

# HZE Particle Radiation Induces Tissue-specific and *p53*-dependent Mutagenesis in Transgenic Animals

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## Abstract

Transgenic animals, with the integrated target gene, provide a unique approach for measuring and characterizing mutations in any tissue of the animal. We are using the plasmid-based *lacZ* transgenic mice with different *p53* genetic background to examine radiation-induced genetic damage resulting from exposure to heavy particle radiation. We measured *lacZ* mutation frequencies (MF) in the brain and spleen tissues at various times after exposing animals to an acute dose of 1 Gy of 1 GeV/amu iron particles. MF in the spleen of *p53*<sup>+/+</sup> animals increased up to 2.6 – fold above spontaneous levels at 8 weeks post irradiation. In contrast, brain MF from the same animals increased 1.7 – fold above controls in the same period. In the *p53*<sup>-/-</sup> animals, brain MF increased to 2.2 – fold above spontaneous levels at 1 week after treatment, but returned to control levels thereafter. Radiation also induced alterations in the spectrum of mutants in both tissues, accompanied by changes in the frequency of mutants with deletions extending past the transgene into mouse genomic DNA. Our results indicate that the accumulation of transgene MF after radiation exposure is dependant on the tissue examined as well as the *p53* genetic background of the animals.

KEYWORDS: Transgenic, mutation, HZE radiation, *p53*.

## 1. Introduction

Exposure to densely ionized charged particle (HZE) radiation poses a significant human health risk in long-term manned space explorations. Published records of biological responses to HZE radiation have centered on either *in vitro* systems or clonogenic populations of cells harvested from *in vivo* model systems. Although it is important to evaluate the particle radiation-induced alterations in the genome at the tissues level in animal models, especially in tissues with limited proliferative capacities eg. the brain, technical limitations due to the lack of selectable somatic target genes and the limited *in vitro* clonogenic potential of most primary cultures have hampered the research in this area. The advent of transgenic rodent mutation model systems has provided a means to rapidly assay, in a statistically reliable way, mutations in the DNA from any tissue of the body over the period of the life span of the animal [1, 2]. These systems can be used to evaluate acute as well as the long-term consequences at the tissue level in response to radiation treatments. The plasmid-based *lacZ* transgenic mouse used in the current study contains multiple copies of the 5.3 kb *lacZ* target pUR288 reporter gene integrated in tandem into the genome of every cell of the animal [3, 4]. The target genes can be recovered with high efficiency and the system is sensitive to the detection of a broad spectrum of mutations, including large deletions extending into the host genomic DNA.

In recent years, a plethora of published literature is available that supports the involvement of *p53*

tumor suppressor gene function in cell-cycle regulation, proliferation, differentiation, oncogenesis and apoptotic signal transduction pathways. We have succeeded in crossbreeding *p53* nullizygous mice with the *p53* wild-type *lacZ* transgenic mice to obtain *lacZ* transgenic animals that are either hemizygous or nullizygous to *p53* gene function. The goal of our study is directed at examining the mutagenic potential of a single acute dose of HZE radiation in the brain and spleen of transgenic mice and address the question of whether functional copies of *p53* in the genome influences the frequency and molecular characteristic of the genetic damage incurred.

## 2. Materials and Methods

### 2.1. Animals

C57BL/6 Line 60/*lacZ* (*p53*<sup>+/+</sup>) transgenic mice were crossbred with *p53* nullizygous mice (Jackson Laboratories, Bar Harbor, ME) to obtain animals that are either *p53* nullizygous/*lacZ* (*p53*<sup>-/-</sup>), or *p53* hemizygous/*lacZ* (*p53*<sup>+/-</sup>).

### 2.2. Radiation

Four mice (2M/2F), between the ages of 8-10 weeks, from each group were whole-body irradiated with an acute dose of 1 Gy of 1 GeV/amu iron particles at BNL. The dose average LET of the beam is 146 keV/μm and characteristics of the beam is described

in Zeitlin et al. [5]. Brain and spleen tissues were harvested from control and exposed animals at 1, 4, 8 and 16 weeks post irradiation.

