Fiscal Year:	FY 2020	**Task Last Updated:**	FY 08/14/2020
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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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:**	01/06/2020
No. of Post Docs:	0	**No. of PhD Degrees:**	3
No. of PhD Candidates:	3	**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
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Flight Program:			
Flight Assignment:	NOTE: Extended to 1/6/2020 per NSSC information (Ed., 2/12/19)		
Key Personnel Changes/Previous PI:	November 2017 Report: Dr. Cheryl Lichti left UTMB (University of Texas Medical Branch) to take a position at Washington University, St. Louis. She is still a collaborator on the project, but is no longer a Co-Investigator and is not receiving salary support since 8/31/17. Ana Nia (MD/Ph.D. Graduate student, joined the project October 2017. 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):	Ullrich, Robert 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 (PI) is actively collaborating with ground-based clinical researchers in HCC research. The computational methods being developed to analyze the vast omic data sets has the potential to revolutionize omic analyses.

Introduction/Background
A primary concern for astronauts during deep space travels is their exposure to ionizing radiation. Galactic Cosmic Rays are primarily composed of protons (85%), helium (14%), and high charge-high energy ions (HZE) such as 56Fe, 28Si, and 16O. Earth bound humans are not normally exposed to these ions due to the shielding of Earth's magnetosphere, but outside of this protection astronauts will be exposed to HZE. Exposure to HZE is a major risk factor for astronauts due to the possibility of HZE induced cancer as well as other potential health risks, i.e., cardiovascular and cognitive effects. This raises the question of what are the risks that will be posed to the astronauts during deep space travel such as a mission to an asteroid or to Mars and back?

HRP Relevancy

Risks: 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. These risks could be compounded with other flight factors such as: microgravity, dehydration, stress, nutrition, microbial contamination, and other factors.

Gaps: This research focused on three radiation carcinogenesis gaps (Ed. note August 2020--gaps have since changed for this project to Cancer-102:Determine the role of radiation quality on carcinogenesis and shared biology with other degenerative diseases (IRP Rev L); Cancer-302:Identify tissue-specific surrogate end-points for space radiation induced pre-malignancy and shared biology with other degenerative diseases (IRP Rev L); Cancer-401:Identify biomarkers for estimating individual susceptibility, risk assessment, and health monitoring (IRP Rev L)).

Cancer 1: How can experimental models of tumor development for the major tissues (lung, colon, stomach, breast, liver, and leukemia's) be developed to represent the major processes in radiation carcinogenesis and extrapolated to human risk projections?

Cancer 3: How can models of cancer risk be applied to reduce the uncertainties in radiation quality effects from SPEs and GCR?

Cancer 7: How can systems biology approaches be used to integrate research on the molecular, cellular, and tissue mechanisms of radiation damage to improve the prediction of the risk of cancer?

Final Study Hypothesis: Since indirect effects are likely a major mechanism of induction of HCC, an integrated omics approach will allow monitoring of the changes in the cellular microenvironment (lipids, mRNA and protein translation/modification) overtime resulting from low dose HZE irradiation.

Microenvironmental alterations will be used to identify: • Risk factors ; • Biomarkers for early detection ; • Mitigation targets

Specific Aims have been condensed for this final report:

1) Develop and implement an integrated omics platform utilizing transcriptomics, proteomics, lipidomics and metabolomics to identify microenvironmental-bystander responses to low dose HZE exposure in mice.

2) The integrated omics data sets will be used to predict alterations in biological/biochemical pathways induced by low dose HZE exposure. Biochemical assays will also be used as validation for the effected pathways.

3) Predictions will be made as to the risk involved for human astronauts during deep space travel who will be exposed to similar doses of HZE and potential countermeasures to combat the HZE induced changes will be presented.

Justification for a Multi-Omics Study of HZE induced HCC: Cellular signaling is known to be a very complex and eloquent process in which many levels of regulation are orchestrated to elicit the proper response (e.g., proliferation, differentiation, migration, survival). DNA contains the instructions that make these processes possible, which are transcribed into RNA to relay the message that is ultimately translated into proteins. Proteins can also be further regulated by post translational modifications. Proteins then go on to play key roles in cellular regulation. Although lipids and metabolites are products of proteins (enzymes) regulated by DNA, the lipids and metabolites themselves are not encoded within the DNA making them an excellent target for microenvironmental change. With this much interconnectivity, it is easy to understand why a multi-omics platform would be beneficial in monitoring the effects of deep space radiation especially in relation to the formation and progression of cancer where dysfunction occurs on many levels. Thus, we applied an integrated omics approach to elucidate information on the interaction between DNA/RNA, proteins, lipids and metabolites induced by low-dose, high charge, high energy (HZE) ions.

