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Environmental Mutagen Society
Assessment of the Technologies for Molecular Biodosimetry for Human Low-Dose Radiation Exposure Symposium
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Mutational and Epigenetic Mechanisms of Susceptibility and Risks for Genetic Diseases
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Final Report, Agenda and Abstracts
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In the event of a radiological accident, the rapid evaluation of the individual absorbed dose is paramount to discriminate those individuals who must receive medical attention. New research with genomic- and proteomic-wide tools is showing that within minutes to hours after exposure to ionizing radiation the cellular machinery is modified. For example: large-scale changes occur in the gene expression profiles involving a broad variety of cellular pathways after a wide range of both low dose (<10 cGy) and high dose (>10 cGy) ionizing radiation exposures. Symposium 12 was organized to address a wide range of biological effects using the latest technologies. To address current models following ionizing radiation exposure, methods in biodosimetry and dose effects the symposia featured a general overview titled "Model Systems and Current Approaches in Biodosimetry" by Matthew A. Coleman, from Lawrence Livermore National Laboratory and a talk entitled "Brief Overview of Biodosimetry Projects in the NIH Rad/Nuc Program" by Dr. Narayani Ramakrishnan, National Institute of Allergy and Infectious Diseases. These two talk set the tone for issues in data and model integration as well as addressing the national need for robust technologies for biological dosimetry.

The first biomarkers of interest involved the use of mRNA transcripts using gene expression analysis technologies. Dr. Sally Amundson, from Columbia University Medical Center discussed "Rapid Assessment of Gene Expression Signatures for Biodosimetry". The team at Columbia University has developed robust expression signatures using both *ex vivo* and *in vivo* human models to identify signatures of ionizing radiation (IR) response (0.5 – 8 Gy) up to 48 hours after exposure. Their profiles included a diverse set of genes capable of distinguishing between multiple doses of ionizing radiation after exposure. The *CDKN1A* transcript of interest showed a similar response in both the *in vivo* and *ex vivo* human studies. In collaboration with Frederic Zenhausern and colleagues at the Center for Applied Nanobioscience of Arizona State University, they are also developing an automated gene analysis chip that can be used in the field for triage. Dr. James D. Tucker of Wayne State University followed with a talk entitled "Persistence of Gene Expression Changes Following Low Doses of Ionizing Radiation". Dr. Tucker's goal was to identify a minimal number of transcripts that were persistent out to 24 hours following low doses (0, 5, 10, 15, 25, 50 and 70 cGy) of ionizing radiation. Dr. Tao Chen, from the National Center for Toxicological Research, U.S. Food and Drug Administration, was selected from submitted meeting abstracts to discuss his work on "Determination of Marker Genes for the Exposure of Aristolochic Acid in Rat Kidney by Cross-Platform Comparison and Biological Function Choice". This talk added insight into down-selecting specific biomarkers of interest based on biological functions within pathways to increase the specificity of predictive genes. This approach is a novel way to integrate array-based findings from other laboratories using different approaches.

Cellular methods for biodosimetry were discussed by Dr Stephen D. Dertinger, of Litron Laboratories in his talk entitled "Flow Cytometric Scoring of Radiation-Induced Micronuclei: Reticulocyte- and Lymphocyte-Based Approaches". His talk detailed the comparison between traditional methods of scoring chromosomal aberrations (dicentrics) and micronuclei in lymph to a novel approach using blood reticulocytes. Dr Dertinger

and co-workers have also developed a rapid flow cytometric assay using TK6 lymphoblastoid cells for rapid clinical assessment using a two dye staining method (EMA followed by SYTOX Green). This method could rapidly sort out dying cells and discriminate between doses from 0.25 - 1 Gy.

