Fiscal Year:	FY 2013	**Task Last Updated:**	FY 11/04/2012
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Project Title:	Evaluation of Space Radiation-induced Myocardial and BM-derived EPC Damage and Assessment of Associated Long-term Degenerative Cardiovascular Risks		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
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) **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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Comments:	NOTE: PI moved to Icahn School of Medicine at Mount Sinai from Temple University in October 2018.		
Project Type:	GROUND	**Solicitation / Funding Source:**	2010 Space Radiobiology NNJ10ZSA001N
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No. of PhD Candidates:	1	**No. of Master' Degrees:**	
No. of Master's Candidates:	2	**No. of Bachelor's Degrees:**	
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Flight Program:			
Flight Assignment:	NOTE: Extended to 5/31/2014 per NSSC information (Ed., 12/4/13)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Yan, Xinhua (Caritas St. Elizabeth's Medical Center Of Boston, Inc.)		
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Task Description:	The main objectives of our proposal is to determine space radiation-dependent short- and long-term degenerative cardiovascular (CV) risks on the molecular, cellular, and tissue levels in the heart as the primary organ and bone marrow (BM)-derived endothelial progenitor cells (EPC) first, as a primary space radiation target and second, as a possible significant contributor to degenerative CV risks, later on in life. Our central hypothesis is that low-dose space radiation-induced DNA damage repair is inefficient in BM-derived EPC and this may lead to increased mutagenesis with subsequent long-term loss of endothelial function of BM-derived EPCs. This may then pose significant degenerative CV risk on physiologic homeostasis in the aging heart and on the regeneration and neovascularization processes in the heart under pathologic conditions such as acute myocardial infarction (AMI). Comparisons will be made between two types of low-dose radiation - proton versus iron and single versus fractionated exposures. In short-term in vitro studies (minutes, hours, and up to 30 days after exposure) will evaluate in ex-vivo expanded EPCs and cardiomyocytes DNA damage and repair as well as radiation-induced bystander effects (irradiated cells emit signals to "un-hit" cells), angiogenic gene expression in EPCs. In short-term in vivo studies in the heart we will evaluate acute damage (inflammation and cell death), DNA damage, and repair kinetics. In our long-term studies (3, 6, 9, and 12 months after exposure) we will evaluate oxidative stress and antioxidant defense in BM-derived EPCs, alterations in several EPC endothelial functions, number of circulating peripheral blood EPCs, and cardiomyocyte contractility. In the last part of our studies we will assess CV risks as a result of low-dose radiation plus aging and CV risks under pathological condition -- radiation plus aging plus adverse CV event (i.e., AMI). Here we will evaluate post-AMI survival, alterations in cardiac physiology (echocardiography), infarct size, inflammation, cardiac regeneration, neovascularization, and mobilization of EPCs from BM. Our studies will address two high-priority research topics of this specific solicitation and NASA research interests for degenerative risks to the heart -- (1) development of murine models to estimate risks for degenerative heart diseases; (2) determine the progression rates and latency periods for space radiation-related degenerative CV risks as a function of radiation type (proton vs. heavy ion), exposure frequency (single vs. fractionated), age, and age plus adverse CV event.