Task Book Report Generated on: 04/24/2024

Firmest, Mark Ph.D. Project Title: Induction of Hepatocellular Carcinoma by Space Radiation: A Systems Biology Study of Causative Mechanisms Division Name: Human Research Program Divisipline— Elements' Subdivisipline— Human Research Program Elements: (1) SR-Space Radiation health Joint Agency Name: TechPurt: No More Space Radiation Carcinogenesis Human Research Program Risks: (1) Cancer-Risk of Radiation Carcinogenesis Space Biology Special Category: None Human Research Program Risks: (1) Cancer-Risk of Radiation Carcinogenesis Space Biology Special Category: None Pl Definite: None Pl Definite: None Pl Definite: None Pl Corganization Type: University of Texas Medical Branch Pl Address 1: UTMB Cancer Research Center Pl Address 2: 301 University Blvd, Rt. 1074 Pl Web Page: City: Galveston State: TX Comments: Project Type: GROUND Solicitation / Funnting Start Date: 01007:2015 End Date: 01007:2019 No. of PhD Candidates: 0 No. of PhD Experts: 0 No. of PhD Candidates: 0 No. of PhD Experts: 0 No. of PhD Candidates: 0 No. of Master's Candidates: 0 No. of	F:I V	EV 2010	Trans. Trans. Car.	FV 11/00/2017
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Division Name: Human Research Program/Discipline: Program/Discipline: UMAN RESEARCH—Radiation health Human Research Program Elements: UD Respace Radiation Human Research Program Risks: UD Research Risk of Radiation Carcinogenesis Space Biology Cross-Element None Space Biology Cross-Element None Pl Email: UNIVERSITY None Pl Corganization Type: UNIVERSITY Phone: 409-747-1943 UNIVERSITY Programication Name: The University of Texas Medical Branch Pl Address 1: UTM Gancer Research Center Pl Address 2: 301 University Blvd, Rt. 1074 Pl Web Page: City: Galveston State: TX Comments: Project Type: GROUND Solicitation / Funding Solicitation / Funding Source: State Date: 0107/2015 Eard Date: 0107/2015 Eard Date: 0107/2015 Eard Date: 0107/2015 Degrees: 0. No. of Pabl Candidates: 0 No. of Master's Candidates: 0 No. of Bachelor's Candidates: 0 Monitoring Center: NASA JNC Contact Emails Elight Program: Elight Assignment: 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 collaboration on the project, but is no longer a Co-Investigator and is not receiving salary support since 82-11.7, Am Niu (MDP)Ph.D. Graduate student, joined the project October 2017. Key Personnel Changes/Previous Pl: Washington University, St. Louis. She is still a collaboration on the project, but is no longer a Co-Investigator and is not receiving salary support since 82-11.7, Am Niu (MDP)Ph.D. Graduate student, joined the project October 2017. Mayer-Bases, Anker Ph.D. (University of Texas Medical Branch) to take a position at Washington University, St. Louis. She is still a collaboration on the project, but is no longer a Co-Investigator and is not receiving salary support since 82-11.7, Am Niu (MDP)Ph.D. Graduate Studies, Joined the project October 2017. Mayer-Bases, Anker Ph.D. (University of Texas Medical Branch) Ni				
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Human Research Program Elements: (1) SRSpace Radiation Human Research Program Risks: (1) Cancer-Risk of Radiation Carcinogenesis Space Biology Cross-Element None Space Biology Cross-Element None PI Email: None PI Email: Program Risks: None PI Email: Program Risks: None PI Canalization Type: UNIVERSITY Phone: 409-747-1943 Organization Type: UNIVERSITY Phone: 409-747-1943 Organization Name: The University of Texas Medical Branch PI Address 1: UTMB Cancer Research Center PI Address 2: 301 University Blvd, Rt. 1074 PI Web Page: City: Galveston State: TX Zip Code: 77555-5302 Congressional District: 14 Comments: Project Type: GROUND Solicitation / Funding Sources Sources: Sources: 0107/2015 End Date: 0106/2019 No. of Past Dost: 0107/2015 End Date: 0106/2019 No. of PhD Candidates: 3 No. of Master' Degrees: 0 No. of PhD Candidates: 3 No. of Master' Degrees: 0 No. of Master's Candidates: 0 No. of Master's Degrees: 0 No. of Master's Candidates: 0 Monitoring Center: NASA JSC Contact Email: lisa C. Simonsen, Lisa Contact Phone: Flight Program: Flight Program: Flight Assignment: November 2017 Report: Dr. Cheyl 