

MEETING REPORT

NASA Radiation Biomarker Workshop September 27–28, 2007

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A summary is provided of presentations and discussions at the NASA Radiation Biomarker Workshop held September 27–28, 2007 at NASA Ames Research Center in Mountain View, CA. Invited speakers were distinguished scientists representing key sectors of the radiation research community. Speakers addressed recent developments in the biomarker and biotechnology fields that may provide new opportunities for health-related assessment of radiation-exposed individuals, including those exposed during long-duration space travel. Topics discussed included the space radiation environment, biomarkers of radiation sensitivity and individual susceptibility, molecular signatures of low-dose responses, multivariate analysis of gene expression, biomarkers in biodefense, biomarkers in radiation oncology, biomarkers and triage after large-scale radiological incidents, integrated and multiple biomarker approaches, advances in whole-genome tiling arrays, advances in mass spectrometry proteomics, radiation biodosimetry for estimation of cancer risk in a rat skin model, and confounding factors. A summary of conclusions is provided at the end of the report. © 2008 by Radiation Research Society

INTRODUCTION

On September 27–28, 2007, the NASA Ames Research Center hosted a workshop on Radiation Biomarkers with

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support and participation from the Space Radiation Project Element (SRPE) of the Human Research Program, NASA Johnson Space Center. The overall goal of the workshop was to provide an update on radiation biomarker research across key sectors of the radiation research community—academia, clinical medicine, DOE laboratories, DOD laboratories and NASA—with an eye toward potential future applications in space. This was a first in a series of workshops to provide periodic updates and to help define research needs for future applications on long-duration human space missions.

With the possible exception of cataracts (1), there are presently no direct human data available for space-type radiation for any of the radiation-induced risks considered of highest priority by NASA for long-duration human space travel, i.e., carcinogenesis, acute and late CNS risks, chronic and degenerative tissue risks, and acute radiation risks (2). Although information exists from Earth-based studies sufficient to recommend crew exposure limits and spacecraft design requirements for missions in low-Earth orbit, there is insufficient knowledge about the health effects of space radiation to provide recommendations for crew exposure limits and design requirements for extended lunar and Mars missions (3). A major focus of the NASA space radiation effort is basic and fundamental research to expand the knowledge base and reduce the uncertainty inherent in current exposure limits and design requirements. At present, this includes a large number of radiobiological research projects performed mostly by peer-reviewed principal investigators in academia using accelerator-based simulated space radiation at the NASA Space Radiation Laboratory, Brookhaven, NY. The study of biomarkers (biodosimetry) is currently embedded within the radiobiology research. It is anticipated that information on the mechanisms and processes involved in space radiation dam-

age and repair will reveal specific indicators of space radiation exposure. The NASA Strategic Program Plan for Space Radiation Health Research (3) indicates that biomarkers/biodosimetry will be specifically pursued during Phase 3 (2010–2023).

Biomarkers to assess radiation response (and dose) in astronauts have been used since the early 1960s. The first such assessments were made by Bender *et al.* (4, 5), who measured dicentric chromosome aberrations in peripheral blood lymphocytes of crew members of Gemini-3 and Gemini-11. These flights lasted only 5 h and 3 days, respectively, and no significant increase in chromosome damage was observed when pre-flight and post-flight samples were compared. More recently, a substantial database has been developed from studies using various cytogenetic methods, including fluorescence *in situ* hybridization (FISH) techniques, to evaluate pre- and post-mission blood lymphocyte samples from crew members on Mir and ISS (6–9). These results indicate that radiation dose accumulated over a period of a few months or more can induce a measurable increase in the yield of chromosome damage. However, shorter missions of a few weeks or less appear to be below the detection limit for cytogenetic effects.

Advances in genomics, proteomics and experimental low-dose radiobiology are providing new opportunities for the development of radiation biomarkers. Given the lead time required for biomarker development and NASA's plan to return humans to the Moon by 2019 and onward to Mars by 2030, it is important to discuss the potential utility of biomarkers in space and the extent to which uncertainties in space radiation risk assessment could be reduced by biomarker-based research studies. Further discussions should include how the large number of animal radiobiology studies supported by several federal agencies could partner with studies of human biomarkers to facilitate cross-species extrapolation and ultimately extrapolation to humans.

THE SPACE RADIATION ENVIRONMENT

The radiation environment in space is complex; it includes charged particles such as hydrogen and iron and a myriad of secondary radiation including neutrons produced by charged-particle interactions with materials (e.g., spacecraft, astronauts, the surface of the Moon). During periods of low solar activity, the major contributor to dose in deep space or on the lunar surface is galactic cosmic radiation (GCR), which is composed primarily of high-energy protons (in the GeV range). GCR also includes heavier charged particles ranging from helium to iron ions (10). During periods of high solar activity (an approximately 11-year cycle), the probability for a significant solar particle event (SPE) is elevated. A large SPE can release a very high flux of charged-particle radiation—about 98% consists of protons, which are typically less than ~150 MeV.

High-energy GCR radiation is very penetrating and is therefore difficult to shield. For example, a 1 GeV proton

has a range of 324 cm in water and a 1 GeV/nucleon ^{56}Fe ion has a range of 27 cm in water. In contrast, solar protons typically penetrate less than about 10 cm in water, and the vast majority penetrate less than 1 cm in water, barely enough to penetrate a lunar extravehicular activity (EVA) spacesuit (11). The highly penetrating high-energy particles from GCR also produce more secondary radiation by spallation reactions in materials. In some cases, these secondary radiations have greater relative biological effectiveness (RBE) than the primary radiations. Thus shielding of GCR radiation poses a challenge for long-term space travel as well as for human habitation of a base on the Moon.

During non-SPE solar minimum conditions, the dose-equivalent rate in interplanetary space is estimated to be in the range of 0.5 to 1.4 Sv/year (12). Due to shielding by the Moon, the dose-equivalent rate on the lunar surface is estimated to be less than that in interplanetary space. However, lunar surface dose modeling is complicated due to the production of secondary radiations (e.g. neutrons) in the lunar regolith. It is estimated that a 6-month stay on the Moon would result in exposure to about 0.15 Sv (13). A 2.5-year roundtrip to Mars may result in an integrated dose equivalent of the order of 1 Sv (12). These dose estimates (which assume no significant SPEs during the mission) are not expected to result in significant short-term risk, but they may increase the long-term stochastic health risks that are associated with radiation exposure.

SPEs pose a different challenge. Although protons from SPEs are of much lower energy than those from GCR (and therefore can be shielded more easily), they pose an acute health risk for astronauts who are exposed during extravehicular activity (EVA). For example, if an astronaut had been participating in an EVA on the Moon during the August 1972 SPE and had received the full radiation from that event, the doses have been calculated to be 15 Sv to the skin, 1.9 Sv to the yellow marrow, and 0.9 Sv to the red marrow (11). These doses would have greatly exceeded the current 30-day dose limits established for LEO of 1.5 Sv for skin and 0.25 Sv for marrow. The dose rates during a large SPE can range from ambient to ~0.5 Sv/h for marrow and to more than 10 Sv/h for skin. Although these dose rates are considered to be high for most radiation protection conditions, they are not considered radiobiologically acute (defined as 1 Gy/min) and are expected to have an effectiveness higher than chronic radiation but lower than acute radiation. Thus the SPE radiation would have the additional complexity of intermediate and varying dose rate and response.

INDIVIDUAL SUSCEPTIBILITY

Dr. William Morgan (University of Maryland) addressed challenges associated with biomarkers of radiation sensitivity and individual susceptibility. He noted that when attempting to identify individuals at risk for developing radiation-induced cancer, one must consider the genetic com-

plexities involved in carcinogenesis. Thus it may be important to identify the genes responsible for the initiation, promotion and progression of cancer. However, the actual variants contributing to such complex diseases are unknown. The most common type of variation in the genome is the single nucleotide polymorphism (SNP). SNPs occur once in every 300–500 nucleotides (14). SNPs give rise to individual gene variants that alter susceptibility to common diseases. Consequently, mapping complex traits requires determining which of the myriad of SNPs influence disease risk. Rapidly developing technologies will facilitate identification of risk-related SNPs. However, any genetic variation may be complicated by gene-gene and/or gene-environment interactions. Each SNP is a rare event. Therefore, studies of a very large human population are required to identify SNPs that may be useful markers of disease susceptibility.