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	In terms of Earth-based applications the information gained from our studies will have benefit for people on Earth who are (1) undergoing therapeutic radiation in the proximity of the heart (breast, esophageal, lung cancers, etc.) for cancer treatment (direct “hit” effect); (2) undergoing therapeutic radiation elsewhere in the body (prostate, colon, skin, liver cancers, etc.) for cancer treatment (non-targeted effects) that may affect heart years and decades after these treatments. In addition, our studies will also provide novel insights into the alterations in cardiac function processes on the molecular and cardiac physiology levels that may allow for estimation of degenerative risks to cardiovascular system in the civilian population exposed to full body low-dose radiation due to accidental exposures (Chernobyl, Fukushima, etc.) and cancer patients undergoing very frequent imaging tests (i.e., full body Computer Tomography, PET Scans, etc.). Our studies will address two high-priority research topics for NASA research interests for degenerative risks to the heart - (1) development of murine models to estimate risks for degenerative heart diseases; (2) determine the progression rates and latency periods for space radiation-related degenerative CV risks as a function of radiation type (proton vs. heavy ion), exposure frequency (single vs. fractionated), age, and age plus adverse CV event.
	We have completed four sub-projects within the scope of main project. Each of the the summaries below represent progress for the particular subproject. SUB-PROJECT 1: Bioequivalent Low Dose Full Body Proton and Iron Irrradiation Mediate Comparable DNA Damage, Apoptosis, and Proliferation Responses in the Heart and BM-derived EPC. Radiation-induced chromosomal instability was demonstrated in the bone marrow (BM) for up to 24 months after full body irradiation with either X-rays or neutrons, indicating that chromosomal instability can be initiated and maintained in vivo. However, there is a significant gap in studies to date assessing full body radiation-induced survival and function of BM-derived endothelial progenitor cells (EPCs) and effects of space radiation on the heart. It was shown for myeloid and lymphoid BM-derived stem and progenitor cells that after space flights the numbers of these cells are reduced to just one-half of their normal levels, suggesting that EPCs may be similarly reduced in the normal EPC population. Neither data on BM-derived EPCs survival and proliferation during and after space flights, nor DNA damage responses of EPCs or heart tissue to space radiation, are currently available. A growing body of evidence indicates that in the heart and other organ-tissues vascular homeostasis does not exclusively rely on proliferation of local endothelial cells (ECs) but also involves BM–derived EPCs. Decrease in the total number of BM-derived EPC or their dysfunction could contribute to the pathogenesis of ischemic and/or peripheral vascular diseases, as well as for maintenance of normal vascular homeostasis in the heart. SUMMARY of sub- project 1: (A) our ex-vivo BM-derived EPC data suggest that early increase in BM-derived EPC apoptosis may be a direct effect of single low dose proton or Iron radiation, whereas later increase in apoptosis and decrease in proliferation could be a result of delayed non-targeted effects. (B) our in vivo heart data reveal that the decay of proton or Iron IR-induced p-H2AX foci are significantly slower in cardiac non-EC (may include cardiomyocytes and BM-derived inflammatory cells) vs. EC; and IR-induced inflammatory response are bimodal and long-lived in heart, suggesting a possibility of inflammatory signaling in bystander-type effects and kinetics of H2AX foci induction and decay in the heart tissue. SUB-PROGECT 2: TNFR2/p75 Signaling Induces Delayed Radiobiological Bystander Responses in BM-derived EPCs: Implications for Development of Countermeasures. Tumor necrosis factor alpha (TNF) binds two receptors TNFR1/p55 and TNFR2/p75 and activate several signaling cascades. Ionizing radiation (IR) increases tissue levels of TNF. TNF signaling regulates numerous cytokines and chemokines that known to mediate radiation-induced non-targeted effects (NTE), a phenomenon where cells that are not directly “hit” by IR exhibit IR effects as a result of signals received from nearby or distant IR cells. Little is known about the role of p55 or p75 in regulating NTE in bone marrow (BM)-derived endothelial progenitor cells (EPCs). It is

