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**Monday, June 30, 2008**

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**Poster Session I**

**Carcinogenesis and  
Genomic Instability I**

**6:30 p.m. – 8:00 p.m.  
Grand Ballroom Salon D**

**Chair:**

**Kathryn Held**

## Direct Visualization of Repair of DNA Double-Strand Breaks Induced by Low-Dose $\gamma$ -rays in Living Human Cells

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DNA double-strand breaks (DSB) repair is essential for preserving genomic integrity. *In situ* immunodetection of various DSB markers, such as  $\gamma$ H2AX, phosphorylated DNA-PKcs, and 53BP1, have widely been used to quantify radiation induced DSBs rejoining. Due to a higher incidence of spontaneous foci, it is difficult to discriminate the foci that are induced by low-dose radiation. Here, we demonstrate the use of exogenously expressed 53BP1, fused to yellow fluorescent protein (YFP), as an *in vivo* marker to study ionizing radiation (IR) induced DSBs repair, and the dynamic behavior of DNA repair proteins at the sites of DSBs in living mammalian cells. Using YFP-53BP1 as a marker, we noticed that the induction of YFP-53BP1 foci in living cells showed a linear dose-response relationship with doses ranging from 1cGy to 1Gy and all the YFP-53BP1 foci observed in living cells co-localized with  $\gamma$ H2AX. Further, the DSBs repair kinetics is similar in cells treated with 1, 5 and 10 cGy of  $\gamma$ -irradiation, indicating that DSB are similarly repaired irrespective of dose. In addition, even at doses down to 10 cGy of  $\gamma$ -radiation, YFP-53BP1 becomes progressively recruited to the sites of DSBs reaching maximum intensity around 20-30 min and diminished thereafter. We also show that the DNA-YFP-53BP1 complex is a dynamic structure and the YFP-53BP1 reversibly interacts with DSBs. Interestingly, the dynamics of YFP-53BP1 at the sites of DSBs is independent of dose. The data presented here are in agreement with current models that the DNA DSBs are efficiently repaired at low and high doses, and offer a new means to directly study both dynamics of repair proteins at the DSB sites and DNA DSBs repair in living cells.

**Chromosomal Instability in Radiation-Induced Mouse Acute Myeloid Leukemia**

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To better assess the risk posed to astronauts of developing radiation-induced Acute Myeloid Leukemia (AML), we have been conducting studies to better define the dose response and RBE for HZE particle exposures, as well as to provide a better understanding of underlying mechanisms in the CBA AML model. For the present studies, we investigated the potential role of chromosomal and telomeric instability in murine AML following exposure to HZE particles (1 GeV/n  $^{56}\text{Fe}$ ) and Gamma ( $\gamma$ )- rays ( $^{137}\text{Cs}$ ). There was no evidence of significant telomeric instability or chromosomal numerical aberrations in the AMLs. However, we did find that AMLs resulting from both radiation types showed increased levels of *chromatid*-type aberrations (those involving only one chromatid of a metaphase chromosome). Because chromatid-type aberrations are obliged to occur after replication in the cell cycle of collection, they are suggestive of on-going instability. We also evaluated and compared background (0 Gy) chromosomal instability in young (8-12 weeks) vs. old (>1 yr) CBA mice and found negligible differences; i.e., age is not a contributing factor to the observed instability seen in the tumors. To determine whether radiation induces an instability that leads to progression of carcinogenesis (whether or not instability occurs as an early event), we employed a spleen colony forming unit (CFU-S) assay. This strategy facilitates elimination of stable aberrations; those seen in every cell (clonal) are not the result of on-going instability. We examined two time points (5 days and 7.5 months) post irradiation (HZE) and found that some colonies displayed (chromatid-type) instability at five days; by 7.5 months this number dramatically decreased. We also examined colonies for the emergence of new translocations involving chromosomes 1, 2 or 3 during colony formation, but found no evidence of this type of chromosomal instability. Interestingly, we observed many fewer AMLs following HZE irradiation than expected. We are investigating possible connections between radiation-induced on-going instability and susceptibility to radiation-induced AML.

This work supported by NASA's NSCOR Radiation Leukemogenesis project (NAG9-1569).

## Human Cell Neoplastic Transformation To Soft Agar Growth By Space Radiation

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### INTRODUCTION

Space radiation contains particles of a wide range of atomic numbers (Z) and energies, corresponding to species with differing linear energy transfer (LET) values. Protons comprise most of the charged particles in space, outnumbering high energy particles of higher atomic number (HZE particles) by several hundred fold. Human cancer induction by radiation exposure is a principal long-term risk of space travel. We are using a human primary cell model system designed to evaluate initial cell alterations that lead to anchorage-independent growth, also called neoplastic transformation. At low doses, the dose-response relations for colony induction (per survivor) in soft agar are linear with radiation dose, thus allowing ready comparison of the transforming efficiency of different radiation species.