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 Col-precision on an in not receiving shalary support since St. Value, Stein, Stein, Jump Ph.D. Graduate student, joined the project Colober 2017. November 2016 report: Dr. Joseph Moskal (Northwestern University) is no longer at longer and collegated with his project and is being removed as Col- on the project, but is no longer a todent with his project and is being removed as Col- on the project, but is no longer a todent with his project and is being removed as Col- on the project hour is no longer a foot-lowesignor and in not receiving shalary support since St. Jan. St. State. Statil a collaborator on the project has the molecular project has being memore and Soling for the project Colverber 2017. November 2016 repo	Program/Discipline Element/Subdiscipline:	HUMAN RESEARCHRadiation heal	lth	
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Space Biology Cross-Element Space Biology Cross-Element Space Biology Special Category: None PI Email: meanmett@umb.edu Fax: FY PI Organization Type: UNIVERSITY Phone: 409-747-1943 Organization Type: UNIVERSITY Phone: 409-747-1943 Organization Type: UNIVERSITY Phone: 409-747-1943 Organization Name: PI Address 1: UTMB Cancer Research Center Organization Name: PI Address 2: 301 University Blvd, Rt. 1074 PI Web Page: City: Galveston State: TX City: Galveston Source: City: GROUND Solicitation / Funding Source: Project Type: GROUND Solicitation / Funding Source: Oli772015 End Date: Oli702015 Dear Desc: On No. of PhD Degrees: On No. of Master' Degrees: On Monitoring Center: No. of Bachelor's Candidates: On Monitoring Center: No. of Bachelor's Candidates: On Monitoring Center: Nowember 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 St. 1/17. Ann Nia (MDP.D. Grandutes Tuben.) Ising Control of St. (Cort Laten.) In Carel In Cort of St. (Cort of Laten.) In Cort of Degrees: On St. (In Cort of Cort	Human Research Program Elements:	(1) SR:Space Radiation		
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Space Biology Special Category: None Space Biology Special Category: None Pt Email: mremmett@utmb.edu Fax: FY	Space Biology Element:	None		
PI Email: mremmetti@utmb.edu Fax: FY Pl Organization Type: UNIVERSITY Phone: 409-747-1943 Organization Name: The University of Texas Medical Branch PI Address 1: UTMB Cancer Research Center PI Address 2: 301 University Blvd, Rt. 1074 PI Web Page: 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: 0107/2015 End Date: 0106/2019 No. of Pab Descress: 0 No. of PhD Candidates: 3 No. of Master' Degrees: 0 No. of PhD Candidates: 3 No. of Master' Degrees: 0 No. of Master's Candidates: 0 No. of Bachelor's Degrees: 10 No. of Bachelor's Candidates: 0 Monitoring Center: NASA JSC Contact Honitor: Simonsen, Lisa Contact Phone: Contact Email: Isa e. simonsen@nasa.gov Flight Program: Flight Assignment: 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. Ann Nia (MD/Ph.D. Graduate student, joined the project and is not receiving salary support since 8/31/17. Ann Nia (MD/Ph.D. Graduate student, joined the project Cotche 2017. November 2016 report: Dr. Cheryl F. Lichti has replaced Dr. Nilsson at 20% Effort) is no longer involved with their project. Dr. Cheryl F. Lichti has replaced Dr. Nilsson at 20% Effort). COI Name (Institution): Meyer-Bases, Anke Ph.D. (Florida State University) of Texas Medical Branch) NNX15AD65G Performance Goal No.:	Space Biology Cross-Element Discipline:	None		
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No. of Master's Candidates: 0	No. of Post Docs:	0	No. of PhD Degrees:	0
Degrees: No. of Bachelor's Candidates: O Monitoring Center: NASA JSC Contact Monitor: Simonsen, Lisa Contact Phone: Contact Email: lisa.c.simonsen@nasa.gov Flight Program: Flight Assignment: 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). Meyer-Baese, Anke Ph.D. (Florida State University) Ullrich, Robert Ph.D. (University of Texas Medical Branch) NNX15AD65G Performance Goal No.:	No. of PhD Candidates:	3	No. of Master' Degrees:	0
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Task Book Report Generated on: 04/24/2024

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.