Nevertheless, there is little question about the role of DNA repair in ameliorating the effects of radiation-induced DNA damage and minimizing the incidence of cancer. Many of the cancer genes identified in family studies have a role in DNA replication and/or repair. For many DNA repair or repair-related genes, the loss of function is incompatible with normal development and often results in embryonic lethality. A more challenging question is the extent to which any alteration in the ability to repair damaged DNA contributes to the sporadic incidence of cancer. Given that most individuals show extensive sequence variation in their DNA repair genes, it is likely that susceptibility will vary among individuals, depending on the particular combination of inherited alleles.

Evidence for the importance of a moderate reduction in DNA repair is accumulating from animal models. BALB/c mice are sensitive to radiation-induced mammary tumors. Genetic linkage analysis indicates that this sensitivity is associated with allelic variation in the catalytic subunit of DNA-PKcs, a gene involved in the non-homologous end-joining pathway (15). In addition, a number of transgenic knockout mouse models have provided direct evidence for a significant role of DNA repair-related gene function in carcinogenesis. For example, mice that are heterozygous for a mutation in *ATM*, the gene involved in the disease ataxia telangiectasia (AT), have heightened susceptibility to cancer. Such mice are more sensitive to high-dose ionizing radiation than are their wild-type counterparts (16). It is estimated that ~1% of the human population is heterozygous for *ATM*. Data for mice suggest that *ATM* heterozygotes are susceptible to radiation-related cancer.

However, a number of *in vitro* experiments using cultured cells from AT heterozygotes have failed to demonstrate enhanced sensitivity to ionizing radiation, particularly at low doses (17). Most techniques are able to detect a shift in the average response of AT heterozygote cells compared with normal cells but with considerable overlap between the two groups. Although it is somewhat controversial, one assay that appears to provide excellent discrimination in-

volves X-irradiating cells in G₂ and quantifying radiation-induced cytogenetic damage (18). Furthermore, haploinsufficiency is only one factor that may induce susceptibility to radiation exposure. Allelic imbalance in gene expression levels can be caused by other factors such as *cis* acting regulatory polymorphisms in coding, intronic or regulatory sequences as well as by differential DNA methylation or histone acetylation. In addition to genetic factors, non-genetic factors may add to the complexity of radiation susceptibility. Such factors include lifestyle, diet, smoking and reproductive history.

While it is likely that one day it will be possible to identify radiation-sensitive/resistant individuals, such radiation responses may be normally distributed within the population. In adequately identifying such a phenotype, one must then consider how radiation responses are modulated by given genetic and epigenetic considerations as well as environmental impacts and must also consider a host of social, ethical and legal issues.

MOLECULAR SIGNATURES OF LOW-DOSE RESPONSES

Dr. Andrew J. Wyrobek (Lawrence Berkeley National Laboratory) discussed the applications of systems biology approaches for investigating the cellular responses to low-dose radiation using genome-wide gene expression technologies and bioinformatics tools. The objective of radiation systems biology approaches is to increase knowledge about early cellular responses to low-dose radiation and to reduce the uncertainties of assessing genetic health risk at low doses levels (19, 20). The early transcriptome profiling studies underscore the complexity of gene expression phenotypes and response pathways that are modulated in cells and tissues after exposure to low doses. Complex gene networks and pathways have been identified for low-dose exposures (21) and for cellular protection mechanisms against radiation-induced cytogenetic damage (22). Bioinformatic analyses have identified similar gene networks after low-dose exposures in both mouse and human models, suggesting that there are robust low-dose radiation responses across tissues and species. Radiation gene expression profiles have also been characterized for the proteome (23).

Low-dose effects on cells have been evaluated experimentally using a variety of exposure regimens, including acute, low-dose-rate or chronic, and adaptive response exposure regimens. Adaptive response regimens are important because they show that under certain circumstances and with some variability not yet understood, low-dose exposure can confer radioprotection against subsequent exposures. Typical adaptive response regimens use a low dose (priming dose) followed some hours later by a high dose (challenge dose) to determine whether the priming dose conferred protection against damaging effects of the challenge dose. Radioadaptive responses have been described for cell killing, DNA damage, chromosomal aberrations,

cancer latency and other cellular phenomena in a variety of biological models, most notably as summarized below.

Transcriptome analyses have been performed in cells of human cell lines irradiated *in vitro*. Analyses of a detailed dose–response curve (1 cGy–4 Gy) in human lymphoblastoid cells from two unrelated individuals identified a set of ~300 low-dose-responsive genes (1–10 cGy), several of which did not have a significant dose slope, consistent with plateau-like responses in the low-dose range. Bioinformatic analyses suggest that low-dose-responsive gene products are associated with cellular homeostasis mechanisms, special signal transduction pathways, and various subcellular locations.

Transcriptome analyses of the cytogenetic adaptive response have also been performed in human cells. More than 100 genes were identified whose expression was associated with the adaptive response (22). This study provided molecular insights into the mechanisms of cellular protection against radiation-induced chromosomal damage. It was hypothesized that the pathways associated with these genes are the basis of an adaptive response molecular switch that controls the degree of protection against radiation-induced chromosome damage in irradiated cells. Research is in progress to test this molecular switch in mice after whole-body irradiation.

Baseline gene expression has been evaluated in the tissues of unirradiated mice. Baseline transcriptional profiles were characterized for preselected genes associated with DNA damage recognition and repair processes among several tissues of healthy adult mice (testis, brain, liver, spleen and heart). Significant tissue variation was found in the baseline expressions of stress response, damage control and DNA repair-associated genes (21). Overall, stress response genes exhibited the greatest variation among tissues, with the highest expression in liver and heart, while DNA repair genes exhibited the least variation among tissues. A multitude of factors, including metabolic activity, immunological and inflammation status and oxidative damage, may affect the expression of stress response genes. Damage control genes associated with cell cycle regulation and DNA repair genes generally had the highest expression in testis. Variations in basal expression of DNA damage recognition and repair-associated genes among healthy tissues provided the foundation for investigating their differential response to genotoxic agents and susceptibility to genetic disease.

Transcriptome analyses have been performed to study the effects of whole-body exposure to radiation on mouse brain tissue. Cellular functions associated with altered transcript profiles were characterized for mouse brain exposed *in vivo* to low-dose γ radiation. Whole-body exposure of male mice to low-LET radiation altered the transcript expression in their central nervous system, with distinct time- and dose-dependent clusters, and identified gene sets unique to low-dose radiation (24). Advanced bioinformatics was applied to identify the major gene networks and biochemical pathways that were uniquely associated with low-dose and

high-dose exposures as well as pathways shared across doses. Brain irradiation modulated the expression patterns of over 1000 genes, of which >800 showed more than 1.5-fold variation. About 30% of genes showed dose-dependent variations, including genes exclusively affected by 0.1 Gy. About 60% of genes showed time-dependent variation, with more genes affected at 30 min than at 4 h. Early changes involved signal transduction, ion regulation and synaptic signaling. Later changes involved metabolic functions including myelin and protein synthesis. Low-dose radiation also modulated the expression of genes involved in stress response, cell cycle control and DNA synthesis/repair. This study demonstrated that doses of 0.1 Gy induced changes in gene expression that were qualitatively different from those at 2 Gy. The findings suggest that low-dose irradiation of the brain induces the expression of genes involved in protective and reparative functions while down-modulating genes involved in neural signaling activity. *In situ* analyses of tissue sections have provided important validation approaches to assess variations in radiation responses among different neuronal cell types [e.g. ref. (25)]. Our results support the model that brain tissue exposed to low-dose radiation responds through unique molecular pathways not observed after high doses, which underscores the problems that will be encountered when using high-dose data to infer low-dose mechanisms and to assess low-dose radiation risks in the CNS.

Dr. Wyrobek concluded that systems radiation biology approaches with advanced bioinformatics are showing substantial promise for improving the molecular understanding of the early cellular responses to low-dose radiation and to help to reduce the uncertainty of assessing risk at low doses. The finding of genes and pathways unique to low-dose radiation in both human cells and mouse brain tissues provides a foundation for identifying risk predictors for genomic instability and disease susceptibility in tissues irradiated *in vivo*.

MULTIVARIATE ANALYSIS OF GENE EXPRESSION

Dr. Nicholas Dainiak (Yale University) presented his work on multivariate analysis of cytokine gene expression after low-dose radiation exposure. He concluded that while DNA microarray analysis may provide insight into gene function within and across biological networks, meaningful data can be generated only when studies are appropriately controlled and when the state of a living system is well defined. Parameters that are critical for the study of radiation effects include radiation quality, dose, dose rate, cell type and tissue type. Owing to the enormity of the data set, appropriate methods of data analysis must be applied, including those that determine inherent grouping (hierarchical cluster analysis, or HCA, and principal component analysis, or PCA) and those that define known class membership (26).

