INDUCTION OF RADIATION SIGNATURE MUTATIONS *IN VIVO* WITH CHARGED PARTICLES AT LOW FLUENCE

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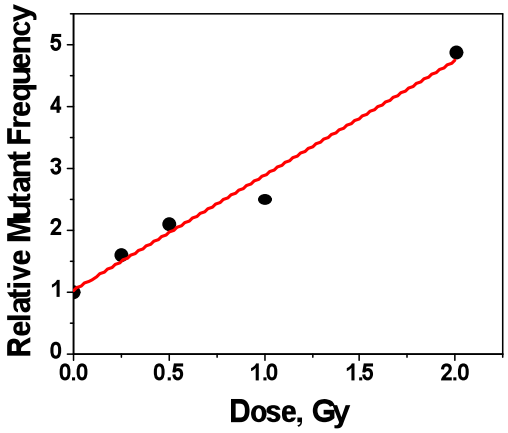
The health risks from exposure to charged particles in the space environment are not well defined, particularly at low fluence. One major exposure risk is cancer, which is associated with charged particle-induced mutations. We use a mouse model to detect autosomal mutations that range in size from small intragenic events (i.e., point mutations) to loss of entire chromosomes. This model is based on isolation of mutant cells from mice that are heterozygous for the selectable *Aprt* locus (*Aprt*$^{+/-}$), located on chromosome 8. *Aprt* mutant cells from charged particle-exposed or unexposed mice are expanded for molecular and cytogenetic analysis to identify radiation signature mutations. Signature mutations from high-dose charged particle exposures (200 cGy Fe ions, 1 GeV/amu; 500 cGy protons, 1 GeV) include large interstitial deletions, non-reciprocal translocations, and discontinuous loss of heterozygosity (LOH) along chromosome 8 [1, 2].

For the current study, we asked whether Fe and Ti ions (1 GeV/amu) at average fluences of 1 or 2 traversals per cell nucleus could induce radiation signature mutations in mouse kidney cells exposed *in vivo*. The doses used were 25 or 50 cGy Fe ions and 20 or 40 Ti ion, respectively. *Aprt* mutant frequencies rose as a function of dose, including at the lowest doses tested for both charged particles. The figure shows *Aprt* mutant frequencies for kidneys exposed to Fe ion doses ranging from 0.25 to 200 cGy; all increases were statistically significant including at 0.25 cGy (p=0.034; 1.6 fold increase). Molecular analysis showed the induction of radiation signature mutations at fluences of 1 or 2 traversals per nucleus, though less frequently than at the highest doses tested for Fe ions (2 Gy) and Ti ions (1.4 Gy). Chromosome painting demonstrated non-reciprocal translocations affecting chromosome 8 in the low fluence-exposed kidney cells. Finally, we found that LOH for mouse chromosome 14 is a marker of radiation effect in charged particle-induced mutants at high doses. This effect was also observed at the lowest fluence tested for both Fe and Ti ions. Our results showed that 23% and 19% of *Aprt* mutants from kidneys exposed to 25 cGy Fe ions and 20 cGy Ti ions, respectively, exhibited LOH for chromosome 14, as compared with 4% of spontaneous mutants.



In sum, we have demonstrated mutagenic effects in normal epithelium *in vivo* from exposure to high-energy heavy ions at an average fluence of 1 traversal per cell nucleus. The types of mutagenic events documented at low fluence are similar to those associated with the etiology of human tumors. These results suggest strongly that no lower threshold exists for autosomal mutations induced by exposure to these heavy ions, which is an important observation for modeling health risks from prolonged space travel.

[1] Turker M.S. et al (2009) *Rad. Res.* 172, 558-566.

[2] Turker M.S. et al (2013) *Rad. Res.* 179, 521-529.