

Molecular mechanisms and cellular consequences of low-dose exposure to ionizing radiation

Andrew J. Wyrobek¹, Francesco Marchetti¹, Xiu Lowe¹, Xiaochen Lu², Terumi Kohwi-Shigematsu¹, Brian Davy¹, Thomas E. Schmid¹, Sylvia Ahn¹, Tarlochan Nijjar¹ Matthew A. Coleman²,

Contact information: ajwyrobek@gmail.com

¹Life Science Division, Lawrence Berkeley National Laboratory, Berkeley, CA,

²BioSciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA.

The objectives of this research are to characterize the genome-wide molecular responses to low-dose ionizing radiation (<10cGy), to identify tissue and cell-type specific differences in pathways responses, and to identify the pivotal molecular pathway responses that control risks to genome integrity and health. This project utilizes mouse *in vivo* and human cell-line models, transcriptome and proteome profiling, bioinformatics and system biology tools for functional annotation and building gene networks, biomarkers of genomic and chromosomal instability, and biomarkers of tissue toxicity and cancer risk.

We analyzed the nature of the dose-response curve of transcript profiles in human lymphoblastoid cells from two unrelated individuals sampled at four hours after graded doses of 1.0, 2.5, 5.0, 7.5 and 10.0 cGy to identify novel radiation-responsive genes and to construct gene-interaction networks of low-dose response functions. A set of 291 low-dose responsive genes was identified (false discovery rate < 0.01) of which 81 genes showed consistent responses across doses and cell lines, with QRT-PCR validation. Most low-dose-inducible genes did not have a significant dose slope, consistent with plateau-like responses across the 1-10 cGy range. In addition, several genes had significantly elevated transcript expression at 1 cGy and significant Y-intercepts of the fold-change regression lines in both cell lines, as evidence for radiation induction at doses below 1cGy. Network analyses indicate that low-dose-responsive gene products are associated with cellular homeostasis (e.g., membrane signaling, molecule transport, immune response, metabolism); signal transduction linked to MYC, FOS and TP53 functions; and associated with specific subcellular locations (e.g., Golgi, mitochondria, and endoplasmic reticulum). In separate studies, we identified >100 genes including TP53 functions in human lymphoblastoid cells that were strongly associated with adaptive cytogenetic responses, providing new molecular insights into the mechanisms of cellular protection against radiation-induced chromosomal damage.

In collaboration with PNNL, LC-MS/MS mass-spectrometry was employed to identify proteins changes that were unique to low dose (10cGy) and others unique to a higher dose (2Gy) in human lymphoblastoid cells. Several of the 10 cGy-induced proteins were also identified in our gene-transcript experiments, suggesting that protein and transcript changes may be linked for certain proteins. The relationship between transcript and protein response is being further explored using proteomic antibody arrays and immunohistochemistry in tissue sections.

We are also conducting experiment to compare the human and murine transcript responses after low-dose exposures and to characterize the variations in expression of radiation-sensitive genes across tissues and cell types to examine the hypothesis that

different cell types employ different radiation-response mechanisms. Prior studies of transcript expression in unirradiated mice showed substantial variation among tissues in transcript expression of stress response genes and less variation among DNA repair genes. In recent studies with irradiated animals, we identified and characterized the time and dose dependencies of hundreds of radiation-sensitive genes in irradiated brain tissue. During the past year, we developed procedures to investigate the distributions of low-dose induced genes in the brains of irradiated animals using *in-situ* RNA hybridizations. Groups of mice were exposed to 0, 1, 10 and 200 cGy of ionizing radiation and tissue sections sampled at 4 hours after exposure were probed for transcript of PCNA and several other genes that had showed 1.2 to 3 fold radiation induction on microarrays. Among animals treated at 1 and 10 cGy, we found 10-50 fold increases in transcript labeling among different layers of the cerebral cortex layers and ~10 fold increases in the basal ganglia of irradiated brains, and found minimal to non-detectable radiation effects in other regions of the brain. Preliminary immunohistochemical analyses of PCNA protein distributions were consistent with the *in-situ* RNA results. SOD-1 also showed ~10 fold increases in transcript labeling in the basal ganglia after 10cGy exposures. Studies are ongoing with probes for other radiation sensitive genes to investigate relative pathway utilization among the different cell types of the irradiated brain and in other organs.

We are applying bioinformatics tools to compare the low-dose transcript responses in human and mouse cells. Common pathways in both systems were associated with TP53, MYC, FOS and YWHAZ (14-3-3, tyrosine 3-monooxygenase; involved in ERK/MAPK, IGF-1 signaling). In addition, four pathways detected in irradiated brain tissue were not evident in irradiated lymphoblastoid cells: JUN, TNF, SP1 and IFNG.

The applications of our project are to improve knowledge of the early cellular responses to low-dose IR and to reduce the uncertainty of assessing risk at low-dose levels. This project continues to identify novel genes and molecular pathways associated with low-dose IR exposure, setting the foundation for understanding risks to genomic integrity and disease susceptibility in tissues irradiated *in vivo*.

[Supported by the U.S. Department of Energy under Contract Nos. W-7405-Eng-48 and DE-AC02-05CH11231 with funding from the DOE Low Dose Radiation Research Program]