HCA uses the entire data set to extract natural clusters

TABLE 1
Comparison of Gene Clusters from PCA and PP^a

Cluster	PCA	PP
1	<i>IL6, TNF</i> <i>TNFRSF1</i>	<i>IL6, TNF</i> <i>TNFRSF1</i>
2	<i>IL8</i>	<i>IL8</i>
3	<i>L8A, CSF1R</i>	<i>IL5RA, IL8RA</i> <i>CSF1R, IL2</i> <i>IL12A, IL6ST</i>
4		<i>IL2, IL2A</i> <i>IL6ST</i>
5		T-cell receptor <i>TNFRSF10A</i>
6		<i>MCPI, IFN</i>
7		<i>IL6R, IL1R2</i> <i>SCYA5, IL1R1</i> <i>TGFR3</i>

^a PP offers advantages over PCA (dimensionality reduction and noise reduction) while compensating for non-linearity. Reproduced with modification from Dainiak *et al.* (29).

without reducing the dimensionality of the data. Consequently, HCA becomes computationally unfeasible with very large data sets that also have an indirect relationship with a covariate. Methods that reduce dimensionality and eliminate non-significant information include PCA and projection pursuit (PP). A disadvantage of PCA is that it is unable to determine the maximum probability for heteroscedastic (i.e. non-uniform) uncertainties that may be correlated with each other. It is difficult for PCA to distinguish noise (i.e. spot variation) from systemic variance (i.e. bias in the microarray). Studies that have large within-group variance may be better analyzed by projection pursuit, a technique that has been used to analyze chemical data sets (27, 28). Accordingly, clustering may be revealed when within-group variance is large.

Whereas for PCA, latent variables are transformed into space according to the singular value decomposition algorithm, PP employs principal component scores to obtain sequential maximized χ^2 indices. The resulting PP factors are used to generate two candidate planes that are evaluated by the method of Posse, and the PP index with the least variance is defined as the new starting plane. The algorithm iterates until the most informative structure is obtained. Since PP seeks departure from normality, it is not sensitive to outliers.

Using an in-house PP algorithm and MATLAB and PP functions (Computational Statistics Toolbox), scores plots were compared from PCA and PP in microarrays prepared from mRNA of human subjects exposed to 0.18–49.00 mSv as a result of the Chernobyl Nuclear Power Plant catastrophe. Improved clustering was obtained when PP was used, both for the groups of identified genes and for intra-cluster variance (29). The data in Table 1 show that PP detects the expression of genes in seven distinct groupings 11–13 years after low-dose exposure.

The biological relevance of clusters identified by PP is

striking. For example, IL8, MCP1, TNF α and IL10 negatively regulate hematopoietic stem/progenitor cell proliferation. It is possible that such cytokines mediate cytopenias that occur after irradiation. In addition, TNFRSF (Fas) and its cognate ligand are overexpressed on the cell surface after irradiation (30, 31), an interaction that is required for radiation-induced apoptosis in lymphocytes (32). Furthermore, identification of overexpressed ligands (i.e. IL8 and TNF α) and their respective receptors in one or more clusters suggests that the pathways in which these molecules participate are involved in the biological response to ionizing radiation. It is unknown whether a gene in a cluster activates another gene of the cluster (i.e., overexpression of the two genes may be independent of each other). Regardless, PP has the potential to identify expression profiles that may explain biological effects of radiation exposure.

In conclusion, microarray technology has revolutionized genome-scale data collection by increasing the throughput of information. The plethora of information provided by microarrays must be assessed with tools that not only account for inherent noise components but also provide sufficiently robust analysis. The application of new tools such as PP and methods that are based on known class membership may address both of these issues and provide structures (i.e. gene clusters) that are more biologically relevant than those provided by traditional methods such as HCA and PCA. Since there is no consensus regarding the best method to analyze a multivariate data set, it is recommended that microarray data be submitted to public databases where information can be re-evaluated and interpreted as new methods for data analysis are applied (33).

BIOMARKERS IN BIODEFENSE

Dr. Ken Turteltaub (Lawrence Livermore National Laboratory, LLNL) discussed his extensive work on biodefense biomarkers. Over the last decade, interest in developing biomarkers for use in measuring hazardous exposures and risk from such exposures and for use in early disease diagnosis has grown exponentially. New technologies allow rapid high-capacity analysis of genes, proteins and most recently small molecules. While significant progress is being made, a number of studies have raised issues about the intra- and interlaboratory reproducibility of results and the influence of confounding factors on the ability to use biomarkers as a diagnostic test in the field.

Approximately 6 years ago, a project was initiated at LLNL to test the feasibility of detecting and diagnosing an infectious disease using changes in the levels of biomolecules in peripheral blood. It was proposed that the underlying biochemistry of an organism changes under the influence of a stressor such as an infectious agent. It was reasoned that these biochemical changes would begin as soon as the host cells began interacting with the pathogen and that a change in physiology would trigger changes in the levels of molecules residing inside cells and body fluids.

Detection of these changes might be used both to detect a developing disease early, possibly in the presyndromic or prodromal period, and to allow more rapid intervention, with the result being reduced morbidity and mortality. Thus a series of studies were conducted in rodents and in human samples to assess the capabilities of several high-throughput technologies such as arrays, mass spectrometry, gel electrophoresis, solution-based multiplexed antibody assays, and RT-PCR. A controlled experiment in rodents was conducted to discover which gene transcripts and proteins in plasma change in expression after rodents are challenged with a virus and when these changes would first be detectable. An apparently healthy human population was then studied to determine how variable a set of blood gene transcripts would be and how stably they were expressed over time. Finally, gene transcript changes and protein levels were measured in several human cohorts, including individuals with HIV, rheumatoid arthritis or bacteremia, dialysis patients, and a group of apparently healthy individuals during a marathon run. These groups simulated a series of potentially confounding factors such as individual variability, pre-existing conditions, and the effects of general physical stress on the levels of protein and nucleic acid transcripts in blood. An individual's response to radiation in space could be influenced by similar factors, including co-exposure to infectious agents, physical stress from living in an extreme environment, and individual differences in response.

The results in inbred laboratory animals suggest that, under controlled laboratory conditions, it is possible to detect pathological states with biomarkers a few days prior to development of overt symptoms. It is also possible to detect overtly ill humans using single biomarkers. Detection of prodromal disease states was much more difficult based on a single biomarker. Use of multiple markers combined into a panel can produce a signature that discriminates people with early pathological states from healthy people in some situations.

A variety of factors made discrimination of early pathological states difficult in the human studies, including sample collection, storage and shipping methods, sites of sample collection from the individual, and the method used for quantification. Significant changes in blood protein levels were found in the marathon runners both during and after the run. Significant differences in gene expression were found in mice depending on whether the blood was collected from the tail vein or by retro-orbital bleeding. Large differences in gene expression patterns were seen among rheumatoid arthritis patients. Thus sample collection, physical stress, pre-existing conditions and the method of analysis can affect the levels of potential biomarkers found in blood. It was concluded that use of molecular signatures to detect and diagnose disease in the presyndromic and prodromal phases of a disease's pathology is possible but that significant attention needs to be paid to understanding factors that affect levels of the potential markers as well as

the factors that affect their analysis. Attention should be focused on finding both sets of molecules that are sensitive to the disease state (which will likely be sensitive to other biological factors) as well as those that are refractory to confounding factors (which may be less sensitive to the disease state). Combining of these two groups of markers into panels may provide a signature that would be useful in (1) assessing exposure to hazardous environments, (2) determining risk from that exposure, and (3) diagnosing a developing disease early.

BIOMARKERS IN RADIATION ONCOLOGY

Dr. Srinivasan Vijayakumar, Dr. Andrew Vaughan and colleagues from the UC Davis Medical Center presented the radiation oncology perspective on biomarkers. Advances in the field of radiation treatment of tumors both clinically and with regard to the general effects of radiation on living systems require an integrated approach that combines studies of basic biological mechanisms and of the physics of dose deposition and measurement with the assessment of relevant end points of acute and chronic biological change. Biological responses have the potential to be applied to treatment of individual patients.

