Mechanisms of Low Dose Radio-Suppression of Genomic Instability

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Summary:

The major goal of this project is to contribute toward the elucidation of the impact of longterm low dose radiation on genomic stability. We have created and characterized novel technologies for delivering long term low dose radiation to animals, and we have studied genomic stability by applying cutting edge molecular analysis technologies. Remarkably, we have found that a dose rate that is 300X higher than background radiation does not lead to any detectable genomic damage, nor is there any significant change in gene expression for genes pertinent to the DNA damage response. These results point to the critical importance of dose rate, rather than just total dose, when evaluating public health risks and when creating regulatory guidelines. In addition to these studies, we have also further developed a mouse model for quantifying cells that have undergone a largescale DNA sequence rearrangement via homologous recombination, and we have applied these mice in studies of both low dose radiation and space radiation. In addition to more traditional approaches for assessing genomic stability, we have also explored radiation and possible beneficial effects (adaptive response), long term effects (persistent effects) and effects on communication among cells (bystander effects), both in vitro and in vivo. In terms of the adaptive response, we have not observed any significant induction of an adaptive response following long term low dose radiation in vivo, delivered at 300X background. In terms of persistent and bystander effects, we have revealed evidence of a bystander effect in vivo and with researchers at and demonstrated for the first time the molecular mechanism by which cells "remember" radiation exposure. Understanding the underlying molecular mechanisms by which radiation can induce genomic instability is fundamental to our ability to assess the biological impact of low dose radiation. Finally, in a parallel set of studies we have explored the effects of heavy iron particle radiation on largescale sequence rearrangements and we have discovered tissue specific differences in sensitivity to homologous recombination. DOE support has given rise to critical new knowledge about the biological impact of low dose rate radiation and about the underlying mechanisms that govern genomic stability in response to radiation exposure. This work has spurred interest in radiation among MIT scientists, and has fostered ongoing research projects that will continue to contribute toward our understanding of the biological effects of low dose radiation exposure.

Detailed Report:

Novel Platform for Continuous Low Dose Rate Irradiation

A major barrier to studies of long term low dose rate irradiation is the lack of infrastructure to support these studies. Most institutes do not have access to appropriate sources, and establishing new delivery systems is often cost prohibitive. With the support of the DOE, we developed a novel platform that delivers continuous low dose radiation to mice over the course of days or even months. Importantly, this technology is relatively straight forward to implement, and thus has the potential to open doors to low dose studies in other institutions.

Specifically, this project provided the opportunity to evaluate ^{125}I as a radiation source for long term animal irradiations. The use of this isotope, and especially in a liquid formulation, offers several advantages for this purpose. ^{125}I decays by electron capture, resulting in the emission of photons with energies 27to 35 keV. At these energies the source can be easily shielded, an essential requirement for sitting in standard animal care facilities. Importantly, a specially-shielded room is not required (unlike the case when ^{137}Cs or ^{60}Co sources are used). The limited penetrability of the ^{125}I photons results in essentially no dose to animal care personnel, an important consideration for long-term irradiations. On the other hand, while the ^{125}I photons are easily shielded by high-Z materials, they have sufficient energy to penetrate the low Z materials between the source and the animal and, further, can penetrate the animal with only a factor of ~2 reduction in dose deposition through the thickness of a mouse.

We found that the use of ^{125}I in a liquid formulation (rather than, for example, the solid seed configuration) allows for great flexibility in both (i) source configuration and (ii) dose rate. The extent and configuration of the radiation source can be modified simply by changing the size and shape of a fillable phantom. For instance, while we used large Nuclear Medicine flood phantoms for the work described here, smaller phantoms could be installed in a cell incubator for cell-culture irradiations. The liquid formulation leads to a uniform radiation field across the phantom (with the exception of edge-effects). Similarly, a large range of dose-rates can be accessed simply by varying the total activity added to the water phantom. We have found it a simple matter to maintain the desired dose-rate by weekly additions of the isotope to compensate for the reduction in activity due to radioactive decay. This ability to maintain any desired dose-rate is not possible when using solid ^{125}I seeds which are available in only finite activities.

