 was found to be induced by IR. Because p53 protein, the major component of the IR-induced apoptosis, is mutated in a large number of prostate tumors, it's important to identify other proapoptotic genes that can function via a p53-independent mechanism to induce the expression of such genes for the control of radio-resistant tumors. It has been shown that in the absence of p53, IR-inducible apoptosis is mediated via TNF-alpha transactivation. One of the ways the transcription activation domains transmit their effects to target genes is protein-protein interaction mediated by functionally and structurally distinct domains. Among these protein modules, the WW domain (also called the WWP or Rsp5 domain).WW domain recognizes and binds to a short oligopeptide called the PPxY, to mediate protein-protein interactions. This motif is present in the transcription activation domains of a wide range of transcription factors suggesting that it plays an important role in transcriptional activation. We have identified the PPxY motif in the transactivation domain of the EGR-1. Our hypothesis was that tyrosine phosphorylation of the PPxY motif of the EGR-1 may play a role in regulating the radiation-induced transactivation of proapoptotic genes in prostate cancer cells. To test our hypothesis we mutated one or both tyrosines in this motif in plasmid construct containing EGR-1 cDNA. We then co-transfected mutant constructs with the reporter construct, EBS-CAT containing three EGR-1-binding sites into p53-null prostate cancer cell line (PC-3), which was found to be moderately resistant to radiation-inducible apoptosis. We have demonstrated that inactivation of even one tyrosine resulted in significant down-regulation of the reporter gene activity in transfected cells. IR further inhibited the reporter gene activity. Our findings demonstrate that WW-binding domain of EGR-1 might play a role in regulating the balance between survival/ apoptosis response to radiation. Western blot analysis and immunohistochemistry studies are underway to determine the expression of prosurvival/ proapoptotic genes.

(PS4165) Gene expression in the spleen of mice after irradiation with middle-dose-rate γ-rays. Takashi Sugihara[1], Hayato Murano[2], Kimio Tanaka[1], Yoichi Oghiso[1]. [1]Institute for Environmental Sciences, Rokkasho, Aomori, Japan, [2]Tohoku Environmental Science Service Corporation, Mutsu, Aomori, Japan.

Serial gene expression after long-term γ-ray irradiation with low-dose-rate (< 0.1 mGy/min) or middle-dose-rate (0.1 - 99mGy/min) in murine tissues remains to be elucidated. Based on the results of our previous microarray analyses using murine NIH3T3/PG13Luc cells, 5 genes, including Trp53-dependent genes (*CyclinG1*/*Ccng1* and *p21*/*Cdkn1a*) and extra-cellular matrix-related genes (*Tnc*, *Col1a2* and *Fbln5*), were selected as markers to evaluate gene expressions in spleens of mice after irradiation. Female C3H mice were irradiated for 1- 20 days at 400 mGy/22 hr/day. Time changes of the gene expression levels were serially observed by the real-time PCR method. The Trp53-dependent genes (*Ccng1*, *Cdkn1a*) were up-regulated in the dose range higher than 0.8 Gy. On the other hand, extra-cellular matrix related-genes (*Col1a2*, *Tnc* and *Fbln5*) were up-regulated in the dose range higher than 4.0 Gy. Gene expression profiles of spleen cells from irradiated mice were quite similar to those in murine cell line irradiated at middle-dose rates. These results indicate that the up-regulation pathways for extra-cellular matrix-related genes in murine tissues are different from those of up-regulation for Trp53-dependent genes after middle-dose-rate γ-ray irradiation. (This work was supported by a grant from Aomori Prefecture, Japan.)

(PS4166) Nf-κB-mediated her-2 overexpression promotes radioresistance. Ning Cao, Ming Fan, Kazi Mokim Ahmed, Jian Jian Li. Purdue University, West Lafayette, IN, USA.

Decreased tumor sensitivity to radiotherapy causes an obstacle for improving the survival rates in cancer patients. The mechanism underlying the adaptive tumor radioresistance remains elusive. Breast cancers with high expression of *HER-2* (human epidermal growth factor receptor-2; a proto-oncogene) show a resistant phenotype associated with rapid cancer progression and poor prognosis. We previously reported that *HER-2* overexpression reduces breast cancer cell sensitivity to radiation and enhances radiation-induced transcription factor NF-κB activity in breast cancer cells. In the present study, we found that both *HER-2* mRNA and HER-2 protein levels were increased in breast cancer MCF-7 and MDA-MB-231 cells after exposure to therapeutic radiation. The newly established radioresistant MDA+FIR clones showed hetero-