Year 3 Results: Project Setbacks/Modifications: Over the past three years, several challenges have arisen that have necessitated alternative research paths to be devised. The P.I. and Co-P.I. have worked together to circumvent these unforeseen occurrences and believe that the research and data has actually benefitted from these changes. The changes will be listed in the results presented below as a "Note."

Results Synopsis: Tissues 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)] have been processed for all omics platforms. Comprehensive Lipid Analysis by FT-ICR LC-MS/MS has been conducted for all lipid extracts and data collected is being analyzed. RNA sequencing was performed by the UTMB sequencing core and the raw data has been received. We are actively analyzing the data to determine statistical significance for each gene. Samples have been processed (extracted) for targeted proteomic analysis, and data acquisition started last week.

Note: Due to an almost month long power outage in the Medical Research Building at UTMB where our animal tissue collection 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.

Third Year Specific Aims Progress.

Specific Aim 1. Determine the microenvironmental changes in hepatic lipids by MALDI-IMS after HZE-irradiation with 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).

Note: Due to the departure of a collaborator from UTMB, the P.I. lost access to the MALDI-IMS instrument that was to be used to collect the imaging data. The PI and Co-PI have agreed that the comprehensive lipid analysis by ultra, high-resolution FT-ICR MS supersedes the low resolution MALDI-IMS. The importance of the MALDI-IMS is primarily for monitoring the lipid microenvironment around a forming tumor. The few tumors seen in these animals were not discovered until the point of tissue harvest, thus the value of the MALDI-IMS has been deemed less important than the comprehensive lipid analysis by FT-ICR MS. In retrospect, the MALDI-IMS should be paired with PET scanning (or other available in vivo imaging technique) of each animal to allow monitoring of tumor formation over time

Specific Aim 2. Determine transcriptional changes in the hepatic microenvironment of HZE- and gamma-irradiated samples, compared to controls. RNA was isolated from left lobe liver tissue samples from all time points and all treatment groups and submitted to the UTMB Next Generation sequencing core for low-read RNA sequencing. All samples have been analyzed and raw data has been received from the core. Approximately 50,000 expressed genes have been identified in each sample. The amount of data obtained from the low-read RNA sequencing is staggering. Analysis is underway to determine significantly altered genes and pathways involved in each treatment group. (See Specific Aim 4)

Note: The P.I. transitioned to collecting transcriptomic data with low-read RNA sequencing. The collaborators at Northwestern University, Evanston, IL, who were to provide the targeted transcriptomic gene array analysis withdrew from academic research and thus from this research project. Low read RNA sequencing provides much more transcriptomic data than could have been obtained by targeted transcriptomic analysis as originally proposed. Although RNA sequencing was not in the budget for this project, the P.I. had to find a replacement for the targeted transcriptomics after the collaborators withdrew. To cover the costs of the low-read RNA sequencing, the P.I. committed non-NASA funds along with other re-budgeting to obtain the transcriptomic data for this NASA project.

Preliminary Transcriptomic Data: Transcriptomic analysis was performed on RNA extracted from two 40 micron left lobe liver slices which were sliced on a cryotome at -20°C. Isolated RNA was sequenced with an Illumina HiSeq 1500 Analyzer in the UTMB Molecular Genomics Core Facility. Transcriptomic reads were aligned to the mouse genome and Star software was used to determine expression levels. Principle component analysis (PCA) plots were used to visualize the initial data.

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. All samples have been processed for both lipidomic and proteomic analysis. FT-ICR LC-MS/MS spectra have been collected for all lipid samples and data analysis of all the files is currently underway. The proteomic analysis started last week and should be completed by December 2017.