Computational Analysis of Transcriptomic Data Sets

The final Specific Aim in the original proposal of this grant had a Computational Analysis component to augment the knowledge-based Ingenuity Pathways Analysis (IPA) of these large omics data sets. Computational mathematical analysis is much less biased than IPA, but there is often still some operator bias in the selection of certain cut-off values. Pure computational analysis will enable interactions of specific species within omics data (transcripts, proteins, lipids, etc.) to be determined mathematically and not rely on previous interpretation of literature to determine these interactions.

Task Progress:

Additionally, computational analysis has the potential to identify interactions between transcripts or proteins that are not identified in the literature and thus are “assigned as undetermined.” Undetermined transcripts which are related to a specific pathway can be exploited to discover novel proteins which could prove to be valuable therapeutic targets.

The results of our computational analysis are highly complementary to our full integrated-omics analysis. The beauty of the computational analysis is that it is totally non-biased and yet still supports the results of the IPA multi-omics analysis presented early in this report. The computational analysis also provides additional information on more transcripts involved in response to HZE radiation and correlates unidentified transcripts to pathways that would have never been assigned with a knowledge-based analysis algorithm.

Two novel computational algorithms were developed using the transcriptomic data generated from the HZE radiated mice and their controls from NNX15AD65G. Both projects have been published and the publications are listed below.

The citations for these publications are (See also Bibliography section below):

1. Anna M. Nia, Tianlong Chen, Brooke L. Barnette, Kamil Khanipov, Robert L. Ullrich, Suresh K. Bhavnani, Mark R. Emmett. Efficient Identification of Multiple Pathways: RNA-Seq Analysis of Livers from 56Fe Ion Irradiated Mice. BMC Bioinformatics, 2020, 21:118-129. DOI: 10.1186/s12859-020-3446-5.

2. Anna M. Nia, Kamil Khanipov, Brooke L. Barnette, Robert L. Ullrich, George Golovko, Mark R. Emmett. Comparative RNA-Seq transcriptome analyses reveal dynamic time-dependent effects of 56Fe, 16O, and 28Si irradiation on the induction of murine hepatocellular carcinoma. BMC Genomics, (2020) 21:453. DOI:10.1186/s12864-020-06869-4.

Final Conclusions of Integrated Omics Approach to Define the Molecular Mechanisms of Low Dose, High Charge, High Energy Irradiation in Liver

• Mitochondria are drastically affected by HZE irradiation in particularly 16O and 56Fe irradiation in C57 wild type mice.

• Mitochondria effects are exhibited in all omics datasets (transcriptomics, proteomics, lipidomics, & metabolomics) and each omics data set supports findings in the other data sets.

• Mitochondria dysfunction was also validated through biochemical assays (Complex I inhibition).

• Computational analysis independently identified mitochondrial dysfunction as a major affected pathway after low dose HZE exposure.

• Activation of Immunological Pathways is the second most affected group of pathways after exposure to low dose HZE. Computational analysis identified immunological pathways as the primary affected pathway after low dose HZE exposure.

• Mitochondria effects by HZE irradiation are novel, real, and important to the health and safety of the astronauts. Not only in liver, but these effects could also be very detrimental in brain and cardiac tissue that have high levels of mitochondria. Mitochondrial dysfunction is most likely the root cause of HZE induced cardiomyopathy and cognitive dysfunction.

• HZE induces mitochondrial effects that are of great risk for deep space flight missions. The HZE effects will be additive to conditions that are already known from low orbit studies in mice. Recent reports discuss effects seen in rodents which travelled on the Space Shuttle and on the International Space Station (ISS). After exposure to microgravity, stress, low dose/low dose rate radiation, high levels of bacterial contamination, dehydration, etc., the authors noted disruption of lipid metabolism in liver and hypothesized that mitochondrial dysfunction could be causing the detrimental effects seen in the rodents (Laiakis, E., et al., [#20337] in NASA Human Research Program Investigators’ Workshop (2020); Blaber, E., et al., Int. J. Mol. Sci, 18, 2062 (2017), doi: 10.3390/ijms18102062; and Beheshti, A., et al., Scientific Reports, 9, 19195, (2019) doi:10.1038/s41598-019-55869-2)). These low orbit biological effects will be compounded when low does HZE is added during a deep space mission.