From the perspective of basic biology, the utility of biological markers of radiation effects may be viewed in different ways. The most direct utility of a biological marker is recording the presence of a radiation exposure at some time in the past. Levels of specific gene transcripts or proteins have been studied by a number of groups, and radiation responsive genes have been identified in a number of tissues, most commonly circulating cells of the blood. However, such studies are complicated by the assumption that the dose to an individual is uniform. Nonuniform dose dramatically increases the complexity of the analysis and thus the ability to predict biological response. In an effort to gain information on regionally defined irradiation, the response of buccal cavity cells was examined in patients undergoing irradiation for head and neck tumors. Such cells comprise the inner lining of the mouth and are easily sampled by gentle brushing of the mouth cavity and immediate extraction of RNA. Samples were taken from four quadrants (upper/lower, back/front) both before and after the first radiation dose of an approximately 30-fraction course. Such dose to the tumor target might peak at around 2 Gy, with doses to the sampling positions within the buccal mucosa ranging from this dose downward. The dose to each quadrant was determined by the planning computers used to configure the treatment and confirmed by MOSFET detectors placed within a mouth guard at the sampling sites. Samples produced RNA of suitable quality for analysis by qPCR. Using 50 cGy as a threshold, 12 genes known to respond to radiation exposure were assessed. Transcripts for *HSPC132A*, *MDM2*, *PCNA*, *CDKN1A* and *CCNG1* were significantly elevated after exposure. This study indicates the potential for buccal cavity transcript monitoring as a

guide to radiation exposure. However, in terms of analysis, issues still remain regarding both the transient nature of transcript elevation and the biological significance of a positive result.

To better address the complexity of the biological response to radiation, an alternative approach was considered. Many tumors, including those of the head and neck as well as the breast and elsewhere, exhibit loss of heterozygosity (LOH) at chromosome 11q23. This location may be the site of one or more tumor suppressor genes. LOH events are a known consequence of DNA double-strand breaks and therefore may be induced by radiation. Using cells of a mucoepidermoid cell line (H292) as a model, cells were irradiated with either 4 or 8 Gy, and the surviving cells were analyzed after two sequential rounds of cloning. LOH at 11q23 was detected using polymorphic markers for either the maternal or paternally derived chromosome that generated specific PCR products. This experiment showed that 11q23 was highly susceptible to LOH after irradiation, with 10–20% of all irradiated clones showing LOH at 11q23 but not elsewhere on chromosome 11. This analysis has distinct advantages over conventional transcript or protein profiling. First, the aberration is a marker linked to the transformation process. Second, the marker allows rapid screening of affected cells using PCR-based techniques. Third, the change is a permanent alteration in the genome (i.e., screening may be carried out on historically exposed individuals). Finally, unaffected cells within the sampled region provide an appropriate internal control. Therefore, this approach measures an individual biological response.

RADIATION BIOMARKERS AND TRIAGE— GENE EXPRESSION

Biomarkers and applications from the DHS perspective were discussed by Dr. Sally Amundson (Columbia University). In the event of a large-scale radiological incident, there would be a critical need for rapid, high-throughput radiation biodosimetry, both because of the need for medical triage and as an active reassurance measure to decrease panic among those not actually exposed. Currently available biodosimetry approaches are not adequate for these needs, so some have suggested the development of gene expression profiles as a biodosimetry approach amenable to development of high-throughput and fieldable assays (34–36).

Microarray analysis was previously used to identify 55 genes responding in human peripheral blood 24 h after *ex vivo* exposure to γ rays and demonstrated linear induction of *CDKN1A*, *DDB2* and *XPC* at doses from 0.2 to 2 Gy 24 and 48 h after exposure (34). Dose-dependent increases in the expression of these genes were also detected 4 and 72 h after exposure, but the increases exhibited less linearity. In a later study, 85 genes responded *in vivo* in humans after the first or second 1.5-Gy fraction of total-body irradiation (TBI) and showed dose-dependent increases of *CDKN1A*,

DDB2, *FCGR1A* and *CXCL10* through successive fractions (36).

More recent studies have used the Agilent whole-genome microarray platform (37). Global gene expression profiles of *ex vivo* irradiated human peripheral blood from unrelated healthy donors were measured at several times after irradiation. This study spanned a range of γ -ray doses relevant to medical decisions in a radiological triage situation and identified hundreds of genes responding to radiation. Quantitative real-time PCR of *CDKN1A* and other responding genes indicated a biphasic dose response similar to that seen previously in ML-1 human myeloid cells (38), with linear kinetics up to 2 Gy and further increases with a decreased slope through 8 Gy. There was also good agreement between gene induction using different irradiation and culture protocols, different donor pools, and different gene expression measurement techniques.

To make such gene expression signatures useful for triage, a collaborative effort is under way to develop microfluidic cartridges (39) to take a blood sample and automatically perform a chemiluminescence-based gene expression assay. The cartridges contain all necessary reagents, pumps, valves and control electronics, do not rely on molecular amplification methods such as PCR, and deliver highly consistent results (CV <10%). A hand-held, microprocessor-controlled prototype has been developed for sample preparation, and a commercial chemiluminescence reader is being modified for the microfluidic cartridges. This biodosimetry concept was tested at the Coyote Crisis Campaign 2006, a disaster preparedness exercise in Scottsdale, AZ.

The stand-alone version of the microfluidic gene expression-profiling cartridge could potentially be adapted to provide rapid turnaround biodosimetry to support extended space exploration missions. If, for instance, a solar particle event (SPE) occurred while astronauts were on the lunar surface, rapidly available biodosimetric information could be used to help determine whether an individual should be restricted to shielded areas for the remainder of the mission or, in extreme cases, whether a mission should be cut short. Targeted biodosimetry studies such as characterizing the gene expression response to SPE spectrum protons and validation at lower doses would also be needed to establish the usefulness of such an approach.

Additional studies are still needed to fully develop gene expression analyses for application in radiation biodosimetry. The *in vivo* responses must be more thoroughly characterized, including understanding the extent to which cancer patients undergoing TBI can be used as a model for healthy individuals. Animal studies, such as some already performed in mice (40) or planned studies in non-human primates, will also be critical. Another important area is determining the radiation specificity of the defined biodosimetry signatures. Since a large proportion of the *in vivo* response to ionizing radiation comprises p53-regulated genes and cytokines and genes involved in immune response (36), we need to be sure that infection, burns, gen-

eral injury responses or exposure to other toxins will not produce false-positive radiation exposure signals. The prototyped gene expression cassettes must also be tested thoroughly and their sensitivity and specificity determined. Despite the remaining questions, current findings strongly support the usefulness of gene expression signatures and a biochip approach for radiation biodosimetry.

RADIATION BIOMARKERS AND TRIAGE— MULTIPARAMETER APPROACHES

William F. Blakely (USU/AFRRI) in his talk entitled “Space Exploration Biodosimetry—Use of Integrated and Multiple Biomarkers” co-authored by his Armed Forces Radiobiology Research Institute (AFRRI) colleagues illustrated the potential dual use of AFRRI’s integrated biodosimetry for space-flight biodosimetry. Using a scenario of a radiation exposure during a Mars mission, Dr. Blakely showed how dynamic, space-flight deployable (41), and integrated multiparameter biodosimetry can provide key contributions in the medical management of acute radiation sickness (ARS). He recommended deployment of these or alternative software applications and consideration for use of blood cell counters and radiation-responsive protein bioassays for use on long-duration and other space-flight missions where radiation overexposures are possible.

Effective medical management of suspected radiation exposure incidents requires the measurement of dynamic medical data and physical dosimetry to provide diagnostic information for the treating physician and dose assessment for personnel radiation protection records. The accepted generic multiparameter and early-response approach includes observing prodromal signs and symptoms, obtaining complete blood counts with white blood cell differential, measuring radioactivity and monitoring the exposed individual, bioassay sampling when appropriate to determine radioactivity contamination, sampling blood for the chromosome aberration bioassay using the “gold standard” dicentric assay for dose assessment, and using other available dosimetry approaches. AFRRI’s Biodosimetry Assessment Tool (BAT) is a comprehensive software application developed for recording diagnostic information after suspected radiation exposures (42). AFRRI is also developing a First-responder Radiological Assessment Triage (FRAT) for use on hand-held personal digital assistant devices that provides data collection templates and the ability to integrate results for analysis of clinical signs and symptoms, lymphocyte counts, physical dosimetry, radioactivity and location-based dose estimates (43).

