Fiscal Year:	FY 2021	**Task Last Updated:**	FY 08/27/2020
PI Name:	Hada, Megumi Ph.D.		
Project Title:	Combined Effects of Simulated Microgravity and Space Radiation on Human Cells		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Cell & Molecular Biology (2) Animal Biology: Vertebrate		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	(1) Cell Culture (2) Translational (Countermeasure) Potential		
PI Email:	mehada@pvamu.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	936-261-3155
Organization Name:	Prairie View A&M University		
PI Address 1:	College of Arts and Sciences, PO BOX 519, MS-2230, New Science Bldg		
PI Address 2:			
PI Web Page:			
City:	Prairie View	**State:**	TX
Zip Code:	77446	**Congressional District:**	10
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2016-17 Space Biology (ROSBio) NNH16ZTT001N-FG. App G: Flight and Ground Space Biology Research
Start Date:	10/26/2018	**End Date:**	10/25/2021
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	1	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA KSC
Contact Monitor:	Zhang, Ye	**Contact Phone:**	321-861-3253
Contact Email:	Ye.Zhang-1@nasa.gov		
Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Wang, Jing Ph.D. (University of Texas MD Anderson Cancer Center) Takahashi, Akihisa Ph.D. (Gunma University Heavy Ion Medical Center, Japan) Fujiwara, Keigi Ph.D. (University of Texas MD Anderson Cancer Center)		
Grant/Contract No.:	80NSSC19K0133		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Space radiation and microgravity are two major environmental stressors for human in space travel. One of the fundamental questions in space biology research is whether the combined effects of microgravity and exposure to cosmic radiation are synergistic. While studies addressing this question have been carried out for half a century in space or using simulated microgravity on the ground, the reported results are conflicting. Although the reason for the variation in results is not known, it is possible that it may be due to the diversity of biological systems used but more importantly to the experimental designs and hardware used in these studies. For the assessment and management of human health risks in future Moon and Mars Missions, it is necessary to obtain more basic data on the molecular and cellular responses to combined effects of radiation and microgravity. To establish a firm baseline database, we propose to undertake a systematic study on cultured mammalian cells' responses to the simultaneous insult of radiation and microgravity (both immediate and long term) to elucidate the molecular signaling pathways that lead to these biological effects. The results of the study will provide cellular and molecular biological bases for the assessment and management of human health risks in space. Recently Dr. Takahashi, co-investigator of this proposal, has developed microgravity-irradiation systems consisting of a 3D clinostat synchronized to the carbon-ion or X-ray irradiation systems. Our new experimental setup allows us to avoid stopping clinostat rotation during irradiation, which was required in all other previous experiments. Gunma University Heavy Ion Medical Center is the only facility in the world where we can expose samples to high-linear energy transfer (LET) irradiation as well as low-LET irradiation under the simulated microgravity condition (i.e., without interrupting clinostat rotation). Our preliminary data obtained from the use of this new device on gene expression in human fibroblasts show that splicing cycle-related genes and cell cycle related genes are significantly up-regulated and S-phase DNA replication and DNA repair-related genes were down-regulated with C-ion irradiation under simulated microgravity. In this proposal we will investigate 3 different endpoints from early to late responses in 2 human cell lines using our new devices to study combined effects of microgravity and space radiation. Human fibroblasts and epithelial cells will be exposed to X-rays and C-ions under the simulated microgravity condition (rotated with 3-D clinostat). Control cells will be irradiated in 1G environment (with the static stage). We will investigate the extent of expression of specific proteins and of the post-translational modification states of signaling proteins (Aim 1), gene expressions and the pathways involved (Aim 2), and the extent of chromosome aberrations (Aim 3) caused by the combined effects of simulated microgravity and radiation. To investigate from the early to late endpoints in the same cell types will provide cellular and molecular biological data that are needed to understand the impact of combined effects of simulated microgravity and space radiation on human health. One of the selected endpoints is chromosome aberration, which is a well-established biomarker for cancer risk and has been used by NASA for the risk assessment of astronauts. Studying this endpoint allows us to compare our results to the astronauts' data after their International Space Station missions. Completion of this proposal will allow us to determine how the combination of microgravity and radiation will affect the transcriptomic, metabolomic, and proteomic states of cells as well as heritable changes in DNA. These finding will allow us to help develop the countermeasures for the future space missions.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Completion of this proposal will allow us to determine how the combination of microgravity and radiation will affect the transcriptomic, metabolomic, and proteomic states of cells as well as heritable changes in DNA. These findings will allow us to help develop the countermeasure for the future space missions.