Task Progress:

well-known that aging is a risk factor for coronary and peripheral artery disease. We have previously reported significant age-associated decrease in the expression of p55 and p75 in human and mouse EPCs. We hypothesized that inhibition of TNF signaling either via p55 or p75 may alter TNF-mediated inflammatory response increasing tissue levels of cytokines/chemokines that may induce NTE.

SUMMARY of Sub-project 2:

(A) Our finding indicate that altered TNF signaling inhibits early non-targeted effects (NTEs) (hours) in BM-derived EPCs. Compared to WT, delayed (5 days) NTE were increased in naïve gamma irradiation conditioned growth media-treated TNF receptor 1 (p55) and 2 (p75) EPCs, suggesting significant role of TNF-TNFR1 and TNFR2 signaling in mediating delayed NTEs, possibly, via activation of NFkB and other stress response transcription factors.

(B) Based on gene expression profiles, prediction of transcription factor activation or inhibition our data revealed a time-dependent pattern of activation of stress response transcription factors at 1hr their disappearance at 5hrs, return at day 1, disappearance at day 3 and return back again at day 5. This “oscillating” pattern of stress response, DNA damage repair and cell cycle arrest transcription factors activation with subsequent increase in gene expression in pathways downstream of DNA damage repair, cell cycle and inflammatory cytokine genes indicates a significant role of TNF-TNFR1 and TNFR2 signaling in DNA damage responses in BM-derived EPCs. We conclude that TNF ligand-receptor axis regulates NTEs in naïve EPCs and suggest that restoring TNF signaling balance could represent a mitigating measure for prevention of delayed NTEs in tissues adjacent or distant from primary irradiation target.

SUB-PROJECT 3: Transcriptional Profiling of Cardiac Cells Reveal an Immense Complexity of Gene Expression Over One Month after a Full Body 0.15 Gy 56Fe but Not 0.9 Gy Proton Radiation.

Cardiomyocytes are the basic contractile cells within the heart, whose contractile function directly influences the pathogenesis of heart disease and the development of myocardial failure. Understanding of complex processes that take place in cardiomyocytes after insults caused by exposure to ionizing radiation, such as proton and high charge and high energy (HZE) particles, is paramount to our understanding of cardiovascular system function during and after exploration-type space missions. We performed full genome transcriptional microarray analyses using primary, ventricular cardiac muscle cells (cardiomyocytes-CM) from control and full body proton or iron irradiated mice. We isolated CM using standard preparation protocol that includes cannulation of the aorta and collagenase digestion followed by a Ca2+ gradient selection.

SUMMARY of sub-project 3:

(A) The analysis of proton-irradiated samples at False Discovery Rate (FDR) of $p<0.05$ did not reveal any changes in RNA transcription. When, FDR for the same samples was decreased ($p<0.1$), there were only 21 genes that exhibited an appreciable increase/decrease in RNA transcription, thus eliminating the need for further bioinformatics analysis of proton irradiated samples.

(B) Very stringent analysis of Iron IR samples after correction for FDR ($p<0.05$) and 2 or more fold change in RNA transcription using Genome Studio, IPA, Gather software revealed 51 genes with a greater than or less than 2-fold-change in RNA transcription with biphasic time course in comparison to controls. This changes included up-regulation at day 7, complete down-regulation by day 14 and a second significant up-regulation by day 28 post IR.

(C) Less stringent analysis unadjusted $p<0.05$, FDR $p<0.2$ and ±1.5-fold change revealed 878 gene list with a clear down-regulation of gene expression on days 1 and 3 compared to control, while an increase was observed on days 7 and 28 relative to control. There was a striking relationship to inflammatory genes seen just by looking at the top 10 up-regulated genes on day 28 ranging 8.0-fold to 15-fold increases.

(D) Functional pathways analysis – Ingenuity software: Functional analysis of the 878 gene list showed a time dependent switch from decreased free radical scavenging on day 3 to an increased activity on day 7. The most striking biological functions related from this data are the time dependent increase in immune/inflammatory categories on days 7 and 28.

(E) Prediction of transcription factor activation/inhibition analysis – Ingenuity software: In line with the above function analysis, prediction of the activation or inhibition of transcription factors based on downstream gene expression in the 878 gene list again shows factors related to immunity and inflammation. Our findings indicate an immense complexity of RNA transcription, regulation of biological pathways, included, but not limited to inflammation, DNA damage/repair, free radical scavenging and immune trafficking post 56Fe irradiation, but NOT bio-equivalent dose of proton.

SUB-PROJECT 4: Full Body Single Dose 0.5 Gy Proton is Beneficial Whereas Single 0.15Gy 56Fe Dose is Deleterious for Acute Myocardial Infarct recovery up to 3 month post-IR.