Protein-based signatures were introduced by Dr. Natalia I. Ossetrova, from the Armed Forces Radiobiology Research Institute. Dr. Ossetrova discussed “Early-Phase Ionizing Radiation Response - Use of Protein Biomarkers for Triage Biodosimetry Applications”. The presentation compared protein expression data from ex vivo human, non-human primates and murine model systems exposed to varying doses of IR (0-7 Gy) with the goal of assessing multiple protein markers such as GADD45A, salivary a-amylase (SAA), IL6, ras-p21, raf-1, p53, and CDKN1A. Protein biomarkers GADD45A, SAA, and IL6 showed time- and dose-dependent increases in the murine model system after whole-body ^{60}Co gamma exposures (0-7 Gy) up to 96 hours after exposure. These protein expression profiles were useful for both predicting radiation exposure as well as distinguishing between the different doses of exposure. Results from the nonhuman-primate irradiation study showed that multivariate discriminant analysis for multiple proteins (p53, CDKN1A (p21 WAF1/CIP1, SAA, IL-6, and CRP) could successfully differentiate between non-irradiated and irradiated animals. As the number of biomarkers increased an enhanced separation between animal groups was observed providing a more accurate assessment of exposure.

The talks were of a high caliber and succeeded in covering biological changes associated with both low and high dose IR effects in their respective model systems. Evaluation of potential gene and protein biomarkers for early and late diagnostic information will be critical for determining the efficacy of genomic and proteomic signatures to both low and high dose IR exposures. It was clear that research is required to identify approaches that enable rapid handling and processing for mass-casualty and population triage scenarios. Development of *in vivo* human and non-human primate model systems will be crucial for validating both the basic biology and the instrumentation for biodosimetry. Such studies will also help further our understanding of the molecular mechanisms of the biological effects of radiation and the differences of responses due to individual genetic variation present in the human population.

Monday, October 22, 2007

1:30 PM–3:30 PM SYMPOSIUM 12: Assessment of the Technologies for Molecular Biodosimetry for Human Low-Dose Radiation Exposure

Chairperson: Matthew A. Coleman, Lawrence Livermore National Laboratory and Narayani Ramakrishnan, National Institute of Allergy and Infectious Diseases

1:30 PM–1:40 PM	Model Systems and Current Approaches in Biodosimetry *Speaker: Matthew A. Coleman, Lawrence Livermore National Laboratory*
1:40 PM–1:50 PM	Brief Overview of Biodosimetry Projects in the NIH Rad/Nuc Program *Speaker: Narayani Ramakrishnan, National Institute of Allergy and Infectious Diseases*
1:50 PM–2:20 PM	Rapid Assessment of Gene Expression Signatures for Biodosimetry *Speaker: Sally Amundson, Columbia University*
2:20 PM–2:35 PM	Persistence of Gene Expression Changes Following Low Doses of Ionizing Radiation *Speaker: James D. Tucker, Wayne State University*

2:35 PM–2:50 PM	Flow Cytometric Scoring of Radiation-Induced Micronuclei: Reticulocyte- and Lymphocyte-Based Approaches *Speaker: Stephen D. Dertinger, Litron Laboratories*
2:50 PM–3:20 PM	*In Vivo* Murine Dose-Response Calibration Curves for Early-Response Exposure Assessment Using Multiple Radiation-Responsive Blood Protein Biomarkers *Speaker: Natalia I. Ossetrova, Armed Forces Radiobiology Research Institute*
3:20 PM–3:30 PM	Determination of Marker Genes for the Exposure of Aristolochic Acid in Rat Kidney by Cross-Platform Comparison and Biological Function Choice *Speaker: Tao Chen, National Center for Toxicological Research, U.S. Food and Drug Administration*

Model Systems and Current Approaches in Biodosimetry. Coleman MA. LLNL, Livermore, CA, United States.

In the event of a radiological accident, the rapid evaluation of the individual absorbed dose is paramount to discriminate the worried but unharmed from those individuals who require immediate medical attention. Physical, clinical and biological dosimetry are usually combined for the best dose assessment. However, because of the practical limits of physical and clinical dosimetry, many attempts have been made to develop a dosimetry system based on changes in biological parameters, including techniques for hematology, biochemistry, immunology, cytogenetics, etc. The steadily increasing sophistication in our understanding of the biochemical responses of irradiated cells and tissues provides the opportunity for developing novel mechanism-based biosignatures of exposure. Evaluation of these potential gene, protein and small molecule biomarkers for early and late diagnostic information will be critical for determining the efficacy of the signatures. Also needed are approaches that enable rapid handling and processing for mass-casualty and population triage scenarios. Development of *in vivo* model system will also be crucial for validating both the biological and the instrumentation required for biodosimetry. Such studies will also help to gain an understanding of the molecular mechanisms associated with biological effects of radiation exposure.