### 2.3. Transgene harvest and analysis

Total genomic DNA was isolated from the brain and spleen tissue using standard techniques and the MF of the integrated target *lacZ* transgene was measured using published techniques [6]. *LacZ* mutant clones were isolated, propagated and the plasmid DNA extracted using commercially available DNA extraction kits (Qiagen, CA). Plasmid DNA was restriction digested using 2 enzyme digestion systems: (1) dual Pst/SacI digestion producing a 3.4 kb and a 1.9 kb restriction bands in the pUR288 plasmid and (2) Rsa I digestion producing a series of 7 restriction fragments in the pUR288 plasmid. Gel separation of digested bands provided information on the size of the deletion in the *lacZ* mutants. In addition, Southern analysis of digested mutant DNA using mouse genomic DNA probes provided information regarding the events that involve the integration of mouse DNA into plasmid sequences.

### 3. Results

We measured *lacZ* mutation frequencies (MF) in the brain and spleen tissues at various times after exposing animals to an acute dose of 1 Gy of 1GeV/amu iron particles. The spontaneous *lacZ* MF for both tissues in all three animal strains with different *p53* genotypes appear to be between  $2-4 \times 10^{-5}$ . We observed temporal dependent changes in MF in the spleen of *p53+/+* animals with a maximum increase of up to 2.6 fold above spontaneous levels at 8 weeks post irradiation. In contrast, brain MF from the same animals increased 1.7 – fold above controls in the same period. MF in the brain and spleen harvested from irradiated *p53* hemizygous animals were not significantly different from spontaneous levels and did not change significantly over the period of 16 weeks after radiation. In the *p53-/-* animals, brain MF increased to 2.2 – fold above spontaneous levels at 1 week after treatment, but returned to control levels thereafter. In contrast, MF in the spleen of irradiated *p53-/-* animals show a progressive increase up to greater than 3 – fold higher than spontaneous level at 16 weeks post irradiation. These results suggest that the accumulation of mutations in the inert non-transcribed *lacZ* transgene varies as a function of time after radiation, and is both tissue – and *p53* genotype – specific.

We cloned a total of 240 mutants from the control and irradiated spleen and brain tissues and characterized the spectrum of deletion mutants in both tissues based on the size of deletion and the

presence of mouse-specific sequences in the *lacZ* plasmid cassettes. Clones with similar deletion patterns and were derived from the same animal were considered to have arisen from clonal expansion and were not included in the spectrum analysis. Consistent with the concept that spleen is a tissue with a high cell turnover, we measured a larger number of clonal expanded mutants in the spleen than in the brain for both control and irradiated samples. The majority of mutants harvested from the control brain involved small changes in the *lacZ* sequence that were not distinguishable from the control plasmid deletion patterns. On the hand, the majority of mutants harvested from the control spleen involved large deletions from 1 – > 3kb in size. The spectrum of spleen mutants harvested from irradiated animals were similar to that obtained from the unirradiated control animals. In the brain, a higher percentage of mutants had large deletions ( > 3 kb) with an increase in the number of mutant that showed positive for mouse sequences. These results suggest that, in addition to changes in *lacZ* MF after iron irradiation, molecular analysis of the nature of genetic damage also point to tissue specific effects.

### 4. Discussion

Our results indicate that the HZE radiation induces *lacZ* transgene mutations and that the magnitude of this induction is dependent on the tissue assayed, and the time of harvest. Genetic damage, as measured in the *lacZ* MF assay, appeared to persistent for weeks after an acute radiation exposure. We have previously demonstrated that, using the same animal, exposure to 1 Gy of iron beam resulted in dramatic immediate response in the hematopoietic system, as indicated by a large induction in micronuclei in circulating reticulocytes (MN-RET) [7]. The magnitude of the initial response and the subsequent recovery from genetic damage in both the *lacZ* mutation assay and the MN-RET are dependent on the *p53* status of the animal.

We have demonstrated that the plasmid-based *lacZ* transgenic mouse model system has the potential to reveal LET dependent characteristics of radiation damage and provide a correlation between the damage induced by ionizing radiation on Earth and in space. With a greater understanding of the fundamental mechanisms that underlie responses to ionizing radiation exposure at the molecular level *in vivo*, it should be possible to improve risk estimates for individuals who are exposed to ionizing radiation of various ionization densities, whether it is environmental, man-made, or cosmic.

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