Our experience with constructing large area sources for long-term irradiations of small animals based on ^{125}I has thus been very successful from the perspective of: (i) unlimited flexibility in targeted dose-rate, (ii) long-term uniformity of dose-rate, (iii) easy positioning in standard animal facilities requiring no room modifications, and (iv) personnel safety. There is one significant drawback to the use of ^{125}I for this purpose, however, and that is the cost of the weekly additions of the isotope, especially when high dose-rates are targeted. Weekly additions are, of course, necessitated by the 60 day half-life of ^{125}I. We therefore undertook an evaluation of a range of other isotopes in search of longer-lived radioactive species that would still provide the same experimental advantages as ^{125}I listed above.

We examined several possibilities in terms of: half-life, emission type and energy, chemical form, cost and availability. We found the most promising isotope for this purpose to be 241-Americium. This is an artificial isotope that decays by alpha-emission and is routinely used, in micro-curie quantities, in smoke detectors. Many of the decays leave the daughter, ^{237}Np, in an excited state, which undergoes prompt emission of both γ-rays (by internal transition) and Np x-rays (following internal conversion). It is these photons (14, 17, 18, 21, 26 and 60 keV) that are of interest here. Thus, like ^{125}I, ^{241}Am is attractive because the emitted photons are low enough in energy to be easily shielded for use in an animal facility or biology laboratory. Further advantages to americium include the fact that it is in a solid form (and thus requires none of the additional safety procedures necessary for liquid sources) and its half-life of 432 years. No regular additions of activity are needed to maintain a constant dose-rate.

To generate an area source of americium large enough to irradiate significant numbers of animals requires significant activity. Few vendors supply americium, which is an artificial element. Negotiations with NRD Inc. (Grand Island, New York) led to the purchase of 2.3 Ci of americium in the form of 32 individual foils, each 3" x 14". With this configuration it will be possible to arrange the strips into many different spatial configurations, greatly increasing the flexibility of the source. Funding for these foils was supplied in part by this DOE program (33%) and in part by J.C. Yanch, collaborator (67%). Efforts are currently underway to characterize the radiation emitted by the foils. Of interest are (i) the dose rate at the surface of the foils, (ii) the homogeneity of the dose-rate across a given foil, (iii) the uniformity of dose rate among all 32 foils, and (iv) the dose-rate as a function of depth through water-equivalent materials (plastics, soft-tissue, growth medium, animals). Characterization will be performed using a combination of Geiger-Muller detector, phosphor imaging plates, and OSL (Optically Stimulated Luminescence) dosimeters. Americium will offers a highly suitable source for very long term mouse irradiations (potentially many months or even years), thus opening the doors to many studies that are relevant to long term health issues, such as cancer and aging. A manuscript describing the ^{125}I irradiator is currently under review.

Biological Impact of Acute versus Chronic Irradiation on Genomic Stability in vivo

To evaluate the biological impact of continuous low dose radiation, we evaluated both gross physiological characteristics and highly sensitive molecular responses. Mice were exposed to levels of radiation that are approximately 300X higher than background levels (0.3 rads/day). The weight of the mouse, and the weight of the spleen and the pancreas were evaluated. There were no changes in these gross parameters. Several sensitive molecular end points were also evaluated. Initial studies were focused on application of a mouse model that we have developed in which homologous recombination events can be detected by emission of a fluorescent signal. The Flourescent Yellow Direct Repeat (FYDR) mice carry an integrated transgene that give rise to a fluorescent signal following a homologous recombination event. Significant effort has gone toward the development of this mouse model for studies of radiation-induced homologous recombination (see below). For example, we demonstrated that recombinant cells accumulate with age, thus demonstrating the utility of these mice for long term studies of the accumulation of sequence rearrangements. In our studies of long term low dose exposure, we found that exposure to 5 weeks of continuous radiation at 300X background did not have any impact on the frequency of homologous recombination events. These results are consistent with normal repair processes being sufficient to prevent radiation-induced DNA damage from giving rise to large scale sequence changes. Importantly, these results also show that any potential adjustments in the steady state levels of DNA repair are not significantly impacted by this level of exposure. As an alternative method for evaluating large scale sequence changes, we also measured the frequency of micronuclei. This assay has been shown to be one of the most sensitive assays available for evaluating DNA stability. We found that we were able to detect an induction of micronuclei when animals were exposed to an acute dose of 10 rads. In contrast, when 10 rads was delivered over the course of 5 weeks (at the level of ~300X background), we did not detect any increase in the frequency of micronuclei. Taken together, these data are consistent with there being no significant increase in double strand breaks in the exposed animals.

