

Fiscal Year:	FY 2012	Task Last Updated:	FY 11/02/2011
PI Name:	Wang, Ya M.D., Ph.D.		
Project Title:	NSCOR: Mechanisms underlying the risk of HZE particle-induced solid tumor development		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Radiation health		
Joint Agency Name:		TechPort:	No
Human Research Program Elements:	(1) SR :Space Radiation		
Human Research Program Risks:	(1) Cancer :Risk of Radiation Carcinogenesis		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Comments:			
Project Type:	GROUND	Solicitation / Funding Source:	2010 Space Radiation NSCOR/Virtual NSCOR NNJ10ZSA002N
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No. of PhD Candidates:	2	No. of Master' Degrees:	
No. of Master's Candidates:		No. of Bachelor's Degrees:	
No. of Bachelor's Candidates:	3	Monitoring Center:	NASA JSC
Contact Monitor:	Cucinott1a, Francis	Contact Phone:	281-483-0968
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Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:			
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	<p>The Emory University-Georgia Health Sciences University NSCOR will investigate the mechanisms by which high charge and energy (HZE) particles, a component of space radiation, induce lung cancer. HZE exposure elicits complex DNA damage, together with a broader cell/tissue stress response that likely includes changes in expression of tumor suppressor proteins, persistent elevation of reactive oxygen species, and alterations in the pattern of DNA methylation. The central hypothesis of this NSCOR is that this broader stress response amplifies the carcinogenic risk from a primary DNA damage event. Preliminary studies suggest that a small noncoding RNA, microRNA-21 (miR-21) plays a key role in coordinating the HZE particle-associated stress response. Center investigators will use genetic, epigenetic, and biochemical approaches to address the role of miR-21 dependent and independent stress responses in HZE particle-induced lung cancer. There are four projects:</p> <ol style="list-style-type: none"> 1. Determine whether the lung cancer suppressor, Gprc5a, protects against HZE particle-induced lung carcinogenesis, and whether miR-21 overexpression blunts this protective effect. 2. Determine whether HZE-particle radiation exposure results in hyper-reliance on error-prone DNA repair pathways, whether miR21 mediates this effect, and whether dysregulation of DNA repair contributes to lung carcinogenesis. 3. Determine the nature of the HZE-particle induced ROS stress response, whether it contributes to HZE particle-induced lung carcinogenesis, and the role of miR-21 in this process. 4. Determine the scope of HZE-particle radiation-induced alterations in DNA methylation patterns, whether these alterations contribute to lung carcinogenesis, and the role of miR-21-dependent targeting of DNA methyltransferase 1 (DNMT1) in this process.
Task Description:	
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	<p>Lung cancer is the most common fatal cancer among men and women worldwide. Lung cancer is believed to be one of the major risks of HZE-particle exposure, although quantitative and mechanistic understanding of this risk is lacking. The EU-GHSU NSCOR will address this important knowledge gap. In addition, our NSCOR team will answer the question concerning whether and how different qualities (LET) of radiation affects lung tumorigenesis. These results will not only provide important information that will aid in the facilitation of the NASA Mars project, but will also provide the public with useful information concerning lung carcinogenesis and the benefits of cancer prevention.</p>
Task Progress:	<p>Progress</p> <ol style="list-style-type: none"> 1. Radiation: this proposal includes two major categories: Animal and cell experiments. We have performed several types of studies, which are described as follows: <ol style="list-style-type: none"> 1). Cell studies: We carried out several experiments using human lung epithelial cells and cells over-expressing miR-21. We are currently preparing mouse lung epithelial cells from miR-21 knock-in or Gprc5a^{-/-} mice. Exposure of those cells to X-ray or HZE- particles is planned for the next year of support. 2). Animal studies are summarized as follows: We already exposed 620 mice (including wild type, miR-21 knock in and Gprc5a knock out) to HZE particle (iron or silicon) or X-ray at either single dose (1 Gy) or fractionation doses (0.2 Gy x 5). Next year we will exposure additional 560 mice ((including wild type, miR-21 knock out, Gprc5a knock out and combined miR-21 knock in and Gprc5a knock out) to HZE particle (iron or oxygen) or X-ray at either single dose (1 Gy) or fractionation doses (0.2 Gy x 5). <p>X-ray exposure was performed at the Department of Radiation Oncology, Emory University, and the HZE-particle exposure was performed at NASA Space Radiation Laboratory (NSRL), Brookhaven National Laboratory (BNL). The mice will be sacrificed at one year following radiation exposure for observing tumorigenesis, particularly in the lung tissue.