genic adaptive radioresistance, and cells with higher radioresistance expressed more HER-2. siRNA-mediated suppression of *HER-2* markedly reduced radiation-induced *HER-2* expression and re-sensitized MDA+FIR clones (derived from therapeutic-mimic ionizing radiation treated MDA-MB-231 cells) to radiation, indicating that HER-2 plays a crucial role in adaptive radioresistance. In addition, we also found that deletion of NF-κB binding site located in the *HER-2* promoter significantly diminished radiation-induced *HER-2* promoter activity. Inactivation of NF-κB with an IKK-β inhibitor (IMD-0354) markedly decreased both basal and radiation-induced *HER-2* promoter activity with substantial reduction of basal and radiation-induced HER-2 protein expression. Together, these results provide the first evidence that the NF-κB-mediated up-regulation of *HER-2* plays an important role in development of the adaptive radioresistance in breast cancer cells. Thus, the NF-κB/HER-2 pathways can be targeted to develop novel therapeutic agents to prevent and re-sensitize HER-2-associated tumor radioresistance.

(PS4167) Analysis of gene expression profiles in mice exposed to low-dose rate radiation. Keiko Taki[1], Bing Wang[1], Tetsuo Nakajima[1], Jianyu Wu[1], Tetsuya Ono[2], Tsuneya Matsumoto[3], Yoichi oghiso[3], Kimio Tanaka[3], Kazuaki Ichinohe[3], Shingo Nakamura[3], Satoshi Tanaka[3], Mitsuru Nenoi[1]. [1]National Institute of Radiological Sciences, Chiba, Japan, [2]Tohoku University, Sendai, Japan, [3]Institute for Environmental Sciences, Aomori, Japan.

Risk assessment of low-dose rate radiation is an important issue. Tanaka et. al. have previously reported the late effects of chronic exposure to low-dose rate gamma rays as assayed by life span using 4000 mice (*Rad. Res.* (2003)160, 376–379). Statistical analyses showed that the life spans of mice of both sexes irradiated with 21 mGy day^{-1} ($P < 0.0001$) and of females irradiated with 1.1 mGy day^{-1} ($P < 0.05$) were significantly shorter than those of the control group.

In this study, we investigate the molecular mechanisms of this shortening of life span under continuous low-dose rate irradiation in mice. We followed experimental conditions of Tanaka et. al. C57BL/6J mice were exposed to gamma rays at the low-dose rates of 32nGy/min (21mGy), 650nGy/min (420mGy), 13uGy/min (8.0Gy) continuously. RNA was extracted from kidney of irradiated or on-irradiated mice, and gene expression profiles were determined using Illumina Sentric Mouse-6 microarrays. The 54 genes have been extracted with a difference in expression by 1.6 times or more after irradiation. The pathway analysis was done for the gene of each 54. In the irradiated groups at 650 nGy/min (total dose 420 mGy) and 13 uGy/min (total dose 8 Gy), 4 genes categorized to the mitochondrial oxidative phosphorylation pathway were activated. These results strongly suggest one possibility that the reactive oxygen produced by activated mitochondrial oxidative phosphorylation after continuous low-dose rate irradiation is a cause for the shortening of life span.

(PS4168) Single versus fractionated doses of radiation lead to differences in gene expression in human tumor cell lines. John A. Cook[1], Mong-Hsun Tsai[2], Gadisetti V.R. Chandramouli[1], William DeGraff[1], C. Norman Coleman[1], Eric Y. Chuang[3], James B. Mitchell[1]. [1]NCI, Bethesda, MD, USA, [2]National Taiwan University, Taipei, Taiwan, [3]National Tawian University, Taipei, Taiwan.

Studies were conducted to determine whether gene expression profiles following a single dose of radiation would yield equivalent gene expression profiles following fractionated radiation in different tumor cell lines. MCF7 (breast), DU145 (prostate), and SF539 (gliosarcoma) cells were exposed to a total radiation dose of 10 Gy administered as a single dose (SD) or by daily multi-fractions (MF) of 5 × 2 Gy. Following radiation treatment, mRNA was isolated at 1, 4, 10, and 24 hr and processed for cDNA microarray analysis. To determine the influence of the tumor microenvironment on gene expression one cell type (DU145) was evaluated growing as a solid tumor in athymic nude mice for both radiation protocols. Unsupervised hierarchical cluster map analysis demonstrated significant differences in gene expression profiles between SD and MF treatments for cells treated in vitro, with MF yielding a more robust induction compared to SD. Genes associated with *p53* were induced in MCF7 cells (normal *p53*) after either SD or MF exposures. Both the SF539 and DU145 showed no *p53* response after either SD or MF exposure. Only 13 out of 463 genes were up-regulated by at least 2 fold in all the cell lines studied, predominately by MF exposure. Seven out of the 13 common genes were *IFN* (interferon)-associated genes including *OAS3*, *G1P3*, *G1P2*, *IFIT2*, *IFITM1*, *BST2*, and *LGALS3PB*. Importantly, *STAT1* (a key signalling gene regulated by IFN) was elevated by MF exposure in the 3 cell lines. MF also up-regulated *TGF-β* associated genes (*EGR1*, *VEGF*, *THBS1*, and *TGFB2*) in the SF539 cells. DU145 cells grown in vivo exhibited a completely different set of genes induced by both SD and MF compared to the same cells exposed in vitro. The results of the study clearly show distinct differences in the molecular response of cells between SD and MF radiation exposures and demonstrate that the tumor microenvironment can significantly influence the pattern of gene expression after radiation exposures.

