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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. (Cedars-Sinai)		
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Task Description:	The goal of the Duke NASA Specialized Center of Research (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, 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.
	During the funding period, the Duke NASA Specialized Center of Research (NSCOR) made significant progress towards determining the acute effects of high charge and energy (HZE) particles on lung progenitor cells. In addition, we developed two robust mouse models of radiation-induced lung cancer, which we used to assess the effect of space radiation exposure on lung cancer risk. Members of the Duke NSCOR attended and presented at the NASA Human Research Program Investigators' Workshop each year of the funding period. Furthermore, two of the graduate students from the Duke NSCOR successfully completed the NASA Space Radiation Summer School. Through these activities and from our research projects, members of the Duke NSCOR gained new information about the effects of space radiation on normal lung tissue and lung cancer development. In total, the Duke NSCOR published 2 papers from this NASA-funded work. Another 5 manuscripts are currently under review or in preparation. Project 1. The role of the tumor suppressor p53 in space radiation-induced lung cancer. David Kirsch, M.D., Ph.D., Lead We proposed to study the role and timing of the tumor suppressor 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 proposed to develop a model of radiation-induced small cell lung cancer (Aim 3). For Aim 1, we analyzed lung tumor development in the lung cancer prone KrasLA1 mice, bearing normal levels of p53 or an extra copy of p53. We observed that an extra copy of p53 suppressed lung tumor initiation in the absence of radiation, without affecting tumor grade or proliferation. Although radiation exposure did not impact lung tumor initiation in mice with wildtype expression of p53, our results suggest that space radiation may increase tumor grade. In contrast, mice with an extra copy of p53 had enhanced lung tumor burden following either terrestrial or space radiation. These results suggest that an extra copy of p53 can promote radiation induced lung tumorigenesis. For Aim 2, we utilized an in vivo knockdown system that enabled temporal regulation of p53 expression in mice. We found that when p53 expression was permanently decreased following irradiation, mice developed soft-tissue sarcomas. Space radiation increased the sarcoma incidence as compared to terrestrial radiation. Furthermore, we showed that

Task Progress:

temporarily blocking p53 expression during radiation exposure was sufficient to ameliorate acute hematologic toxicity while simultaneously reducing lymphoma development. Our results suggest that the p53 response to radiation promotes radiation-induced lymphomagenesis and that inhibiting p53 could be a promising approach to prevent hematopoietic injury following exposure to large doses of radiation.

For Aim 3, we developed a mouse model of radiation-induced small cell lung cancer. Using this model, we found that space radiation was more effective than terrestrial radiation at accelerating lung and brain tumor development. Radiation dose and quality also impacted tumor incidence and histological subtype. Consistent with data in humans, our results suggest that female mice may have a higher excess relative risk of lung and brain tumor development following irradiation.

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

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

For Aim 1, we irradiated and analyzed tumor formation in CC10-CreER; lsl K-RasG12D mice. When mice were exposed to fractionated space radiation, they had more extensive lung tumor formation, but no change in tumor number or distribution as compared to unirradiated controls. Using microarray data from the tumors of mice described above, we also identified the potential targets EphA3, Zbtb16, and Pla2g7 that may be responsible for the differences in tumor initiation and progression that we observe. Analysis of tumors formed following terrestrial radiation exposure is ongoing.

For Aim 2, we generated K5-CreER; lsl K-RasG12D mice and treated them with tamoxifen. Unfortunately, these mice quickly developed tumors in the forestomach and lip causing respiratory occlusions and morbidity. Therefore, we have not been able to characterize the impact of space radiation in this model.

For Aim 3, we generated SPC-CreER; lsl K-RasG12D mice and treated them with tamoxifen. Unfortunately, this Cre-driver was leaky and many of the mice developed confluent tumors in the lung prior to radiation exposure. Therefore, we have not been able to characterize the impact of space radiation in this model.

