Fiscal Year:	FY 2020	**Task Last Updated:**	FY 08/13/2021
PI Name:	Grabham, Peter Ph.D.		
Project Title:	A Determination of Bioactive Proteins Secreted by the Human Vasculature in Response to Low Dose Space Radiation		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **SR**:Space Radiation		
Human Research Program Risks:	(1) **Cardiovascular**:Risk of Cardiovascular Adaptations Contributing to Adverse Mission Performance and Health Outcomes		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	10032-3702	**Congressional District:**	13
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2017 HERO 80JSC017N0001-Crew Health and Performance (FLAGSHIP1, OMNIBUS). Appendix A-Flagship1, Appendix B-Omnibus
Start Date:	08/01/2018	**End Date:**	07/31/2020
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No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA JSC
Contact Monitor:	Zawaski, Janice	**Contact Phone:**	
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 7/31/2020 per NSSC information (Ed., 1/29/2020)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Brown, Lewis Ph.D. (Columbia University)		
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Task Description:	The specific purpose of the research proposed here is to determine the proteins that are potentially released into the blood stream by the lining of the human vasculature in response to exposure to space radiation. This would create a useful database for radiobiology studies and comparisons with the proteins secreted in astronaut blood. Such proteins have the potential to cause pathological processes such as inflammation, they are also spread around the body in the blood, and are important factors in many pathologies. The microvasculature permeates all tissues at the microscopic level so the whole body is a target for charged particles. A single heavy ion particle would be expected to traverse many microvessels as it passes through the body causing a more widespread response. Studies on the effect of different charged particles on human 3D microvessel models shows that both developing and mature microvessels lose structure and function after exposure to very low doses of various charged particles. Mature microvessels lose structure detectible as low as 1.25 cGy. Angiogenesis, the growth of new vessels, is inhibited by light ions and heavy ions detectible at 1.25 cGy. Even more striking, the combined effect of each ion has a synergistic effect detectible as low as 0.6 cGy. The low fluence of these doses indicates a bystander effect where the response is transmitted to other cells and such a mechanism would involve the secretion of molecules by the target cell. We propose to use proteomics and other techniques to determine the proteins secreted by the human microvessel models. A database of these proteins secreted by human tissue models would not only be of great use to a number of researchers investigating a diverse number of pathologies related to space radiation but also provide insights into the mechanisms of the vascular response to charged particles.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Provides a comprehensive database of proteins secreted by micro-vessels in response to space radiation.
Task Progress:	Damage or dysfunction of blood micro-vessels (e.g., capillaries) is a major contributor to both acute and long-term cardiovascular, neurological, and other pathologies. The microvasculature is a major target for radiation since it permeates all tissues at the microscopic level to form a vast network. Thus, the whole body is potentially an endothelial target for charged particles. The endothelium that lines the blood vessels is the main component of the microvasculature and forms the interface between the blood and the tissues. The endothelium is also known to secrete bioactive proteins such as immune response molecules, growth factors, cell adhesion molecules, cytokines and more, in response to various stress factors including radiation. These stress molecules would potentially be released into the blood stream and many are important in the progression of degenerative disease. Preliminary data shows that human developing and mature 3D tissue models of micro-vessels are extremely sensitive to charged particle radiation. The hypothesis for this study is: A determination of the proteins secreted by micro-vessels in response to low doses of specific charged particles would be highly useful in determining the risks of space radiation in causing cardiovascular and other neurodegenerative pathologies during long-term space flight. It would also provide a useful database of proteins that are potentially released from the endothelial cells of the human vasculature in response to space radiation. Such a database would be useful to other researchers investigating other specific degenerative disorders. Mature micro-vessel tissue models lose structure after exposure to heavy ions detectible at a dose as low as 1.25cGy time (low LET (linear energy transfer) ions are not effective on the mature structures up to 4 Gy)). For developing micro-vessels -- angiogenesis, the growth of new vessels, is inhibited by light ions (3 KeV/n or below) and heavy ions (8 KeV/n or above) each detectible at a dose of 1.25cGy although the mechanisms of inhibition are distinct. Even more striking, the combined effect of each ion affecting the early stages of angiogenesis by light ions and later stages by heavy ions has a synergistic effect that is detectible as low as 0.6 cGy. Hypothesis & Specific Aims We hypothesized that low doses of space radiation directly target the endothelium causing the secretion of bioactive proteins eventually leading to increased risk to the health of the space crew during long-range missions. Aim 1. Identify the proteins secreted by the endothelial cells in human 3D micro-vessel tissue models in response to space radiation. Carry out proteomics analysis and follow up with ELISA to determine the secreted proteins from developing micro-vessels after exposure to a mixture of low and high LET particle radiation. Aim 1a. Identify the proteins secreted in response to gamma radiation. The timing of beam time requests at the NASA Space Radiation Laboratory (NSRL) dictates that an initial period without beam access is likely. This time will be used to examine gamma radiation for a reference radiation and for a crucial development of the assay before NSRL runs. Aim 1b. Identify the Proteins secreted by mature micro-vessels in response to a low dose of high LET charged particles. To determine the proteins secreted by mature micro-vessels, which are the majority of micro-vessels in the body at any one time. Aim 1c. Identify the Proteins secreted by developing micro-vessels in response to a low dose of a space -relevant mixture of low and high LET charged particles. To determine the proteins secreted by developing vessels that are still not connected. Rationale: Human 3D microvessel models are much closer to microvessels in vivo than 2D monolayers. The fluence estimates are low enough to indicate that the effect is through a bystander response mediated by secreted bioactive proteins. A database of proteins secreted by endothelial cells is the research product of this proposal and is of practical use to the NASA Human Research Program (HRP) program for a number of reasons. 1) Create a resource for NASA researchers to examine the changes in secreted proteins important to various specific pathologies. 2) Form the basis of future databases that would include other associated cell types. 3) Provide information relevant to proteins secreted into the blood in vivo (mice and astronauts). 4) Provide mechanistic insights into our own research on the specific effects of radiation on mature vessels and developing vessels. 5) Provide research leads on the synergistic effects of mixed heavy and light ions. By understanding the effect of space radiation on the secretion of bioactive proteins from the endothelium, we will gain the knowledge to understand potential pathologies and develop interventional strategies to block or repair the deleterious effects of space travel.