(PS4169) Low-dose rate photons and simulated solar particle event protons: gene expression in liver. Daila S. Gridley, Asma Rizvi, Adeola Y. Makinde, Xian Luo, Jian Tian, Melba Andres, George B. Coutrakon, Michael J. Pecaut. Loma Linda University & Medical Center, Loma Linda, CA, USA.

Introduction/Purpose. Low-dose rate background radiation combined with a solar particle event (SPE) during space exploration can result in exposure to relatively large radiation doses. The goal of this study was to evaluate gene expression in the liver, an organ that is vital in many biological processes.

Experimental Procedures. C57BL6 mice (n = 44) were whole-body irradiated with simulated SPE protons (sSPE), both with and without low-dose rate photons (LDR). There were 4 groups: a) 0Gy control, b) LDR, c) sSPE and d) LDR+sSPE. The LDR was delivered to 0.049 gray (Gy) at 0.024 cGy/hr using 57-Co flood sources; sSPE was delivered to 2Gy over 36h at 25–215 MeV in 10 MeV increments. The liver was excised from subsets/group immediately after irradiation (day 0) and on day 21 thereafter and analyzed for 84 genes involved in hypoxia-related signaling using a real-time polymerase chain reaction (RT-PCR) array. Genes up-/down-regulated by >2-fold were noted.

Results. On day 0, few genes were up-regulated: LDR - Birc5 (survivin); sSPE - Id1, Mylpf; LDR+sSPE - Bax, Id1, Snrp70. Genes down-regulated in all 3 irradiated groups at this same time point were Arnt2, Chga, Lct, Mt3 and Th; decreased expression was noted in additional genes that were dependent on radiation regimen. By day 21, more genes were affected in all irradiated groups than on day 0. Exposure to LDR+sSPE resulted in up-regulation of genes completely different than those in mice irradiated with either LDR or sSPE alone (LDR - Cstb, Dctn2, Snrp70; sSPE - Dctn2, Khsrp, Man26, Snrp70; LDR+sSPE - Casp1, Col1a1, Hspcb, Il6st, Nos2, Rpl28, Spnb2). Down-regulated genes in all irradiated groups on day 21 were Dr1, Lct, Lep and Mybl2; great variation was seen in the 13, 22 and 16 additional genes suppressed in the LDR, sSPE and LDR+sSPE groups, respectively.

Conclusions. The results demonstrate that whole-body irradiation with LDR photons and time after exposure had a great impact on hepatocellular gene response to a simulated SPE. The affected genes are important in hypoxia-induced signaling pathways, immune response, apoptosis and metabolism.

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(PS4170) Modulation of nuclear factor kappa B dependent gene expression in human cells after microbeam irradiation with accelerated alpha particles. Christa Baumstark-Khan[1], Christine E. Hellweg[1], Luis F. Spitta[1], Andrea Arenz[1], Roland Ruscher[1], Klaus-Dieter Greif[2], Ulrich Giesen[2], Guenther Reitz[1].

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Cellular stress protection response leads to transcription of genes via modulation of transcription factors. Activation of the NF-κB pathway as a possible anti-apoptotic route represents an important cellular response. A previously developed screening assay for NF-κB-dependent gene activation makes use of *HEK/293* cells stably transfected with a receptor-reporter-construct carrying the destabilized enhanced green fluorescent protein variant d2EGFP as reporter under the control of the NF-κB responsive element. The cell system has already been shown to respond after exposure to ionizing and non-ionizing radiation. Using the microbeam facility at PTB-Braunschweig, cells were exposed to 2.1 MeV α particles (LET ~160 keV/μm). Hits were either delivered to only the cell nucleus or, for comparison, statistically distributed over the whole cell population. Gene expression and proteomics data show the complexity of cellular radiation response. When, as it is the case for microbeam experiments, only limited numbers of cells are available and the number of genes to be analysed has to be restricted, quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) is the method of choice, since it confers high sensitivity. After microbeam radiation cell survival was determined via the colony forming ability test, and time-dependent activation of the NF-κB dependent anti-apoptotic pathway was followed using expression of the reporter gene d2EGFP by flow cytometry and expression of selected NF-κB target genes (IκBα and GADD45β) by quantitative RT-PCR analysis. One nuclear α particle traversal reduces survive to ~0.75. 12 hours after exposure with 1 to 10 nuclear hits NF-κB dependent d2EGFP fluorescence can be seen, a maximum is reached after 36 h. After exposure with 5 nuclear hits (surviving level ~0.4) maximal NF-κB activation is achieved. Exposure to two α particles per nucleus results already in up regulation of GADD45β gene expression. Diffuse irradiation increases the transcription of GADD45β and IκBα. The latter might be involved in the termination of the radiation-induced NF-κB activation. As activation of the NF-κB pathway plays a prominent role in the negative regulation of apoptosis, it might favour survival of cells with DNA damage, especially after low doses of densely ionising radiation.

