

## Low Dose Ionizing Radiation and HZE Particle Effects on Adult Hippocampal Neurogenesis and mRNA Expression

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Most of our knowledge about low dose radiation effects relates to DNA damage and chromosomal aberrations that result in cell death or alterations in genetic programs leading to malignancy. In addition to direct DNA damage, there is accumulating evidence that radiation induced alterations in the microenvironment can have significant effects on programs of cell replication and differentiation such as neurogenesis in adult mammalian brain. Adult neurogenesis in the hippocampus is postulated to play an important role in learning and memory and manipulations that alter neurogenesis, including inhibition following radiation exposure, have been linked to changes in animal behavior. Our overriding hypothesis is that production of new neurons in the rodent brain represents a sensitive measure of CNS microenvironmental changes following radiation exposure. We further postulate that radiation elicits alterations in gene expression patterns, which may impact neurogenesis. We previously reported dose-dependent effects of both gamma ray and high-LET (HZE; <sup>56</sup>Fe) particle exposure on hippocampal neurogenesis, including decreased BrDU incorporation following HZE irradiation at doses as low as 1 cGy. Here we report initial results arising from our microarray analysis of hippocampal gene expression following exposure to gamma rays and high-LET (HZE; <sup>56</sup>Fe) particles.

Male C57BL/6J mice at 8-10 weeks of age were subjected to single-dose whole body radiation exposure, without anesthesia, at Brookhaven National Lab. For gamma rays, a total of 336 mice were exposed to a static <sup>137</sup>Cs source and were placed at various distances from the source in order to administer an estimated set of doses ranging from 1 to 300 cGy (1, 3, 10, 30, 100 and 300), with dose rates ranging from 2 to 37 cGy/min. An additional set of 48 mice was placed in the source room, but not exposed to radiation for use as controls. Of note, dosimetry for gamma radiation exposure was verified using the OneDose<sup>TM</sup> dosimetry system (Sicel Technologies, Morrisville, NC) with phantom mice run in parallel to live mice exposures. This revealed higher than expected delivered doses of 1.02, 3.06, 10.6, 36.1, 137, and 411 cGy. HZE particle exposure (<sup>56</sup>Fe, 1000 GeV) was conducted at the National Space Radiation Laboratory with 288 mice receiving 1, 3, 10, 30 or 100 cGy at dose rates ranging from 1 to 100 cGy/min. An additional group of 48 control animals were sham irradiated. Logistically, the two sets of exposures were carried out at different times, but variables were minimized as much as possible by using the same animal vendor as well as identical housing and handling conditions. For mRNA analysis, the dentate gyrus was microdissected from 6 mice for each dose at 8 h, 48 h, and 30 d following radiation exposure. RNA was extracted with Trizol and samples representing 0, 3.06, 36.1 and 137 cGy gamma or 0, 3, 30, and 100 cGy HZE at each of the time

points (144 samples total) were submitted for microarray analysis using the Illumina MouseRef-8 arrays (Asuragen, Austin TX). High-quality expression profiles were obtained from 141 samples. Data were analyzed with Partek (St. Louis, MO) software, including quantile normalization, principal components analysis, hierarchical clustering, analysis of variance (ANOVA), and t-tests.

There were 16,445 probes with signals above the background noise in at least one of the conditions. For 2,406 of these there was a "significant" (nominal  $P < 0.01$ , i.e.  $P$  not corrected for multiple comparisons) radiation type x dose x time interaction. A simpler set of ANOVAs (dose effects within each radiation type and time point condition) yielded the following numbers of differentially expressed genes at nominal  $P < 0.01$ : 1,187 for HZE at 8 h; 3,814 for HZE at 48 h; 1,824 for HZE at 30 d; 1,607 for gamma radiation at 8 h; 2,265 for gamma radiation at 48 h; 1,614 for gamma radiation at 30 d. Even at the lowest dose of radiation there were many differentially expressed genes ( $P < 0.01$  for dose effect by ANOVA and nominal  $P < 0.01$  by t-test for comparison of 0 and 3 cGy): 93 for HZE at 8 h; 864 for HZE at 48 h; 947 for HZE at 30 d; 154 for gamma radiation at 8 h; 200 for gamma radiation at 48 h; 294 for gamma radiation at 30 d. If the requirement for a significant effect in the overall ANOVA is removed, there are even more effects at the low dose. The majority of the largest effects ( $>1.5$ -fold) at 8 hours were increased expression whereas most of the larger changes were decreased expression (relative to nonirradiated controls) at 48 hours. Functional relations among differentially expressed genes are being explored with Gene Set Enrichment Analysis (GSEA), Database for Annotation, Visualization and Integrated Discovery (DAVID), and Ingenuity Pathways Analysis (IPA). Preliminarily, these tools have revealed that many biological processes are affected by radiation but that the lists are quite different for the various dose, time, and radiation conditions.

In conclusion, we have profiled gene expression patterns in the dentate gyrus and find significant effects at even the lowest doses tested (3 cGy). Further analysis of our extensive microarray data is underway to better understand the microenvironmental changes elicited by radiation that influence neuronal cell genesis and survival.

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