From Mice and Men to Earth and Space: Joint NASA-NCI Workshop on Lung Cancer Risk Resulting from Space and Terrestrial Radiation

Jerry W. Shay[1],[*], **Francis A. Cucinotta**[2], **Frank M. Sulzman**[2], **C. Norman Coleman**[3], and **John D. Minna**[1]

[1]University of Texas Southwestern Medical Center, Dallas, TX

[2]NASA, Lyndon B. Johnson Space Center, Houston, TX

[3]National Cancer Institute, Radiation Research Program, Bethesda, MD

Abstract

On June 27–28, 2011 scientists from the National Cancer Institute (NCI), NASA, and academia met in Bethesda to discuss major lung cancer issues confronting each organization. For NASA – available data suggest lung cancer is the largest potential cancer risk from space travel for both men and women and quantitative risk assessment information for mission planning is needed. In space the radiation risk is from high energy and charge (HZE) nuclei (such as Fe) and high energy protons from solar flares and not from gamma radiation. By contrast the NCI is endeavoring to estimate the increased lung cancer risk from the potential wide-spread implementation of computed tomography (CT) screening in individuals at high risk for developing lung cancer based on the National Lung Cancer Screening Trial (NLST). For the latter, exposure will be x-rays from CT scans from the screening (which uses "low dose" CT scans) and also from follow-up scans used to evaluate abnormalities found during initial screening. Topics discussed included the risk of lung cancer arising after HZE particle, proton, and low dose Earth radiation exposure. The workshop examined preclinical models, epidemiology, molecular markers, "omics" technology, radiobiology issues, and lung stem cells (LSC) that relate to the development of lung cancer.

Meeting Overview

Charles Bolden, NASA Administrator (Washington, DC), indicated the biggest challenges to future space missions are advanced propulsion and medical effects of space irradiation. NASA needs to develop protectors and mitigators of radiation exposure, learn more about inter-individual differences, and determine if early detection biomarkers may reduce the overall risk to fatal cancer from long-term exposure to irradiation. Peter Greenwald, Associate Director for Prevention at the NCI, provided an overview of the NLST to determine if low dose CT scans could detect and reduce the mortality of lung cancer.

NASA and Radiation Risks from Space Flight – Reason for the NASA Lung Cancer Consortium

Francis Cucinotta, Chief Scientist, (NASA Space Radiation Program, Houston, TX) provided an overview of the risk of space radiation in the development of lung cancer. NASA formed a Lung Cancer Consortium to develop and test realistic experimental human

[*]corresponding author: Jerry W. Shay, UT Southwestern Medical Center, Dallas, TX 75390-9039, Jerry.Shay@utsouthwestern.edu, Tel 214-648-3282.

and mouse models to examine lung cancer risk after exposure to simulated space radiation (HZE and protons). Dr. Cucinotta explained the difference between GCR (galactic cosmic rays) and solar particle events (SPE containing high energy protons). About 5–10 SPEs per year are predicted, and while proton irradiation can be shielded, over 9 feet of shielding are required to significantly reduce GCR exposure to astronauts, which is impractical. Accurate methods to project individual based risk are needed, which rely on extrapolation from experimental data to humans. At the NASA Space Research Laboratory at Brookhaven National Laboratory, scientists expose human lung cells and mice to simulated space radiation to measure the frequency of malignant transformation, and aid in developing countermeasures/mitigators to reduce cancer risk. Astronauts are largely never-smokers (NS) which is a critical factor for risk assessment.

Workshop Goals

John Minna (UT Southwestern, Dallas, TX)) and Norman Coleman (NCI, Bethesda, MD) co-organizers of the workshop (along with Francis Cucinotta (NASA, Houston, TX) and Frank Sulzman (NASA, Houston TX)), highlighted the goals of the workshop to: 1) share information and methodology between NASA and NCI; 2) discuss state of the art models and methodologies investigating the initiation and progression of lung cancer; 3) identify important new research directions; and 4) identify future areas of collaborations to achieve common goals.

Meeting Highlights

Daniela Gerhard (NCI, Bethesda, MD) indicated that early gene changes and other molecular data on lung cancer already exist. She reviewed The Cancer Genome Anatomy (TCGA) Project that provides a publically available source for mining of well-annotated data, including mutational, chromosomal rearrangement, and epigenomics data through NCI. Mitchell Anscher (Virginia Commonwealth University, Richmond, VA) indicated in the past 20 years, medical radiation exposure in the US has increased 5-fold, primarily reflecting the increased utilization of CT scans. Nevertheless, the average per person exposure dose in the US remains very low. The available data indicate the excess relative risk (ERR) from the low doses used in diagnostic imaging is very small, increases with cumulative dose, and accounts for <1% of all cancers occurring in the U.S.A. During radiation therapy reactive oxygen and reactive nitrogen species are produced in tissues, resulting in addition to killing of tumor cells, damage to the vasculature, injury and death of parenchymal cells (potentially leading to fibrosis), recruitment of inflammatory cells, and release of pro-inflammatory and pro-fibrotic cytokines. Radiation-induced malignancy can occur in cancer survivors in locations exposed to high doses, while recent evidence suggests that there is a small ERR for second cancers developing in regions exposed to low doses (in the range of 1–5 Gy).