(PS4171) Dosimetric evidence of cell extra nuclear sensitivity to alpha irradiation? Nicolas Chouin[1], Manuel Bardies[1], Michel Cherel[1], Alain Faivre-Chauvet[1], Christos Apostolidis[2], Alfred Morgenstern[2], Albert Lisbona[3], Jacques Barbet[1], Karine Bernardeau[1], Francois Davodeau[1]. [1]INSERM U601, Nantes, France, [2]ITU, Karlsruhe, Germany, [3]CRLCC Nantes-Atlantique Rene Gaudducheau, St. Herblain, France.

Initial results of a microdosimetric analysis of in vitro α-irradiation of a cell line (Adam) show an unexplained high sensitivity that could be explained by a cytoplasm or membrane sensitivity.

The aim of the present study was to verify this hypothesis based on statistical dosimetric calculations by clearly assessing the different uncertainties involved in the calculation.

In order to eliminate uncertainties on the cell geometry during the irradiation, two sets of experiments were carried out. In one case, cells were lying at the bottom of the culture well and in the other, culture wells were agitated continuously. Monoclonal antibodies radiolabeled with ^{213}Bi were then added in the solution. They specifically targeted MHC/peptide complexes present at the cell surface. Cell survival was measured with a [^{3}H]-Thymidine incorporation assay. Dosimetric calculations were carried out on more than 200 experimental points with a microdosimetric model presented previously. Uncertainties on the number of radiolabed antibodies taken up by cells and distribution of cell radii based on measurements were taken into account.

Cell survival represented as a function of mean absorbed dose within the cell nucleus was coherent between both configurations with a D_0 of 0.25 ± 0.07 Gy.

For both configurations, the percentage of mortality was higher than the percentage of hit nuclei for almost every experimental point and up to 40%. Error bars associated with each experimental point that represent the uncertainty on the number of radiolabeled antibodies taken up by cells show that this observation was not due to statistical errors. On contrary, dosimetric calculations carried out for the whole cell show that percentage of hit cells was higher than percentage of mortality and thus seem to confirm the possibility of a significant extra cellular sensitivity.

A certain number of uncertainties are still present in this kind of experiments. Errors during the experiments or errors inherent to the cell survival measurement can have an important influence on results. These errors are believed to be random errors and should not influence results in just one way.

The present study questions the limits of dosimetric calculations in a context of *in vitro* cell irradiation and indicates the importance of cell extra nuclear sensitivity to α-irradiation.

(PS4172) Mechanisms of adaptive response induction by low dose and low dose rate: modeling approach. Olga A. Smirnova. Research and Technical Center of Radiation-Chemical Safety and Hygiene, Moscow, Russian Federation.

An adaptive response in mammals (mice), which is induced by low level single/protracted preirradiation and manifested in reduced mortality of pre-exposed specimens after challenge acute irradiation, is theoretically studied. Specifically, mechanisms of the adaptive response are revealed by making use of biologically motivated mathematical models of the major hematopoietic lines. Here, we originate in both, the radiobiological concept of a critical body system and the fact that death of animals in the relevant experiments was caused by the hematopoietic subsyndrome of the acute radiation syndrome. The developed models constitute the systems of nonlinear differential equations for concentrations of functional blood cells and their bone-marrow precursors, the dose and dose rate being variable parameters in them. Modeling results show that the adaptive response induced in mice by low dose single preirradiation is caused either by acquired radioresistance of lymphopoietic and/or thrombocytopoietic systems, if challenge exposure takes place in an early period after preirradiation, or by acquired radioresistance of granulocytopoietic system, if challenge exposure takes place in a late period after preirradiation. In turn, the adaptive response, which is induced in mice by low dose rate protracted (more than 1 month) preirradiation and revealed by subsequent challenge exposure, is due to acquired radioresistance of granulocytopoietic system. Modeling assessments of durations of the early period, the late period, and the period of protracted preirradiation coincide with their experimental estimations. Furthermore, the mechanisms of the acquired radioresistance, which is induced in the above-mentioned hematopoietic lines by low level single/protracted preirradiation, are also elucidated. The developed models can be employed in detailed studies of the adaptive response in mammals and in the planning of the prospective experiments in this field.

(PS4173) Three-dimensional model of tissue and heavy ions effects. Artem L. Ponomarev[1], Alamelu Sundaresan[2], Janice L. Huff[1], Francis A. Cucinotta[1]. [1]NASA JSC, Houston, TX, USA, [2]UT Medical School, TSU and UH, Houston, TX, USA.