K-RasG12D mutant mice, especially those in Aims 2 and 3, developed widespread tumors causing death of the mouse within 24 weeks post tamoxifen administration. As an alternative approach, we crossed the small cell lung cancer model (developed by the Kirsch lab) with the CC10-CreER (Aim 1); K5-CreER (Aim 2); and SPC-CreER (Aim 3) genes in order to assess the effects of radiation in a less penetrant model. The Rbfl/fl;p53fl/+ irradiated mice, as well as their sham controls, are currently being monitored for tumor formation.

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

The focus of this project was to compare effects of terrestrial and space radiation radiation on the clonogenic behavior and repair capacity of lung epithelial progenitor cells and to determine the impact of p53 deficiency on these responses. We have developed novel mouse models to functionally investigate progenitor cell behavior both in vivo and in vitro following radiation exposure.

For Aim 1 we used in vivo lineage tracing and novel in vitro models that recapitulate epithelial-stromal interactions seen in small airways, to determine how radiation exposure impacts clonal expansion of epithelial progenitor cells. Lineage tracing coupled with morphometry in tissue sections showed that exposure to radiation was associated with dose-dependent increases in clone sizes within airways. By utilizing a whole-mount imaging system to quantitate patch size across an entire lung lobe, we found that terrestrial gamma-ray exposure increased the frequency of medium, but not large patches, whereas exposure to HZE particles increased both the total size and frequency of medium and large patches. However, in vivo clonal expansion of epithelial progenitor cells was not associated with a significant change in the epithelial proliferative index.

Our ability to couple lineage tracing of epithelial progenitor cells with an in vitro assay in which epithelial cells are co-cultured with stromal support cells in a 3D matrix has provided a sensitive measure of moderate- to low-dose effects. We found that both terrestrial and space radiation exposures caused a dose-dependent decrease in freshly isolated airway epithelial cells immediately following radiation. When we isolated airway epithelial cells two months after space, but not terrestrial, radiation we saw that colony forming efficiency was still significantly decreased. Using qRT-PCR and immunofluorescent staining for markers of senescence, we found that HZE particle exposure leads to senescence of progenitors, which leaves fewer progenitor cells to maintain the lung, leading to larger clonal expansion.

For Aim 2, we used both in vitro and in vivo experiments to assess the role of p53 airway epithelial repair following radiation exposure. We used lineage tracing methods in mice deficient for p53 and assessed clonal expansion following space radiation exposure. We found that, while wild type mice had significantly increased clone sizes post-radiation, p53 deficient mice did not undergo clonal expansion, indicating that the senescence-induced patch expansion we observe following radiation exposure is p53-dependent. Additionally, we exposed p53 deficient cells to terrestrial radiation, plated them in our in vitro 3-D co-culture assay, and assessed colony forming ability as compared to wild type controls. p53 null cells had a higher colony forming ability at baseline as well as following radiation exposure. Lastly, through collaborative interaction with the Kirsch lab, we demonstrated that p53 gene dose dramatically impacts homeostatic behavior of epithelial progenitor cells, suggesting that inter-individual differences in regulation of the p53 pathway may impact epithelial behavior in the normal lung that influences responses to radiation exposure.

Core A: Administrative Core. David Kirsch, M.D., Ph.D., Lead

The Administrative Core (Core A) provided overall management of the NSCOR award by ensuring that projects made satisfactory progress. During the fifth year of funding, the Administrative Core monitored project progress by conducting Duke NSCOR meetings once a month and multiple teleconferences with NASA funded investigators. Minutes were recorded at these meetings in order to ensure that tasks were completed in a timely manner. In addition, the Administrative Core worked with Project leads and Core leads to consider the strengths of our NSCOR to develop ideas and concepts for the competing renewal application, which is now pending.

