Fiscal Year:	FY 2017	**Task Last Updated:**	FY 11/08/2016
PI Name:	Emmett, Mark Ph.D.		
Project Title:	Induction of Hepatocellular Carcinoma by Space Radiation: A Systems Biology Study of Causative Mechanisms		
Division Name:	Human Research		
Program/Discipline:			
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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City:	Galveston	**State:**	TX
Zip Code:	77555-5302	**Congressional District:**	14
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2013-14 HERO NNJ13ZSA002N-RADIATION
Start Date:	01/07/2015	**End Date:**	01/06/2019
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	2	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA JSC
Contact Monitor:	Simonsen, Lisa	**Contact Phone:**	
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Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:	November 2016 report: Dr. Joseph Moskal (Northwestern University) is no longer affiliated with academia nor involved with this project and is being removed as Co-I on the project. November 2015 report: Dr. Carol L. Nilsson (Co-I, 10% Effort) is no longer involved with the project. Dr. Cheryl F. Lichti has replaced Dr. Nilsson at 20% Effort. Two advanced graduate students, Brooke L. Barnette and Shinji K. Strain, will replace the TBA senior scientist (50% Effort).		
COI Name (Institution):	Meyer-Baese, Anke Ph.D. (Florida State University) Ullrich, Robert Ph.D. (University of Texas Medical Branch) Lichti, Cheryl Ph.D. (University of Texas Medical Branch)		
Grant/Contract No.:	NNX15AD65G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Exposure to high-energy heavy ions (HZE) during space travel is a health risk for astronauts. Even at low doses, exposure to HZE can lead to cancer. To better understand the molecular mechanisms of HZE induced carcinogenesis we will use a mouse model of HZE-induced hepatocellular carcinoma to study microenvironment changes after exposure to low level HZE. A comprehensive systems biology approach consisting of transcriptomics, lipidomics, proteomics, and metabolomics with novel data analysis will be used to build detailed biological pathways and identify molecular mechanisms that drive carcinogenesis. This work will further our understanding of risk at a mechanistic level and allow the development of new models for estimating human risk.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	It is anticipated that there will be crosstalk between the molecular changes involved in HZE induced hepatocellular carcinoma (HCC) and environmentally induced HCC seen on Earth. The Principal Investigator (P.I.) is actively collaborating with ground based clinical researchers in HCC research.
Task Progress:	Year 2 Results: Tissues have been harvested from all time points (30, 60, 120, 270, & 360) for all groups (600 MeV/n 56Fe ions (0.2 Gy), 1 GeV/n 16O (0.2 Gy), and 350 MeV/n 28Si (0.2 Gy) and 137Cs gamma rays (1 and 3 Gy). Due to an almost month long power outage in the Medical Research Building at University of Texas Medical Branch (UTMB) where our animal sacrifice is performed (main buss bar failure), the 240 day time point had to be postponed by 30 days, thus moving the fourth time point from 240 days to 270. The final 360 day tissues were recently harvested on October 10, 11, and 17 of 2016. NOTE: The first liver tumors were observed in some of the 270 and 360 day, 3 Gy Gamma, and HZE irradiated mice. Based on previous work done by the Ullrich Group, it is expected that more tumors will develop with time, but there is no budget to extend the studies out another six months (total of 540 days post irradiation). The progress on the specific aims for this project are below. Specific Aim 2. Determine transcriptional changes in the hepatic microenvironment of HZE- and gamma-irradiated samples, compared to controls. All tissue samples have been collected. Extraction of RNA for transcription analysis is underway. The P.I. has transitioned to collecting transcriptomic data from these samples with low read RNA sequencing. Low read RNA sequencing will provide much more transcriptomic data than could have been obtained by targeted transcriptomic analysis as originally proposed. The main drawback of RNA sequencing is the high cost of analysis. Although RNA sequencing was not in the budget for this project, the P.I. had to find a replacement for the targeted transcriptomics that was going to be performed in the laboratories of collaborators at Northwestern University in Evanston, IL. These collaborators started a small pharmaceutical company and withdrew from academic research. RNA sequencing is a “cutting edge” technology, but is also an expensive technology. The RNA sequencing will be performed at the UTMB Molecular Genomics Core. The RNA sequencing originally was projected to come at no cost to the project, but due to cost increases there will be costs for these analysis which will require some re-budgeting by the P.I. The P.I.’s laboratory is working with the UTMB Molecular Genomics Core to determine the quality and quantity of RNA produced from small samples of liver tissue. Preliminary results demonstrated that the RNA was of high enough quality and quantity to perform low read RNA sequencing. To keep RNA sequencing costs to a minimum, all samples are being processed in the P.I.’s laboratory and will be analyzed in blocks to maximize the efficient use of the RNA Libraries with the Illumina HiSeq 1500 Analyzer in the UTMB Genomics Core Facility. Because not all transcripts are translated into protein, this data will be validated by targeted proteomic studies. Pilot proteomic studies have verified that FULL proteomic data sets can be obtained from small samples of liver tissue.and thus, will provide adequate protein concentrations for our targeted proteomic analysis. Once the RNA sequencing studies have been completed, we are ready to proceed with the targeted proteomics (Targeted Proteomics is part of Specific Aim 3). Specific Aim 3. Determine comprehensive ultra high-resolution lipidomic alterations as well as high-resolution targeted proteomic microenvironment changes in hepatic tissue from tissue punches of HZE- and 137Cs gamma ray-irradiated animals as well as non-irradiated controls The lipid extraction methodology has been optimized for small samples of liver tissue without having to thaw the sample multiple times. All sampling for lipidomics, transcriptomics, and proteomics are being collected by this method, which preserves sample integrity. This provides a more uniform representation of what is occurring on the molecular level across the whole tissue instead of being localized to a specific area, i.e., from a tissue punch. Lipid samples are homogenized in 155mM ammonium acetate which allows a total protein concentration can be determined using a Bradford protein assay before lipid extraction, which denatures the protein pellet. Determination of total protein concentration permits a uniform sample load for each sample and allows for consistent quantification. Lipid extractions have been conducted on the 30, 60, and 120 day time point and are currently underway for the 270 and 360 day time points. A data dependent method for automated collection of MS/MS spectra has been optimized using a combination of lipid standards such as GM1, GD1a, sphingosine-1-phosphate, 3-sn-phosphatidic acid sodium salt, etc., along with biological samples obtained from murine synaptosomes. MS/MS data was obtained for GM1 at 45.0V. Other standards such as GD1a also required 45.0V to produce adequate fragmentation, whereas lipids, such as 3-sn-phosphatidic acid sodium salt, only required 20.0V. The automated MS/MS with collision energies 45.0V and 20.0V has also been optimized with samples from biological samples (synaptosomes derived from mouse brain). Since these collision energies have been successful with both lipid standards and biological samples, they are currently being applied for the starting points for MS/MS analysis of the liver lipids in this project. MS/MS data for the first three time points of this study have been collected and data analysis is underway. Specific Aim 4. Correlate large ‘omic datasets by use of Ingenuity Pathways’ Knowledge based software and unique algorithms developed by our collaborators to construct biological pathways that elucidate molecular mechanisms of HCC carcinogenesis induced by HZE irradiation. New data analysis regimes have continued to be evaluation and progress has been made on efficient lipid identification and quantification which greatly surpass the manual lipid data analysis and identifications used previously by the P.I. Dr. Cheryl Lichti and Brooke Barnette have continued to evaluate new software packages for lipidomic data analysis, and have been working closely with some of the developers. These software packages are primarily designed for peak picking and identification of small molecule metabolites not large lipid ions. Dr. Lichti has also adapted available proteomic software packages to aid in peak picking of the higher m/z lipids. Software packages currently being evaluated and modified are: 1) Bruker Daltonics Target Analysis. This software is proposed to be used with the P.I.’s lipid library (>25,000 entries). Lipid identifications will be made based on accurate mass and ms/ms fragmentation patterns.