As an alternative approach for evaluating DNA damage, we collaborated with the laboratory of Prof. Peter Dedon to measure the levels of DNA base damage. We were interested in a lesion

that is known to be directly induced by radiation (8-oxo-2'deoxyguanosin) as well as lesions that are induced by stimulation of the inflammatory response (1,N(6)-etheno-2'-deoxyadenosine and 2'-deoxyinosine). Our hypothesis was that low dose radiation may not directly induce detectable increases in DNA damage, but it may lead to changes in steady state repair capacity, or it may modulate the immune response. Using liquid chromatography isotope dilution tandem mass spectrometry, the levels of 2'-deoxyinosine from nitrosative deamination, 8-oxo-2'-deoxyguanosine from oxidation and 1,N(6)-etheno-2'-deoxyadenosine from lipid peroxidation were measured in spleens from mice exposed to 5 weeks of 0.3 rads/day. These three lesions are known to be biological significant. While 8-oxoguanine and 2'deoxyinosine are highly mutagenic, 1,N(6)-etheno-2'-deoxyadenosine inhibits DNA replication and is thus cytotoxic. No significant change in DNA damage levels was observed. In addition, we evaluated the levels of these three lesions in animals exposed to an acute dose of 10 rads (equal in total dose to the 5 week exposure condition), and we did not observe any increase in the levels of these three lesions, which is consistent with rapid repair by DNA glycosylases. These studies are important for two reasons. First, the most sensitive assay for DNA damage was used to evaluate the genomes of mice exposed to long term low dose radiation, and even with these methods, no significant change was observed. Second, liquid chromatography isotope dilution tandem mass spectrometry is sufficiently sensitive to measure spontaneous levels of DNA damage. Knowing the limits of sensitivity lends weight to the statement that long term low dose radiation delivered at 0.3 rads per day does not lead to any increase in the levels three biologically important base lesions.

Finally, as an alternative approach to evaluate genotoxic stress, we measured the transcript levels of several genes known to be sensitive indicators of radiation exposure. The genes found to be most sensitive and consistently affected by radiation are part of the DNA damage response and repair network: Cdkn1a, Gadd45a, Mdm2, Atm, and Ddb2. Using RT-PCR, we evaluated the levels of these genes in mice exposed to 5 weeks at 0.3 rads/day. We did not observe any increase in the levels of these genes in the exposed cohort. To explore the sensitivity of the assay in our hands, we evaluated the levels of these genes in control animals and animals exposed to 10 rads of acute irradiation (equal to the total dose of the mice exposed for 5 weeks). We again did not observe any significant changes in gene expression. However, if samples were analyzed in a paired fashion (e.g., the same mouse was analyzed twice – once before exposure, and once after), then it was possible to discern significant changes in gene expression. Although not the main objective of these studies, these results call attention to the importance of inter-animal variation and the extent to which biological response can be masked. In line with the major objectives, importantly, these results show that there is no significant change in gene expression even when animals are exposed to ~300X background for 5 weeks, suggesting that this dose rate is below a threshold rate required to elicit a biological response.

The possibility that low dose radiation can give rise to protective effects is of great interest, and there has been extensive work done in vitro to show that adapting doses of radiation protect mammalian cells against the genotoxic effects of a subsequent challenge dose. We hypothesized that the animals exposed to long term low dose radiation might be changed in a subtle way and that this change may become 'amplified' if animals were tested for sensitivity to a challenge. Previous studies had shown that mammalian cells become resistant to homologous recombination events induced by a crosslinking agent (mitomycin C) if they are first exposed to a low dose of radiation. To test for an adaptive response in the mice, we allowed mice to live for 5 weeks at 0.3 rads/day and subsequently challenged them with exposure to an acute dose of

mitomycin C. There was no significant difference in the mice with and without pre-exposure to the long term low dose radiation. These results are consistent with the results described above, indicating that there is not any significant change in gene expression for several of the most sensitive indicators of genotoxic stress. Taken together, these studies are all internally consistent and point to a benign impact when animals are exposed to ~300X background radiation for 5 weeks.