Task Progress:	Post-translational modification of proteins: Human fibroblasts (1BR-hTERT) were exposed to simulated μG for 0, 15, 30, 60, 120, 240, 480, and 1440 min at Gunma University and total 37 protein samples were collected (4-5 samples for each time points). All samples were shipped to MD Anderson Cancer Center. Reverse-phase protein arrays analysis was performed using a panel of 453 antibodies. 36 proteins whose levels of phosphorylation or expression were statistically different between cells exposed vs not exposed to simulated microgravity for 15 – 480 min. A total of 10 reverse-phase protein array (RPPA) events were downregulated while 26 were upregulated. Interestingly, the protein events that are known to promote cell motility and actin cytoskeleton dynamics were activated at early time points. Our study so far indicates that 1) Cells appear to adapt to microgravity within hours, 2) phosphorylation of proteins that promote cell migration increases transiently when cells are exposed to microgravity, and 3) actin dynamics is sensitive to gravity and may be involved in gravity sensing. These results were presented as a poster at the 2020 NASA Human Research Program Investigators' Workshop, and a manuscript is under preparation describing these findings. Gene expressions: Human fibroblasts (1BR-hTERT) were maintained under standing or rotating conditions for 3 or 24 h after synchronized C-ion or X-ray irradiation at 1 Gy as part of a total culture time of 2 days. Among 57,773 genes analyzed with RNA sequencing, we focused particularly on the expression of 82 cell cycle-related genes after exposure to the radiation and simulated μG. The expression of cell cycle-suppressing genes (ABL1 and CDKN1A) decreased and that of cell cycle-promoting genes (MKI67, KPNA2, CCNB1, STMN1, and MCM4) increased after C-ion irradiation under μG. The cell cycle may pass through the G1/S and G2 checkpoints with DNA damage due to the combined effects of C-ions and μG, suggesting that increased genomic instability might occur in space. Manuscript with these results has been published in Int J Mol Sci (see Cumulative Bibliography). On the basis of our RNA-seq results, we are proceeding data analysis focusing on human aging-related genes. Specifically, 84 genes encoding key molecules involved in human aging were selected with information of RT2 Profiler PCR Array panel (Qiagen, Hilden, Germany) including several function (e.g., genomic instability, inflammatory response, cellular senescence, cytoskeleton regulator, oxidative stress, transcriptional regulation, and epigenetics alterations). By narrowing down the genes according to statistical criteria, pathway analysis is ongoing. Chromosome aberrations: Using μG-irradiation system, human lymphoblast TK6 were exposed to X-rays (0.5, 1.1, and 1.5 Gy) and carbon ions (0.25 and 0.5 Gy) under the simulated μG condition, and chromosomes were collected with the premature chromosome condensation method in the first mitosis. Chromosome aberrations (CA) were quantified by the 3-color fluorescent in situ hybridization method. Cells exposed to irradiation under the simulated μG condition showed a higher frequency of both simple and complex type of CA compared to cells irradiated under the static condition by either X-rays or carbon-ions. Manuscript has been prepared with these results and submitted to Life (Ed. note: now published; see Bibliography section).

Bibliography Type:	Description: (Last Updated: 02/07/2024)
Abstracts for Journals and Proceedings	Takahashi A, Ikeda H, Muratani M, Hidema J, Hada M, Fujiwara K, Souda H, Yoshida Y. "Combined effects of radiation and microgravity: RNAseq analysis." Presented at the 33rd Annual Meeting of Japanese Society for Biological Science in Space, Chiba, Japan, September 21-22, 2019. Abstract book. 33rd Annual Meeting of Japanese Society for Biological Science in Space, Chiba, Japan, September 21-22, 2019. , Sep-2019
Abstracts for Journals and Proceedings	Yamanouchi S, Rhone J, Takahashi A, Hada M. "Chromosome Aberrations in lymphoblastoid cells exposed simultaneously to simulated microgravity and radiation." Presented at the 33rd Annual Meeting of Japanese Society for Biological Science in Space, Chiba, Japan, September 21-22, 2019. Abstract book. 33rd Annual Meeting of Japanese Society for Biological Science in Space, Chiba, Japan, September 21-22, 2019. , Sep-2019
Abstracts for Journals and Proceedings	Fujiwara K, Hada M, Takahashi A. "Gravity sensing by cells and effects of simultaneous exposure of cells to microgravity and irradiation on chromosomes." Presented at the 9th Annual World Congress of Molecular and Cell Biology-Exploring the Essence of Life for a Better Future, Singapore, Oct. 25 – 27, 2019. Abstract book. 9th Annual World Congress of Molecular and Cell Biology-Exploring the Essence of Life for a Better Future, Singapore, Oct. 25 – 27, 2019. , Oct-2019