• Premature aging may be one of the major contributing factors of HZE irradiation that results in mitochondria dysfunction which occurs earlier with HZE irradiation as compared to gamma irradiation

Future Directions

Studies That Will Require New Animal Experiments: • Delve deeper into mitochondria and immunological effects induced by HZE; • Higher n# of mice and focus on “wild type” animals; • Earlier time point (ex. Day or week post-irradiation) to better understand early ROS and drivers of early onset immune pathways; • Later time point (18 month, i.e. tumor development); • Mitochondria respiration via seahorse system (measures oxygen consumption rate and can quantify multiple parameters of mitochondria respiration); • Mitochondria specific transcriptomic, proteomics (mitochondrial formyl-peptides that drive the cytokine storm) and lipidomics; • Mitochondrial effects in other tissues: brain, cardiac; • PET scan system to monitor for potential tumor growth in animals prior to sacrifice; • Test proposed countermeasure compounds. Proposed mitochondria and inflammation centered therapeutics and supplements will be tested in vivo and specific mitochondrial, immunological, and metabolic endpoints will be monitored to determine their effectiveness at alleviating effects induced by exposure to simulated GCR; • Correlate data with cognitive behavioral studies Studies That Can Be Performed on Banked Samples; • Delve deeper into mitochondria effects induced by HZE; • Comprehensive analysis of changes in the non-polar lipid fraction (banked).

Of immediate interest: plasmologens (endogenous antioxidants) and resolvins (tightly linked with inflammation, promote normal cellular function after inflammation); • Mitochondria specific transcriptomics, proteomics (mitochondrial formyl-peptides that drive the cytokine storm), and lipidomics; • Mitochondrial effects in other tissues: brain, cardiac…(banked); • Correlate observed mitochondrial dysfunction (in liver) with cognitive changes induced by HZE (as measured by chemical LTP studies); • Further mine data at hand (enhanced computational analysis).

Proposed Countermeasures and Biomarkers: The novel countermeasures (one FDA approved drug, one in clinical trials, and four approved dietary supplements) have been identified based on the multi-omic data sets and biochemical pathway identifications based on and supported by the multi-omic data sets. The identified countermeasures are primarily targeted at improving mitochondrial function and reducing inflammatory pathways. Additionally, two potential lipid biomarkers have been identified.

Bibliography Type:	Description: (Last Updated: 04/10/2021)
Articles in Peer-reviewed Journals	Nia AM, Chen T, Barnette BL, Khanipov K, Ullrich RL, Bhavnani SK, Emmett MR. "Efficient identification of multiple pathways: RNA-Seq analysis of livers from 56Fe ion irradiated mice." BMC Bioinformatics. 2020 Mar 20;21(1):118. https://doi.org/10.1186/s12859-020-3446-5 ; PMID: 32192433; PMCID: PMC7082965 , Mar-2020
Articles in Peer-reviewed Journals	Nia AM, Khanipov K, Barnette BL, Ullrich RL, Golovko G, Emmett MR. "Comparative RNA-Seq transcriptome analyses reveal dynamic time-dependent effects of 56Fe, 16O, and 28Si irradiation on the induction of murine hepatocellular carcinoma." BMC Genomics. 2020 Jul 1;21(1):453. https://doi.org/10.1186/s12864-020-06869-4 ; PMID: 32611366; PMCID: PMC7329445 , Jul-2020
Articles in Peer-reviewed Journals	Nia AM, Shavkunov A, Ullrich RL, Emmett MR. "137Cs γ ray and 28Si irradiation induced murine hepatocellular carcinoma lipid changes in liver assessed by MALDI-MSI combined with spatial shrunken centroid clustering algorithm: A pilot study." ACS Omega. 2020 Oct 6;5(39):25164-74. https://doi.org/10.1021/acsomega.0c03047 ; PMID: 33043195; PMCID: PMC7542585 , Oct-2020
Articles in Peer-reviewed Journals	Laiakis EC, Shuryak I, Deziel A, Wang YW, Barnette BL, Yu Y, Ullrich RL, Fornace AJ Jr, Emmett MR. "Effects of low dose space radiation exposures on the splenic metabolome." Int J Mol Sci. 2021 Mar 17;22(6):3070. https://doi.org/10.3390/ijms22063070 ; PMID: 33802822; PMCID: PMC8002539 , Mar-2021
Dissertations and Theses	Nia A. "Efficient Identification and Comprehension of Molecular Pathways Associated with Irradiation Induced Hepatic Carcinogenesis." Dissertation, The University of Texas Medical Branch, March 2020. , Mar-2020
Dissertations and Theses	Barnette BL. "An Integrated Omics Approach to Define the Molecular Mechanisms of Low Dose, High Charge, High Energy (HZE) Irradiation in Liver." Dissertation,The University of Texas Medical Branch, July 30, 2020. , Jul-2020