Rapid Assessment of Gene Expression Signatures for Biodosimetry. Amundson SA. Columbia University Medical Center, Center for Radiological Research, New York, NY, United States.

Introduction: In the event of a large-scale radiological incident, there will be a critical need for rapid, high-throughput radiation biodosimetry for triage purposes. To address this need, we are developing an integrated biochip that will perform a chemoluminescence-based gene-expression assay directly from a blood sample. The biochips will contain all necessary reagents, pumps, valves and control electronics and will not rely on molecular amplification methods such as PCR. Methods: We have used Agilent whole genome microarrays to profile the gene expression response of human peripheral blood to ionizing radiation. We *ex vivo* irradiated human peripheral blood from healthy donors with gamma-ray doses from 0.5 to 8 Gy, and monitored global gene expression at 6, 24 and 48 hours after exposure. Advanced bioinformatics approaches are also being developed to reduce the computational overhead associated with the assay, allowing determination of dose from an individual sample without reference to a control or standard RNA sample. We have developed a hand-held, microprocessor-controlled prototype microfluidic cartridge. Results: Real-time PCR of *CDKN1A* revealed a biphasic response, with linear kinetics to 2 Gy and further increases through the highest dose used. A dose-response relationship was also evident within the microarray data. Analysis revealed sets of genes that distinguish between untreated controls and four different doses of irradiation at the various times. We have also used patients undergoing total body irradiation (TBI) as a model to test our gene expression signatures *in vivo*. *CDKN1A* expression *in vivo* after a single TBI fraction (1.5 Gy) showed induction ratios similar to the *ex vivo* 2 Gy samples. Our *ex vivo* dosimetry signatures were also able to discriminate control from irradiated patient samples, although differences from the healthy donors were also evident. Discussion: This biodosimetry concept was tested at the Coyote Crisis Campaign 2006, a disaster preparedness exercise in Scottsdale, Arizona. While additional studies are needed, our current findings strongly support the usefulness of gene expression signatures and our biochip approach for radiation biodosimetry. Supported by NIAID grant U19 AI067773.

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Abstract Not Available.

Flow Cytometric Scoring of Radiation-Induced Micronuclei: Reticulocyte- and Lymphocyte-Based Approaches. Dertinger S[1], Hyrien O[2], Chen Y[3]. [1]Litron Laboratories, Rochester, NY, United States, [2]University of Rochester Medical Center, Dept. Biostatistics & Computational Biology, Rochester, NY, United States, [3]University of Rochester Medical Center, Dept. Radiation Oncology, Rochester, NY, United States.

In instances of accidental radiation exposure or radiological terror events, incomplete or missing physical dosimetry will be the norm. Biodosimetry is therefore needed to make rational clinical management decisions, and for long-term carcinogenic risk assessments. Traditional biodosimetry techniques rely on cytogenetic damage endpoints (dicentrics and micronucleated lymphocytes), however these methods are too cumbersome to monitor large populations. Here we describe work with two flow cytometry-based assays that enhance the rate at which cytogenetic damage can be measured. In the first system, micronuclei are scored in blood reticulocytes. Mouse experiments suggest that radiation-induced micronucleus frequencies become saturated at approximately 1 - 2 Gy, depending on strain. Whereas this characteristic limits the assay's effective dose range, we will present data that suggests that the assay's utility can be extended beyond 1 - 2 Gy by combining the micronucleated reticulocyte endpoint with reticulocyte counts. This was achieved by modeling the joint distribution of these two endpoints with a parametric multivariate statistical model, with the dose estimated by the method of maximum likelihood. A second approach is based on flow cytometric scoring of micronuclei in blood lymphocytes. To date, this assay development work has primarily occurred with the human lymphoblastoid cell line TK6. The specimen handling and flow cytometric analysis procedures will be described. Results of experiments with low-LET irradiated cultures will be presented, together with parallel microscopy-based measurements. Technical challenges and future directions will be discussed.