Identification and validation of early-phase radiation biomarkers are needed to provide enhancement of the ability biological dosimetry to assess individuals who may have been exposed to ionizing radiation (44). This is important in providing quantitative indications for early initiation (20 h after radiation exposure) of cytokine therapy in individuals exposed to life-threatening radiation doses as well as

to provide effective triage tools for first responders in mass-casualty radiological incidents (45). Monitoring of radiation exposure by biological dosimetry is complementary to physical dosimetry, since it can weigh radiation quality and dose rate according to biological efficacy. Molecular biomarkers are used as diagnostic end points in environmental health and cancer. Hofmann and colleagues (46) reported radiation-induced increases in serum amylase in 41 patients after either whole-body irradiation or irradiation of the head and neck region. Mal’tsev and colleagues (47) measured serum C-reactive protein (CRP) in Chernobyl radiation victims within 1–9 days after exposure and correlated its levels to the severity of ARS. Dr. Blakely’s research group’s working hypothesis is that hematological changes, gene expression and encoded protein biomarkers detected in biological samples (peripheral blood) can (a) distinguish the concerned public from individuals exposed to radiation and (b) triage exposed individuals by assessing radiation dose and injury. Their research strategy involves use of both *ex vivo* (human) and *in vivo* (murine, non-human primate) radiation model systems. They have employed quantitative methodology to measure changes in blood cell (e.g. lymphocytes, neutrophils) counts, multiple gene expression and encoded-protein targets as well as blood serum enzyme activities. Blood cell counts were measured using a clinical hematology analyzer. Gene expression targets [*GADD45A*, *DDB2*, *BAX*, *BCL2*, *CDKN1A* (p21/Waf/Cip)] were quantified by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) (48, 49). Encoded proteins (ras p21, raf, Gadd45a, Bax, Bcl2, p21/Waf/Cip, IL6, CRP) were detected by the enzyme-linked immunosorbent and microsphere (Luminex[™])-based assays (41, 50). Blood serum levels of amylase activity were measured using conventional commercial reagents used in blood chemistry analyzers (42). Radiation-induced changes in peripheral blood cell counts as well as up-regulation of gene expression and protein targets from various pathways (DNA damage and repair, cell cycle checkpoints, apoptosis/anti-apoptosis, cytokine, etc.) and radiosensitive tissue (salivary gland) from selected radiation model systems will be presented (41, 48–51). These results support the proof of concept that the use of multiple early-response biomarkers can provide useful diagnostic indices for medical management of radiation casualties.

ADVANCES IN WHOLE-GENOME TILING ARRAYS

Dr. Viktor Stolc (NASA Ames Research Center) discussed the cutting-edge genomics capabilities of the Ames Genome Research Facility (<http://phenomorph.arc.nasa.gov/>) and their recent work on high-density oligonucleotide tiling arrays. Identification of the transcribed regions in model organisms as well as the human genome is one of the major challenges of postgenomic biology for understanding human physiology. Empirical transcriptome mapping using whole-genome tiling microarrays has been shown to be the most comprehensive and unbiased ap-

proach. This novel method uses high-density oligonucleotide microarrays with probes chosen uniformly from both strands of the entire genomes including all gene-coding and intergenic regions. By hybridizing the microarrays with tissue-specific or pooled total RNA samples, a genome-wide picture of transcription can be derived. The comprehensive transcriptome analysis enables identification of the genetic basis of biological phenotypes and revealed transcribed sequences not detected by other methods. Advancement of microarray design to enable probing of polygenetic samples will significantly improve medical diagnostics and the efficacy of treatment in human diseases.

Comprehensive genome-wide analysis of transcription can be performed rapidly with high-density oligonucleotide microarrays designed to encode unique sequences that hybridize only to their intended complement RNA sequences. Significant computational resources are required to compute all possible sequence variants for the microarray design. The NASA Ames Genome Research Facility in collaboration with several academic laboratories produced optimized specificity and sensitivity of the oligonucleotide probes for detection and discrimination of very low-abundance transcripts (52–55). For example, in the sea urchin embryo, they were able to detect and discriminate the differential expression of very low-abundance RNA transcripts even for genes known to be expressed at low levels in only a few cells (52). Dr. Stolc stated that a recently completed high-resolution map of the mouse transcriptome produced using the same technique that they applied to map the human genome (53) and the sea urchin embryo (52) revealed significant regions of novel RNA expression that are syntenous between the mouse and human genomes. This comparative analysis also resulted in identification of short transcribed regions in the human genome, previously undetected solely from the human data due to lower statistical significance. Thousands of previously uncharacterized transcripts in the mouse genome enabled identification of several hundred previously undescribed human transcripts (55).

ADVANCES IN MASS SPECTROMETRY PROTEOMICS

Dr. Julie Leary (University of California, Davis) provided an overview of the state of mass spectrometry (MS) proteomic analysis. Mass spectrometric data from proteomic analysis were presented of both large molecular clusters and small peptides from proteins containing post-translational modifications (56, 57). New developments involving organic derivatizing agents can be used to unambiguously determine the sites of post-translational sulfation on proteins and peptides (56). In a somewhat related fashion, metal affinity columns are extremely successful enriching proteins and peptides containing low-level phosphorylation (57). Since phosphorylation and sulfation are isobaric, this combination of solution sample preparation and mass spec-

trometry can be paramount in distinguishing these important changes.

Mass spectrometry technology can be very effective for the analysis of various biomarkers, particularly peptides and proteins. Modern advances in this field have now produced instrumentation that is capable of analyzing large-molecular-weight (megadalton) multi-subunit architecture. This can be very powerful for investigating how proteins interact in both normal and diseased tissue as well as measuring stoichiometric changes to these proteins during radiation exposure. One can easily envision isolating protein biomarkers from serum samples of exposed individuals and tracking both the various post-translational modifications that may differ as well as comparison and contrast of proteins and peptides that may differ in exposed and nonexposed individuals.

RADIATION BIODOSIMETRY FOR ESTIMATION OF CANCER RISK IN A RAT SKIN MODEL

Dr. Fredric Burns (New York University) presented his work on radiation biodosimetry of cancer induction in rat skin. DNA double-strand breaks (DSBs) are relevant to carcinogenesis because they may rejoin, most frequently by non-homologous end joining, to create chromosome rearrangements and elevated genomic instability (58–61). Although DSBs occur spontaneously, their highly efficient induction by ionizing radiation is probably unique among carcinogenic agents (62). DSBs are also strongly linked to the cytotoxic action of ionizing radiation which competes with carcinogenesis, particularly at higher radiation doses (63, 64).

Radiation ionizations either occur within dense tracks (e.g. heavy ions) or are scattered randomly (e.g. X rays). As LET increases in dense tracks, DSB rejoining becomes more frequent because of the lower average distance between DSBs. For randomly distributed DSB rejoining increases with radiation dose to the power of 2, because each DSB is produced in proportion to radiation dose. The above considerations lead to an expression describing the expected yield of DSB rejoining for any type of radiation as follows:

$$\text{Yield}(D, L) = CLD + BD^2, \quad (1)$$

where L is LET, D is radiation dose, and B and C are to be evaluated empirically. Equation (1) is the well-known linear-quadratic function, except for the L in the linear term.

While it is plausible that Eq. (1) might correctly describe the yield of DSB rejoining, a leap is required to imagine that this same functional form might also be applicable to cancer induction. Surely the extensive genomic alterations associated with cancer progression would obliterate all traces of any initial DNA damage with causative relevance to the cancers. But what if Eq. (1) fits both cancer and DSB rejoining across a broad range of LETs and doses? Would that not be a contradiction of initial lesion obliteration and

raise the possibility that DSB rejoining is the long-sought, causative DNA alteration of radiation carcinogenesis? At present, the latter is an unproven postulate in any other organ or species, but in rat skin it appears to be well-founded based on carcinoma yields and estimates of DSB rejoining in surrogate keratinocytes. One explanation of how Eq. (1) could explain both cancer and DSB induction would be that the cancers originate in a small fraction of irradiated cells most probably stem cells, with just the right rejoining that permits long-term survival with elevated levels of genomic instability. It is the latter that eventually produces the additional genomic alterations required for malignancy.

