LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

UCRL-TR-202389

FEASIBILITY STUDY TO ESTIMATE PERSON-TO-PERSON STABILITY OF mRNA SIGNATURES OF RADIATION EXPOSURE IN HUMANS

C. Hartmann-Siantar, C. F. Manohar, M. A, Coleman, H. Kapur, C. Ramon, F. Hill, P. Gu, D. Nelson, J. Lehmann, A. J. Wyrobek

February 18, 2004

This work was performed under the auspices of the U.S. Department of Energy by University of California, Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.

LDRD Project Final Report

FEASIBILITY STUDY TO ESTIMATE PERSON-TO-PERSON STABILITY OF mRNA SIGNATURES OF RADIATION EXPOSURE IN HUMANS

Tracking Code number 03-FS-029
Account Number: 3939-55

Purpose:

The purpose of the research is to conduct two studies that are important for establishing the feasibility of gene expression profiles as biodosimeters of exposure to ionizing radiation.
Aim 1. To measure person-to-person variability in gene transcript response to radiation.
Aim 2. Measure the effects of time after radiation on gene expression profiles.

Background:

Gene expression is known to change with time and dose after exposure suggesting that it can be used to assess exposure dose in individuals after an exposure event. However, there is insufficient information to assess the applicability of this approach to the general population. Understanding the effects of time after radiation and gene-expression variability among individuals is important in assessing the feasibility of mRNA expression profiles as biological dosimeters of exposure to radiation. We proposed to investigate gene expression variation in mRNA signatures after exposure to ionizing radiation using a well- characterized national collection of human lymphoblastoid cell lines obtained from a diverse population of adults. The overall goal is to understand the biological variation as well as temporal aspects specific to the adaptive response process over the course of 48 hours.

Human Lymphoblastoid (HLB) cells were obtained from the NIH Human Genetic Cell Repository. The Human Genetic Cell Repository resource is comprised of cell lines from 450 unrelated individuals, male and female designed to reflect the diversity in the human and facilitates finding genetic variants in the entire human population from a random sample of residents of the United States.

We utilized gene transcript microarrays representing approximately 22,000 human genes per array to develop standard curves and characterize the effects of variables important for the application of radiation dosimetry: exposure dose, time after exposure, and individual variation. The primary focus of this study was to assess inter-individual variation at several selected doses and time points after exposure to help develop biodosimeters.

Activities:

Experimental Design and wet lab work:

Aims 1 and 2 Are integrated into a single study design that improves the efficiency and minimizes the DNA chip costs. Six cell lines: two adaptive, three non-adaptive, and one that was "synergistic" as determined in the study of Sorenson et al., 2002.

- Three exposures: 0 cGy, 10 cGy and 200 cGy.

- Post-exposure time points: 4 hours, and 24 hours for all cell lines.

- Eight post-exposure time points (15', 30', 1h, 2h, 4h, 8h, 24h, 48h) for one adaptive and one non-adaptive cell line were investigated.

Progress Report:

Vials of human lymphoblastoid cells were thawed and grown in culture until they were expanded and irradiated using a cesium source. Sixty milliter of cells in suspension culture, in a plastic tissue culture flask, were irradiated at specific doses following which they were incubated at 37^0C for defined times and then harvested. Cells were spun down, washed with Phosphate Buffered Saline, aliquoted into 6 tubes each and spun down. Dry pellets were flash frozen in liquid nitrogen, and transferred to -80^0 C. RNA is isolated from frozen cell pellets using standard protocols, and quantified/checked for quality with the Agilent Bioanalyzer and Spectrophotometer.

Samples Collected : Biodosimetry Project: 2 X 3 X 8 = 48 samples ; Interindividual Variation Project: 6 X 3 X 2 = 36 samples.Experimental control and test target messenger RNA were labeled using a T7amplification kit (Arcturus Inc.). Amplification followed by biotin labeling was performed to generate targets. Targets were next purified and fragmented according to the Affymetrix protocol. Labeled and fragmented RNA was checked to ensure quality. Microarrays were hybridized, washed and scanned for signal following the Affymetrix protocol using Affymetrix human GeneChips (HGU133A). MAS-5 Chip reports were generated and quality metrics performed to ensure quality of hybridizations.

Statistical & Bioinformatic Analyses: Data analysis proceeded in 3 stages.
Phase1: *Quality Assurance*: First, the data was quality checked and normalized with the best available algorithms. Our current approach is to use RMA (robust multiarray averaging) from the affymetrix package on the BioConductor web site.
Phase2 : Second, the data is currently being filtered using robust linear model techniques to obtain sets of genes exhibiting statistically meaningful expression differences across time points and cell lines.
Phase3: *Exploratory and Discovery Techniques*: The resulting subset of the data will then be analyzed using a variety of exploratory and model-based methods to discover set of genes with common patterns of expression and statistically meaningful interactions and correlations across time points. Inter-cell line variation will be assessed using random and mixed effect linear models. Genes that show robust response patterns across cell lines will become candidates for biodosimeters and validation by single-gene methods. Genes that show person-to-person differences will be evaluated as indicators of differential individual response.
Phase 1 of the Statistical Analyses is completed, Phase 2 and 3 are in process.