### METHODS AND APPROACHES

To determine the transforming efficiency of different HZE particles, we studied their effectiveness in inducing soft agar growth [1-3]. We initiated primary cultures from human neonatal foreskins, using only early passage cells in all irradiation experiments. Cells were irradiated with particles at the NASA Space Radiation Laboratory at BNL, or—for low LET radiation—by the 100 kVp X-ray source in BNL's Medical Department. We tested the transforming efficiency of the following HZE particles: 1 GeV/n Fe ions (LET, 151 keV/ $\mu$ ), 1.1 GeV/n Ti ions (LET, 108 keV/ $\mu$ ), 600 MeV/n Si ions (LET, 50.8 keV/ $\mu$ ) and 290 MeV/n C ions (LET, 12.88 keV/ $\mu$ ). We also tested whether the energy (and thus the LET) of species of the same Z affected the transforming efficiency, using Fe ions at 300, 600 or 1000 MeV/n as well as protons of 1 GeV, 500, 250 or 100 MeV. After cells were irradiated and released from the NSRL, they were returned to Biology Department, plated and allowed to grow on solid surfaces for determination of clonogenic survival, or plated and grown in semi-solid medium for measurement of anchorage-independent colonies.

### RESULTS

We determined the number of transformed soft-agar colonies per survivor for increasing doses (0-30 cGy). Each ion was tested least in two independent experiments, generally in different NSRL runs. For each experiment, 5-6 replicate determinations were carried out for both anchorage-dependent clonogenic survival and for soft agar growth. The results from the multiple experiments were then summarized, and the slopes and errors for each slope determined. On a per particle basis, the transforming efficiency (transformants/ $10^5$  survivors/ion/ $\text{cm}^2$ ) increased with increasing LET; however, the ions chosen also increased monotonically with Z. To determine the role of LET in determining transformation efficiency, we examined the transforming efficiency of beams of one HZE particle (Fe) as well as proton beams of different energies. The transforming efficiency of Fe ions increased as the LET increased, corresponding to decreased energy of the ions. We also determined the transforming efficiency of proton beams of 250, 500 and 1000 MeV. Even though—on an absolute basis, the LETs of these proton beams are similar—higher LET protons were more effective in inducing anchorage-independent growth. We are extending these studies to the energies and dose rates found in space radiation, including solar particle events (SPEs).

### REFERENCES

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### ACKNOWLEDGMENTS

<sup>1</sup>This research was supported by Human Research Program of the Exploration Systems Mission Directorate of NASA to BMS; Low Radiation Dose Program of DOE and the Human Research Program of the Exploration Systems Mission Directorate of NASA to BMS, and NSBRI (PI, A. Gewirtz; Co-PI, BMS). We thank Drs. Adam Rusek, Michael Sivertz and I Hung Chiang (NSRL Physics) for dosimetry and untiring help, and Keith Thompson, Biology Department statistics consultant, for guidance on statistical analysis of data.

### **Characterization of Chromosome Damage in Dermal Fibroblasts Irradiated *In Vitro* Versus *In Situ* In a 3D Human Skin Model**

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As part of ongoing research jointly funded by NASA and DOE, we are investigating cytogenetic and submicroscopic damage to the genomes of human fibroblasts irradiated *in vitro*, as compared to fibroblasts recovered from irradiated artificial skin constructs. To this end, we have been studying transmissible IR-induced structural changes to chromosomes in FT200 and FT300 skin constructs (MatTek Corp). The constructs mimic the biological properties of human skin, consisting of basal, spinous, granular, and cornified epidermal layers. The cells of these constructs are both metabolically and mitotically active, and express markers of differentiation specific to epidermis. The dermal compartment, which is separated from the epidermis by a well-developed basement membrane, is composed of a collagen matrix containing viable dermal fibroblasts, which constitute the focus of our investigation.

We developed a method of physically separating the fibroblast-containing dermal layer from the epidermal layer, and have successfully produced viable single-cell suspensions of the fibroblasts contained within. Results regarding basic radiobiological properties of dermal fibroblasts derived from this artificial skin model will be presented that compare *in vitro* versus *in situ* gamma ray exposure in terms of survival and chromosome aberrations in long-term bulk cultures. To date we have collected and stored several hundred 24-color mFISH images from cells irradiated *in vitro* and *in situ* (i.e. within intact skin), which we are in the process of systematically karyotyping.

Our original intent was to isolate independent clonal derivatives from irradiated skin constructs, as a prelude to molecular and cytogenetic analysis. A major impetus for this approach was that any chromosomal damage found in the progeny of singly irradiated cells should be homogeneous within all cells of a particular clone, and thus would be amenable to submicroscopic analysis by high density CGH DNA microarrays (array-CGH; aCGH). In particular, we are interested in determining the dose response for submicroscopic interstitial deletions, which, because of their proposed dose and frequency distributions, should allow us to make direct measurements of damage following very low doses (e.g.,  $\leq 10$  cGy).

The amount of clonal expansion of dermal fibroblasts required by such a procedure rendered the majority of skin-derived clones reproductively senescent by the time the requisite number of cell divisions had taken place. Consequently, we have been actively investigating the possibility of assembling a "custom" skin model, in which the dermal fibroblast compartment of the MatTek model is replaced by hTERT-immortalized fibroblasts. Results from first-generation "custom" skin constructs have been quite encouraging, yielding viable fibroblasts that, in theory, should be immortal, and hence capable of unlimited expansion post irradiation for cells carrying stable chromosome aberrations, which will be harbored by each and every cell within a given clone.