***In Vivo* Murine Dose-Response Calibration Curves for Early-Response Exposure Assessment Using Multiple Radiation-Responsive Blood Protein Biomarkers.** Ossetrova N, Blakely W. AFRRI/USUHS, Bethesda, MD, United States.

The present need to rapidly identify severely irradiated individuals who require prompt medical treatment in mass-casualty incidents, as well as exposed vs. non-exposed individuals in population-monitoring radiation scenarios, prompted a murine *in vivo* dose- and time course-dependent study to evaluate the potential utility to use radiation-responsive blood protein biomarkers for exposure assessment purposes. Protein targets were measured by enzyme linked immunosorbent assay (ELISA) in male BALB/c mice (6-8 weeks old) blood plasma after whole-body ^{60}Co gamma exposure (10 cGy/min) to a broad dose range (0-7 Gy) and time-points (4-96 h). Our research strategy involves the use of human, non-human primate, and murine models involving *ex vivo* and *in vivo* radiation exposure to identify and validate radiation-responsive protein biomarkers. Using an *ex vivo* model of human peripheral blood lymphocytes as well as an *in vivo* murine model, we earlier reported radiation-responsive changes in the expression of proteins *ras*-p21, *raf*-1, GADD45a, p53, and p21WAF1/CIP1, each with a progressive time- and radiation dose-dependent increase. These results also revealed dose-dependent correlations among this subset of protein biomarkers, demonstrating their utility to identify potentially exposed individuals during the early assessment of radiation exposure. In addition, we recently presented similar data from non-human primates exposed to whole-body 6-Gy 250-kVp x-irradiation and 6.5-Gy ^{60}Co gamma-irradiation. Data analyzed with use of multivariate discriminant analysis established very successful separation of animal groups before and after irradiation. Here we present results from on-going murine *in vivo* studies demonstrating time- and dose-dependent increases in multiple blood protein biomarkers (i.e., GADD45a, IL-6, serum amyloid A). The use of multiple protein targets was evaluated using multiple regression analysis to provide dose-response calibration curves to enhance radiation sensitivity. Results from these *ex vivo* and *in vivo* validation studies establish an initial proof-of-concept that radiation protein biomarkers could provide useful diagnostic information for radiation-exposure assessment. [AFRRI supported this research under work unit BD-10.]

Determination of Marker Genes for the Exposure of Aristolochic Acid in Rat Kidney by Cross-Platform Comparison and Biological Function Choice. Chen T, Mei N, Guo L. National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR, United States.

Aristolochic acid (AA) is the active component of herbal drugs derived from *Aristolochia* species that have been used for medicinal purposes since antiquity. AA induced nephropathy and urothelial cancer in humans and malignant tumors in the kidney of rodents. Although our previous study found that AA induced more than two thousand differentially expressed genes in rat kidney, a reasonable number of signature genes for the AA exposure have not been determined. In this study, we explore the selection methods for multigene predictors for AA exposure. Lists of differentially expressed genes for AA treatment and riddelliine treatment (as a control for testing the specificity of the AA predictors) were generated from different microarray platforms: Affymetrix (Rat Genome 230 2.0), Applied Biosystems (Rat Genome Survey Microarray), GE Healthcare (RatWhole Genome Bioarray,300031) and Agilent (Whole Rat Genome Oligo Microarray, G4131A, used for generating testing data). Affymetrix was performed at two independent test sites, one for building the predictors and the other for generating testing data. Sets of 16 genes were selected by fold-change ranking when $p < 0.0001$ from the common genes among Affymetrix, Applied Biosystems and GE Healthcare, and from each individual platform with or without biological function selection. The sensitivity and specificity of these predictors were determined by evaluating their predictive percentages for the testing data. We found that the biomarker genes from cross-platform comparison were more accurate for predicting the AA exposure than those from an individual platform; and that the biological function selection increased the specificity of the predictive genes. Our results suggest that such an integrated analysis promises to be a valuable method for generating biomarker genes.