A three-dimensional tissue model was incorporated into a new Monte Carlo algorithm that simulates passage of heavy ions in a 'tissue box'. The tissue box was given as a realistic model of tissue based on confocal microscopy images. The action of heavy ions on the cellular matrix for 2- or 3-dimensional cases was simulated. Cells were modeled as a cell culture monolayer in one example, where the data were taken directly from microscopy (2-d cell matrix), and as a multi-layer obtained from confocal microscopy (3-d case). Image segmentation was used to identify cells with precise areas/volumes in an irradiated cell culture monolayer, and slices of tissue with many cell layers. The cells were then inserted into the model box of the simulated physical space pixel by pixel. In the case of modeled tissues (3-d), the tissue box had periodic boundary conditions imposed, which extrapolates the technique to macroscopic volumes of tissue. For the real tissue (3-d), specific spatial patterns for cell apoptosis and necrosis are expected. The cell patterns were modeled based on action cross sections for apoptosis

and necrosis estimated from current experimental data. A spatial correlation function indicating a higher spatial concentration of damaged cells from heavy ions relative to the low-LET radiation cell damage pattern is presented. The spatial correlation effects among necrotic cells can help studying microlesions in organs, and probable effects of directionality of heavy ion radiation on epithelium and endothelium.

(PS4174) Effects of X-ray micro beam irradiation on the function of neuronal network. Takahiro Kuchimaru[1], Fuminobu Sato[1], Tomohisa Fujita[1], Toshiji Ikeda[2], Kikuo Shimizu[3], Yushi Kato[1], Toshiyuki Iida[1]. [1]Graduate School of Engineering, Osaka Univ., Osaka, Japan, [2]The Institute of Science and Industrial Research, Osaka University, Osaka, Japan, [3]Radioisotope Reasearch Center, Osaka Univ., Osaka, Japan.

Micro beam technique has been applied to novel experiments for studying radiation effects in living cell such as bystander effect or behavior of repair proteins at the site of DNA lesion. We developed tabletop a X-ray micro beam system for single cell irradiation. The system was constructed of micro focus X-ray tube (voltage:~50 kV, current:~1 mA, target:Rh), an X-ray optics, automatic sample stage and an inverted microscope. X-ray micro beam in a diameter of 10 μm was delivered to a sample cell in the air and dose rate of ~50 mGy/s. The dominant deposited energy of X-ray micro beam on a sample cell ranged from 2 to 10 keV. Radiation effects in neuronal network have been investigated with this X-ray micro beam system. We used rat pheochromocytoma PC12 cell which is differentiated to neuron-like cell with nerve growth factor (NGF). The activity of PC12 neuronal network was measured by fluorescent monitoring of intracellular Ca^{2+} concentration. X-ray micro beam was irradiated to some single cells in the network with various doses (~20 Gy). After irradiation, Ca^{2+} fluorescent intensity of irradiated cells and other cells in the network were measured at 15 minutes interval. In addition, DNA double-strand breaks induced by X-ray micro beam irradiation were evaluated with indirect immunofluorescent stain of γ-H2AX focus which followed DNA double-strand breaks with the immediate and substantial phosphorylation of histone H2AX. We have also focused the response of neuronal differentiation to ionizing radiation in PC12 cell. It is reported that Interleukin-6 (IL-6) is expressed in the irradiated cell. In this paper, correlations between radiation effects such as a DNA double-strand break and an expression of IL-6 induced by X-ray micro beam irradiation and the activity of neuronal cell network have been discussed.

(PS4175) New perspectives in modeling of carcinogenesis induced by ionizing radiation. Igor Akushevich[1], Galina Veremeeva[2], Aliaksandr Kulminski[1], Svetlana Ukraintseva[1], Konstantin Arbeev[1], Alexander Akleyev[2], Anatoli Yashin[1]. [1]Duke University, Durham, NC, USA, [2]Urals Research Center for Radiation Medicine, Chelyabinsk, Russian Federation.