Preliminary data: Existing data indicates that the effect of different charged particles on human 3D microvessel models show that both mature and developing microvessels lose structure and function after exposure to very low doses of various charged particles. Data on developing particles is now published (Wuu et al. 2020). The low fluence of these doses indicates a bystander effect where the response is transmitted through secretion of factors by the target cell to other cells. In the case of endothelial cells this would include secretion into either the surrounding tissue or into the blood stream. Therefore, it is highly likely that low doses of charged particles induce the secretion of bioactive proteins from the microvasculature.

Changes to Specific Aims: After consultation with NASA, a change in the specific Aims was suggested. This mainly concerned substituting the high and low LET charged particles with the newly available simplified Galactic Cosmic Radiation simulation (sim GCR sim). The sim GCR sim is a mixture of ions, energy, and doses determined by a NASA consensus formula that consists of 5 ions: protons at 1000 MeV, 28Si at 600 MeV/n, 4He at 250 MeV/n, 16O at 350 MeV/n, 56Fe at 600 MeV/n, and protons at 250 MeV, in the following proportions: Protons 100 MeV at 34.8%, Si at 1.1%, He at 18%, O at 5.8%, 56Fe at 1%, and Protons 250 MeV at 39.3%. This mixture is the simplified version of the full GCR simulation and represents the proportions of ions found in space and thus translates to exploratory class missions. Thus, the simGCRsim supersedes individual ions and would be more relevant for use in this study. Due to budget limitations Gamma irradiated samples were eliminated from the study. Proteomic analysis was successful in both developing and mature microvessel cultures and the change in secreted proteins was not the same for each. Notably, protein disulfide-isomerases were differentially expressed in mature vasculature cultures but were unchanged in developing microvessels. Ongoing analysis should reveal more insights into the changes in protein revealed in this study. Data will be submitted to the NASA database.

Summary mature microvessels

§ Identifications were returned 1,687 proteins with a 1% false discovery rate (predicted error).

§ More than 200 secreted proteins were detected.

§ A variety of proteins were differentially expressed in 50 cGy radiation treated including Platelet endothelial cell adhesion molecule (known to be radiation induced), Protein disulfide-isomerase A1, A3, A4, A6, 40S ribosomal protein S8, Gamma-glutamyl hydrolase, Endoplasmin, Peptidyl-prolyl cis-trans isomerase B, HLA class I histocompatibility antigen, Cw-18 alpha chain, Prothymosin alpha, Vesicular integral-membrane protein VIP36.

§ Chaperone proteins and ribosomal proteins were enriched in the 50 cGy secretome.

Summary developing microvessels

§ Identifications were returned 1,291 proteins with a 1% false discovery rate (predicted error).

§ About 200 secreted proteins were detected.

§ A variety of proteins were differentially expressed in 50 cGy radiation treated including Neuroblast differentiation-associated protein AHNAK, ATP-citrate synthase, ATP-dependent 6-phosphofructokinase, platelet type, 40S and 60S ribosomal protein, Trifunctional purine biosynthetic protein adenosine-3, Alpha-centractin.

§ A variety of proteins were enriched in the 50 cGy secretome including translational protein, ribosomal protein, actin-binding cytoskeletal protein, chaperones, and vesicle coat proteins.

References (see also Bibliography section)

Yen-Ruh Wuu, Burong Hu, Hazeem Okunola, Amber M. Paul, Elizabeth A. Blaber, Margareth Cheng-Campbell, Afshin Beheshti* and Peter Grabham*. LET dependent low dose and synergistic inhibition of human angiogenesis by charged particles: Validation of miRNAs that drive inhibition. IScience 23, 22020.

Bibliography Type: Description: (Last Updated: 03/04/2024)

Articles in Peer-reviewed Journals

Wuu Y-R, Hu B, Okunola H, Paul AM, Blaber EA, Cheng-Campbell M, Beheshti A, Grabham P. "LET-dependent low dose and synergistic inhibition of human angiogenesis by charged particles: Validation of miRNAs that drive inhibition." iScience. 2020 Dec 18;23(12):101771. https://doi.org/10.1016/j.isci.2020.101771 ; PMID: 33376971; PMCID: PMC7756138 , Dec-2020

Articles in Peer-reviewed Journals

Afshinnekoo E, Scott RT, MacKay MJ, Pariset E, Cekanaviciute E, Barker R, Gilroy S, Hassane D, Smith SM, Zwart SR, Nelman-Gonzalez M, Crucian BE, Ponomarev SA, Orlov OI, Shiba D, Muratani M, Yamamoto M, Richards SE, Vaishampayan PA, Meydan C, Foox J, Myrrhe J, Istasse E, Singh N, Venkateswaran K, Keune JA, Ray HE, Basner M, Miller J, Vitaterna MH, Taylor DM, Wallace D, Rubins K, Bailey SM, Grabham P, Costes SV, Mason CE, Beheshti A. "Fundamental biological features of spaceflight: Advancing the field to enable deep-space exploration." Cell. 2020 Nov 25;183(5):1162-84. Review. https://doi.org/10.1016/j.cell.2020.10.050 ; PMID: 33242416; PMCID: PMC8441988 , Nov-2020