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PI Name:	Kirsch, David M.D., Ph.D.		
Project Title:	Duke NSCOR: Lung Cancer Risk from HZE Ions		
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Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Radiation health		
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Contact Monitor:	Cucinott1a, Francis	**Contact Phone:**	281-483-0968
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Key Personnel Changes/Previous PI:	Kouros Owzar is no longer a CoInvestigator (FY2012 report/November 2011).		
COI Name (Institution):	Bloom, Rochelle Ph.D. (Duke University) Yoshizumi, Terry Ph.D. (Duke University) Onaitis, Mark M.D. (Duke University) Stripp, Barry Ph.D. (Duke University)		
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Task Description:	The goal of the Duke NSCOR is to understand mechanisms of high charge and energy (HZE) ion-induced lung cancer. To accomplish this goal, the Duke NSCOR has brought together experts in radiation biology, lung cancer development, lung injury and repair, radiation dosimetry, statistics, and education. We will combine sophisticated mouse genetics, in vivo lineage tracing, ex vivo isolation of lung epithelial progenitor cells, and analyses of lung cancers induced by HZE nuclei to dissect mechanisms of HZE ion-induced lung cancer. We will integrate 3 separate projects to understand how the cell of origin influences lung cancer development after HZE ion exposure, identify mechanisms of cellular response to HZE ions in different progenitor populations in the lung, and define how and when the p53 tumor suppressor, which is the most commonly mutated gene in human lung cancer, regulates HZE ion-induced carcinogenesis in the lung. We anticipate that our hypothesis-based research will ultimately lead to the development of better models for HZE ion carcinogenic risk assessment for individual astronauts and novel approaches to prevent HZE ion-induced lung cancer through biological countermeasures.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Lung cancer causes more than one million cancer deaths each year and is the leading cause of cancer death worldwide. Despite advances in the detection and treatment of lung cancer, lung cancer prevention presents a major unmet need. While many lung cancer cases are preventable as they are due to smoking, it is estimated that 25% of lung cancer cases worldwide involve never smokers. Though multiple risk factors including exposure to radiation from radon gas have been implicated, no clear-cut cause has emerged to explain the relatively high incidence of lung cancer in never smokers. Lung cancers arising in never smokers demonstrate different mutation patterns and frequencies when compared to cancers arising in smokers, suggesting that lung cancer arising in never smokers is a clinically distinct disease. Indeed, lung cancers in never smokers respond differently to targeted cancer therapies. Further research into the mechanism of lung cancer development in never smokers is needed so that more successful strategies for prevention and treatment of lung cancer can be developed. By studying the mechanisms of lung cancer initiation and development, the Duke NSCOR is generating new knowledge that can be used to develop novel approaches for the prevention and treatment of lung cancer. Lung cancer can be divided into two major forms: small-cell lung cancer and non-small cell lung cancer. Both non-small cell lung cancers and small cell lung cancers have developed in survivors of the atomic bombs in Japan. Similarly, both types of lung cancer arise in smokers. Cancers arising in never smokers preferentially develop in the distal airways and are of the adenocarcinoma histological subtype, which is a type of non-small cell lung cancer. Recently, genomic sequencing technology has been utilized to identify the most commonly mutated genes in adenocarcinomas. Based on this analysis, the two most commonly mutated genes in adenocarcinomas are Trp53, which encodes the tumor suppressor p53, and the oncogene Kras. The Duke NSCOR is utilizing sophisticated genetically engineered mouse models to study the role of p53 and Kras in non-small cell lung cancer. For example, we are studying