

Fiscal Year:	FY 2016	Task Last Updated:	FY 01/07/2016
PI Name:	Cornforth, Michael Ph.D.		
Project Title:	Molecular Characterization of Transmissible Chromosome Aberrations Produced By Ions of Intermediate and High Atomic Number		
Division Name:	Human Research		
Program/Discipline:			
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) Cancer :Risk of Radiation Carcinogenesis		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	77555-5302	Congressional District:	14
Comments:			
Project Type:	GROUND	Solicitation / Funding Source:	2013-14 HERO NNJ13ZSA002N-RADIATION
Start Date:	03/11/2015	End Date:	03/10/2019
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:	0	No. of Bachelor's Degrees:	0
No. of Bachelor's Candidates:	0	Monitoring Center:	NASA JSC
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Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:	January 2016: No changes		
COI Name (Institution):	Loucas, Bradford Ph.D. (University of Texas Medical Branch, Galveston)		
Grant/Contract No.:	NNX15AG74G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	<p>During deep space exploration, personnel will be exposed to charged particles of intermediate and high atomic number, often collectively referred to as densely ionizing radiations. For a given dose these are almost certainly more likely to cause cancer than the sparsely ionizing types of radiation typically encountered on Earth, such as x- and gamma rays. Since it is not possible to determine directly the carcinogenic potential of such radiations, it becomes necessary to rely on surrogate experimental systems to provide this information. For a number of reasons, the formation of nonlethal (transmissible) chromosome aberrations, mainly reciprocal translocations and inversions, is considered by many to represent the best surrogate endpoint. And yet, only recently have we begun to really understand the molecular processes governing their formation, including possible differences that probably exist in the way that aberrations produced by sparsely- versus densely-ionizing radiations are formed. We propose using advanced molecular methods, including genome sequencing, to characterize structural changes to the DNA of human cells that accompany the formation of transmissible chromosome aberrations caused by exposure to various types of radiation likely to be encountered in deep space.</p>
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	<p>Radiation-induced reciprocal chromosome translocations and inversions are particularly important in that regard as it relates to manned space activities. In addition to causing cancer, their appearance also accompanies ongoing genome instability processes associated with its progression. The fact that these particular chromosome aberrations are transmissible (non-lethal) also makes them ideal candidate biomarkers of accumulated radiation exposure. We argue that molecular analysis of breakpoint junctions formed as the result of translocations and inversions is vital to understanding the process of exchange aberration formation, since it is here where underlying repair/misrepair pathways leave their "molecular fingerprints." Regarding relevance to NASA's concerns, the study of chromosome aberrations stands to tell us much about mechanisms underlying the cancer process itself. The relationship between particle energy/track structure and radiogenic changes to the genome represents an important first step in understanding 1) basic dose-response relationships at low fluences and 2) fundamental carcinogenic processes that may ultimately form the basis for subsequent mitigation strategies.</p>
Task Progress:	<p>Objective 1: Isolation and cytogenetic characterization of clones. In addition to the gamma ray clones we had already isolated, we have now collected and cryopreserved 18 human cell clones that represent the survival of single cells exposed to 0.2 Gy of 1.5 MeV ⁷Li ions delivered at NASA Space Radiation Laboratory (NSRL). These are being analyzed for translocations via mFISH and for inversions via dGH. For inversions, we now have recently developed a directional genomic hybridization (dGH) probe set capable of simultaneously detecting inversions in chromosomes 1, 2, and 3. This should greatly increase the sensitivity of the assay, thus providing us more inversion-bearing clones from which to choose for subsequent molecular analysis, as compared with the original chromosome 3-specific probe set. Because of issues related to Objective 2, one particular clone (K1-400 C4) was further analyzed by G-banding at Emory University, in order to more accurately assign the cytogenetic location of translocation breakpoints. G-banding localized the translocation breakpoints as occurring between chromosomes 3 and 4. A paracentric inversion involving chromosome 3 that was too small to be seen by either G-banding or mBAND was discovered using directional genomic hybridization (dGH), a cytogenetic approach we developed specifically for the detection of inversions.</p> <p>Objective 2: Molecular characterization of clones. DNA libraries were made from five clones, each containing a particular radiation induced translocation, and one clone control clone. These were analyzed independently (by whole genome sequencing; WGS) by laboratories at Oregon Health & Science University (OHSU) and UTSW (University of Texas Southwestern Medical Center). Despite experimenting with various established algorithms, neither laboratory was initially able to identify discordant reads that were consistent with the breakpoint locations in any of the cytogenetically verified rearrangements. The decision was made to concentrate sequencing efforts on clone K1-400 C4, which mFISH analysis showed to contain a gamma-ray-induced t(3;4) reciprocal translocation, and which dGH showed to also contain a prominent paracentric inversion in chromosome 3. We were eventually able to identify a putative translocation breakpoint between chromosomes 3 and 4 of this clone, and oligonucleotide primers that were designed that flanked the presumptive breakpoint location yielded a PCR product of anticipated size. Unfortunately sequencing analysis showed this to be a false positive position in the genome. Moreover, all of the presumptive inversions tentatively identified by WGS were inconsistent with the known cytogenetic position of the inversion as well. The biggest problem we faced was the preponderance of false-positive calls to the reference genome.</p> <p>At OHSU, a deeper-coverage genomic library was made from clone K1-400 C4. It was sequenced using a different bioinformatic algorithm, and calls were stringently filtered against (normal background) structural variants from large panel of normal samples. This allowed us to correct for any reference mapping artifacts. As a result, we were able by WGS to presumptively identify the cytogenetic location of this reciprocal translocation. Although it will require further verification, it is likely that we have successfully identified the genomic location of the reciprocal translocation in clone 400 C4.</p> <p>Our success with mapping this translocation notwithstanding, and in order to move our research forward at a faster pace, we plan to employ sequencing of longer insert libraries, which has higher specificity for the identification of the genomic regions where the breakpoints are located. We plan to implement this strategy by using mate-pair sequencing, combining this data with that from short read paired-end sequencing.</p> <p>Objective 3: Cytogenetic Effects of Abrogating CtIP activity. During final negotiations with NASA program management, this objective was deleted from the proposal.</p>
Bibliography Type:	Description: (Last Updated: 03/17/2023)
Abstracts for Journals and Proceedings	<p>Cornforth MN. "Molecular Characterization of Transmissible Chromosome Aberrations Produced by Ions of Intermediate and High Atomic Number." 2015 NASA Human Research Program Investigators' Workshop, Galveston, TX, January 13-15, 2015.</p> <p>2015 NASA Human Research Program Investigators' Workshop, Galveston, TX, January 13-15, 2015. , Jan-2015</p>
Articles in Peer-reviewed Journals	<p>Loucas BD, Shuryak I, Cornforth MN. "Three-color chromosome painting as seen through the eyes of mfish: another look at radiation-induced exchanges and their conversion to whole-genome equivalency." <i>Frontiers in Oncology</i> (submitted, 12/2015). , Dec-2015</p>