Note: The ultra, high-resolution 12T FT-ICR MS system used to collect the lipidomic and targeted proteomic data was down for over six months during the last year (December 2016-May 2017). The loss of this crucial instrument during this time necessitated that the P.I. devise alternative analysis strategies to complete the analysis outlined in the proposal. The targeted proteomics analysis has been moved to a Sciex 5600 triple-TOF instrument in the UTMB proteomics core. For our targeted proteomics analysis, we will be using a novel application of SWATH data independent acquisition. Preliminary Lipidomic Data: Preliminary lipid data interpretation based on the distribution of lipid classes in each treatment show that the lipid classes identified are dependent upon radiation type. This was true for both mice strains. Chi Squared tests were then used to look at the frequency of the different lipid classes compared to non-irradiated control group of the respective strain. At a P value of .05 frequencies for 56Fe, 3Gy gamma, & 16O were all

Task Progress:

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significantly different compared to the control for C3H strain of mice. For the C57 mice, the frequencies of control lipid classes compared to 1Gy & 3 Gy gamma irradiation were not significantly different, whereas they were for the 56Fe, 16O, and 28Si irradiated animals. Progress on Targeted Proteomic Data: Since RNA sequencing has been completed we are moving toward the targeted proteomic analysis of the samples. All samples have been processed for proteomic analysis and the first LC-MS/MS analysis began last week.

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. We have continued to evaluate different software packages for data analysis regimes of lipid identification and quantification which greatly surpass the manual lipid data analysis. We have determined that the peak picking algorithm used within Elements does not perform well enough with our data files, and has trouble distinguishing between the isotopic distributions from isobaric lipids within the same family. We have also experimented with the program mzMine, and determined that while the program seemed promising, it could not handle the size of our data files. Our group is also in contact with developers of new lipid analysis software and is in the process of gaining access to the software for interfacing with our in-house developed lipid database. We have also been working toward writing our own script for lipid data analysis.

We have currently collected transcriptomic and lipidomic data. Anna Nia (M.D.-Ph.D. student) joined the PI's group in October 2017. Miss Nia is a computational biologist and has started working on the large data set analysis of both the lipidomic and transcriptomic data sets. After several different filtering steps, analysis will be performed on a more manageable list of genes/transcripts, and different combinations of pairwise comparison will be used to identify transcripts that are significantly affected by different treatments. Multilevel modeling has been rarely implemented in the context of transcriptomic and lipidomic data. Our focus in this type of modeling would be on individual genes that have shown a significantly different behavior across different experimental parameters. Specifically, Miss Nia is working to identify a list of genes (using different computational approaches) and then perform a multilevel analysis. Multilevel models can allow for dynamic analysis of all data points, regardless of their behavior patterns. We believe this will provide further insight into future gene expression data based on different parameters. We hope to be able to extend the same type of analysis to the lipidomic data set as well. These analysis are unique in that they are purely mathematically based and thus are not influenced by any external bias.

Another computational Ph.D. student, John Miller, is currently rotating through the P.I.'s laboratory. Mr. Miller is pursuing a more traditional approach to analysis of the large transcriptomic data sets. The analysis of transcriptomic data will be done using a statistical R package (EdgeR). EdgeR is one of the most commonly used packages for differential gene expression analysis. EdgeR enables the comparison of the variation in the read counts from the different RNA-seq sample groups to determine which genes are differential expressed (genes that have a log fold change = 2, and an FDR value < 0.10). Using these parameters we will be able to analyze the differential gene expression through individual group analysis (i.e., comparison of individual groups), multi-group analysis (i.e., comparison of a treatment group across all time points), and whole-system analysis (i.e., comparison of the entire dataset). The three levels of analysis will allow for maximum data collection from the dataset given. Our goal is to determine how the changes in mRNA levels are affected based on the time, treatment, and strain of the sample groups used in the experiment. Finally, once a list of significantly altered genes for the model(s) has been determined we will import the list into IPA along with other data sets to determine affected pathways.

Presentations: During this year, preliminary data from this work was presented at the 65th ASMS Conference in Indianapolis, Indiana, in a poster entitled "Systems Biology Approach to Define the Molecular Mechanisms of Galactic Cosmic Ray Induced Hepatocellular Carcinoma." Data was also presented at 28th Annual NASA Human Research Program Investigators' Workshop Integrated Pathways to Mars in Galveston, TX, and will be presented at the 29th Annual NASA Human Research Program Investigators' Workshop in January 2018. Finally, it is anticipated that the first publications from this work will be submitted during 2018.

Summary of Project Status:

Despite several major setbacks, the PI and Co-PI are confident that the project is on-track and on schedule. The final year of primarily large "omics" data set analysis are already in progress. The addition of a computational mathematician MD/Ph.D. student and our Florida State University collaborator (computational mathematician) are crucial to integrating these omics data sets into a full systems biological analysis of the effects of HZE irradiation on induction of hepatocellular carcinoma.

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