**NEOPLASTIC TRANSFORMATION IN VITRO: A PRELIMINARY REPORT OF FINDINGS  
WITH 232 MeV PROTONS AND 1 GeV IRON IONS**

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Over the past several years we have established that low doses of low-LET radiation suppress the frequency of neoplastic transformation in vitro to levels less than that seen spontaneously using the HeLa x skin fibroblast human hybrid cell assay system. Included among the various low-LET radiation sources used was 232 MeV protons delivered at the intermediate dose-rate of 20 mGy/min at the High Energy Proton Facility at Loma Linda University Medical Center (LLUMC). In this study we found evidence for suppression of neoplastic transformation at doses below 100 mGy [1]. We are currently examining the effect of reducing the dose-rate to 200 mGy/h which corresponds to peak dose-rate that may be encountered during a solar particle event (SPE). To date four experimental runs, also carried out at LLUMC, have been completed. The combined results indicate no significant suppression or induction of neoplastic transformation up to a dose of 1000 mGy. However, we are planning to repeat these studies as parallel high dose-rate experiments did not give as high a transformation frequency at high doses (1000 mGy) as we would have expected based on our previous data.

We have established a dose-response curve (0 – 1000 mGy) for neoplastic transformation induced by 1 GeV/amu iron ion irradiation. These experiments were performed at the NSRL at BNL where we have now carried out three experimental runs. The data show a very interesting and apparently reproducible feature which is that at low doses (10 and 50 mGy) the response is similar to that seen for low-LET radiation, i.e. there is an apparent suppression of neoplastic transformation. Since at doses <100 mGy the majority of cells are not traversed by an iron ion track, and the low-LET delta ray penumbra extends several cell diameters [2], then it is feasible that the low-LET delta ray component may be suppressing any inducing effect of the iron ions. Such an effect is not out of the question since the induction of an adaptive response by low doses of x-rays against alpha particle-induced transformation has previously been reported [3]. We have also carried out parallel experiments with Cs-137 gamma radiation in an effort to establish an RBE at the higher doses used. Very preliminary data indicate a value of 1.6 at a dose-level of 1000 mGy Cs-137 gamma radiation.

Our first run with a “mixed beam” (protons followed by iron) schedule was carried out in the Fall of 2007. Preliminary data indicate that 10 cGy of 1 GeV/amu MeV protons induces an adaptive response against the effect of 100 cGy 1 GeV/amu iron ions.

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**Persistence of  $\gamma$ -H2AX and 53BP1 foci in proliferating and non-proliferating human mammary epithelial cells after exposure to gamma rays or Fe ions.**

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We have measured  $\gamma$ -H2AX and 53BP1 foci in proliferating and non-proliferating human mammary epithelial cells (HMEC) after exposure to sparsely and densely ionizing radiation. Foci numbers were quantified with computer based analysis at various time points. Our results reveal that the disappearance of radiation induced  $\gamma$ -H2AX and 53BP1 foci in HMEC have different dynamics as a function of radiation quality and proliferation status. Fe-ion induced  $\gamma$ -H2AX foci in non-proliferating cells were still present at 72 hr after exposure, while 53BP1 foci were back to control levels at 48 hr. However, in proliferating HMECs both  $\gamma$ -H2AX and 53BP1 foci were removed during the 24–48 hr time interval after irradiation. Foci numbers after  $\gamma$ -ray irradiation were removed faster and returned to control levels at 12 h at 37°C regardless of marker and cell proliferation status. We speculate that the persistent  $\gamma$ -H2AX foci in Fe-ion irradiated non-proliferating cells could be due to the fact that not all DSB repair pathways for rejoining complex DSBs are available to the cell in G0/G1-phase and/or that damaged cells in a cycling population could be eliminated more efficiently via an apoptotic pathway. Another possibility is that the remaining  $\gamma$ -H2AX foci does not represent open DSBs.

To further elucidate the mechanism of foci removal in replicating and non-replicating cells, we have started to measure apoptosis in proliferating and non-proliferating HMECs after exposure to different radiation qualities. Further experiments are necessary to help understanding why we see remaining  $\gamma$ -H2AX foci in non-proliferating cells while 53BP1 foci return to control levels within 48h after Fe ion exposure. We have started to analyze co-localization data for these two proteins. Additional studies are underway using HMEC that form 3D acini when cultured on top of an extracellular matrix (Cultrex).

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## **Influence of Genetic Background in individual Rederived Inbred Mice on the induction of Radiation-Induced Chromosome damage in Haemopoietic Cells**

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### **INTRODUCTION**

Genomic instability is effectively induced by ionising irradiation. This process may be influenced by heterogeneity in individual responses, possibly due to alterations in proteins responsible for the maintenance of genomic integrity or altered oxidative metabolism. Recently, evidence has accumulated, supporting a relationship between genetic background and the induction of radiation-induced genomic instability (Watson *et al.* 1997; Kadhim 2003). Our previous *in vivo* study in CBA/H mice exposed to whole body X-ray irradiation (Watson *et al.* 2001), demonstrated radiation-induced chromosomal instability in bone marrow from inbred CBA/H mice. In that study, we also observed inter-individual variation in the level of chromosomal instability within this mouse strain.