As surrogates for rat keratinocytes *in vivo*, rat skin keratinocytes were irradiated with either ^{56}Fe ions (LET = 125 keV/ μm), X rays (LET = ~ 0.4 keV/ μm) or protons (LET = 25 keV/ μm), and DSBs were quantified by the *in situ* γ -H2AX antibody technique. The results for ^{56}Fe ions showed that many DSBs (H2AX-positive foci) were aligned along straight, parallel tracks oriented in the beam direction for at least 4 h after exposure. Interestingly, straight tracks persisted in parallel formation as nuclei rotated slowly relative to their orientation at time zero. The γ -H2AX antibody technique provides a way to estimate the yield of DSB rejoining so that quantitative comparisons with cancer yields for the same doses and radiation types become possible. The linear term in the DSB version of Eq. (1) was estimated based on total DSBs per track for the three radiation types studied as follows: two doses (1.5 and 3.0 Gy) of ^{56}Fe ions, roughly equi-carcinogenic doses (4.5 and 9.0 Gy) of X rays and Bragg-peak protons (0.3 and 1.1 Gy). At higher doses, every epithelial cell is expected to exhibit multiple DSBs, while the cancer probability is only of the order of 1 per 10^6 cells exposed. It is an advantage for biodosimetric purposes that DSBs are very frequent in comparison to the cancers, but proportionality between the two end points must be verified empirically throughout the dose and LET ranges typical of space radiation.

When DSB rejoining in surrogate keratinocytes and skin cancer yields for three diverse LET values was plotted as a function of radiation dose, an almost exact superimposition was apparent when proportionality was fixed at 100 DSBs per keratinocyte = 1.0 carcinoma per rat at 1 year. A plot of the cancer and DSB yields on the same coordinates showed cancer yields falling within the error bars of the DSB estimates at all six available doses. If both DSBs and cancers show dose and LET dependences as described by Eq. (1), a useful tool for predicting carcinogenic outcomes of various, possibly even complex, radiation exposure scenarios might become available on the basis of a comparatively simple short-term *in vitro* assay.

CONFOUNDING FACTORS

Dr. Terry C. Pellmar (Armed Forces Radiobiology Research Institute) discussed factors that may confound bio-

marker analyses after radiation exposure. Many factors influence biodosimetric assessments. The dose and time dependence of a biomarker clearly must be addressed for accurate biodosimetry. While some biomarkers might increase throughout the radiation dose range of interest, others might level off or even begin to decrease as the dose increases. Time of sampling can significantly affect measurements as well (41). Some markers increase slowly and are sustained; others increase quickly but only transiently. The quality of the radiation also affects the dose-response curve for biomarkers. For example, the calibration curves for cytogenetic biodosimetry using dicentric chromosomes show that fission neutrons are more effective than γ rays for an equivalent absorbed dose (65). Accurate interpretation of the assay requires information on radiation quality. In addition, the possibility of partial-body exposure needs to be considered. High radiation doses to small areas may not be revealed by a biomarker that reflects whole-body changes. Differentiation of partial-body from total-body exposures will be important for treatment decisions.

The specificity of a biomarker and the variability within the population must also be considered in the development of biodosimetric assays. For example, the prodromal symptoms, nausea and vomiting, are excellent indicators of radiation exposure but are also symptoms of other common conditions. In addition, the interindividual variability in the emetic response to radiation is large (66). Some people may vomit early after a relatively low dose while others may not vomit at all despite a serious exposure. Biomarkers that respond robustly to radiation may also respond to other conditions. Baseline levels of the biomarkers may be widely disparate in the population, making small changes difficult to discern (43). Changes in biomarkers may be altered by the health status of an individual and by any drugs that have been taken (36). Furthermore, any injuries that occur in addition to the radiation exposure could have an impact on biomarker levels.

Biomarkers are very useful in defining a radiation exposure but many confounding factors exist that must be considered in their interpretation.

DISCUSSION AND CONCLUSIONS

The workshop presentations stimulated the following discussion and conclusions.

Cytogenetic analyses in peripheral blood lymphocytes have been used to reconstruct radiation dose to astronauts in space [e.g., see refs. (4–9)]. Although physical radiation monitoring is employed on all human missions in space and more sophisticated technologies will be available for return to the Moon, the advantage of biomarkers is measurement of the biological response in the individual, which includes contributions from dose, dose rate, radiation quality and biologically based modifiers of response such as DNA repair. Thus biomarkers provide a measurement that would be expected to correlate better with health outcome than a

physical dosimeter alone. An accurate biomarker (biodosimeter) response could be critical for treatment management if an astronaut has received a large acute exposure from an SPE.

It would be particularly helpful for long-duration human space exploration to have biomarkers that measure individual susceptibility to the major health risks associated with radiation exposure in space: carcinogenesis, acute and late CNS risks, chronic and degenerative tissue risks, and acute radiation risks (2). Such markers could be used, for example, to select astronauts for long-duration missions who may have low susceptibility to the major radiation-induced health risks. The risk that can presently be estimated from biomarkers (biodosimetry) is related to average population risk, not individual risk, and therefore cannot be used to select resistant individuals. However, available biomarkers can be used to identify individuals with unusually high radiosensitivity, such as those with certain known DNA repair deficiencies.

Astronaut biodosimetry and biomarker evaluations in astronauts are facilitated by the relatively small number of individuals on a space mission and by the ability to obtain pre- and post-flight samples (and perhaps during mission samples on very long missions). However, these advantages are tempered by possible confounding factors. For long-duration missions, the temporal stability of the biomarker would be particularly important. Temporal instability can result from both biological and physical factors. In the case of a lunar mission, during a large SPE, when an accurate assessment of dose may be most urgently needed, the SPE proton dose will be highly skewed toward the first few centimeters of tissue due to the low proton energies. This inhomogeneous dose distribution will tend to result in (a) reduced initial biomarker frequency (compared with uniform whole-body dose) due to dilution with unirradiated biomarkers in more shielded body compartments (this phenomenon is clearly observed in partial-body exposures) and (b) variable time-course profiles of changes in biomarker concentration over time due to differential stem cell doses. This would be the case even for biomarkers that are stable after a uniform whole-body dose (67).

Given that most individuals show extensive sequence variation in their DNA repair genes, it is likely that susceptibility will vary between individuals depending on the particular combination of alleles inherited. For example, mice heterozygous for a mutation in ATM have heightened susceptibility to cancer and are more sensitive to ionizing radiation. However, other factors may cause increased or decreased sensitivity to radiation (e.g., allelic imbalance in gene expression).

Systems biology approaches are showing substantial promise for improving the molecular understanding of the early cellular responses to low-dose radiation and to help to reduce the uncertainty of assessing risk at low doses. The finding of genes and pathways unique to low doses in both human cells and mouse brain tissues lays the founda-

tion for identifying risk predictors for genomic instability and disease susceptibility in tissues irradiated *in vivo*.

While DNA microarray analysis may provide insight into gene function within and across biological networks, meaningful data can be generated only when studies are appropriately controlled and when the state of a living system is well defined. Since there is no consensus regarding the best method to analyze a multivariate data set, it is important that microarray data be submitted to public databases where information can be re-evaluated and interpreted as new methods for data analysis are applied.

Additional studies are needed to fully develop gene expression for any radiation biodosimetry application. The *in vivo* responses must be characterized more thoroughly, including understanding the extent to which cancer patients can be used as a model for healthy individuals. Animal studies will also be critical. Also, since a large proportion of the *in vivo* response to ionizing radiation comprises p53-regulated genes and cytokines and genes involved in immune response (29), we need to be sure that infection, burns, general injury responses, or exposure to other toxins will not produce false-positive radiation exposure signals.

Studies in support of biodefense programs clearly illustrate the normal variability in both gene expression and proteins and demonstrate the need for multiple simultaneous markers. Multiple early-response biomarkers can also provide useful diagnostic indices for medical management of radiation casualties. Results from studies in rat skin carcinogenesis indicate that enumeration of DSBs in surrogate cells *in vitro* may, when properly calibrated, become a biodosimetric tool for estimating cancer risks associated with space radiation.

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REFERENCES

1. F. A. Cucinotta, F. K. Manuel, J. Jones, G. Iszard, J. Murrey, B. Djojonegro and M. Wear, Space radiation and cataracts in astronauts. *Radiat. Res.* **156**, 460–466 (2001).