Kyoji Furukawa (Radiation Effect Research Foundation, Hiroshima, Japan) reviewed the Japanese atomic-bomb survivor data for late effects of ionizing radiation exposure at low/moderate doses. The 1,803 first primary lung cancer incident cases diagnosed among 105,404 cohort members between 1958 and 1999 were classified into histological types, and individual smoking histories and radiation dose estimates were used to characterize the combined effects of radiation and smoking on the risk of each of the major subtypes of lung cancer. Using Poisson regression methods, both smoking and radiation exposure significantly increased the risk for each: the ERR associated with 50 pack-years of smoking were significantly larger for small cell and squamous cell carcinomas than for adenocarcinomas. The gender-averaged ERR/Gy for NS (at age 70 after exposure at age 30) were estimated as 1.5 (95% confidence interval: 0.1–4.6) for small cell carcinoma, 0.75 (0.3–1.3) for adenocarcinoma, and 0.27 (0–1.5) for squamous cell carcinoma. The smoking-

adjusted radiation effect tended to be larger for moderate-smokers than for heavier-smokers. Survivors who smoked 10 cigarettes per day increased the risk of developing lung cancer. However, survivors who smoked 20 or more cigarettes per day were already at high risk for lung cancer and the addition of irradiation exposure did not significantly increase that risk.

Mark Little (NCI, Bethesda, MD) provided an overview of multi-stage modeling and integration of lung cancer risk using data from the Colorado Plateau uranium miner cohort and the Japanese atomic bomb survivors. Dr. Little concluded that a three mutation model fits the uranium miner data best and that the interactions of radon and smoking may be submultiplicative but superadditive. Lung cancer mortality risks in both the Japanese and Colorado survivors suggests similar time and age of exposure trends which is very close to the ratio suggested by purely dosimetric considerations. The largely γ-ray exposures from the A-bomb produced qualitatively similar effects to the α-particle exposure in the mines.

Mahadevappa Mahesh (Johns Hopkins University, Baltimore, MD) gave an overview of the NLST comparing low dose spiral (helical) CT (1.5mSV whole body and about 4mGy to the lungs) to standard chest X-rays. Since 2007, there have been 68 million CT scans in the US, or about 6mSv per capita. The NLST screening trial involved 33 institutions, enrolling 53,454current and former heavy smokers ages 55–74 over a 20 month period. Christine Berg (NCI, Bethesda, MD) provided an overview of the initial NLST analysis. There were 20% fewer lung cancer deaths seen among those who were screened with low dose spiral CT compared to chest X-rays. These results were statistically convincing, so the trial was stopped. She discussed concern about the cumulative effects of radiation from multiple CT scans, and that the majority of CT detected findings turn out not to be cancer, producing significant expense, patient anxiety and potential risk from diagnostic procedures. Also the population enrolled in the NLST was highly motivated, urban, and screened at major medical centers, they may not be representative of the entire population. The NLST results should help determine risk cohorts best suited to CT-screening, permit optimization and standardization of screening strategies, and facilitate development of molecular markers for risk assessment.

Robert Ullrich (University of Texas Medical Branch in Galveston, TX) reviewed differences between irradiation effects on solid tumors versus leukemia. Charged particle irradiations cause more damage than photon radiation. This may result in hematopoietic stem cell death in mouse models susceptible to leukemogenesis. The increase in incidence showed an inverse dose effect (where lower or fractionated doses were more leukemogenic compared to single higher doses). While in the past the dose response was assumed to be linear, recent data from a variety of sources suggest otherwise. There are issues about non-targeted effects including ROS, inflammatory responses, cytokine pathways, persistent DNA damage signaling and the effects of irradiation on the microenvironment. The inverse dose effect appears to be in the 0.5 -1Gy range. It is not known if these effects will hold up at much lower doses and when given in fractions as would happen on along-term space mission.

Adi Gazdar (University of Texas Southwestern, Dallas, TX) reviewed lung cancer arising in never smokers (NS), the 7th leading cause of cancer deaths in the US (10–15% of lung cancers), mostly adenocarcinomas, and higher in East Asians (35–50%). NS lung cancers have fewer mutations, more EGFR mutations, more copy number changes, and more mitochondrial DNA mutations than lung cancers in smokers.

Avrum Spira (Boston University, Boston, MA) focused on the early molecular detection of lung cancer. He discussed the "field of injury" concept and using brushings from the upper airway has identified an mRNA biomarker signature that distinguishes smokers with and without lung cancer (sensitivity of 80% and a specificity of 84%). The biomarker signature

was 90% sensitive for stage 1 lung cancer and can separate smokers with or without chronic obstructive pulmonary disease (COPD). Ongoing studies easily obtained brushings from nasal epithelium to assess field injury effects.