One of the major long-term effects of ionizing radiation on humans is the increase of risk of cancer development. An important practical task is the assessment of individual risks for persons subject to protracted low-dose irradiation. Calculation of individual risks requires using methods of mathematical modeling and generalization of existed approaches. In this report we review the current state of the art of modeling efforts in tumorigenesis relying on a multi-stage hypothesis. Possible schemes of application of the population and bio-molecular multi-stage models of carcinogenesis to data on population exposed to ionizing radiation as well as their perspectives and limitations are discussed. The concept of barrier mechanisms is presented as a basis for a new multiple pathway model of carcinogenesis development in which cell malignization occurs due to inefficient operation of, a part of, or all barrier systems such as antioxidant defense, repair systems, apoptosis, and more. Experimental observations supporting the hypotheses that carcinogenic effects at the cell level are related to the quality of operation of complex of the barrier systems, which are in complex interaction with each other, are described. Mathematical formalism for exact and approximate solutions of the model is presented. One advantage of the modeling approach is in natural combining of the two types of measures expressed in terms of model parameters (age-specific hazard rate and age-specific means of state of each barrier). Another advantage is in application to the case of protracted irradiation, when barrier systems directed to reparation of genetic damages or elimination of cells carrying unrepared damages play a special role. Results of simulation studies allowing us to conclude that the model can be estimated using current population epidemiological data and newly collected data on biomolecular measurements of state of barrier systems of exposed population are presented. Further generalization of the model such as including other barrier mechanisms (telomerase, growth arrest, etc.) and incorporating the model of IR induced genomic instability, are discussed.

(PS4176) Auger electron therapy. Rebecca Hinrichsen, Helge Thisgaard, Mikael Jensen, Michael Lyngkjær, Lars Martiny. Risø National Laboratory, Roskilde, Denmark.

Auger Electron Therapy

We have developed an *in vitro* cell system to test the biological effect of Auger electron emitters and thereby determine their potential in cancer therapy. Auger electron therapy is a useful strategy for specific tumor cell killing of micrometastases and small tumors in addition to a low level of damage to surrounding cells, originating from cascades of electrons with subcellular ranges and thus highly localized energy depositions. The biological damage from such radionuclides is highly dependent on the precise location of decays within cells. Auger emitters decaying within the cell nucleus, close to the DNA, are extremely radiotoxic with observed biological effects of high-LET character, but the same type of decay outside the nucleus is much less toxic with effects characteristic for low-LET radiations [1, 2].

In our method radionuclides are applied to mammalian cells by microinjection, using an advanced computer assisted microinjection system (AIS 2). The precision of the system allows us to specifically deliver radionuclides into the cell cytoplasm or the cell nucleus and thereby analyze the effect of cellular localization of the emitter [3]. The biological effects are evaluated using a clonogenic assay, which is the preferred method for studying the effectiveness of specific agents on the proliferation of cells (cell reproduction).

By this method it is possible to measure the radiotoxicity and identify the Relative Biological Effectiveness (RBE) for Auger electron emitters and compare this to known α- and β-emitters.

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(PS4177) Automatic unstained cells recognition for single-cell irradiations. Marcin Skoczylas, Roberto Cherubini, Silvia Gerardi. INFN-Laboratori Nazionali di Legnaro, Legnaro, Italy.

Cell recognition is a fundamental task to be accomplished when single-ion cell microbeam irradiations are performed. At INFN-LNL facility [Gerardi et al. Radiat. Res. 164 (2005), 586–590] cell visualization system is based on a phase-contrast optical microscope, without the use of any cell dye. Unstained cells detection and recognition in phase contrast optical microscope images on dirty background is normally achieved manually by an expert operator. Nevertheless, this procedure is time consuming and sometimes it is not sufficient as the amount of living cells to be irradiated could be large. To decrease the time needed to recognize cells on the Petri dish, an automatic, parallel client-server system was implemented on the cluster machine using C++/MPI and Java. Circular pixel-spots taken from the captured grayscale image are firstly pre-classified and potential cell markers for the segmentation

algorithm are obtained. Segmented objects are additionally classified to categorize cell bodies from other structures considered as dirt. As a result, cell coordinates are passed to the LabVIEW program to place cells in front of the beam and perform the irradiation.

This work is partially supported by European RTN-Marie Curie "CELLION" Project.

(PS4178) New improvements of the Krakow single ion hit facility for cells irradiation. Oleksandr Veselov[1], Janusz J. Lekki[1], Rasa Ugenskiene[2], Zbigniew Stachura[1], Kateryna Lebed[1], Wojciech M. Kwiatek[1]. [1]Institute of Nuclear Physics PAN, Krakow, Poland, [2]Jagiellonian University, Medical College, Krakow, Poland.

Since 2005, the Krakow single ion hit facility (SIHF) is a fully operational system for the investigation of the effects induced in biological cells by a controlled number of ions. The technical design of this facility has been described in detail elsewhere [1], including such crucial features as the beam control (proton counting and blanking) system, the on-line optical system for cells visualization and positioning, and the self-developed, flexible software for automatic cells recognition.

In spite of the satisfactory results achieved by the SIHF, it is being continuously modified and upgraded. One of the latest changes in the SIHF setup is the newly designed microprobe control system, using two independent microcomputers linked in a master-server configuration, which enhances the safety and convenience of irradiation experiments. The sample illumination at the irradiation stage was improved by introducing the Philips Lumiled LUXEON emitter, being an energy efficient and ultra compact light source with a long lifetime. This modification enables the on-line cells observation in the transmitted light with a better contrast. However, the character of cell images, changed due to this modification, caused the necessity to introduce additional procedures in automatic cell recognition procedure. The problem has been solved basing on the methods of the Fuzzy Logic analysis applied to image processing. As an illustration, typical examples are provided together with recent results of the irradiation of living cells performed with a controlled number of protons.