The aim of the present study is to investigate the frequency of chromosomal aberrations in control and 0.5Gy alpha-particle irradiated bone marrow cells from individual CBA/CaH and C57BL/6J mice, rederived at the Mary Lyon Centre, Harwell. The sampling of control animals from both strains at two time points may establish a constant background level of chromosomal aberrations. Results will be shown for individual animals to enable an in-depth study of possible variation in inbred mouse strains and link to radiosensitivity

### **MATERIAL AND METHODS**

Femoral bone marrow was extracted from 10-12 week old male CBA/CaH and C57BL/6J mice bred at the Mary Lyon Centre, Harwell. Cells from individual animals were either sham irradiated or irradiated at 0.5Gy, using the MRC Plutonium-238 alpha-particle source. Immediately afterwards, cells were washed and re-suspended with fresh media. Control cells were plated at  $1 \times 10^5$  and irradiated cells at  $2 \times 10^5$  in 45mm Petri dishes with 2ml of 'complete' MEM alpha media supplemented with appropriate growth factors and 0.3% low-melting point agarose. Dishes were then incubated at 37°C, 5% CO<sub>2</sub> for 6-7 days prior to metaphase preparation from the CFU-A colonies for chromosomal analysis. Bone marrow cells from both mouse strains were also cultured for 2 or 6/7 days in liquid cultures using the same growth factors as previously described and metaphase preparations analysed.

### **RESULTS**

Data was analysed using variance ratio and arithmetic methods. For both strains, the chromosomal analyses indicated inter-individual variation in the non-irradiated CFU-A colonies. Results suggest that CBA/CaH is radiosensitive to alpha particle irradiation. However, culture methods may also influence the frequency of cytogenetic damage.



## Yields of Clustered DNA Damages Induced by Ionizing Radiation Species of the Same Z and Different Energies

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### INTRODUCTION

Ionizing radiation induces a variety of DNA alterations, including bistranded clustered damages—two or more closely-opposed strand breaks, oxidized bases or abasic sites within a few helical turns. These DNA damages are difficult to repair and thus probably constitute a source of genomic instability, involved in such cellular processes as cell death, carcinogenesis and ageing.

Previous studies on DNA in non-radioquenching solution showed that the damage spectrum—the relative levels of double strand breaks compared to abasic clusters and oxypurines clusters—is different in DNA irradiated with photons vs. DNA exposed to charged particles, including high energy protons [1]. Also, in a non-quenching environment the clustered damage yields, determined as the number of clustered damages per unit absorbed dose, decreased with increasing linear energy transfer (LET). However, in these studies, the kinetic energy of the HZE radiations used varied and the species of HZE ions increased in atomic number. Interestingly, these results are in “apparent” disagreement with the observed increased biological effect induced by radiation with increasing LET, as expressed by the RBE above unity

### EXPERIMENTAL PROCEDURES

To study the influence of the radiation parameters—energy and atomic number—on the induction of bistranded clusters, we determined the yields of clustered DNA damages induced by irradiation of megabase pair genomic DNA with ionizing radiation species of the same Z and different energies, which varies the LET. Radiation energies were selected to allow comparison of at least two radiation species of different Z and the same energy. DNA samples were irradiated in non-quenching conditions at the NASA Space Radiation Laboratory with ion beams of particles of different Zs: protons (Z=1) at 1 GeV, 500 MeV and 250 MeV; silicon (Z= 14) at 600 MeV/nucleon and 300 MeV/nucleon; and iron (Z=26) at 1 GeV/nucleon, 600 MeV/nucleon and 300 MeV/nucleon. The frequencies of two types of clustered DNA damages—double strand breaks and oxypurine clusters—were determined using gel electrophoresis, electronic imaging and number average length analysis. To analyze the relationship between the DNA clustered damage induction and LET, the yields of DSB and oxypurine clusters were calculated as a function of the absorbed dose and as a function of the number of particles.cm<sup>-2</sup>.

### RESULTS AND CONCLUSIONS

Our results showed minor differences in the absolute yield values of double strand breaks induced by irradiation with species of the same Z and different kinetic energy. In the case of different radiation species with the same energy, the observed differences in the double strand break yields were related to their corresponding LET value. The function of the double strand breaks yields vs. LET showed a negative slope in the case of yield calculated per unit of absorbed dose but it was positive for the yield ascertained per number of particles.cm<sup>2</sup>. The determination of the oxypurine yields for each HZE species is in progress.

### REFERENCES

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### ACKNOWLEDGMENTS

<sup>1</sup>This research was supported by Human Research Program of the Exploration Systems Mission Directorate of NASA to BMS; Low Radiation Dose Program of DOE and the Human Research Program of the Exploration Systems Mission Directorate of NASA to BMS, and NSBRI (PI, A. Gewirtz; Co-PI, BMS). We thank Drs. Adam Rusek, Michael Sivertz and I Hung Chiang (NSRL Physics) for dosimetry and untiring help.

### **Radiation-Induced Leukemia Mouse Model: Applicability to Radiation Quality, Biological Countermeasures and Biomarker Discovery Studies**