Development of a mouse model for detecting radiation-induced homologous recombination

In addition to development of a novel exposure platform, efforts have also gone toward further development of a mouse model for detecting homologous recombination. In particular, we explored the utility of the mouse for studies of recombination in different cell types of particular interest to radiation studies (e.g., hematopoietic stem cells from animals, and hematopoietic and stromal cells from long term bone marrow cultures). We developed methods for studying homologous recombination in several tissues, we compared spontaneous recombination rates among tissues, and we also compared susceptibility of different tissues to radiation-induced homologous recombination. Finally, we collaborated with mechanico optical engineers to create a novel imaging platform that permits quantification of clonal expansion within intact tissue.

Studies of Radiation-Induced Persistent and Bystander Effects

Of great interest are mechanisms of genetic change that do not involve direct DNA damage. For example, it is well established that following exposure to radiation, there can be long term changes that render cells genetically unstable for many cell doublings following exposure. In addition, it has long been known that irradiated cells can induce DNA damage in naive bystanders. We have explored these two important biological processes both in vivo and in vitro. In collaboration with researchers at the University of Lethbridge, we have demonstrated for the first time that bystander effects occur across an animal and that there are epigenetic changes induced in naive tissues. In addition, we have recently performed extensive studies to reveal for the first time the underlying mechanism by which cells remember radiation exposure. Understanding the underlying molecular mechanisms by which radiation can induce genomic instability is fundamental to our ability to assess the biological impact of low dose radiation.

Studies of space radiation

In addition to the studies of long term low dose radiation we also explored the effects of galactic cosmic rays on DNA damage and repair in vivo. We are able to detect DNA double strand break repair by homologous recombination in mouse tissues using a mouse model, as described above. In a recent study we reported that these mice can be used to detect the effects of aging and DNA damaging agents on homologous recombination frequency in various mouse tissues in vivo. Here we investigated if 56Fe ions induce an increase in DNA double strand break repair due to a radiation induced increase in DNA double strand breaks. We exposed mice to 56Fe ions at a dose of 1Gy, at a dose-rate of 1Gy per minute and analyzed homologous recombination repair frequency in the pancreas. Interestingly, we did not detect an increase in HR frequency in exposed animals as compared to non-irradiated control littermates. This result indicates that a

dose of 1Gy 56Fe ions administered at 1 Gy per minute does not induce a significant amount of DNA double strand breaks in the pancreas. Another interpretation of this finding is, that there are tissue specific differences in radiation sensitivity. In particular cell turnover has been shown to be a critical determinant of DNA damage sensitivity. We therefore also investigated esophageal tissue, a highly proliferative epithelial tissue, for homologous recombination frequency. Importantly, we found an increase in esophageal HR frequency in exposed animals, that was on the verge of statistical significance (p= 0.056). Taken together, our studies on the effects of space radiation on DNA damage and repair show that the effects of galactic cosmic rays vary in a tissue-dependent fashion, potentially dependent on cell turnover.

Concluding Comments

Novel technologies have been developed for radiation studies, both for delivery and for analysis. In addition, we have revealed new biological responses to radiation and we have discovered the underlying mechanism by which radiation leads to long term transmissible changes in genomic stability. For space radiation, we have new data suggesting important tissue specific differences in susceptibility to radiation-induced sequence changes. For studies of long term radiation, we show that exposure to ~300x background does not lead to any detectable increase in genotoxicity, even when evaluated with some of the most sensitive cutting edge assays. In conclusion, it is anticipated that the novel technologies we have developed will be broadly useful for studies of radiation biology, and that our deeper understanding of the effects of radiation will contribute to a more solid foundation upon which to evaluate the impact of radiation exposure. Finally, we hope that our studies of long term low dose radiation in vivo will have an important impact on policy makers involved in regulatory decisions, as they consider the potential dangers of radiation exposure in the work place, or under conditions of environmental contamination.

Publications

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