Previous epidemiologic data in radiotherapy patients (breast, head and neck cancer), non-occupational exposure (Life Span Study), occupational exposure (radiologists/technologists, radon-exposed miner, nuclear workers) for radiation-induced circulatory diseases demonstrate that cardiovascular (CV) morbidity may occur within months or years, and CV mortality may occur within decades, after initial radiation exposure. The effect of cosmic radiation during and after space flights on CV system is unknown. The majority of space flight-associated risks identified for the CV system to date were determined shortly after International Space Station (ISS) flight missions that include: serious cardiac dysrhythmias, compromised orthostatic CV response, and manifestation of previously asymptomatic CV disease. These symptoms were determined to be a consequence of adaptation to microgravity, and are not risk factors causatively related to space radiation that could be ameliorated by post-mission exercise program. During the future Moon and Mars missions astronauts will be exposed to higher total doses of space radiation (~0.4-0.5Gy from galactic cosmic rays (GCR), especially during Mars mission that is currently estimated to be 30 to 36 months. During an exploration-class space mission to Mars, astronauts will not have access to comprehensive health care services for periods of at least 2 years. Since the majority of experienced astronauts are middle-aged (average age is 46, and the range is 33 to 58 years), they are at risk for developing serious cardiovascular events, which may be life-threatening for the astronauts and mission-threatening for NASA. In this sub-project we hypothesized that: (1) low-dose space radiation-induced biological responses may be long-lasting and are radiation type-dependent; and (2) radiation may increase CV risks of physiologic homeostasis in the aging heart (Irradiation+Aging) and affect processes of cardiac repair and regeneration due to acute myocardial infarct (Irradiation+Aging+Acute Myocardial Infarct).

In Irradiation+Aging group – no significant difference was observed between non irradiated control and proton or 56Fe

irradiated mice 1 and 3 months post-IR in cardiac function and remodeling as assessed by Echocardiography (ECHO) and Hemodynamic (HEMO) heart measurements. There was a small but statistically significant ($p<0.04$) improvement of % Ejection Fraction (amount of the blood pumped out of the heart) in proton-IR vs. control mice.

In IR+Aging+Acute Myocardial Infarct (AMI) group – there was no difference in post-AMI mortality in any of the three groups. Four weeks after AMI, HEMO and ECHO revealed that proton-AMI mice had better cardiac functional recovery compared to control-AMI and 56Fe-AMI mice; whereas % Ejection Fraction, an independent predictive factor for increased mortality after AMI, was most decreased in 56Fe-AMI mice among the AMI groups, suggesting that 56Fe-AMI hearts may have developed cardiac De-compensation and heart failure. Masson's trichrome staining of mid-myocardial A special staining for fibrosis (scar tissue) revealed that AMI led to small transmural (full thickness of LV) infarct in control-AMI mice, large transmural infarct in 56Fe-AMI mice and small superficial infarct in proton AMI mice, suggesting that low dose proton IR may improve, whereas 56Fe IR is deleterious for post-AMI recovery.

SUMMARY for sub-project 4: Our results reveal that low dose full body single proton or 56Fe IR-induced effects on myocardium are of long duration but they do not affect CV homeostasis under normal conditions. Further, we found that a single proton IR 3 months prior to adverse CV event (Acute Myocardial Infarct) is beneficial, whereas 56Fe deleterious for AMI recovery, strongly suggesting that low dose HZE particle radiation have long-lasting negative effect on degenerative CV risks in case of adverse CV event (e.g., AMI). Therefore, despite of possible "healthy worker factor" for astronauts our findings necessitate further extensive studies of underlying molecular mechanisms of HZE particle radiation in the heart and circulatory system that should include new studies with one or more combination of ions including simulated SPE or GCR cosmic rays, as well as combination of radiation with other confounding factors such as microgravity, history of second hand smoke exposure, etc.

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