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Project Title: The Effects of Space Radiation on Stem Cells and Vascular and Cardine Disease Project Title: The Effects of Space Radiation on Stem Cells and Vascular and Cardine Disease Program/Discipline:	Fiscal Year:	FY 2015	Task Last Updated:	EV 03/23/2015
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Task Description:

The integrity of organs and tissues is maintained through continued cell production to replace damaged or senescent cells. In particular stem cells are pivotal to this process providing the primary source for production of functional cells. In the heart, cardiac stem cells (CSCs) reside in close proximity to stromal cells or mesenchymal stem cells (MSCs) that produce proteins that control the growth and development. Stem cells are quiescent cells that cycle through self replication very slowly. This decreases the ability of these cells to repair damage to DNA and may lead to increased risks of vascular and heart disease. In this application we will evaluate the effects of exposure of stem cells to spaceflight-relevant radiation.

Methods: The methods to be used involve exposing stem cells to radiation and evaluating the performance of the stem cells in models of vascular and cardiac disease.

Significance: These studies will provide insights into the potential of increased risks for vascular and cardiac disease due to radiation during spaceflight. The completion of this work will provide models of stem cell damage that can be used to define the underlying mechanisms and possible treatment.

Rationale for HRP Directed Research:

Research Impact/Earth Benefits:

The proposed studies will provide critical information on the effects of radiation on cardiac stem cells and cardiac derived stromal cells.

Results according to the statement of work:

Isolation, preparation and maintenance of the cells: To determine the effect of spaceflight relevant radiation on stem cells, stem cell populations were exposed to radiation in vitro. Two types of stem cell populations were used for these experiments including:

- a) Human Fetal Heart (HFH) derived plastic adherent CSCs: The heart tissue was digested with collagenase and cells cultured in standard plastic culture flasks in media supplemented with recombinant human stem cell factor (rhSCF). Over 1 to 2 weeks, adherent cells are grown from the tissue as CSCs. These CSCs were passaged using trypsin treatment.
- b) Bone Marrow (BM) Derived Mesenchymal Stem Cells (MSCs): The human MSCs were isolated from BM from normal donors and expanded using standard culture conditions and stored frozen in liquid nitrogen. The MSCs have a standard phenotype expressing CD105, CD90 and CD73 but negative for CD45.

Early passage (P3 to P5) cells were thawed and cultured in T162 cm2 culture flasks and were finally grown in T75 flasks, T25 flasks, chamber slides, e-plates, and 6 well plates for irradiation at Brookhaven National Laboratory (BNL) for irradiation.

Radiation exposure systems:

Gamma-radiation: An exposure system was designed for in vitro studies where the effects of continuous low dose rate gamma-radiation exposure on cell cultures were investigated. A low activity Cesium 137 source, originally used for survey meter calibration was placed inside an incubator and the dose rates at different shelf positions and with different attenuator combinations were determined. Dosimetry was performed using several methods such as nano dots measurements, parallel plate ion chambers, and Gafchromic EBT3 film. Experiments were performed with dose rates of 50mR/h and 100mR/h with continuous exposure of cells for 12h.

In addition, four radiation exposure systems were used that include protons and three types of heavy ions at NASA Space Research Laboratory Radiation Facility at BNL, NY in April 2014.

Heavy ions: For HZE particle radiation, 56Fe, 48Ti, and 28Si radiation were performed. The energy of Fe and Ti ions were 1GeV/nucleon corresponding to a LET of 150keV/microm and 108keV/microm in H2O respectively. The energy of Si was 300 MeV/n. The in vitro doses that were used in this proposal are: single fractions of 0.1 and 0.2 Gy with dose rate of 0.5Gy/min.

Protons: Proton beam at energy level of 1 GeV/n and a dose rate of 0.25Gy/min were used for the studies. The dose used for the study were 0.1, 0.2, 0.5, 1.0 Gy. This was given as single dose.

- a) Effect of radiation on activation of repair genes: To study the effects of radiation on activation of repair genes and induction of DNA damage, cells were analyzed for gamma-H2AX staining by flow cytometry and immunofluorescence. Gamma-H2AX marks sites of double-strand breaks. CSCs or MSCs grown either in 6 well plates or in 2 well chamber slides were exposed to radiation at BNL. For immunofluorescence, 15 minutes, 1, 2, 6, 12, 18, and 24 hours after, cells were fixed in cold acetone: methanol (1:1). Media was collected at each time for cytokine analysis later. The fixed cells will be analyzed for DAPI (red-nucleus) and immunofluorescence staining with gamma-H2AX antibody (green). For flow cytometry, cells were trypsinized and stained with FITC conjugated gamma-H2AX antibody and propidium iodide (PI) at 15 minutes, 1, 2, 6, 12, and 18 hours after radiation using a method by Muslimovic et al. and analyzed. HFH74 cells showed a 2-4 fold increase in the staining up to 2h following 100 cGy of proton irradiation. At later time points, this increase was lost. Following HZE ion irradiation however, an increase in gamma-H2AX was observed at 6h or 18h. Significant induction of gamma-H2AX was not observed in NHMSC cells following proton irradiation; however, an increase in gamma-H2AX was observed at 6h or 18h following Iron irradiation. In the case of Iron irradiation, a significant increase in cell death was observed up to 2h following 20 cGy exposure in both the cell lines. However, it appears that cells were able to recover from this initial damage. Since flow cytometry provides an average number of positive events in a cell population, currently we are analyzing gamma-H2AX staining using immunofluorescence.
- b) Effect of radiation on colony forming (CFU-F) potential and cell growth and proliferation: One potential effect of spaceflight-relevant radiation may be to induce aging of stem cells and therefore we compared the CFU-F formation potential of cells after radiation exposure. In NHMSC cells following Iron irradiation (10cGy), a surviving fraction (SF) of 0.43 ± 0.0 was observed. Following continuous gamma irradiation for 12h, a SF of 0.91 ± 0.1 at 50mR/h and 0.8 ± 0.1 at 100mR/h was observed. HFH74 cells showed a plating efficiency of only 7% even in matrigel coated plates, therefore the growth of the cells with and without radiation was followed in real time using cell electronic sensing system (ACEA Biosciences). As expected, with all types of irradiation in HFH74 cells increased growth delay was observed with increasing radiation dose. However, in NHMSC cells, except following proton irradiation, significant

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	differences in growth were not observed with the increasing doses of radiation. Reference: Muslimovic A, Ismail IH, Gao Y, Hammarsten O. An optimized method for measurement of gamma-H2	
	in blood mononuclear and cultured cells. Nature Protoc 3(7):1187-93, 2008.	
Bibliography Type:	Description: (Last Updated:)	