Fiscal Year:	FY 2014	**Task Last Updated:**	FY 12/24/2013
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Project Title:	Targeting NO/IKK Signaling to Counteract Hemodynamic Flow-Dependent Endothelial Dysfunction and Vascular Damage after Space Radiation		
Division Name:	Human Research		
Program/Discipline:	NSBRI		
Program/Discipline--Element/Subdiscipline:	NSBRI--Cardiovascular Alterations Team		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **HHC**:Human Health Countermeasures		
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:	78229-3901	**Congressional District:**	21
Comments:			
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No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	1	**Monitoring Center:**	NSBRI
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:	NOTE: Period of performance change per NSBRI; previous POP was 9/1/2012-8/31/2015 (Ed., 11/13/12)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Prihoda, Tom (The University of Texas Health Science Center at San Antonio) Mohan, Sumathy (The University of Texas Health Science Center at San Antonio) Blakely, Eleanor (Lawrence Berkeley National Laboratory)		
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Task Description:	Space travelers are irradiated with cosmic rays at a dose rate considerably higher than that received on Earth. At the dose and dose rates encountered in space, one of the important risks is the induction of subclinical vascular abnormalities during the mission that leads to cardiovascular disease. Endothelial dysfunction is regarded as a primary sub-clinical condition that could progress into cardiovascular diseases over the life of the astronauts. The proposed hypothesis is that space radiation at low doses may impair the interplay between three key proteins (eNOS, Hsp-90, and IKK-ß), and cause functional alterations of endothelial cells. This dysfunctional endothelium fails to regulate vascular healing processes and negate cell migration/motility. When unchecked, this may predispose the vascular bed to become a sustained pro-inflammatory milieu for the initiation of cardiovascular abnormalities. We proposed to address these above concerns by investigating how these molecular mediators are functionally interrelated and how they coordinately provide a niche for the development of cardiovascular abnormalities upon high LET radiation exposure. The proposed specific aims are: Aim 1: To study the significance of low doses (0, 0.2, 0.4, or 0.8 Gy) of high-LET radiation exposure on vascular bed damage, negation of cell migration/motility, and impairment of vascular healing processes. Aim 2: To determine the mechanism of high LET radiation-initiated functional alterations of endothelial cells that are sustained to cause progressive cardiovascular diseases. Aim 3: To assess in vitro, ex vivo and in vivo the potential of targeting eNOS and IKK-ß independently and in combination as a promising countermeasure to limit endothelial dysfunction. The specific aims originally proposed were not altered. The key findings are: (a) In year 1, we established the flow shear system at NSRL and cultured human primary endothelial cells under simulated high (16 dynes/cm2) hemodynamic shear stress - a local environment in which the endothelial cells are in constant physiological shear stress due to continuous blood flow. Experiments were performed to determine the response of endothelial cells to radiation after parallel plate flow versus static culture. This was carried out first at low LET radiation exposures using gamma irradiator at the P.I.'s institution. This allowed us to optimize the conditions and prepared for the heavy ion exposure at NSRL. Also, this approach allowed us to generate data from low LET radiation for comparison with heavy ion exposures. A significant difference, both in cell morphology and radiation-induced gene expression profile examined by real-time Q-PCR micro-array, were observed in cells grown on matrigel (simulated extra cellular matrix) and experiencing physiological hemodynamic flow shear stress (that occurs in normal blood vessels) compared to static culture. Next, we confirmed the differential regulation of key proteins involved in NO signaling pathway after both high and low LET radiation exposures at doses ranging from 0.1 to 1.6 Gy. Sustained activation of IKK-beta/NF-kB pathway was validated in cells exposed to 56Fe (600 MeV/amu) and 137Cs gamma radiation. Second, time- and dose-dependent impaired regulation of nitric oxide was measured in terms of intracellular accumulation of nitric oxide by FACS analysis. Third, to determine whether radiation-induced IKK binds with heat shock protein (Hsp-90), a Mammalian two hybrid system was established. This approach proved the favorable binding of IKK with Hsp-90. Next, to examine whether this binding of IKK competitively uncoupled eNOS binding to Hsp-90, both cell-free system and intra-cellular competitive binding assays were performed. To further confirm any increase in IKK activation after radiation exposure is responsible for decreased bioavailability of nitric oxide, a blocking assay was performed to block IKK and measured a time-dependent reactivation of nitric oxide in the exposed cells. Cells