Lung Stem Cells (LSC) in Mouse and Man

Piero Anversa (Brigham and Women's Hospital, Boston, MA) reported that the human lung had a pool of rare undifferentiated c-Kit+ stem-like cells located in niches in the distal airways. These cells were observed to be self-renewing, clonogenic and multipotent *in vitro*. After injection in regions next to cryogenically damaged mouse lung, these putative human lung stem (HLSC) formed human bronchioles, alveoli, and pulmonary vessels integrated structurally and functionally within the mouse lung. The c-Kit+ putative lung stem cells also express the transcription factors Nanog, Oct3/4, Sox 2, and KLF4, which are expressed in human embryonic SCs and the same factors that convert adult differentiated cells into induced pluripotent stem (iPS) cells. Dr. Anversa believes these HLSC may be critical determinants of the turnover and repair of bronchiolar and alveolar epithelial cells, vascular and non-vascular smooth muscle cells, and endothelial cells of the distal airways. While these data are novel, others expressed healthy skepticism and mentioned there is no evidence for a common progenitor for both epithelial and vascular lineages.

Jerry Shay and John Minna (University of Texas Southwestern, Dallas, TX) reported that human adult bronchiole epithelial cells (HBECs) derived from the central airways immortalized with telomerase and cdk4 exhibited the capacity for multipotent differentiation. Under specific conditions the HBECs produced both ciliated and mucous secreting cells (a characteristic of the central airways). When the HBECs were placed in Matrigel™ overlying a fibroblast feeder layer, they formed cyst-like structures that progressively grew and expressed surfactant proteins (SPA and SP-C) as well as CC10 a Clara cell specific protein (both characteristics of the peripheral airways). When experimentally transformed by genetic manipulation, the immortalized HBECs produced tumors when injected orthotopically into the NOD/SCID mouse lung including squamous cell carcinoma (characteristic of the central airway), and large cell carcinoma and adeno/squamous carcinomas (characteristics of the peripheral airway). These results indicate that the adult human lung has multipotent progenitor cells.

Barry Stripp and Mark Onaitis (Duke University Medical Center, Durham, NC) discussed mouse LSC. A series of proliferative kinetic, chimera and lineage tracing experiments showed abundant and widely distributed progenitors exist that contribute to normal mouse lung epithelial maintenance but also rare "chemically resistant" epithelial SCs that can function as a reserve pool of progenitors. Their results imply that in the mouse there is not a multipotent LSC, but region-specific progenitor cells. This is in contrast to previous investigations indicating that a rarer cell called a bronchioalveolar SC (BASC) contributed to the lung epithelium maintenance. While there remains controversy over the LSC, from NCI and NASA's perspective the central question is whether terrestrial and space radiation will affect lung stem/progenitor pools and if the critical genes when mutated leads to an increased incidence and progression of lung cancer.

NASA Lung Cancer Consortium

David Kirsch (Duke University Medical Center, Durham, NC) and Ya Wang (Emory University, Atlanta, GA) provided overviews of the approaches to address the risk of lung cancer from exposure to space irradiation. Minh To (University of California, San Francisco, CA) from Alan Balmain's group working with Mary-Helen Barcellos-Hoff (New York University, New York, NY) discussed genomic approaches using novel genetically engineered mouse models to study the molecular mechanisms of lung cancer development

and progression. While early, their results emphasize the necessity of studying the system as a whole to understand how perturbation of networks by radiation results in cancer. Dr. Ilona Linnoila (NCI, Bethesda, MD) reviewed the varying mouse models of small cell lung cancer (SCLC).

John Minna (University of Texas Southwestern, Dallas, TX) presented a perspective on integrating and translating molecular and functional definitions of lung cancer. He described how working with the NCI Special Program of Research Excellence (SPORE) and NCI Cancer Target Development and Discovery Network (CTD^{2}N) multiple groups are developing a comprehensive genetic, epigenetic, and proteomic understanding of the molecular pathogenesis of lung cancer. From this information "molecular portraits" ("clades") of lung cancer subtypes are being developed through genome wide siRNA and shRNA libraries, and large scale chemical library screens. Systematic approaches that allow identification of genes that when knocked down kill lung cancer cells but not normal lung epithelial cells were also discussed. Dr. Minna emphasized the need to have a complete genomic understanding of lung cancers that arise on Earth compared to those that would arise after HZE particle radiation in both human cell systems and in mice so that we can understand their similarities and differences.

Lung Cancer Genomics, Epigenomics, and Integration

Finally, there were discussions about epigenetics and lung cancer (Steven Belinsky, Lovelace Respiratory Research Institute, Albuquerque, NM), the NCI Cancer Genome Anatomy Project (Marcin Imielinski, Massachusetts General Hospital, Boston, MA), integrated databases for lung cancer risk assessment (Mervi Heiskanen, CaBIG-NCI, Bethesda, MD), and systems biology analysis (Thomas Deisboeck, Massachusetts General Hospital, Boston, MA).

Workshop Outcomes

It was agreed that we needed to improve the use of computational biology to better model risks of lung cancer from irradiation exposure. Two emerging questions were raised: Is lung cancer from irradiation a single disease or several different diseases and how good are mouse and human cell culture models for extrapolating to human disease? The group discussed how to integrate and allow better mining of already obtained genomics data, and emerging "omics" technologies, including sharing information and methodologies between NASA and NCI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.