Core A program coordinator Ms. Cooley made travel arrangements for the Duke NSCOR team to travel to Brookhaven National Laboratory biannually in order to expose mice to 56Fe and 28Si ions. Travel arrangements were also made for the annual Radiation Research Society Meeting. Moreover, the Administrative Core arranged for the NASA Human Research Program (HRP) Investigators' Workshop.

	Duke NSCOR administrators served as liaisons between the project groups to guide BNL (Brookhaven National Laboratory) and Duke training and credentialing of new investigators, ensure timely and accurate submission and renewal of IACUC protocols, NSCOR progress reports, as well as applications for NASA Space Radiation Laboratory (NSRL) Beam Time. Core A provided budget oversight for the Duke NSCOR. Lisa Hall monitored project expenditures. Mrs. Hall met monthly with Dr. Kirsch to review spending and fiscal matters for each NSCOR project and Core. Marcia Painter assisted with the financial accounting for the Duke NSCOR. Core B: Physics Core. Terry Yoshizumi, Ph.D., Lead The Physics Core (Core B) provided comprehensive measurements of radiation dose (dosimetry) and oversaw 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 provided quality control for radiation exposure experiments. Members of the physics core participated and presented physics reports at regularly-scheduled NSCOR meetings. The Core ensured the timely incorporation of new dosimetry technology to provide state-of-the-art dosimetry support. The Physics Core successfully collaborated with the X-ray Irradiator manufacturer addressing kV x-ray dosimetry. Core C: Education Core. Rochelle Schwartz-Bloom, Ph.D., Lead The Education Core (Core C) developed a problem-based unit (Raising Interest in Science Education: Research & Development) to teach high school students about radiation in space by incorporating principles of physics, chemistry, and biology. The unit contains 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 also learn how mutations in p53 genes can increase the risk of cancer, especially of the lung. The astronauts will meet some “virtual” scientists (the Principal Investigators of projects 1-3) who study these topics and whose research findings are crucial to the development of a successful space program that includes a trip to Mars. This past year after the final revisions of the unit (and after its beta-testing by high school students), the website was developed for dissemination. The URL is http://rise.duke.edu/ [Ed. note 3/11/21: URL no longer connects; suggest contacting PI for further information].