This study was supported by the project CELLION of the EU 6th Framework Programme, No MRTN-CT-2003–503923.

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(PS4179) A fast analytical model for assessing biological effectiveness of light ion beams in radiotherapy. Pavel Kundrat. Institute of Physics, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

A detailed probabilistic model of biological processes is used in conjunction with a simple analytical model of light ions' Bragg peaks to predict the biological effectiveness along penetration depth of ion beams in water or tissue for hadron radiotherapy applications. The physical model is based on energy-loss tables generated by the SRIM code, on phenomenological energy-loss straggling formulas, and on total nuclear cross-sections to estimate the reduction in primary particle fluence due to nuclear reactions. Fragmentation products are not followed at all. This simple model enables to satisfactorily reproduce experimental depth-dose distributions for protons and light ions with ranges in water up to approx. 15–20 cm, while the neglect of secondary particles formed in nuclear reactions is a crude approximation for heavier ions (such as neon) at higher energies.

The biological module is based on the probabilistic two-stage model (Kundrat et al., Phys. Med. Biol. 2005, 2006). Two classes of damage to DNA are distinguished according to their severity. Repair success probabilities can be incorporated, too. Parameters of the biological model are derived from survival data under mono-energetic irradiation and used to assess cell survival along the penetration depth of the ion beam. Model predictions are in excellent agreement with published experimental data, indicating potential applications of the model scheme in treatment planning applications in hadrontherapy. Fast calculations based on the present semi-analytical scheme might be especially useful for truly biological optimization of multiple ports and/or intensity modulated ion beams.

(PS4180) Podcasting information in the radiological sciences to health care professionals. Carl D. Elliston, David J. Brenner, Nitin Gumaste, John Zimmerman, Eric J. Hall. Columbia University, New York, NY, USA.

Particularly in the context of the potential for a large-scale radiological event, there is a growing necessity to train health care professionals at all levels in the radiological sciences. Correspondingly, there is increasing demand from health care professionals, most of whom have no background in the radiation sciences, for such information in an accessible format.

We have created an online educational resource to address this need. Our goal is to provide convenient access to the resource and to ensure that the user makes efficient use of his or her time during the learning activity. Another challenge is the potential long time span from the initial learning encounter until the user needs to apply the information; therefore, the educational strategy is to effect a long-lasting attitudinal change in the learning, while providing concise summary information for review and easy retrieval of detailed knowledge that may be required in an emergency.

Our solution to these challenges involves enhanced podcasting. A podcast is a media file that is distributed over the internet for playback on portable media players, such as an iPod, or on a personal computer. If the user subscribes to a podcast, all new files are automatically downloaded. Enhanced podcasting incorporates audio, images, links, and chapters in a podcast.

Our podcasts are based on a semiannual training course entitled *Radiological Science in the Context of Radiological Terrorism* which has been developed and implemented by faculty at Columbia, Harvard, and elsewhere. It covers a broad spectrum of topics to help participants understand 1) the nature of ionizing radiation; 2) how radiation is damaging to people; 3) how we know what we know about radiation risks; 4) potential radiological terrorist scenarios; and 5) emergency preparedness for a radiological event.

Each podcast consists of about 40 minutes of audio, playable on any iPod or similar player, optionally synched with accompanying Powerpoint slides viewable on a video iPod or a PC, as well a PDF handout giving an outline of the key topics covered.

A web page has been created to allow users to subscribe to the material in a simple, straightforward manner. The URL is: http://cmcr.columbia.edu/training/podcasts.html

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(PS4181) Characterization of a pre-cell hit detector to be used in single cell irradiation experiments at the Lund Nuclear Microprobe. Charlotta Nilsson[1], Jan Pallon[1], Göran Thungström[2], Natalia Arteaga-Marrero[1], Mikael Elfman[1], Per Kristiansson[1], Christer Nilsson[1], Marie Wegdén[1]. [1]Lund Institute of Technology, Lund, Sweden, [2]Mid Sweden University, Sundsvall, Sweden.

Recently, the Lund Nuclear Microprobe (NMP) at Lund Institute of Technology has been modified to include a Single Ion Hit Facility for biological irradiation experiments on single, living cells with a counted, low number of protons or alpha particles. Non-targeted cell irradiation has already been carried out in an ongoing collaboration with the Biomedical Centre at Lund University.

So far, the ion hits have been detected after the cell dish using a p-i-n diode, however, the aim is to replace the diode with a pre-cell hit detector, which would facilitate on-line imaging of the cell dishes during irradiation, thus enabling targeted irradiation. An on-line microscope as well as cell recognition software is already available. The proposed pre-cell detector is an ultra-thin silicon semiconductor detector, with a thickness of less than 9 μm and an area of 1×1 mm^2.