2. NASA Bioastronautics Roadmap, 2005. [<http://bioastroroadmap.nasa.gov/index.jsp>]
3. The NASA Space Radiation Project Element, Human Research Program, Johnson Space Center. [http://hacd.jsc.nasa.gov/projects/space_radiation_research_radioplans.cfm; http://humanresearch.jsc.nasa.gov/elements_radiation.asp]
4. M. A. Bender, P. C. Gooch and S. Kondo, The Gemini-3 S-4 space-flight radiation interaction experiment. *Radiat. Res.* **31**, 91–111 (1967).
5. M. A. Bender, P. C. Gooch and S. Kondo, The Gemini XI S-4 space-flight radiation interaction experiment: the human blood experiment. *Radiat. Res.* **34**, 228–238 (1968).
6. B. Fedorenko, S. Druzhinin, L. Yudaeva, V. Petrov, Y. Akatov, G. Snigiryova, N. Novitskaya, V. Shevchenko and A. Rubanovich, Cytogenetic studies of blood lymphocytes from cosmonauts after long-term space flights on Mir station. *Adv. Space Res.* **27**, 355–359 (2001).
7. I. Testard, M. Ricoul, F. Hoffschir, A. Flury-Herard, B. Dutrillauz, B. Fedorenko, V. Gerasimenko and L. Sabatier, Radiation-induced chromosome damage in astronauts' lymphocytes. *Int. J. Radiat. Biol.* **70**, 403–411 (1996).
8. T. C. Yang, K. George, A. S. Johnson, M. Durante and B. S. Fedorenko, Biodosimetry results from space flight Mir-18. *Radiat. Res.* **148** (Suppl.), S17–S23 (1997).
9. K. George, M. Durante and F. A. Cucinotta, Chromosome aberrations in astronauts. *Adv. Space Res.* **40**, 483–490 (2007).
10. J. A. Simpson, Elemental and isotopic composition of the galactic cosmic rays. *Annu. Rev. Nucl. Part. Sci.* **33**, 323–382 (1983).
11. J. W. Wilson, B. M. Anderson, F. A. Cucinotta, J. Ware and C. J. Zeitlin, Spacesuit radiation shield design methods. In *SAE Transactions 2006*. SAE 2006-01-2110, SAE, Warrendale, PA, 2006.
12. R. A. Mewaldt, A. J. Davis, W. R. Binns, G. A. de Nolfo, J. S. George, M. H. Israel, R. A. Leske, E. C. Stone, M. E. Wiedenbeck and T. T. von Rosenvinge, The cosmic ray radiation dose in interplanetary space—present day and worst case evaluations. In *Proceedings of the 29th International Cosmic Ray Conference*, Pune, India, 2005.
13. J. H. Adams, M. Bhattacharya, Z. W. Lin, G. Pendleton and J. W. Watts, The ionizing radiation environment on the moon. *Adv. Space Res.* **40**, 338–341 (2007).
14. L. Kruglyak and D. A. Nickerson, Variation is the spice of life. *Nat. Genet.* **27**, 234–236 (2001).
15. Y. Yu, R. Okayasu, M. M. Weil, A. Silver, M. McCarthy, R. Zabriskie, S. Long, R. Cox and R. L. Ullrich, Elevated breast cancer risk in irradiated BALB/c mice associates with unique functional polymorphism of the Prkdc (DNA-dependent protein kinase catalytic subunit) gene. *Cancer Res.* **61**, 1820–1824 (2001).
16. C. Barlow, M. A. Eckhaus, A. A. Schäffer and A. Wynshaw-Boris, Atm haploinsufficiency results in increased sensitivity to sublethal doses of ionizing radiation in mice. *Nat. Genet.* **21**, 359–60 (1999).
17. D. E. Weeks, M. C. Paterson, K. Lange, B. Andrais, R. C. Davis, F. Yoder and R. A. Gatti, Assessment of chronic gamma radiosensitivity as an *in vitro* assay for heterozygote identification of ataxia-telangiectasia. *Radiat. Res.* **128**, 90–99 (1991).
18. K. K. Sanford and R. Parshard, Detection of cancer prone individuals using cytogenetic response to X-rays. In *Chromosome Aberrations, Basic and Applied Aspects* (G. Obe and A.T. Natarajan, Eds.), pp. 113–120. Springer-Verlag, Berlin, 1990.
19. A. L. Brooks, M. A. Coleman, E. B. Double, E. Hall, R. E. Mitchell, R. L. Ullrich and A. J. Wyrobek, Differential transcript modulation of genes associated with diverse biological pathways after low versus high doses of ionizing radiation. In *Advances in Medical Physics* (A. B. Wolbarts, R. G. Zamenhof and W. R. Hendee, Eds.), pp. 255–286. Medical Physics Publishing, Madison WI, 2006.
20. D. J. Brenner and E. J. Hall, Computed tomography—an increasing source of radiation exposure. *N. Engl. J. Med.* **357**, 2277–2284 (2007).
21. L. M. Tomascik-Cheeseman, M. A. Coleman, F. Marchetti, D. O. Nelson, L. M. Kegelmeyer, J. Nath and A. J. Wyrobek, Differential basal expression of genes associated with stress response, damage control, and DNA repair among mouse tissues. *Mutat. Res.* **561**, 1–14 (2004).
22. M. A. Coleman, E. Yin, L. E. Peterson, D. Nelson, K. Sorenson, J. D. Tucker and A. J. Wyrobek, Low-dose irradiation alters the transcript profiles of human lymphoblastoid cells including genes associated with cytogenetic radioadaptive response. *Radiat. Res.* **164**, 369–382 (2005).
23. F. Marchetti, M. Coleman, I. Jones and A. J. Wyrobek, Candidate protein biomarkers of human exposure to ionizing radiation. *Int. J. Radiat. Biol.* **82**, 605–639 (2006).
24. E. Yin, D. Nelson, M. Coleman, L. Peterson and A. Wyrobek, Gene expression changes in mouse brain after exposure to low-dose ionizing radiation. *Int. J. Radiat. Biol.* **79**, 759–775 (2003).
25. X. R. Lowe, X. Lu, F. Marchetti and A. J. Wyrobek, The expression of Troponin T1 gene is induced by ketamine in adult mouse brain. *Brain Res.* **1174**, 7–17 (2007).
26. S. K. Schreyer, L. V. Karkanitsa, J. Albanese, V. A. Ostopenko, V. Y. Shevchuk and N. Dainiak, Analysis of radiation-associated changes in gene expression using microarray technology. *Br. J. Radiol.* **26** (Suppl.), 131–141 (2002).
27. V. Schoonjans, N. Taylor, B. D. Hudson and D. L. Massart, Characterization of the similarity of chemical compounds using electrospray ionization mass spectroscopy and multivariate exploratory techniques. *J. Pharm. Biomed. Anal.* **28**, 537–545 (2002).
28. V. Schoonjans and D. L. Massart, Combining spectroscopic data (MS, IR): exploratory chemometric analysis for characterizing similarity/diversity of chemical structures. *J. Pharm. Biomed. Anal.* **26**, 225–235 (2001).
29. N. Dainiak, S. K. Schreyer and J. Albanese, The search for mRNA biomarkers: global quantification of transcriptional and translational responses to ionizing radiation. *Br. J. Radiol.* **27** (Suppl.), 114–122 (2005).
30. J. Albanese and N. Dainiak, Ionizing radiation alters Fas antigen ligand at the cell surface and on exfoliated, membrane-derived vesicles: Implications for apoptosis and intercellular signaling. *Radiat. Res.* **153**, 49–61 (2000).
31. J. Albanese and N. Dainiak, Regulation of TNFRSF6 (Fas) expression in ataxia telangiectasia cells by ionizing radiation. *Radiat. Res.* **154**, 616–624 (2000).
32. C. Belka, P. Marini, W. Budach, K. Schulze-Osthoff, F. Lang, E. Gulbins and M. Bamberg, Radiation-induced apoptosis in human lymphocytes and lymphoma cells critically relies on the up-regulation of CD95/Fas/APO-1 ligand. *Radiat. Res.* **149**, 588–595 (1998).
33. J. Albanese, K. Martens, L. V. Karkanitsa, S. K. Schreyer and N. Dainiak, Multivariate analysis of low-dose radiation-associated changes in cytokine gene expression profiles using microarray technology. *Exp. Hematol.* **35**, 47–54 (2007).
34. S. A. Amundson, S. Shahab, M. Bittner, P. Meltzer, J. Trent and A. J. Fornace, Jr., Identification of potential mRNA markers in peripheral blood lymphocytes for human exposure to ionizing radiation. *Radiat. Res.* **154**, 342–346 (2000).
35. S. A. Amundson, M. Bittner, P. Meltzer, J. Trent and A. J. Fornace, Jr., Induction of gene expression as a monitor of exposure to ionizing radiation. *Radiat. Res.* **156**, 657–661 (2001).
36. S. A. Amundson, M. B. Grace, C. B. McClelland, M. W. Epperly, A. Yeager, Q. Zhan, J. S. Greenberger and A. J. Fornace, Jr., Human *in vivo* radiation-induced biomarkers: gene expression changes in radiotherapy patients. *Cancer Res.* **64**, 6368–6371 (2004).
37. M. N. Kronick, Creation of the whole human genome microarray. *Expert Rev. Proteomics* **1**, 19–28 (2004).
38. S. A. Amundson, K. T. Do and A. J. Fornace, Jr., Induction of stress genes by low doses of gamma rays. *Radiat. Res.* **152**, 225–231 (1999).
39. R. H. Liu, J. Yang, R. Lenigk, J. Bonanno and P. Grodzinski, Self-contained, fully integrated biochip for sample preparation, polymerase chain reaction amplification, and DNA microarray detection. *Anal. Chem.* **76**, 1824–1831 (2004).