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The development of safer and more effective radioprotectors is critical to protecting astronaut and military personnel from unintended radiation exposure. Radioprotective agents are needed to protect personnel not only from acute, early arising (radiation syndrome) effects, but from late arising (cancer) radiation pathologies as well. There have been relatively few attempts to develop biological countermeasures or “chemopreventive drugs” to prevent these late arising radiation pathologies. Identification of a countermeasure is hindered by the lack of an effective late effects *in vivo* model and mechanistic information regarding the action of the potential countermeasure. A murine model for the development of acute myeloid leukemia (AML) has been developed by Duhrsen and Metcalf which allows an analysis of the indirect effects of irradiation without the complicating mutagenic effects on hematopoietic cells. In this model, cells of the IL-3-dependent line, FDC-P1, consistently transformed to myeloid leukemia cells when injected into DBA/2 mice gamma-irradiated with 350 cGy. Changes in the microenvironment favor the outgrowth of preexistent abnormal cells and promote their definitive leukemic transformation. This model was applicable to other types of radiation and to heavy metals. Results showed that mice exposed to internalized depleted uranium (DU), an alpha particle emitter, also developed myeloid leukemia after injection of factor-dependent FDC-P1 cells. Concomitant to the development of leukemia, we also measured changes in rasP21 oncoprotein serum levels and chromosomal aberrations in chromosomes 2 and 11. These biomarker changes during development of leukemia may be effective tools to assess individual sensitivity to radiation and the resultant leukemia. Radiation quality studies with neutrons and  $^{60}\text{Co}$  demonstrated that  $^{60}\text{Co}$  gamma radiation induced a significantly lower incidence of leukemia (50% at 350 d) and the latency to leukemia development was significantly longer (50% leukemic at 350 d) than that for neutrons (100% at 350 d and 50% leukemic at 91 days). DNA Methylation and genomic instability studies are underway to evaluate leukemogenesis mechanisms.

Recently our laboratory has been testing the phenyl fatty acids, phenylacetate (PA) and phenylbutyrate (PB) for their effectiveness as biological countermeasures to  $^{60}\text{Co}$ -induced leukemia. These relatively non-toxic agents have demonstrated significant clinical and *in vivo* effectiveness as anti-tumor agents. Our goal was to test their ability to prevent or inhibit the development of  $^{60}\text{Co}$ -induced leukemia using a post-radiation treatment regimen. Data demonstrate that in comparison to radiation controls (2.5 Gy), PA (5.0 mg/kg) was effective at reducing the development of radiation-induced leukemia by increasing latency to 30% leukemic animals (2.1 +/- 0.3-fold) and decreasing total number of leukemic animals at day 250 (90% versus 30%, respectively). Proteomic analysis of serum and chromosomal aberration assays are currently underway.

The authors intend to assess whether this model may be applicable to other types of radiation exposures relevant to NASA.

## Loss of *PU.1* on Chromosome 2 in CBA/CaJ and C57BL/6 Mice after Irradiation with HZE Iron Ions, Protons, or Gamma-rays.

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Deletion of a region of chromosome 2 encompassing the murine homologue of the *PU.1* gene (*Sfp11*) occurs in more than 95% of radiation induced acute myeloid leukemia (AML) in mice. While this early event is necessary, it is not sufficient, as a mutation in the other allele is required for the development of these leukemias. The spontaneous incidence of AML in CBA/CaJ mice is negligible (<1%), but they are susceptible to radiation-induced AML. C57BL/6 mice do not develop AML either spontaneously or after irradiation. In the present study, susceptible (CBA/CaJ) or resistant (C57BL/6) mice were irradiated with 0, 0.1, 0.2, 0.4, and 1.0 Gy doses of 1 GeV/n iron ions at the NASA Space Radiation Laboratory, and 1 day, 30 days or 1 year later we harvested bone marrow cells 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 sampled bone marrow cells at similar times. In addition we have further results following irradiation of both mouse strains with 1, 2, and 3 Gy of 150MeV protons.

Without irradiation about 1% of mitotic bone marrow cells from both mouse strains appear to have lost one allele of *PU.1*. At 1 day after irradiation the loss of *PU.1* for the HZE Fe ions was greater over the entire dose range and the frequency of loss for CBA bone marrow cells was nearly twice that for cells from the C57BL/6 strain. For gamma-rays the *PU.1* loss was also greater in the CBA than in C57BL/6 mice, but doses about 3.5 times greater were required to yield equal levels of effect. For proton irradiation the dose responses for *PU.1* loss was also linear with the CBA having a 2-fold higher sensitivity than C57BL/6 mice. The protons were about 1.7 times (CBA) or 1.6 times (C57BL/6) more effective per unit dose than gamma-rays.

By 1 month after irradiation the proportion of *PU.1* deleted cells in C57BL/6 mice returned to background levels, for both HZE Fe ion and gamma-ray irradiations. In CBA/CaJ mice elevated levels of *PU.1* deleted cells persisted in HZE irradiated mice, although these were lower than for 24 hour samples. One possible explanation is the elimination with time of sub-populations of cells with collateral but independently produced lethal chromosomal aberrations that were observed at relatively high frequencies in the 1 day samples. For gamma-ray exposures, frequencies of CBA cells with *PU.1* loss at one month were higher by a factor of about 1.7 than we observed 1 day after irradiation. For protons the *PU.1* loss was somewhat lower (about 0.6) than we observed 1 day after irradiation. For proton irradiated C57BL/6 mice the *PU.1* loss had, again, returned to background levels.

One year after the HZE irradiations the *PU.1* loss in C57BL/6 mice remained at background levels but for the CBA/CaJ strain the levels were even higher than observed for the 1 day samples, again suggesting a growth advantage for a sub-population of surviving cells containing *PU.1* deletions.

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### **Dose-rate Effects of Protons on the Induction of Genomic Instability *in vivo***

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Protons are the most abundant component of solar particle events in space. Information on biological effects of protons delivered at low dose rates is limited. This study is designed to determine the dose-rate effects of protons on *in vivo* induction of genomic instability (expressed as delayed chromosomal damage). This approach is highly relevant for assessing cancer risks; however, it has not been applied with proton exposure. We will characterize the type, the frequency, and the distribution of initial and subsequent chromosome aberrations, and the expression of genomic instability induced *in vivo* in bone marrow (BM) cells after exposure of mice to 100 MeV/amu protons at the dose and dose rates encountered in space. A novel genome-wide multicolor fluorescence *in situ* hybridization (mFISH) of mouse chromosomes will be used to detect and characterize chromosome aberrations in metaphase cells prepared from BM cells of exposed mice collected at different times (up to 6 months) post-irradiation.