To function in single ion experiments on single cells, the detector would have to have a reliable efficiency of close to 100 %,

and, in the case of pre-cell detection, at the same time cause a minimum of beam spreading. The detector is also intended to function simultaneously as vacuum window, thus the vacuum tightness is also of interest. These characteristics have been investigated at the Lund NMP, using protons in the range of 2 MeV - 2.55 MeV, as well as an alpha source with ions of higher energies. The efficiency of the thin silicon detector was investigated by setting up a ΔE-E detector telescope, with the thin detector as ΔE detector and a p-i-n diode (assumed to have 100 % efficiency) as stop detector behind. The experiments show that the efficiency increases, approaching 100 %, with decreasing proton energy, as expected from theory. The energy loss in the detector is roughly 260 keV for 2 MeV protons and 220 keV for 2.55 MeV protons, in accordance with SRIM simulations, which means that the 2 MeV proton signal can more easily be separated from the noise. The promising results from these experiments will be presented.

(PS4182) Monte Carlo Microdosimetry for targeted Irradiation of Individual cells using a microbeam facility. Fredrik L. Andersson, Sebastien Incerti, Odile Boissonade, Philippe Barberet, Carlos Furtado, Claire Habchi, Philippe Moretto, Duy Thuy Nguyen, Thomas Pouthier, Hervé Seznec. Centre d'Etudes Nucleaires de Bordeaux Gradignan, Gradignan, France.

In the frame of broad beam cellular irradiation experiments, accurate instrumental techniques of dosimetry exist to measure the particle fluence delivered in irradiated areas. Nevertheless, the dose actually deposited in the cells is calculated on the basis of different rough assumptions in terms of cell-dependant geometrical factors or seeding density. On the other hand, for microbeam cellular irradiation, the beam is accurately controlled in the sense that the exact number of particles delivered in individual cells is counted, but for comparison with broad beam random irradiation, it is of primary interest to evaluate the actual dose the cells receive. In addition, the fact that the cell morphology, for instance the nucleus thickness, is a function of elapsed time after seeding, adds more difficulty to this evaluation

For those reasons, we propose a new simulation software under development at the microbeam facility available at the Centre d'Etudes Nucléaires de Bordeaux-Gradignan (CENBG) in France, based on the Geant4 Monte Carlo Simulation toolkit.

In this software, the cell geometry and the element composition model are based on a high-resolution voxelized 3D phantom of a human keratinocyte cell line built from confocal microscopy images and from quantitative chemical analysis using a focused ion beam at a sub-micron level.

Microdosimetry calculations are presented under single cell irradiation conditions with 3 MeV incident alpha particles. Deposited dose dependence results are given for different cell morphologies and chemical compositions measured at CENBG and are compared with calculations on reference cells.

The results show the importance of taking into account in a model the complex chemical composition as well as the morphology state of the targeted cell. In addition, this study shows the remarkable possibilities of the Geant4 toolkit for the modeling of high-resolution 3D phantoms at the cellular level for microbeam irradiation.

(PS4183) Development of nanotechnology based high spatial and temporal resolution cellular and small animal irradiation systems. Sha X. Chang, Jian Zhang, Sigen Wang, David Bordelon, Eric Schreiber, Sarah Graboski, Adrienne D. Cox, Otto Zhou. University of North Carolina, Chapel Hill, NC, USA.

Encouraging advances today in basic cancer biology, pharmacology, and nanomedicine will no doubt produce promising biomarkers/biosensors for better and earlier cancer detection, treatment, and treatment response measurement. These exciting advances underscore the need to develop new research tools that can facilitate comprehensive and clinically relevant studies that will be required to evaluate the immediate and long-term effects of these agents on both cellular and tissue level pre-clinically. Today there is a significant shortage of high spatial and high temporal resolution irradiation systems specialized for cellular and small animal irradiation to facility the above studies. We have proposed a novel cellular irradiation system and a micro-CT-RT (RadioTherapy) system based on carbon nanotube (CNT) field emission technology to provide unprecedented high-resolution for cancer biology and animal model research. The CNT cellular irradiation system uses low LET electron pixel beams with a large dynamic dose rate range. The system can simultaneously irradiate multiple individually selected single cells and allows real time microscopic observation of cellular response. The CNT micro-CT-RT system is a high spatial and temporal resolution image-guided irradiation system that is the analogy of the state of the art clinical image-guided radiotherapy (IGRT) system but at the mouse's spatial and temporal scale. Using the proposed x-ray pixel beam array technology the micro-CT-RT can electronically and instantly form arbitrary shaped and intensity-modulated fields with 2mm and 1ms or better resolution. In this presentation we will show some of the promising results from the feasibility study of both the CNT cellular and micro-RT system development.

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