how mutations in Kras in different kinds of cells in the lung affect lung cancer development with exposure to space radiation. We are also studying mice with an additional copy of p53 or inducible p53 suppression to investigate the timing and mechanism by which p53 suppresses Kras-driven lung adenocarcinoma progression after space radiation exposure. In addition, we are developing a mouse model of radiation-induced small-cell lung cancer. Together these studies will provide new insights into how lung cancer forms, where lung cancers develop, and how Kras and p53 mutation promote lung cancers. As we answer these questions using experiments with space radiation, we expect that our results will not only help us understand how lung cancer develops on earth, but will also provide new insights into preventing and treating lung cancer. In addition to studying lung cancer development, the Duke NSCOR is also studying lung progenitor cell injury and repair after exposure to either terrestrial or space radiation. Injury and inflammation of the lung are key components of many diseases in people including emphysema, asthma, and lung fibrosis. Furthermore, patients receiving radiotherapy for either primary lung cancer or other neoplasms of the thoracic region (e.g. breast cancer) undergo lung tissue remodeling and declining lung function that is directly related to the dose and location of radiation exposure. By exploring which lung cells are injured by space radiation and how these injured lung cells are repaired, we anticipate that this knowledge may also lead to a better understanding of how lung diseases besides cancer develop and strategies that may be employed to moderate the effects of radiotherapy on lung tissue remodeling. This information may ultimately be used to develop novel approaches for the prevention and treatment of these lung diseases, and the improvement of public health.
	In our first year of funding, the Duke NASA Specialized Center of Research (NSCOR) initiated the experiments proposed in our application. We obtained strains of mice and bred mice for our experiments. Members of the Duke NSCOR also completed training at the Brookhaven National Laboratory (BNL), which enabled us to expose mice to space radiation at BNL in June and November of 2011. A review of our planned experiments was performed on March 23, 2011 by our internal advisory committee, which is made up of distinguished scientists and physicians at Duke. In addition, members of the Duke NSCOR interacted with members of other NSCORs and NASA investigators at the following meetings: (1) Visit by David Kirsch, M.D., Ph.D., Mark Onaitis, M.D., and Barry Stripp, Ph.D. to UTSW NSCOR on March 16, 2011 ; (2) Visit by David Kirsch, M.D., Ph.D. to Emory NSCOR on July 15, 2011 ; (3) Presentation by Chang-Lung Lee and David Kirsch, M.D., Ph.D. at the International Conference on Radiation Research in Warsaw, Poland in August 2011 ; (4) Presentations by David Kirsch, M.D., Ph.D., Mark Onaitis, M.D., and Barry Stripp, Ph.D. in a joint NASA-National Cancer Institute meeting on radiation-induced lung cancer in Bethesda, MD on June 27, 2011 ; (5) Presentations by David Kirsch, M.D., Ph.D., Mark Onaitis, M.D., and Barry Stripp, Ph.D. at the annual NSCOR meeting in League City, TX on September 18, 2011 ; (6) Presentations by David Kirsch, M.D., Ph.D., Mark Onaitis, M.D., and Barry Stripp, Ph.D., and Everett Moding at the 22nd Annual NASA Space Radiation Investigator’s Workshop in League City, TX in September 2011. Through these activities, members of the Duke NSCOR learned about space radiation and established collaborations with NASA investigators in other NSCORs. We are now applying this knowledge in our projects described below, which focus on 2 genes that are frequently mutated in human lung cancer: the tumor suppressor p53 and the oncogene K-ras. Project 1. The role of the tumor suppressor p53 in space radiation-induced lung cancer. David Kirsch, M.D., Ph.D., Lead We have proposed to study the role and timing of p53 in radiation-induced lung cancer using mice with an extra copy of p53 (Aim 1) and reversible knockdown of p53 (Aim 2). In addition, we are developing a model of radiation-induced small cell lung cancer (Aim 3).