treated with sodium salicylate, a specific inhibitor of IKK, showed an increased bioavailability of nitric oxide even after 3, 6, 12, and 24 h post-exposure. From year 1 study we concluded that: (i) for in vitro mechanistic studies in response to radiation the endothelial cells should be cultured under their micro-environmental niche -hemodynamic flow shear, and (ii) space radiation can alter nitric oxide signaling through sustained activation of IKK signaling pathway. The activation of IKK competitively binds to Hsp-90 and prevents eNOS binding and subsequent phosphorylation of eNOS by AKT signaling pathway. These sequential events after radiation are responsible for decreased bioavailability of nitric oxide that could lead to abnormal vascular homeostasis. In the next year study during year 2, we will be investigating whether the impaired regulation of nitric oxide signaling alters the physiological functioning of the endothelium that leads to vascular abnormalities including inflammation, vascular permeability, and vasomotor function. This will be carried out both in vitro system under hemodynamic flow shear stress and in vivo animal models.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Therapeutic radiation, while alleviating cancer burden can simultaneously be involved in redevelopment of the disease at the treatment site, which may account for the tumor recurrence and risk of metastasis at distant site. The relationship linking the altered responses that could occur simultaneously in cancer cells versus surrounding normal tissue and also the molecular alterations occur after radiation exposure and re-growth of tumor remain elusive and unknown. Ascertaining those altered responses and selectively targeting the responsible mediators might significantly improve radiotherapy outcome. The approach proposed to NSBRI once established will help to test whether in remnant cancer cells that survive treatment where key proteins are de-nitrosylated due to aberrant regulation of altered eNOS/NO. The functionally redefined proteins are responsible for tumor re-growth, invasion, and metastasis. The overall outcome from this proposed study will add a significant advancement towards achieving disease-free survival of cancer patients.
Task Progress:	The development of subclinical vascular abnormalities, which have been known to occur during space missions, is largely due to functional alterations of endothelial cells (inner lining of the vessels). Endothelial dysfunction is regarded as a primary sub-clinical condition that could progress into cardiovascular diseases over the life of the astronauts. Our hypothesis is that space radiation at low doses may impair the interplay between three key proteins (eNOS, Hsp-90, and IKK-ß), and cause functional alterations of endothelial cells. When unchecked, this may predispose the vascular bed to become a sustained pro-inflammatory milieu for the initiation of cardiovascular abnormalities. We proposed to address these above concerns by investigating how these molecular mediators are functionally interrelated and how they coordinately provide a niche for the development of cardiovascular abnormalities upon high LET radiation exposure. The results are compared with low LET radiation at the same total dose and dose rate specified for high LET radiation. (a) In year 1, we established the flow shear system at NSRL and cultured human primary endothelial cells under high (16 dynes/cm2) hemodynamic shear stress. Experiments were performed to determine the response of endothelial cells to radiation after parallel plate flow versus static culture. (b) Next we confirmed the differential regulation of key proteins involved in NO signaling pathway after both high and low LET radiation exposures at doses ranging from 0.1 to 1.6 Gy. Sustained activation of IKK-beta/NF-kB pathway was validated in cells exposed to 56Fe (600 MeV/amu) and 137Cs gamma radiation. Second, time- and dose-dependent impaired regulation of nitric oxide was measured in-terms of intracellular accumulation of nitric oxide by FACS

	analysis. Third, to determine whether radiation-induced IKK binds with heat shock protein (Hsp-90), a Mammalian two hybrid system was established. This approach proved the favorable binding of IKK with Hsp-90. Next, to examine whether this binding of IKK competitively uncoupled eNOS binding to Hsp-90, both cell-free system and intra-cellular competitive binding assays were performed. To further confirm any increase in IKK activation after radiation exposure is responsible for decreased bioavailability of nitric oxide, a blocking assay was performed to block IKK and measured a time-dependent reactivation of nitric oxide in the exposed cells. From year 1 study we concluded that space radiation can alter nitric oxide signaling through sustained activation of IKK signaling pathway. In year 2, we will be investigating whether the impaired regulation of nitric oxide signaling alters the physiological functioning of the endothelium that leads to vascular abnormalities including inflammation, vascular permeability, and vasomotor function. This will be carried out both in vitro system and in vivo animal models.
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