2) Skyline (MacCoss Lab Software) is an open source program. This software is currently only able to import transition lists which will allow it to be used for quantification, but the species identification must be completed elsewhere. We are working with the developers to establish a library import feature to work with the P.I.'s lipid library for identification.

3) Elements for Metabolomics (Proteome Software). This software has the capability of searching the LipidMaps database which is the gold standard for lipid database searching. A drawback of this software is that there is relatively no control over chromatographic alignment. Our lab is working directly with the programmers of the software and the program now accepts raw .d data files. The programmers are also addressing issues with data size and these changes are to be included in the next updated release.

4) Progenesis QI (Nonlinear Dynamics, Waters Corporation). This software utilizes the raw .d format of the data files, and control of chromatographic alignment. This software can search LipidMaps for lipid identification. We have encountered problems with the peak picking algorithm in this software which does not find the high m/z lipids such as gangliosides. We are working with Nonlinear Dynamics to update their algorithms to correct this problem. In the interim, Dr. Lichti has cleverly used the Progenesis proteomics software to find these peaks in the data.

Discussion: Even with multiple setbacks, the P.I. and Co-I are confident that the project is on-track and on-schedule. The P.I.'s lab has made several proposal changes that were necessitated by loss of our transcriptomics collaborator which dictated the move to low read RNA sequencing, and a major power outage (lab was out of power for almost one month), which necessitated a change from 240 to 270 day time point. The transcriptomic change dictated that new methodology needed to be developed. The advent of, optimization of, and use of the small samples of liver tissue all sample processing (lipid, RNA, and protein) has greatly enhanced our flexibility and preserved sample integrity across all sample analysis platforms. The move to RNA sequencing also greatly increased costs of the crucial Aim 2 project, there will be costs for these analysis that will require re-budgeting. Even with this re-budgeting, the P.I. is confident that the project can stay within the original budget.

During this year, preliminary data from this work was presented at the 64th American Society for Mass Spectrometry Conference in San Antonio, TX in a poster entitled "The application of lipidomics to the study of Heptocellular Carcinoma (HCC) induced by low dose, high-energy, high-charge ions (HZE)." Data was also presented at 27th Annual NASA Human Research Program Investigators' Workshop Integrated Pathways to Mars in Galveston, TX and will be presented at the 28th Annual NASA Human Research Program Investigators' Workshop in January 2017. Finally, it is anticipated that the first publications from this work will be submitted during 2017.

Bibliography Type:	Description: (Last Updated: 04/10/2021)