Abstracts for Journals and Proceedings	Yamanouchi S, Rhone JR, Takahashi A, Hada M. "Increased chromosome aberrations in lymphoblast cells exposed to radiation under simulated microgravity." Presented at the 62nd Annual Meeting of the Japanese Radiation Research Society, Kyoto, Japan, November 11-16, 2019. Abstract book. 62nd Annual Meeting of the Japanese Radiation Research Society, Kyoto, Japan, November 11-16, 2019. , Nov-2019
Abstracts for Journals and Proceedings	Hada M, Rhone JR, Beitman A, Ikeda H, Plante I, H. Souda H, Yoshida Y, Held KD, Fujiwara K, Saganti PB, Takahashi A. "Increased Chromosome Aberrations in cells exposed simultaneously to simulated microgravity and radiation." Presented at the 35th Annual Meeting of the American Society for Gravitational and Space Research, Denver, CO, November 20-23, 2019. Abstracts. 35th Annual Meeting of the American Society for Gravitational and Space Research, Denver, CO, November 20-23, 2019. , Nov-2019
Abstracts for Journals and Proceedings	Takahashi A, Ikeda H, Muratani M, Hidema J, Hada M, Fujiwara K, Souda H, Yoshida Y. "Expression profile of cell cycle-related genes in human fibroblasts exposed simultaneously to radiation and simulated microgravity." Presented at the 35th Annual Meeting of the American Society for Gravitational and Space Research, Denver, CO, November 20-23, 2019. Abstracts. 35th Annual Meeting of the American Society for Gravitational and Space Research, Denver, CO, November 20-23, 2019. , Nov-2019
Abstracts for Journals and Proceedings	Takahashi A, Kambe R, Suzuki K, Tsuruoka C, Morioka T, Takeshima T, Yoshida Y, Nakamura A, Hada M, Nagamatsu A, Ohira Y, Kakinuma S. "Research on combined effects of space radiation and variable gravity." Presented at the 34th Symposium on Utilization of the Space Environment, Sagamihara, Japan, January 21-22, 2020. Abstract book. 34th Symposium on Utilization of the Space Environment, Sagamihara, Japan, January 21-22, 2020. , Jan-2020
Abstracts for Journals and Proceedings	Yamanouchi S, Rhone J, Takahashi A, Hada M. "Increased chromosome aberrations in lymphoblastoid cells exposed simultaneously to simulated microgravity and radiation." Presented at 2020 NASA Human Research Program Investigators' workshop, Galveston, TX, January 27-30, 2020. Abstracts. 2020 NASA Human Research Program Investigators' workshop, Galveston, TX, January 27-30, 2020. , Jan-2020
Abstracts for Journals and Proceedings	Takahashi A, Ikeda H, Muratani M, Hidema J, Fujiwara K, Souda H, Yoshida Y, Hada M. "RNA sequencing of cell cycle-related genes in human fibroblasts exposed simultaneously to radiation and simulated microgravity." Presented at 2020 NASA Human Research Program Investigators' workshop, Galveston, TX, January 27-30, 2020. Abstracts. 2020 NASA Human Research Program Investigators' workshop, Galveston, TX, January 27-30, 2020. , Jan-2020
Abstracts for Journals and Proceedings	Fujiwara K, Takahashi A, Wang J, Ju Z, Yoshida Y, Yamanouchi S, Hada M. "Increased protein phosphorylation and actin dynamics as early response in cells exposed to gravity changes." Presented at 2020 NASA Human Research Program Investigators' workshop, Galveston, TX, January 27-30, 2020. Abstracts. 2020 NASA Human Research Program Investigators' workshop, Galveston, TX, January 27-30, 2020. , Jan-2020
Abstracts for Journals and Proceedings	Yamanouchi S, Rhone J, Takahashi A, Hada M. "Chromosome aberration assays in lymphoblastoid cells using a 3D clinostat with synchronized irradiation systems." Presented at 2nd Living in Space Meeting, Tokyo Japan, February 27-29, 2020. Abstract book. 2nd Living in Space Meeting, Tokyo Japan, February 27-29, 2020. , Feb-2020
Articles in Peer-reviewed Journals	Furukawa S, Nagamatsu A, Nenoi M, Fujimori A, Kakinuma S, Katsube T, Wang B, Tsuruoka C, Shirai T, Nakamura AJ, Sakaue-Sawano A, Miyawaki A, Harada H, Kobayashi M, Kobayashi J, Kunieda T, Funayama T, Suzuki M, Miyamoto T, Hidema J, Yoshida Y, Takahashi A. "Space radiation biology for 'Living in Space.' " Biomed Res Int. 2020 Apr 8;2020:4703286. Review. https://doi.org/10.1155/2020/4703286 ; PMID: 32337251; PMCID: PMC7168699 , Apr-2020
Articles in Peer-reviewed Journals	Yamanouchi S, Rhone J, Mao JH, Fujiwara K, Saganti PB, Takahashi A, Hada M. "Simultaneous exposure of cultured human lymphoblastic cells to simulated microgravity and radiation increases chromosome aberrations." Life (Basel). 2020 Sep 10;10(9):E187. https://doi.org/10.3390/life10090187 ; PMID: 32927618 , Sep-2020

Articles in Peer-reviewed Journals	Takahashi A, Yamanouchi S, Takeuchi K, Takahashi S, Tashiro M, Hidema J, Higashitani A, Adachi T, Zhang S, Guirguis FNL, Yoshida Y, Nagamatsu A, Hada M, Takeuchi K, Takahashi T, Sekitomi Y. "Combined environment simulator for low-dose-rate radiation and partial gravity of Moon and Mars." Life (Basel). 2020 Nov 6;10(11):E274. https://doi.org/10.3390/life10110274 ; PMID: 33172150; PMCID: PMC7694743 , Nov-2020