Bibliography Type:	Description: (Last Updated: 03/11/2021)
Abstracts for Journals and Proceedings	Rampersad RR, Onaitis MW. "High LET radiation leads to increased progression of K-Ras mutant lung adenocarcinoma." Presented at the 2016 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 8-11, 2016. 2016 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 8-11, 2016. , Feb-2016
Abstracts for Journals and Proceedings	Farin A, Kondu B, Stripp BR. "Exposure to high-LET radiation results in p53-dependent airway epithelial progenitor cell depletion and tissue remodeling." Presented at the 2016 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 8-11, 2016. 2016 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 8-11, 2016. , Feb-2016
Abstracts for Journals and Proceedings	Castle KD, Moding EJ, Lee CL, Reinsvold M, Williams N, Luo L, Ma Y, Kirsch DG. "Exposure to HZE Particles Enhances Lung Tumor Development in a Mouse Model of Small Cell Lung Cancer." Presented at the 2016 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 8-11, 2016. 2016 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 8-11, 2016. , Feb-2016
Abstracts for Journals and Proceedings	Kirsch DG, Onaitis MW, Stripp BR. "Duke NASA Specialized Center for Research: Lung Cancer Risk from HZE Ions." Presented at the 2016 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 8-11, 2016. 2016 NASA Human Research Program Investigators’ Workshop, Galveston, TX, February 8-11, 2016. , Feb-2016
Abstracts for Journals and Proceedings	Kirsch DG. "An Extra Copy of p53 Suppresses Initiation of Kras-driven but Not Radiation-Induced Tumors." Presented at the 15th International Congress of Radiation Research, Kyoto, Japan, May 25-29, 2015. 15th International Congress of Radiation Research, Kyoto, Japan, May 25-29, 2015. , May-2015
Abstracts for Journals and Proceedings	Lee CL. "The Tumor Suppressor p53 Acts During Total-body Irradiation to Promote Lymphoma Development." Presented at the 15th International Congress of Radiation Research, Kyoto, Japan, May 25-29, 2015. 15th International Congress of Radiation Research, Kyoto, Japan, May 25-29, 2015. , May-2015
Articles in Peer-reviewed Journals	Moding EJ, Min HD, Castle KD, Ali M, Woodlief L, Williams N, Ma Y, Kim Y, Lee CL, Kirsch DG. "An extra copy of p53 suppresses development of spontaneous Kras-driven but not radiation-induced cancer." JCI Insight. 2016 Jul 7;1(10). http://dx.doi.org/10.1172/jci.insight.86698 ; PubMed PMID: 27453951; PubMed Central PMCID: PMC4955525 , Jul-2016
Articles in Peer-reviewed Journals	McConnell AM, Konda B, Kirsch DG, Stripp BR. "Distal airway epithelial progenitor cells are radiosensitive to High-LET radiation." Sci Rep. 2016 Sep 23;6:33455. http://dx.doi.org/10.1038/srep33455 ; PubMed PMID: 27659946; PubMed Central PMCID: PMC5034250 , Sep-2016
Articles in Peer-reviewed Journals	Barcellos-Hoff MH, Blakely EA, Burma S, Fornace AJ Jr, Gerson S, Hlatky L, Kirsch DG, Luderer U, Shay J, Wang Y, Weil MM. "Concepts and challenges in cancer risk prediction for the space radiation environment." Life Sci Space Res (Amst). 2015 Jul;6:92-103. http://dx.doi.org/10.1016/j.lssr.2015.07.006 ; PMID: 26256633 , Jul-2015
Articles in Peer-reviewed Journals	McConnell AM, Yao C, Yeckes AR, Wang Y, Selvaggio AS, Tang J, Kirsch DG, Stripp BR. "p53 regulates progenitor cell quiescence and differentiation in the airway. " Cell Rep. 2016 Nov 22;17(9):2173-82. https://doi.org/10.1016/j.celrep.2016.11.007 ; PubMed PMID: 27880895 , Nov-2016

Articles in Peer-reviewed Journals	Asselin-Labat ML, Rampersad R, Xu X, Ritchie ME, Michalski J, Huang L, Onaitis MW. "High-LET radiation increases tumor progression in a K-Ras-driven model of lung adenocarcinoma." Radiat Res. 2017 Nov;188(5):562-70. https://doi.org/10.1667/RR14794.1 ; PubMed PMID: 28952911 , Nov-2017
Articles in Peer-reviewed Journals	Oldham M, Newton J, Rankine L, Adamovics J, Kirsch D, Das S. "How accurate is image guided radiation therapy (IGRT) delivered with a micro-irradiator?" J Phys Conf Ser. 2013;444:12070. https://doi.org/10.1088/1742-6596/444/1/012070 ; PMID: 24454521; PMCID: PMC3894105 , Jan-2013
Articles in Peer-reviewed Journals	Lee CL, Castle KD, Moding EJ, Blum JM, Williams N, Luo L, Ma Y, Borst L, Kim Y, Kirsch DG. "Acute DNA damage activates the tumour suppressor p53 to promote radiation-induced lymphoma." Nature Communications. 2015 Sep 24;6:8477. http://dx.doi.org/10.1038/ncomms9477 ; PubMed PMID: 26399548 ; PubMed Central PMCID: PMC4586051 , Sep-2015
Articles in Peer-reviewed Journals	Farin AM, Manzo ND, Kirsch DG, Stripp BR. "Low- and high-LET radiation drives clonal expansion of lung progenitor cells in vivo." Radiation Research. 2015 Jan;183(1):124-32. http://dx.doi.org/10.1667/RR13878.1 ; PubMed PMID: 25564721; PubMed Central PMCID: PMC4409869 , Jan-2015