In this newly funded research, we will give BALB/cJ mice a whole-body exposure to graded doses of 100 MeV/amu protons delivered at 1.0 and 2.0 cGy/min or <sup>137</sup>Cs  $\gamma$  rays [the low linear energy transfer (LET) radiation normally used as a reference radiation]. The total whole-body dose will range from 0 to 1.0 Gy. A reference radiation is required for the comparison of hazards following exposure due to very limited information on *in vivo* biological effects of protons. Bone marrow cells will be collected for the analysis at different times from 3 hrs up to 6 months post-irradiation. The frequencies of chromosomal damage in BM collected at early times reflect the sensitivity of BM cells to radiation and the repair rate after receiving radiation; while those found in BM cells collected at late times post-irradiation represent the dose and dose-rate effects of radiation on the karyotypic progression and genomic instability.

To increase the reliability of risk assessments, a much improved understanding of the molecular basis for dose-rate effects of protons is essential. In this study, levels of activated nuclear factor-kappaB (NF- $\kappa$ B) (a highly important transcription factor) and expression of selected NF- $\kappa$ B target proteins will be measured in BM cells collected at early times after irradiation. This will be coupled with the quantitation of kinase activity of the DNA-dependent protein kinase (DNA-PK) and ataxia telangiectasia mutated (ATM) protein kinase. We anticipate that these two kinases will be crucial for NF- $\kappa$ B activation after irradiation, leading to the activation of proteins involved in inflammatory response and cell survival. Thus, activated NF- $\kappa$ B may play a pivotal role in the maintenance of cells carrying genomic instability. We will then determine the correlation among these biological endpoints. The results will provide insights into *in vivo* molecular mechanisms for biological consequences from exposure to 100 MeV/amu protons that would contribute to the reduction of uncertainties in risk assessment. The data will also illustrate how molecular responses to 100 MeV/amu protons differ from those seen in mice exposed to <sup>137</sup>Cs  $\gamma$  rays.

Our research will be the first to use integrative *in vivo* endpoints (both cellular and molecular) for evaluating dose-rate effects of protons. The results will deliver information that is of high priority for NASA. Our data will not only contribute to reducing the uncertainties in the assessment of cancer risk from exposure to protons during space flights, but will also improve the understanding of the molecular basis for dose-rate dependencies of proton-induced genomic instability. *Research funded by NASA Grant #NNX07AP88G*

## **RESVERATROL REDUCES RADIATION-INDUCED CHROMOSOME ABERRATIONS AND GENE LOSS IN MOUSE BONE MARROW**

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Resveratrol is a secondary metabolite found in a large number of plants including grapes and peanuts. It has been shown to act as a cancer preventive in several model systems. With a wide range of molecular targets including those involved with induction of apoptosis and cell cycle arrest, resveratrol has potential to reduce radiation-induced chromosome aberrations and gene loss. To determine if resveratrol has potential to reduce radiation-induced cytogenetic damage in vivo and potentially reduce risks for radiation-induced acute myeloid leukemia (AML), 10 week old, male CBA mice were divided into groups of 10 mice each. Control groups consisted of no treatment, resveratrol only (100 mg/kg), muscadine grape only (5.7 µg/kg of resveratrol), and radiation only (3-Gy) with end points of 1, 7, and 30 days post-irradiation. Experimental groups were administered resveratrol (100 mg/kg) starting before irradiation with bone marrow collected at 1 and 30 days, resveratrol (100 mg/kg) initiated after irradiation with collection at 1, 7, and 30 days, and resveratrol (1.5-100 mg/kg) or muscadine (0.9-10.7 µg/kg) at varying doses given before irradiation with collection at 1 day. Collected bone marrow was processed for cytogenetic analysis and fluorescence in situ hybridization. Resveratrol (100 mg/kg) significantly ( $p < 0.05$ ) reduced mean chromosome aberrations at all end points when initiated before or after irradiation. Because of the mechanistic link between deletion of the PU.1 gene and the development of radiation-induced AML, loss of the PU.1 gene was also examined. Deletions in the PU.1 gene were significantly ( $p < 0.0001$ ) reduced at 1 and 30 days with pre-irradiation resveratrol. The optimal dose for reducing chromosome aberrations at day 1 for resveratrol alone was 6.25-25 mg/kg and for resveratrol in muscadine was 2.1-7.1 µg/kg.

**CONCLUSIONS:** When initiated pre-irradiation, resveratrol effectively reduced chromosome aberrations and PU.1 loss at 1 and 30 days, indicating radioprotection. Cancer prevention was implied by the post-irradiation resveratrol reduction in chromosome aberrations at 1, 7, and 30 days. The microgram dose level of resveratrol found in muscadine was significantly more effective than the mg dose of resveratrol alone implying that other compounds in muscadine may function synergistically with resveratrol. This potential synergism makes muscadine use practical and effective in the protection of human populations. Its role in protection against radiation-induced AML is under investigation.

