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Genome Wide Evaluation of Normal Human Tissue in Response to Controlled, In vivo Low-Dose Low LET Ionizing Radiation Exposure: Pathways and Mechanisms
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During course of this project, we have worked in several areas relevant to low-dose ionizing radiation.

In-Vivo Human Time Course Data

We have three papers from data derived from the human in-vivo low-dose studies, two published and one still under review. The first (Goldberg et al. 2005) evaluated differences in gene expression due to dose. Human skin samples from men exposed to 0, 1, 10, and 100 cGy during the initial radiotherapy treatment for prostate cancer were analyzed for gene expression using the Affymetrix HGU133 Plus 2.0 platform. This study was funded under the predecessor grant to this one (DOE DE-FG03-01ER63237), as were several important methods papers that were used in the subsequent projects from the present grant. These included Berglund et al. (2007), Lehmann et al. (2006), Lei and Rocke (2005), and Rocke et al. (2005).

The second (Berglund et al. 2008) examined transient changes in expression over time from a low dose. Using the same protocol and analysis methods, we analyzed nineteen gene groups and seven pathways for transient response, defined here as a transcriptional response that is elevated or depressed at 3 and 8 hours compared to the baseline at 0, and at 24 hours. We found nine gene groups that showed such transient differential expression.

The third (Kalanetra et al. 2013) is a more detailed examination of time-course behavior of gene expression from in-vivo exposed skin. In this new data set, we have split observations at 0, 3, 8, and 24 hours post irradiation by 10cGy (eight samples per subject) and eight patients with usable samples. We obtained comprehensive gene expression data using the Illumina platform and utilizing a complex data analysis strategy we obtained 1766 differentially expressed genes. We found that pathways associated with apoptosis and survival, development, cell adhesion, DNA damage, immune response, signal transduction, cytoskeleton remodeling, G-protein signaling, cCTP/CTP and cUTP/UTP metabolism, cell cycle, ATP and ITP metabolism, GTP and XTP metabolism, and CFTR folding and maturation were all significantly affected. This is in review.

MatTek EpiDermFT Skin Plugs

Despite being widely investigated, cell lines grown as monolayers provide a limited insight on the biological processes occurring under a given condition. EpiDermFT is a three-dimensional skin model which is composed of normal human epidermal keratinocytes (NHEK) and normal human dermal fibroblasts (NHDF). This skin model is mainly utilized for studying human skin responses to cosmetics and topical agents. We

aimed to test and validate it by assessing its gene expression following LDIR exposure against known responses of actual human skin irradiated in vivo. The plugs were irradiated with 0, 10, and 100 cGy at a dose rate of 50 cGy/min. Skin plugs were harvested at 0, 3, 8, and 24 hours post irradiation, with duplicates at each combination of dose and time, for a total of 32 samples. RNA was extracted and gene expression assessed using the Illumina platform. Results from this analysis appear in Yunis et al. (2012) and identify pathways responsive to radiation in this model. A further development of the methodology for analysis of such studies was provided in Ray et al. (2012) in which a graph-based approach was used.

Ex-Vivo Irradiated Human Skin

We have used discarded surgical skin to evaluate the transcriptional response to ionizing radiation. This model is less realistic than the in-vivo-irradiated skin, but more realistic than the MatTek skin-plug model. We have Illumina expression data from two subjects at baseline, and at doses of 5 and 500 cGy at 2, 8, and 30 hours following irradiation. There were 3–4 replicates per person per condition, resulting in 80 samples total. We have identified sets of differentially expressed genes by time by dose and by subject, and have identified the pathways and gene groups that are most significantly represented in the differentially expressed genes. The results of this study appear in Albrecht et al. (2012).

Other Areas

In connection with additional funding, we studied the proteomic interaction between oxidative damage from low dose radiation and from arsenic exposure (Berglund et al. 2009). We identified proteins that were differentially responsive to LDIR and arsenic, which helps elucidate the different responses to stimuli which share many common features.

We continue the development of analytical methodology, especially for the analysis of complex gene array experiments as well as other relevant assays. An example of such an effort is Xi and Rocke (2008).

Papers and Publications

Albrecht, Huguette, Blythe Durbin-Johnson, Reem Yunis, Karen M. Kalanetra, Shiquan Wu, Rachel Chen, Thomas S. Stevenson, and David M. Rocke (2012) “Transcriptional response of ex vivo human skin to ionizing radiation: comparison between low and high dose effects,” *Radiation Research*, **177**, 69–83.

Berglund, Susanne R., David M. Rocke, Jian Dai, Chad W. Schwietert, Alison Santana, Robin L. Stern, Joerg Lehmann, Christine L. Hartmann Siantar, and Zelanna Goldberg (2008) “Transient Genome-Wide Transcriptional Response to Low-Dose Ionizing Radiation In-Vivo in Humans,” *International Journal of Radiation Oncology, Biology, Physics*, **70**, 229–234.

Berglund, Susanne R., Alison Santana, Dan Li, Robert H. Rice, David M. Rocke, and Zelanna Goldberg (2009) "Proteomic Analysis of Low Dose Arsenic and Ionizing Radiation Exposure on Keratinocytes," *Proteomics*, **9**, 1925–1938.

Kalanetra, Karen M., Susanne R. Berglund, Reem Yunis, Huguette Albrecht, Shiquan Wu, Ruixiao Lu, Joerg Lehmann, Heather N. Witt, Robin L. Stern, and David M. Rocke (2013) "Gene Expression Time-Course Response of Human Skin Exposed in Vivo to Low-Dose Ionizing Radiation," submitted for publication.

Ray, Monika, Reem Yunis, Xiucui Chen, and David Rocke (2012) "Comparison of Low and High Dose Ionising Radiation using Topological Analysis of Gene Coexpression Networks," *BMC Genomics*, **13**:190.

Xi, Yuanxin and David M. Rocke (2008) "Baseline Correction for NMR Spectroscopic Metabolomics Data Analysis," *BMC Bioinformatics*, **9**:324.

Yunis, Reem, Huguette Albrecht, Karen M. Kalanetra, Shiquan Wu, and David M. Rocke (2012) "Genomic characterization of three dimensional skin model following exposure to ionizing radiation," *Journal of Radiation Research*, **53**, 860–875.

Other References

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Lehmann, Joerg, Robin L. Stern, Thomas P. Daly, David M. Rocke, Chad W. Schwietert, Gregory E. Jones, Michelle L. Arnold, Christine L. Hartmann-Siantar, and Zelanna Goldberg (2006) "Dosimetry for quantitative analysis of the effects of low-dose ionizing radiation in radiation therapy patients," *Radiation Research*, **165**, 240–247.

Rocke, David M., Zelanna Goldberg, Chad Schwietert, and Alison Santana (2005) "A Method for Detection of Differential Gene Expression in the Presence of Inter-Individual Variability in Response," *Bioinformatics*, **21**, 3990–3992.

Zhou, Lei and David M. Rocke (2005) "An Expression Index for Affymetrix GeneChips Based on the Generalized Logarithm," *Bioinformatics*, **21**, 3983–3989.