</p> 2. Each project: This proposal has four projects and project's progress is described as follows: <p>Project 1: It is known that EGFR could promote miR-21 expression through stimulating Stat3, and miR-21 could in turn positively affect EGFR activity. However, it remains unclear how to link the miR-21-EGFR positive loop to radiation-induced tumorigenesis. Recently, we found that the miR-21-EGFR loop is over-activated in DNA double strand break (DSB) repair deficient cells and mice, which is stimulated by endogenous DNA DSB formation that occurs during DNA replication. In addition, we found that the increased level of miR-21 and EGFR is correlated with the increased frequency of radiation-induced tumorigenesis. These results demonstrate for the first time that DNA DSBs have a functional link with the up-regulation EGFR-miR-21 loop. These findings provide an important explanation for why DNA DSB repair deficient mice have a high frequency for spontaneous tumorigenesis, and also provides an additional explanation concerning why radiation-induced DNA DSBs enhance tumorigenesis. These data will further facilitate the experiments as described in project 2. At present, we are also collaborating with Dr. Doetsch and H. Wang (project 3) to study the effects of miR-21 on the generation of radiation-induced reactive oxygen species (ROS).</p> <p>Project 2: It has been proposed that exposure to HZE particle radiation influences pathway choice and, in particular, that it may create an unfavorable intracellular environment for classical non-homologous end joining (C-NHEJ) by releasing small DNA fragments that interfere with the function of Ku protein, an essential participant in C-NHEJ. To further investigate the effect of HZE exposure on pathway choice, we examined its effect on repair of a subsequent I-SceI-induced DSB. We used four independent reporter cell lines. Two of them are designed to express eGFP when an I-SceI-induced DSB is repaired by homologous recombination (HR). The other two are designed to report end-joining events. Accurate joining of nearby I-SceI sites on the same chromosome results in eGFP expression, whereas joining of unlinked I-SceI sites results in dsRed expression. Sequencing of the joints allows discrimination between C-NHEJ and the less accurate process of alt-NHEJ. We exposed all four cell lines separately to 1 GeV/u Fe or 300 MeV/u Si particles during the spring and summer 2011 NSRL beam runs. We repeated the 1 GeV/u Fe exposures a third time during the fall 2011 beam run. We tested the capacity for HR and end-joining repair at intervals following recovery. Data analysis is in progress.</p> <p>Project 3: DNA damage inflicted by radiation or chemotherapeutic drugs induces a cellular stress response by still undefined mechanisms and consequences for cell survival and genomic stability. The goals for Project 3 are to determine the nature of the HZE-particle induced stress response and the resulting DNA damage and genomic</p>

instability that contributes to HZE-particle induced lung carcinogenesis and the role of miR-21 in this process. In the first year we have focused on establishing methodologies and techniques proposed in Aim 1 to measure ROS, DNA damage and genomic instability in response to X-Ray and HZE-particles. Using flow cytometry, we have detected recurrent increases in superoxide and hydrogen peroxide levels in response to a single exposure to dosing as low as 1 Gy radiation. These elevations correlate with increased oxidative DNA damage detected with the alkaline comet assay and with chromosomal mis-segregation during the first mitosis measured by a micronucleus formation assay. ROS levels remain elevated, persisting up to several weeks in the progeny of surviving cells. Initial experiments show that cell exposure to 0.25Gy or 1Gy Fe particles increases the magnitude and the length of this chronic response. Our initial studies on the role of miR-21 show that transient expression at the moment of exposure increases the ROS response and micronucleus formation, pointing to a role in promoting genomic instability. For the next year of support we will analyze changes in gene expression associated with chronically elevated ROS levels to identify the mechanisms involved in their generation and the physiological consequences of a pro-oxidant status induced by both low and high-LET radiation.