## TUMOR INDUCTION IN MALE CBA MICE AFTER EXPOSURE TO Cs<sup>137</sup> GAMMA RAYS AND 1Gev/n IRON PARTICLES

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The CBA mouse strain has served as a valuable model for radiation-induced acute myeloid leukemia (AML) with respect to dose response relationships as well as underlying mechanisms. These mice have a very low spontaneous incidence of AML (<1%) but have been shown to be quite sensitive to induction of AML following exposure to gamma rays, x-rays and neutrons, with an RBE of 3-5 for fission spectrum neutrons. Another advantage is that few competing causes of death have been reported to occur in irradiated CBA mice. Their spontaneous frequency of other tumors is also relatively low except for liver tumors which have a frequency of approximately 12%. However, there have been no reports of an increase in liver tumor incidence following radiation exposure. This study was designed to compare the effects of Cs<sup>137</sup> gamma rays with 1 GeV/n iron particles on the dose response for induction of AML in male CBA mice. Doses for gamma rays were based on previous studies and ranged from 1-3 Gy. For 1 GeV/n iron, doses selected were based on the assumption that the RBE for iron would be near that for neutrons, i.e., 3-5. On this basis doses ranging from 0.1 to 1 Gy were selected. As might be expected, the RBE for life shortening was estimated to be 3.7. However, unexpectedly, the RBE for AML induction by 1 GeV/n iron particles approximated 1. In addition, unlike previous studies and the present study which found very little effect of gamma-ray irradiation on the frequency of hepatocellular carcinomas, a large increase in the incidence of these tumors was found after iron irradiation where an approximate RBE of 35 was observed. These data suggest a difference in effects of HZE iron ions on the induction of leukemia compared to solid tumors suggesting potentially different mechanisms of tumorigenesis. The basis for these differences and the impact of these results on risk estimates for space travel are under investigation.

## **CROSS TALK BETWEEN DNA REPAIR PATHWAYS AND EGFR SIGNALING PATHWAYS IN RADIATION RESPONSE**

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Radiation may induce cell proliferation, apoptosis, differentiation and contributes to cancer initiation or progression. These changes not only depend on radiation type, but also on cell lineage and the tissue microenvironment. Modification of growth factors and their receptors signaling pathways are the early step in cancer development. Epidermal growth factor receptors (EGFR) are highly expressed or mutated at their kinase domain in many human cancers. Therefore, the EGFR deregulated pre-malignant cells may exist in adult health persons and act as a sensitive target of radiation. To address the effect of EGFR on radiation risk, we examined radiation response in carcinoma human epithelial cells (A549), normal human fibroblast cell line (AG1522), and a null EGFR Chinese hamster ovary (CHO) cells, in which wild type EGFR constructs were introduced using a retrovirus vector to express EGFR, and EGFR kinase inhibitor (AG1478) was used to inhibit EGFR kinase activity. We found that exogenous EGFR did not affect cell growth in normal cell culture condition, but enhanced cellular proliferation following exposure to gamma radiation. Clonogenic survivals of exogenous EGFR expressing cells were mildly increased following gamma irradiation. Inhibition of EGFR kinase significantly reduced the survival of cells expressing EGFR at high doses of radiation. To examine the effect of EGFR on DNA damage formation and DNA repair, we detected the foci formation of phosphorylated H2AX (a surrogate marker of DNA double strand breaks (DSBs), p53BP1 (DSB sensing protein), and DNA repair proteins including phosphorylated ataxia Telangiectasia mutated (ATM) and DNA-PK. Our results show that ATM and DNA-PK foci are increased in EGFR cells at the early phase of radiation, while less gamma H2AX foci and p53 foci remained in EGFR cells compared to that of its partner cells at the late phase. Furthermore, EGFR expression slightly increased homologous recombination in CHO cells. These suggest that interaction of EGFR signaling pathway with DNA repair pathway contributes to radiation response in pre-malignant cells, and likely will play a role in cancer progression.

## RADIATION LEUKEMOGENESIS NSCOR WORKS IN PROGRESS

M.M. Weil<sup>1</sup>, J.S. Bedford<sup>2</sup>, Y. Peng<sup>3</sup>, F.A. Ray<sup>4</sup>, X. Liu<sup>5</sup>, J.W. Bacher<sup>6</sup>, L. Nagarajan<sup>7</sup>, M.D. Story<sup>8</sup>, S. Bouffler<sup>9</sup>, H. Bielefeldt-Ohmann<sup>10</sup>, P. Le<sup>11</sup>, X. Liu<sup>12</sup>, P.C. Genik<sup>13</sup>, S.M. Bailey<sup>14</sup>, and R.L. Ullrich<sup>15</sup>  
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### INTRODUCTION

Radiation exposure is a risk factor for acute myeloid leukemia (AML). The goal of the Leukemogenesis NSCOR is to provide the information required to develop a rational scientific basis for estimation of risks for leukemogenesis in humans from exposure to radiation during space flight. Individual projects within the NSCOR explore HZE radiation leukemogenesis in murine model systems and extend the findings to AML in humans. Additional findings relevant to solid tumor risks have emerged from the murine studies.

