

## A Systems Genetics Approach to Low Dose Radiation

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The ORNL Low Dose Radiation SFA targets the need for research that identifies *Genetic factors that affect individual susceptibility to low dose radiation*, one of five research areas targeted in the Original Research Program Plan developed when the DOE Low Dose Program was established ([http://www.lowdose.energy.gov/about\\_originalplan.aspx](http://www.lowdose.energy.gov/about_originalplan.aspx)). The question of genetic susceptibility to low dose radiation exposure is addressed using mouse recombinant inbred strain panels that replicate the genetic variation of a population. These model systems enable two simultaneous and complementary outcomes when used in a systems genetics framework: 1.) *de novo* identification of genetic variants that mediate low dose sensitivity, and 2.) extraction of multiscale networks that span from genetic variation through coordinated gene expression networks to overlying cellular and tissue level adaptations and the resultant systemic effects.

The overarching hypothesis that guides the ORNL SFA is that individual sensitivity to low dose radiation (LDR) can be traced to differences in gene coexpression networks that are altered by radiation exposure. Accordingly, a population could be subdivided based on the types of networks that are activated to adapt to radiation exposure, and ultimately the individuals in each subgroup would exhibit differential radiation outcomes because of these differences in molecular adaptation. Initially we are using the BXD (C57BL/6J X DBA/2J) panel of 79 mouse recombinant inbred strains as our model system to test this hypothesis. The BXD strains have been extensively genotyped, enabling rapid genetic association studies. In addition, the two BXD parental strains have established differences in radiation sensitivity. Physiologically, our emphasis is on LDR effects on the immune system due to both its known radiosensitivity and its impact on many disease processes, such as carcinogenesis and cardiovascular disorders. The SFA includes focus effects on consequences of low dose exposure to the immune system, development of expanded computational and statistical algorithms for systems genetics (see abstract from Langston et al.), transcriptomic profiling in tissues of the immune and nervous systems, and creation of a tissue and sample bank that will be accessible to interested collaborators.

We have begun by first establishing the genetic architecture that underlies immunophenotypes in the BXD panel, reasoning that the coexpression networks that control radiation sensitive cell types are also likely to exhibit radiation sensitivity, and that genetic variation in these networks may mediate genetic susceptibility to radiation outcomes. We identified QTL models that explain the majority of variation in peripheral abundance of total numbers of B and T lymphocytes, and of helper (Th) and cytotoxic (Tc) lymphocyte subpopulations and their relative ratio (Th:Tc). Further, we identified two strong candidate genes, acid phosphatase 1 (*Acp1*) and protein tyrosine phosphatase, receptor type, K (*Ptprk*), for Th:Tc, each of which encodes a phosphatase that has been implicated in susceptibility to inflammatory disorders and cancer. Using spleen RNA from irradiated mice, we determined that each gene is also differentially expressed in response to low dose (10 cGy) exposure. Using graph algorithms we found that *Acp1* is part of a large coexpression clique that appears to be involved in cell cycle control. We are now working to link changes in *Acp1* and *Ptprk* expression to other genes within the LDR response network and to identify intracellular targets of their phosphatase activities in irradiated cells.

We have used the BXD strain set as a population-based model with which to test the hypothesis that LDR enhances immune function, as has been suggested by a number of *in vitro* studies. The ability

of peripheral blood neutrophils to phagocytose bacteria was selected as a functional immune endpoint that could be assayed *ex vivo*, without the need for tissue culture. Blood was collected from male and female BXD mice 48 hours after irradiation, and phagocytosis of labelled *e. coli* was measured using flow cytometry. We found that a single dose of 10 cGy from a  $^{137}\text{Cs}$  source significantly increased both the numbers of neutrophils engaging in phagocytosis and the numbers of bacteria engulfed by each phagocytic cell. While there was a main effect of radiation ( $p < 0.05$ ), we found no statistical evidence of an interaction between treatment and strain, suggesting that enhanced phagocytosis is robust to genetic variation. Total superoxide dismutase (SOD) activity and glutathione levels were measured in spleens of the same mice to determine if the anti-oxidant response to LDR showed heritable differences across the population. Unlike phagocytosis, there was a highly significant statistical interaction between strain and treatment ( $p < 0.001$ ), indicating that SOD activity in response to LDR depends upon genetic background. We used QTL mapping to identify genetic loci that were linked to differential SOD activity in irradiated mice, which uncovered a significant relationship between genetic variation on Chr 17 and SOD activity in LDR but not sham-exposed mice. To our knowledge, this is the first time that genetic sensitivity to low dose radiation has been associated with a specific chromosomal region using QTL mapping. We integrated SOD activity with microarray data collected from spleen of unirradiated mice from the same set of BXD strains to identify genes that showed significant correlation with SOD activity in irradiated but not control mice. Interestingly, we found a significant correlation between expression of mitochondrial Sod enzyme (*Sod2*) with LDR SOD activity ( $r = 0.475$ ,  $P = 0.0026$ ) but not with sham SOD activity ( $r = 0.0293$ ,  $P = 0.8611$ ). This relationship suggests that within a population, individuals with higher levels of *Sod2* expression might be less sensitive to the oxidative stress of LDR due to an enhanced ability to upregulate SOD activity. In addition to *Sod2*, baseline expression of apoptosis-inducing factor, mitochondrion-associated 2 (*Aifm2*) was differentially correlated with LDR SOD activity ( $r = 0.552$ ,  $P = 0.0003$ ) but not with sham SOD activity ( $r = 0.156$ ,  $P = 0.3497$ ). *Aifm2* is a DNA-binding protein with oxidoreductase activity and was originally described as a caspase-independent inducer of apoptosis, functions that have clear relevance to radiation response.

Finally, we used microarray expression profiling to assess the differences in LDR response between the parental strains at the molecular level, and to determine if differential sensitivity to radiation previously described for these strains at higher radiation doses extended into the low dose range. RNA was isolated from spleens collected from sham-exposed controls and from mice exposed to either 10 cGy or 1 Gy ionizing radiation 24 hours prior to sample collection. At 1 Gy exposure, we identified 562 genes differentially expressed compared to controls (q-value  $< 0.05$ ). As expected, this set of genes was significantly enriched in KEGG pathways for cell cycle ( $P = 2.17\text{E-}5$ ), p53 signaling ( $P = 2.01\text{E-}4$ ) and DNA replication ( $P = 5.90\text{E-}4$ ), among other pathways. Only five genes exhibited a significant strain\*radiation interaction (q-value  $< 0.05$ ), indicating that the molecular response to a 1 Gy dose was consistent between the two strains. In stark contrast, 1200 genes showed a significant strain\*radiation interaction after a 10 cGy exposure (q-value  $< 0.05$ ; fold-change  $> 1.5$ ). Gene ontology (GO) analysis indicated significant enrichment in functions related to cell cycle, apoptosis and immune response, among other functions.

We are now beginning follow-on studies directed to identification of the mechanisms through which LDR enhances neutrophil phagocytosis as well as expanding these studies to include effects of LDR on cell migration, chemotaxis and bacterial killing. As part of the larger SFA effort, we are also repeating irradiation of the BXD strain panel to include additional time points of exposure and exposure paradigms (e.g., repeated dose) and to create a tissue bank accessible to other investigators who may be interested in collaboration. The value of using systems genetics is amplified as additional physiological and phenotypic domains are studied, enabling a true systems level assembly of radiation sensitivity. Interested investigators are invited to contact Dr. Voy for further details.