Project 4: The primary goal of this project is to define the epigenetic determinants of HZE radiation exposure induced lung carcinogenesis and the extent to which this is mediated by miR-21. Our preliminary results had indicated that exposure of liver cells to high (Fe) vs. low (X-ray) LET ionizing radiation led to both unique and shared changes in DNA methylation. The fact that such changes were observed at several passages after the initial exposure led us to propose that there may be an epigenetic 'memory' of high LET radiation exposure wherein alterations in DNA methylation resulting from acute exposure and local DNA damage have the potential to become 'fixed' if they are subsequently replicated, leading to a now permanent change in DNA methylation and potentially gene expression. Over the last ten months, we have focused our efforts on testing this hypothesis. Immortalized human bronchial epithelial cells (3-KT) were exposed to varying doses (0.3 GY, 1 Gy) and sources (Si, Fe) of high LET radiation at the BNL facility in June 2011. After one day, a 24 hr exposure time point was collected, and the remaining cells shipped back to the lab in Georgia, where they were maintained in continuous culture for 50 population doublings (~4 months) with weekly collection of cells for genomic DNA, RNA, and protein isolation. Cultures that were not exposed underwent the same handling/ shipping procedures and were maintained in parallel. In preliminary studies we have examined the expression of ~10 genes in cells that have been maintained in continuous culture for 32 population doublings (~8 weeks) after exposure to 0.3 or 1.0 Gy Si particles. We found that DNMT1 and JunD expression show a persistent, dose-dependent, up-regulation (~2-fold) in the Si particle exposed cells, whereas several other genes (CHD1, VIM, DNMT3B, BRSK2, FAK) are unaffected. Interestingly, we identified IL-8 as a strongly, and persistently, upregulated gene; IL-8 expression was upregulated by >18-fold in cells that had been exposed to 1.0Gy Si even after 8 weeks in culture. These data suggest that the persistent activation cytokines, through genetic or epigenetic means, may be one consequence of high LET radiation exposure, and further, that the carcinogenic effects of prior exposure might occur by eliciting a chronic inflammatory state. IL-8 is known to be post-transcriptionally regulated by several miRNAs, several of which are controlled by CpG island containing promoters. One goal over the next funding period will be to determine whether epigenetic silencing of these pri-miRNAs might lead to persistent deregulation of IL-8 transcript levels. In addition, we are now poised to attack our primary goal which is to determine the scope of HZE particle induced DNA methylation changes genome-wide by subjecting DNA samples from acutely exposed and long-term 'memory' series to Illumina Infinium 450K Methylation analyses. We will be comparing the alterations in the DNA methylation profile induced by HZE particle exposure in an acute setting (24 hr after exposure) with those that survive many cell divisions, and their relationship to gene expression. Data generated from this platform will allow us to identify both global and site-specific DNA methylation changes that persist many months after the initial insult which may ultimately be useful as markers for risk assessment.

Bibliography Type:	Description: (Last Updated: 07/07/2021)
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Abstracts for Journals and Proceedings	Shi Y, Yu X, Zhang X, Wang P, Tang X, Wang H, Wang Y. "MiR-21 is a sensitive marker in the brain tissue from irradiated mice." Presented at 22nd Annual NASA Space Radiation Investigators' Workshop, League City, Texas, September 18-21, 2011. 22nd Annual NASA Space Radiation Investigators' Workshop, League City, Texas, September 18-21, 2011. , Sep-2011
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Articles in Other Journals or Periodicals	Shi Y, Zhang X, Wang P, Tang X, Wang H, Wang Y. "MiR-21 is continually elevated long-term in the brain following ionizing radiation." Radiation Research. In Press, October 2011. , Oct-2011