

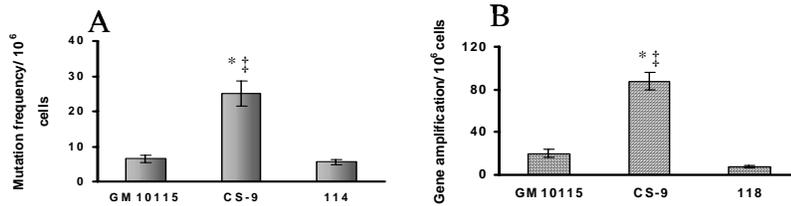
# Hydrogen peroxide significantly contributes to radiation-induced genomic instability

Disha Dayal<sup>1</sup>, Sean M. Martin<sup>1</sup>, Sujatha Venkataraman<sup>1</sup>, Charles L. Limoli<sup>2</sup>, Douglas R Spitz<sup>1</sup>.

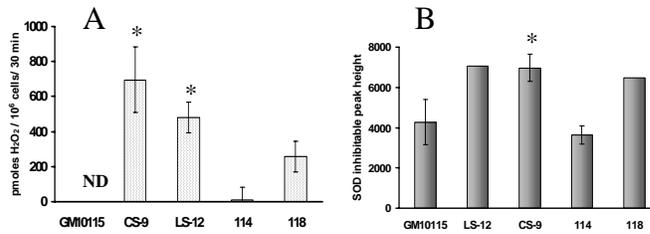
<sup>1</sup>*Free Radical and Radiation Biology Program, The University of Iowa, Iowa City, IA-52246,*

<sup>2</sup>*Department of Radiation Oncology, The University of California, Irvine, CA-92697*

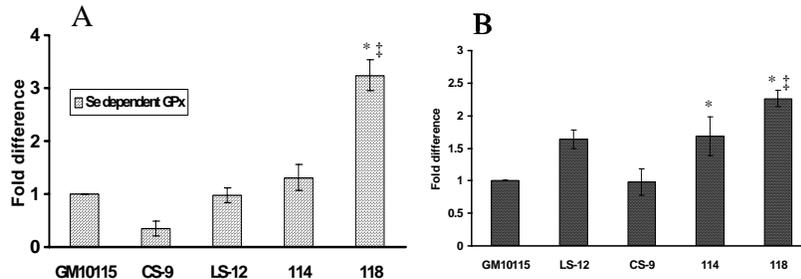
Chronic metabolic oxidative stress is associated with genomic instability following exposure to ionizing radiation (IR). Mitochondria have long been known to be a major source of reactive oxygen species (ROS) capable of causing oxidative stress. *We hypothesized that radiation damages mitochondria, leading to oxidative stress and eventually genomic instability.* This hypothesis is based on preliminary studies in parental hamster fibroblasts (GM10115) as well as genomically unstable (CS-9, LS-12) and stable (114, 118) clones isolated following exposure of GM10115 to 8 Gy IR. Preliminary observations show that CS-9 and LS-12 cells demonstrate increased genomic instability as determined cytogenetically as well as a 2-fold increase in spontaneous mutation rate at *hprt* locus and *CAD* gene amplification relative to the 114, 118 and GM10115 cells. In addition, the unstable cells show evidence of oxidative stress due to increased steady-state levels ROS *i.e.*, superoxide and hydrogen peroxide. Specifically, the CS-9 and LS-12 demonstrate a 2- to 3- fold increase in catalase-inhibitable extracellular hydrogen peroxide production as measured by the p-HPA dimer fluorescence assay. These cell lines also show a 3-fold increase in superoxide dismutase-inhibitable superoxide production from isolated mitochondria as detected by electron paramagnetic resonance, indicative of dysfunctional mitochondria as well as oxidative stress. Furthermore, catalase and glutathione peroxidase activities were found to be 2-fold elevated in the stable 114 and 118 cells, relative to wild type GM10115 and the unstable CS-9 and LS-12 cells. These results indicate that the CS-9 and LS-12 cells have elevated steady-state levels of intracellular pro-oxidants (presumably hydroperoxides) and lower levels of peroxide scavenging enzymes, relative to the wild-type GM10115 and genomically stable 114 and 118 cells. Treatment of the unstable CS-9 cell line with polyethyleneglycol conjugated catalase and adenovirus GPx reduced the mutation frequency and gene amplification by 50% providing strong evidence for the involvement of hydroperoxides in the induction of genomic instability end-points following irradiation. These results support the hypothesis that IR induces metabolic oxidative stress in mammalian cells creating an intracellular pro-oxidant environment that facilitates the propagation of the genomically unstable phenotype in cells post-irradiation.



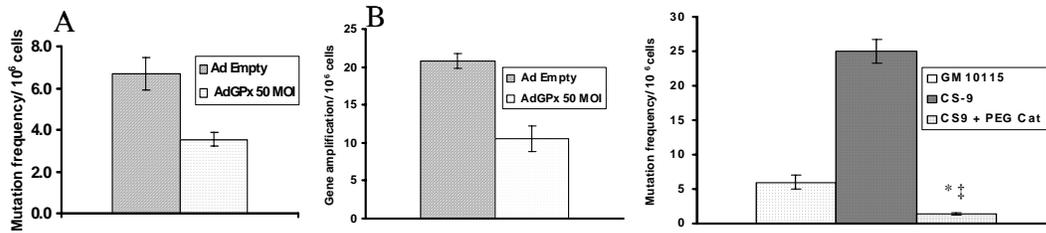
**Fig 1:** CS-9 cells show increased mutation frequency at the hprt locus (**Panel A**) and CAD gene amplification frequency (**Panel B**) as measured by clonogenic survival in 6-thioguanine (for mutation frequency) and media containing N-phosphonacetyl-L-aspartate (for gene amplification). Error bars represent  $\pm 1$  SD from 3 separate dishes.  $p < 0.01$  \* vs wild-type, † vs stable.



**Fig 2: Panel A:** The unstable clones CS-9 and LS-12 produce more catalase-inhibitable extracellular hydrogen peroxide as detected by p-HPA dimer product formation. There was no detectable catalase-inhibitable hydrogen peroxide produced in the wild-type GM10115. The error bars represent  $\pm 1$  SD of 6 independent experimental dishes.  $p < 0.05$  \* vs wild-type. **Panel B:** The unstable clones CS-9 and LS-12 produce more SOD-inhibitable superoxide as detected by EPR using DMPO spin trap. The error bars represent  $\pm 1$  SD from 3 independent experiments.  $p < 0.05$  \* vs wild-type.



**Fig 3:** The stable clones 114 and 118 show 1.5-2-fold increases in the activities of selenium-dependent GPx (**Panel A**) and/or catalase (**Panel B**) as compared to the wild-type control. The error bars represent  $\pm 1$  SD from 3-4 different experiments.  $p < 0.05$  \* vs wild-type, † vs unstable clones.



**Fig 4:** Mutation frequency (**Panel A**) and CAD gene amplification (**Panel B**) can be suppressed by 50% in the unstable clone CS-9 using adenovirus GPx (50 MOI). The error bars represent  $\pm 1$  SD from 3 separate dishes of 1 experiment.

**Fig 5:** Mutation frequency can be drastically suppressed in the unstable clone CS-9 using PEG catalase (100 u/mL). The error bars represent  $\pm 1$  SD from 4 separate experiments.  $p < 0.01$  \* vs wild-type, † vs CS-9.