DEVELOPMENT OF A RISK ASSESSMENT MODEL FOR LUNG CANCER PATHOGENESIS AFTER EXPOSURE OF HUMAN AND MOUSE LUNG EPITHELIAL CELLS TO HZE PARTICLE RADIATION

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OVERALL GOALS: The goals of our NSCOR research program are to discern the molecular and cellular events associated with and participating in the multi-step process of lung cancer pathogenesis, after exposure to the unique radiations associated with long-term manned space exploration. The data generated here, as well as other data from the UTSW NSCOR, are being used to develop a more accurate model of lung cancer risk from HZE radiation exposure.

Approach: We are using molecular and cell biologic approaches in human and mouse models systems to address this problem. These include: 1. Development of a unique panel of immortalized human large and small airway epithelial cells derived from over 50 individuals (human bronchial epithelial cells, HBECs, and human small airway epithelial cells, HSAECs) that have also been genetically manipulated with defined oncogenic changes found in lung cancer to derive isogenic variants representing the preneoplastic steps found in lung cancer; 2. Development of 2D and 3D *in vitro* systems permitting the study of the effects of HZE particle radiation on HBECs and HSAECs in tissue like structures; 3. Characterization of the response and survival of these cells to various types of γ and HZE particle radiation; 4. Detailed genome wide molecular profiling studies (mRNA, miRNA, DNA copy number, methylation, protein) of these cells before and at different time points after radiation; 5. Cell biologic tests of the progression of these cells toward the malignant phenotype including soft agar colony growth and the development of epithelial to mesenchymal (EMT) transition; 6. *In vivo* tests of tumorigenicity; 7. Use of novel transgenic mouse models of lung cancer to explore the development of lung cancer in a whole animal model after different types, doses, and schedules of HZE particle and γ radiation; 8. Establishment of an integrated database of the large volume of data accumulated; 9. Statistical and modeling analysis of the data to establish quantitative elements of the risk of progressing towards lung cancer after HZE particle and γ radiation.

MAJOR QUESTIONS BEING ADDRESSED: We have focused on several major questions. 1. Is there an increased risk of progression toward lung cancer in human and mouse tissues with HZE particle radiation and, if so, what are the quantitative risk estimates for this by dose and schedule? 2. What are the molecular correlates of space radiation induced tumor progression and how do these compare with non space radiation induced lung tumorigenesis? 3. How do the findings of lung cancer development in human tissues and in the whole mouse model compare? 4. What does a model look like that integrates all of these findings?

MAJOR FINDINGS: 1. HZE Particle Radiation Induces Malignant Transformation: From human lung epithelial cell culture (HBEC) and *in vivo* mouse studies we have quantitated how protracted/fractionated HZE particle irradiation increases lung cancer progression toward malignancy with several ion types. **2. HZE Particle Radiation Activates Signaling Pathways that Promote Malignancy:** From studies of HBEC and mouse models of lung cancer, we have determined quantitatively that altered signaling for ROS metabolism, inflammatory responses and DNA damage signaling can distinguish terrestrial from space irradiation and that similar pathways are activated in the most aggressive human lung cancers. **3. Importance of Rad51-dependent Homologous Recombination following HZE Particle Radiation:** We have determined that DNA lesions induced by HZE particle are preferentially repaired by Rad51-dependent homologous recombination and that this repair is actually down-regulated under conditions similar to those found in tissues. **4. Identification of Key "Hub Genes" Following Exposure to HZE Particle Radiation:** We have developed computational biology tools and approaches that allow us to identify a small number of genes which act as master regulators ("hub genes") of gene expression programs in human lung cells following g and HZE particle radiation providing a roadmap for detailed functional and mechanistic studies. **5. Establishment of Database, Datasets, and Biospecimens to Facilitate NASA Collaborations:** We have develop a robust database for sharing among our NASA team and others in the NSCOR lung consortium to permit sharing of biospecimens and integrative molecular analyses - using Super-SPACE model to predict space risk (561 human HBEC microarrays and 144 mouse microarrays of γ and HZE irradiations)

(Risks Estimates and Mechanisms of Lung Cancer Pathogenesis after Space Radiation NASA NSCOR NNX11AC54G)