Task Progress:

For Aim 1, we have performed experiments to confirm increased induction of p53 transcriptional targets in mice with an additional copy of p53 (super p53 mice) after x-ray radiation. We have also established breeding cages to produce mice predisposed to lung cancer in mice with 2 or 3 copies of p53. Thirteen of these mice were sent to Brookhaven National Lab (BNL) and exposed to space radiation with iron. These mice are currently being monitored for lung tumor development. An additional 40 mice will be shipped to BNL for iron exposure in November. In parallel, we have irradiated two cohorts of mice with x-rays for comparison to the mice exposed to space radiation.

For Aim 2, we have performed experiments to quantify p53 knockdown in lung epithelial cells isolated from mice. We have observed p53 knockdown using a systemically inducible p53 shRNA, and we are currently breeding mice with a lung-targeted p53 shRNA. These mice will be used for pilot experiments to determine the best system to investigate the temporal role of p53 in high LET radiation-induced lung cancer.

For Aim 3, we have bred mice to investigate initiation of small cell lung cancer by space radiation. We have performed pilot experiments to determine the best fluorescent reporter to confirm deletion of these conditional genes in lung tissue. Twenty mice will be sent to BNL in November for exposure to space radiation. In addition, we have irradiated two cohorts of mice with x-rays and are monitoring them for the development of lung tumors.

Project 2. The role of cell of origin in space radiation-induced lung cancer. Mark Onaitis, M.D., Lead

We have proposed to study the cell of origin of K-RasG12D-induced lung cancer in response to high-LET radiation. Our aims include studying the effects of high LET radiation on mice in which K-rasG12D is inducibly expressed in different cell types of the lung: Clara cells (Aim 1), basal cells (Aim 2), and Type II cells (Aim 3).

For Aim 1, we have set up breeding cages to obtain many mice with the necessary group of genes. Five mice were sent to Brookhaven, exposed to space radiation with iron particles, and returned to Duke. These mice were sacrificed seven weeks after exposure to space radiation and analyzed for tumor formation. Additional mice will be exposed to space radiation in November at BNL.

In the next year, we plan to expose many, many more of these mice to space radiation. We also plan to isolate lung cells from these mice for in vitro colony-forming assays and to transplant these cells into the lungs of mice lacking an immune system.

For Aim 2, we have set up breeding cages and plan to irradiate many of these basal cell-specific K-rasG12D mice over the next year.

For Aim 3, we have set up multiple breeding cages and generated multiple litters with Type II cell specific K-rasG12D that will be ready for exposure to space radiation in the future. All of the assays described above will be performed.

Project 3. Effects of space radiation and p53 signaling on lung progenitor cells. Barry Stripp, Ph.D., Lead

We have proposed to determine how space radiation impacts epithelial cells that line the airways of the lung. Many different progenitor cell types reside in conducting airways. These progenitor cells normally contribute to replacement of specialized epithelial cell types that function in gas exchange and defense against environmental insults. However, the relative sensitivity of these progenitor cells to radiation effects is not known. We will determine the responses of progenitor cells to radiation injury and repair using in vivo and in vitro assays in normal mice and mice lacking the tumor suppressor p53.

For Aim 1, we have established a method to introduce genetic tags in airway progenitor cells to follow their expansion in vivo following radiation exposure. Our experiments indicate that mice exposed to radiation show evidence of increased clonal expansion of epithelial progenitor cells in vivo. Interestingly, in vitro studies indicate that the number of progenitor cells decreases following radiation exposure. We are applying histopathological methods to quantify these changes and determine the relative effects of X-rays (“terrestrial radiation”) and space radiation. Initial experiments suggest that different epithelial progenitor cells have different sensitivity/resistance to radiation exposure.

For Aim 2, we will genetically tag progenitor cells in mice that are deficient in the p53 tumor suppressor gene. These mice are currently being bred with the expectation that experiments with these mice lines will begin next year. Another goal of experiments in this aim is to generate new genetically engineered “reporter” mice to allow us to identify individual cells that have breaks in their DNA after space radiation exposure. In order to generate these mice, we are first testing the reporter gene constructs in vitro in model epithelial cell culture systems.