### AML AND SOLID TUMOR INCIDENCE IN HZE IRRADIATED MICE

AML sensitive CBA/CaJ mice were irradiated with 1 GeV/n <sup>56</sup>Fe particles at NSRL and with <sup>137</sup>Cs gamma-rays at Colorado State University and followed to 800 days of age for the development of AML. 1 GeV/n <sup>56</sup>Fe ions do not appear to be substantially more effective than gamma-rays for the induction of AML. However, <sup>56</sup>Fe irradiated mice had much higher risk for hepatocellular carcinoma than gamma-ray irradiated mice

### CHARACTERIZATION OF HZE- AND GAMMA-INDUCED AML

Molecular and cytogenetic analyses of HZE- and gamma-induced AML, including assays for chromosomal aberrations, *PU.1* deletion, gene expression, array CGH and microsatellite instability are ongoing.

### HZE EFFECTS ON MYELOID PROGENITOR CELLS

Deletion of the *PU.1* gene on mouse chromosome 2 (Chr. 2) is a critical step in the murine model of radiation leukemogenesis. We have found that AML sensitive CBA/CaJ mice have a higher incidence of Chr. 2 deletion in bone marrow cells following <sup>56</sup>Fe irradiation than AML resistant C57BL/6 mice and the persistence of Chr. 2 deleted bone marrow cells one month post-irradiation may be a biomarker for AML risk. The study is being refined to individually assay hematopoietic stem cells and myeloid progenitor cells for *PU.1* deletion, and it is being extended to <sup>28</sup>Si and proton irradiated mice.

### HUMAN RADIATION LEUKEMOGENESIS

We are investigating which recurrent cytogenetic aberrations are found in radiotherapy patients who develop AML. Chromosome 5 and chromosome 7 deletions appear to be most common, but +8, Inv16 and t(15;17) have also been identified in these patients.

In this poster we present some of the most recent findings from these projects.

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## **M-BAND study of radiation-induced chromosome aberrations in human epithelial cells: Radiation quality and dose rate effects**

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The advantage of the multicolor banding in situ hybridization (mBAND) technique is its ability to identify both inter- (translocation to unpainted chromosomes) and intra- (inversions and deletions within a single painted chromosome) chromosome aberrations simultaneously. To study the detailed rearrangement of low- and high-LET radiation-induced chromosome aberrations in human epithelial cells (CH184B5F5/M10) *in vitro*, we performed a series of experiments with Cs-137 gamma rays of both low and high dose rates, neutrons of low dose rate and 600 MeV/u Fe ions of high dose rate, with chromosome 3 painted with multi-binding colors. We also compared the chromosome aberrations in both 2- and 3-dimensional cell cultures.

Results of these experiments revealed the highest chromosome aberration frequencies after low dose rate neutron exposures. However, detailed analysis of the radiation-induced inversions revealed that all three radiation types induced a low incidence of simple inversions. Most of the inversions in  $\gamma$ -ray irradiated samples were accompanied by other types of intra-chromosomal aberrations but few inversions were accompanied by inter-chromosomal aberrations. In contrast, neutrons and Fe ions induced a significant fraction of inversions that involved complex rearrangements of both inter- and intra-chromosomal exchanges.

The location of the breaks involved in chromosome exchanges was analyzed along the painted chromosome. The breakpoint distribution was found to be randomly localized on chromosome 3 after neutron or Fe ion exposure, whereas non-random distribution with clustering breakpoints was observed after  $\gamma$ -ray exposure. Our comparison of chromosome aberration yields between 2- and 3-dimensional cell cultures indicated a significant difference for gamma exposures, but not for Fe ion exposures. These experimental results indicated that the track structure of the radiation and the cellular/chromosome structure can both affect radiation-induced chromosome aberrations.

## **Comparison of protein expression profile changes in human fibroblasts induced by low doses of gamma rays and energetic protons**

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Extrapolation of known radiation risks to the risks from low dose and low dose-rate exposures of human population, especially prolonged exposure of astronauts in the space radiation environment, relies in part on the mechanistic understanding of radiation induced biological consequences at the molecular level. While some genomic data at the mRNA level are available for cells or animals exposed to radiation, the data at the protein level are still lacking. Here, we studied protein expression profile changes using Panorama antibody microarray chips that contain antibodies to more than 200 proteins (or modified proteins) involved in cell signaling that included mostly apoptosis, cytoskeleton, cell cycle and signal transduction. Normal human fibroblasts were cultured till fully confluent and then exposed to 2 cGy of gamma rays at either low (1 cGy/hr) or high (0.2 Gy/min) dose-rate, or to 2 cGy of 150 MeV protons at high dose-rate. The proteins were isolated at 2 and 6 hours after exposure and labeled with Cy3 for the irradiated cells and with Cy5 for the control samples before loaded onto the protein microarray chips. The intensities of the protein spots were analyzed using ScanAlyze software and normalized by the summed fluorescence intensities and the housekeeping proteins. Comparison of the overall protein expression profiles in gamma-irradiated cells showed significantly higher inductions at the high dose-rate than at the low dose-rate. The protein profile in cells after the proton exposure showed a much earlier induction pattern in comparison to both the high and low dose-rate gamma exposures. The same

expression patterns were also found in individual cell signaling cascades. At 6 hours post irradiation, high dose-rate gamma rays induced cellular protein level changes (ratio to control  $>2$ ) mostly in apoptosis, cell cycle and cytoskeleton, while low dose-rate gamma rays induced similar changes with smaller fold-change values. In comparison, protons induced protein changes mainly in the cell cycle category. Thus, at the total dose of 2 cGy, high dose-rate gamma rays may generate more cellular responses through protein level and modification changes to regulate cell signaling and cell-cell communication. Protons presented the less effect, possibly due to the different track distribution compared with gamma radiation.