40. H. K. Dressman, G. G. Muramoto, N. J. Chao, S. Meadows, D. Marshall, G. S. Ginsberg, J. R. Nevins and J. P. Chute, Gene expression signatures that predict radiation exposure in mice and humans. *PLoS Med.* **4**, e106 (2007).
41. W. F. Blakely, A. C. Miller, M. B. Grace, C. B. McLeland, L. Luo, J. M. Muderhwa, V. L. Miner and P. G. Prasanna, Radiation biodosimetry: applications for spaceflight. *Adv. Space Res.* **31**, 1487–93 (2003).
42. R. C. Sine, I. H. Levine, W. E. Jackson, A. L. Hawley, P. G. S. Prasanna, M. B. Grace, R. E. Goans, R. C. Greenhill and W. F. Blakely, Biodosimetry Assessment Tool: a postexposure software application for management of radiation accidents. *Military Med.* **166**, 85–87 (2001).
43. W. F. Blakely, N. I. Ossetrova, G. L. Manglapus, C. A. Salter, I. H. Levine, W. E. Jackson, M. B. Grace, P. G. Prasanna, D. J. Sandgren and G. D. Ledney, Amylase and blood cell-count hematological radiation injury biomarkers in a rhesus monkey radiation model—use of multiparameter and integrated biological dosimetry *Radiat. Meas.* **42**, 1164–1170 (2007).
44. W. F. Blakely, C. A. Salter and P. G. S. Prasanna, Early-response biological dosimetry—recommended countermeasure enhancements for mass-casualty radiological incidents and terrorism. *Health Phys.* **89**, 494–504 (2005).
45. J. K. Waselenko, T. J. MacVittie, W. F. Blakely, N. Pesik, A. L. Wiley, W. E. Dickerson, H. Tsu, D. L. Confer, N. Coleman and N. Dainiak, Strategic National Stockpile Radiation Working Group, Medical management of the acute radiation syndrome: Recommendations of the Strategic National Stockpile Working Group. *Ann. Intern. Med.* **140**, 1037–1051 (2004).
46. R. Hofmann, G. A. Schreiber, N. Willich, R. Westhaus and K. W. Bogl, Increased serum amylase in patients after radiotherapy as a probable bioindicator for radiation exposure. *Strahlenther. Onkol.* **166**, 688–695 (1990).
47. V. N. Mal'tsev, A. A. Ivanov, V. F. Mikhailov and V. K. Mazurik, The individual prognosis of the gravity and of the outcome of radiation disease on immunological indexes. *Radiat. Biol. Radioecol.* **46**, 152–158 (2006).
48. M. B. Grace, C. B. McLeland, S. J. Gagliardi, J. M. Smith, W. E. Jackson, 3rd and W. F. Blakely, Development and assessment of a quantitative reverse transcription-PCR assay for simultaneous measurement of four amplicons. *Clin. Chem.* **49**, 1467–75 (2003).
49. M. B. Grace and W. F. Blakely, Transcription of five p53- and Stat 3-inducible genes after ionizing radiation. *Radiat. Meas.* **42**, 1147–1151 (2007).
50. A. C. Miller, L. Luo, W. K. Chin, A. E. Director-Myska, P. G. Prasanna and W. F. Blakely, Proto-oncogene expression: a predictive assay for radiation biodosimetry applications. *Radiat. Prot. Dosimetry* **99**, 295–302 (2002).
51. N. I. Ossetrova, A. M. Farese, T. J. MacVittie, G. L. Manglapus and W. F. Blakely, The use of discriminant analysis for evaluation of early-response multiple biomarkers of radiation exposure using non-human primate 6-Gy whole-body radiation model. *Radiat. Meas.* **42**, 1158–1163 (2007).
52. The ENCODE Project Consortium, The ENCODE (ENCyclopedia Of DNA Elements) Project. *Sci.* **306**, 636–640 (2004).
53. P. Bertone, V. Stolc, T. E. Royce, J. S. Rozowsky, A. E. Urban, X. Zhu, J. L. Rinn, W. Tongprasit, M. Samanta and M. Snyder, Global identification of novel transcribed sequences in humans using high-resolution genome tiling arrays. *Science* **306**, 2242–2246 (2004).
54. V. Stolc, M. P. Samanta, W. Tongprasit and W. Marshall, Genome-wide Transcriptional analysis of flagellar regeneration in *Chlamydomonas reinhardtii* identifies orthologs of ciliary disease genes. *Proc. Natl. Acad. Sci. USA* **102**, 3703–3707 (2005).
55. M. P. Samanta, W. Tongprasit and V. Stolc, In-depth query of large genomes using tiling arrays. *Methods Mol. Biol.* **377**, 163–174 (2007).
56. Y. Yu, A. J. Hoffhines, K. L. Moore and J. A. Leary, A novel method for the determination of the sites of sulfation in peptides and proteins. *Nat. Methods* **4**, 583–588 (2007).
57. E. Damoc, C. S. Fraser, M. Zhou, H. Videler, G. L. Mayeur, J. W. B. Hershey, J. A. Doudna, C. V. Robinson and J. A. Leary, Structural characterization of the human eukaryotic initiation factor 3 protein complex by mass spectrometry. *Mol. Cell. Proteomics* **6**, 1135–1146 (2007).
58. K. D. Mills, D. O. Ferguson and F. W. Alt, The role of DNA breaks in genomic instability and tumorigenesis. *Immunol. Rev.* **194**, 77–95 (2003).
59. C. Hsu and Y. Li, Aspirin potently inhibits oxidative DNA strand breaks: implications for cancer chemoprevention. *Biochem. Biophys. Res. Commun.* **293**, 705–709 (2002).
60. R. Stewart, Two-lesion kinetic model of double-strand break rejoining and cell killing. *Radiat. Res.* **156**, 365–378 (2001).
61. E. Dikomey, I. Brammer, J. Johansen, S. Bentzen and J. Overgaard, Relationship between DNA double-strand breaks, cell killing, and fibrosis studied in confluent skin fibroblasts derived from breast cancer patients. *Int. J. Radiat. Oncol. Biol. Phys.* **46**, 481–490 (2000).
62. K. Prise, N. Gillies and B. Michael, Evidence for a hypoxic fixation reaction leading to the induction of ssb and dsb in irradiated DNA. *Int. J. Radiat. Biol.* **74**, 53–59 (1998).
63. F. J. Bums, Y. I. Jin, S. J. Garte and S. Hosselet, Estimation of risk based on multiple events in radiation carcinogenesis of rat skin. *Adv. Space Res.* **14**, 507–519 (1994).
64. F. J. Burns, T. Rossman, R. Zhang, F. Wu, A. N. Uddin and R. E. Shore, Radiation carcinogenesis: mechanisms of induction and prevention. In *Environmental and Occupational Medicine*, 4th ed. (W. N. Rom and S. Markowitz, Eds.), pp. 1262–1276. Lippincott Williams & Wilkins, Philadelphia, 2006.
65. P. G. S. Prasanna, H. Loats, H. M. Gerstenberg, B. N. Torres, C. W. Shehata, K. L. Duffy, R. S. Floura, A. W. Khusen, W. E. Jackson and W. F. Blakely, *AFRRRI's Gamma-Ray, X-Ray, and Fission-Neutron Calibration Curves for the Lymphocyte Dicentric Assay: Application of a Metaphase Finder System*. SP02-1, ADA403812, Armed Forces Radiobiology Research Institute, Bethesda, MD, 2002.
66. *Medical Management of Radiological Casualties Handbook*. Armed Forces Radiobiology Research Institute, Bethesda, MD, 2003.
67. T. Straume and M. A. Bender, Issues in cytogenetic biological dosimetry: Emphasis on radiation environments in space. *Radiat. Res.* **148** (Suppl.), S60–S70 (1997).