Core A: Administrative Core. David Kirsch, M.D., Ph.D., Lead. Duke NSCOR Administrator: Michelle Cooley

The Administrative Core (Core A) provides overall management of the NSCOR award by ensuring that projects make satisfactory progress. During the first year of funding, the Administrative Core has monitored project progress by conducting biweekly Duke NSCOR meetings, an annual Internal Advisory Committee Meeting, and multiple teleconferences with NASA. Minutes were recorded at these meetings in order to ensure that tasks were completed in a timely manner. Core A made travel arrangements for the Duke NSCOR team to travel to Brookhaven National Laboratory in June and November 2011 in order to expose mice to space radiation. Travel arrangements were also made for all of the meetings described above to facilitate communication between the Duke NSCOR and other NASA investigators. Core A also provided administrative support for credentialing Duke NSCOR investigators to work at BNL and for submitting and renewing the animal protocols at Duke and BNL. Core A provided budget oversight for the Duke NSCOR. Project expenditures were monitored by Erin Dillard. Ms. Dillard met monthly with David Kirsch M.D., Ph.D. to review spending and fiscal matters for each NSCOR project and Core. Marcia Painter assisted with ordering supplies and financial accounting for the Duke NSCOR.

Core B: Physics Core. Terry Yoshizumi, Ph.D., Lead

The Physics Core (Core B) provides comprehensive measurements of radiation dose (dosimetry) and oversees the radiation safety of experiments performed by investigators in the Duke NSCOR for experiments with X-rays. By performing routine dosimetry measurements on the standard small animal X-Ray irradiator, the Physics Core provides quality control for radiation exposure experiments. In addition, members of the physics core commissioned a new small animal irradiator (XRAD C225 Cx from Precision X-Ray), which has the capability to perform CT scans on mice and to deliver radiation not only to the entire mouse, but also to volumes as small as 1 cubic millimeter.

Core C: Education Core. Shelly Schwartz-Bloom, Ph.D., Lead

	We are developing an online problem-based unit to teach high school students about radiation in space by incorporating principles of physics, chemistry, and biology. The unit will contain a hypothetical scenario in which a group of young astronauts are selected to travel to Mars in the year of 2040. The astronauts must learn about the types of radiation they will encounter in space (compared to on earth), the damage these high energy particles and cosmic rays can cause to their DNA molecules, how their bodies can deal with the damage using a protein called p53, and what would happen if their p53 gene has a mutation. They will also learn how mutations in p53 genes can increase the risk of cancer, especially of the lung. The astronauts will meet some "virtual" scientists who study these topics and whose research findings are crucial to the development of a successful space program that includes a trip to Mars.
Bibliography Type:	Description: (Last Updated: 03/11/2021)
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Abstracts for Journals and Proceedings	Stripp BR. "Stem/Progenitor Cells that Maintain the Lung Epithelium." Refresher Lecture: Stem Cell Biology. Presented at the 22nd Annual Space Radiation Investigators' Workshop, League City, TX, September 18-21, 2011. 22nd Annual Space Radiation Investigators' Workshop, League City, TX, September 18-21, 2011. Abstract #7052. http://www.dsls.usra.edu/meetings/radiation2011/pdf/7052.pdf , Sep-2011
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Abstracts for Journals and Proceedings	Kirsch DG, Onaitis M, Stripp B. "Duke NSCOR: Lung Cancer Risk from HZE Ions." Tissue Specific Models of Cancer Risk. Presented at the 22nd Annual Space Radiation Investigators' Workshop, League City, TX, September 18-21, 2011. 22nd Annual Space Radiation Investigators' Workshop, League City, TX, September 18-21, 2011. Abstract #7065. http://www.dsls.usra.edu/meetings/radiation2011/pdf/7065.pdf , Sep-2011
Articles in Peer-reviewed Journals	Newton J, Oldham M, Thomas A, Li Y, Adamovics J, Kirsch DG, Das S. "Commissioning a small animal irradiator using 2D and 3D dosimetry techniques." Medical Physics. 2011, in press. , Nov-2011