AUTOSOMAL MUTANTS OF PROTON-EXPOSED KIDNEY CELLS DISPLAY LOSS OF HETEROZYGOSITY ON OTHER CHROMOSOMES

D. Grygoryev[1], C. Dan[1], S. Gauny[2], B. Eckelmann[1], A.P. Ohlrich[1], M. Connolly[1], M. Lasarev[1], G. Grossi[3], M. Turker[1,4], A. Kronenberg[2]

[1]Center for Research on Occupational and Environmental Toxicology (CROET), Oregon Health & Science University, Portland, OR 97239

[2]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

[3]Physics Department and Center for Radioprotection and Health Physics, Universita Federico II, Napoli, I-80126, Italy

[4]Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR 97239

Space exploration involves exposure to ionizing radiations not commonly encountered on Earth. High-energy protons are the most prevalent ion in the galactic cosmic radiation and their impact on human health is a concern with regard to cancer risk. Most human solid cancers occur in epithelial tissues and require mutations at autosomal loci.

We have utilized a mouse model (C57BL/6 x DBA/2) heterozygous for the *Aprt* locus to study proton-induced changes in normal kidney epithelium following whole body irradiation, or in kidney epithelial cells from the same hybrid strain grown on plastic. The mouse *Aprt* locus is located on chromosome 8. Proton exposures (1 GeV, LET=0.24 keV/μm, 0-5 Gy) were performed at the NASA Space Radiation Laboratory at Brookhaven National Laboratory. Mutant and non-mutant clones were isolated from kidneys harvested 3-9 months post-irradiation or from cultured kidney cells one week after exposure. Spontaneous mutants were used as controls. We employed a loss of heterozygosity (LOH) analysis on DNA from each mutant clone using thirteen polymorphic microsatellite loci on chromosome 8. Proton irradiation led to *Aprt* mutations that involve chromosome breakage and mis-repair, including multi-locus deletions, apparent mitotic recombination (MR) events, and discontinuous loss of heterozygosity (DLOH) on chromosome 8 [1].

We extended this study to test the hypothesis that some *Aprt* mutants isolated from proton-exposed kidney cells arose via a genome-wide incident that caused LOH-generating mutations on multiple chromosomes (termed here genomic LOH). Eleven additional chromosome pairs were surveyed using a PCR-based microsatellite analysis. Initial screening used one set of markers per chromosome pair while more detailed analyses used multiple marker sets per chromosome pair (typically 5 or 6 loci per set). The groups of clones that were analyzed included proton-induced *Aprt* kidney mutants (4 or 5 Gy), non-mutant clones that survived the same exposures, and spontaneous *Aprt* mutants from non-irradiated kidneys. Genomic LOH was common among proton-induced *Aprt* mutants. For mutant clones from exposed kidneys, genomic LOH increased as a result of proton irradiation ($p=0.004$ for 4 Gy vs. control; $p<0.001$ for 5 Gy vs. control). If we considered the total number of affected loci, more genomic LOH was found among proton mutants ($p=0.001$ for 4 Gy, $p<0.001$ for 5 Gy). *Aprt* mutants with apparent MR events or loss of chromosome 8 (CL) were enriched for genomic LOH ($p=0.005$ for CL events, $p<0.001$ for MR events). Only two non-mutant clones, one each at 4 and at 5 Gy proton exposure, had an LOH event elsewhere in the genome ($p<10^{-7}$ for 4 Gy, $p<2\times10^{-12}$ for 5 Gy), thus proton irradiation *per se* did not cause high levels of genomic LOH.

Genomic LOH events were also enriched amongst proton-induced *Aprt* mutants from kidney cells irradiated in two-dimenstional culture on plastic, compared with spontaneous mutants. The effect was marginal on a per clone basis ($p=0.09$), but if the number of affected loci was considered, the effect was significant ($p=0.045$). Mutant clones were more likely to demonstrate genomic LOH than non-mutant clones from irradiated cultures ($p=0.005$). Thus, genomic LOH could be stimulated by high doses of protons shortly after irradiation. However, a key finding of this study is that the tissue environment and long-term growth *in situ* enrich for genomic LOH following proton exposure.

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