Fiscal Year:	FY 2007	**Task Last Updated:**	FY 08/03/2006
PI Name:	Wiese, Claudia Ph.D.		
Project Title:	A role for homologous recombination in complex DSB repair after HZE particles		
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:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Comments:	For information purposes only--PI moved in June 2014; however, funding did not go with her. As of 6/1/14, Colorado State University, Department of Environmental and Radiological Health Sciences, 485 MRB - 1618 Campus Delivery, Fort Collins, CO 80523-1618, Office: (970) 491 7618, Email: Claudia.Wiese@colostate.edu		
Project Type:	Ground	**Solicitation / Funding Source:**	2004 Radiation Biology NNH04ZUU005N
Start Date:	10/01/2005	**End Date:**	09/30/2009
No. of Post Docs:	0	**No. of PhD Degrees:**	
No. of PhD Candidates:	0	**No. of Master' Degrees:**	
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:	1	**Monitoring Center:**	NASA JSC
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:	NOTE: End date changed to 9/30/2009, per K. Willison (3/07) NOTE: Question re end date, per JSC; changed back to 9/30/2006 (1/07) NOTE: project extended for full length of proposal, per J. Dardano (2/06)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):			
Grant/Contract No.:	NNJ05HI36I		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Overall Rationale: The overall goal of this proposal is to investigate whether homologous recombinational DNA repair (HRR) contributes to the repair of double-strand breaks (DSBs) generated by the radiation types found in the space radiation environment. This proposal is a continuation of our previous work demonstrating that recombination is induced in human cells exposed to Fe ions. Here, we aim to directly assess the role of HRR in the repair of DNA damage after high linear energy transfer (LET) radiation in mammalian cells, an investigation that has never been carried out before. High LET charged particles deposit large amounts of energy along the ion trajectories, leading to the induction of highly localized DNA damage. These spatially correlated DSBs rejoin with slower kinetics and to less completeness than DSBs induced by low LET radiation. In mammalian cells, X-ray induced DSBs are primarily repaired by non-homologous end joining (NHEJ) in G1, but HRR plays a critical role in S- and G2-phases of the cell cycle. Several reports indirectly suggest that HRR, generally a precise form of DNA repair, plays an important role in the repair of correlated DSBs. Importantly, radiation-induced human tumors arise through a multi-step process of genetic change, and defects in HRR leading to the stimulation of error-prone DNA repair pathways may accelerate this process. For this reason, it is important to investigate directly whether alterations in the ability to perform HRR can sensitize humans to HZE particles. Approach: We will determine in syngeneic human cells whether defects in HRR affect the extent of cell killing and the mechanism of mutagenesis by densely ionizing Fe ions. RNA interference technology will be used to impair HRR (targeting XRCC3, Rad51D or Rad51) in the human lymphoid cell line WTK1. For comparison purposes, NHEJ will also be targeted, and X-rays will be used to test high vs. low LET radiation effects. The Fe particle-induced mutation frequencies will be determined at the autosomal TK1 locus and at the X-linked HPRT locus for parental WTK1 cells and for one representative derivative of WTK1 cells with a 'loss-of-function' phenotype for HRR and for NHEJ. Sets of TK1 mutants will be collected and the Fe ion-induced, X-ray-induced and spontaneous mutation spectra will be compared to discriminate between recombinational and deletional events. Furthermore, we will investigate in hamster and in human mutant cells whether impaired HRR enhances the cytotoxic effects of Fe ions. Wild-type CHO cells and CHO mutant cell lines impaired in HRR (Rad51D, XRCC3) or in NHEJ (DNA-PKcs) will be compared. Both asynchronous and synchronous cell cultures will be used and the relative contributions of both DNA repair pathways to cell survival will be assessed. DSB repair in CHO mutant cell lines and wild-type cells will be measured using a recently described assay that quantifies DSB rejoining by gH2AX foci formation. The effect of the loss of XRCC3 on the cellular sensitivity to Fe particles in an hTERT-immortalized human fibroblast strain will also be determined.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	
Task Progress:	The overall goal of this Research Project is to investigate whether homologous recombinational DNA repair (HRR) contributes to the repair of double-strand breaks (DSBs) generated by the radiation types found in the space radiation environment. We hypothesize that correlated, clustered radiation damage, as induced by iron ions, requires the resection of the damaged DNA and the HRR pathway, and therefore is not channeled into the non-homologous endjoining (NHEJ) pathway. We are investigating syngeneic near-normal human cells that are either HRR-proficient or HRR-impaired. In this study, ablation of HRR is induced by gene-specific knockdown using RNA interference. Homologous recombination (HR) is conserved in all organisms and a prerequisite for both DNA repair and the resumption of stalled replication forks. As replication fork stalling occurs during most cycles of replication, proteins required for HRR generally are essential and cannot easily be deleted in human cells. Therefore, RNA interference is the method of choice for attenuating HRR in near-normal human cells. Importantly, HRR is a DNA repair pathway with close ties to cancer biology and crucial for maintaining genomic stability, limiting mutagenesis and preventing carcinogenesis. It is well established that conditions promoting reduced levels of HRR compromise the fidelity of DSB repair and correlate with an elevated cancer risk. The risk of developing cancer is increased in individuals exposed to space radiation, and defects in HRR, leading to the stimulation of error-prone DNA repair pathways, are likely to contribute to this process. Therefore, it is a necessity to establish the relevance of HRR to HZE radiation, both for better prediction of the astronaut's sensitivity to radiation carcinogenesis and for proper radiation risk assessment. Using syngeneic human cells, this Research Project is assessing whether defects in HRR affect the extent of cell killing (Aim 1) and the levels and mechanisms of mutagenesis (Aim 2) by densely ionizing Fe ions. RNA interference is used to impair HRR (targeting proteins essential for HRR: XRCC3, RAD51, RAD51D, RAD51AP1) in the human lymphoblastoid cell line TK6 (Aims 1&2). TK6 cells were not part of the original grant application due to their inherently lower ability to perform HRR. However, the reviewers strongly recommended also including a wild type p53-expressing cell appropriate for mutation studies (i.e. TK6 cells) into this Research Project in order to be able to obtain results relevant to the risk estimate for normal human tissues. The Fe particle-induced mutation frequencies will be determined at the autosomal TK1 locus and at the X-linked HPRT locus for one representative derivative of TK6 cells with a 'loss-of-function' phenotype for HRR (Aim 2) and these mutation frequencies will be compared to parental TK6 cells studied in our earlier investigation. Sets of TK1 mutants will be collected and the Fe ion-induced, X-ray-induced and spontaneous mutation spectra will be compared to discriminate between recombinational and deletional events (Aim 2). As previously mentioned and in response to the reviewers' critiques, we have decided to redirect one major part of the proposed research within this Research Project to be able to employ the wild type p53 human TK6 cell line instead of its related WTK1 cell line that expresses mutant p53. We now have proceeded to 1) develop retroviral vectors highly competent for the expression of shRNAs targeting HRR genes in near-normal (i.e. TK6 cells and hTERT-immortalized human cells) and finite lifespan (i.e. normal) human cells, and 2) accumulate data using TK6 cells depleted for several HRR proteins individually using the plasmid-based system for the expression of shRNAs. Since the retroviral vectors are reported to yield higher transduction efficiencies and therefore more extensive knockdown of the proteins of interest, we are anticipating only using the retroviral system in future experiments. This approach also allows us to make progress independent of the proposed but difficult generation of the XRCC3-/- knockout from hTERT-immortalized human fibroblasts (currently carried out by our collaborator Dr. L.H. Thompson, LLNL). Among the several endpoints that we are planning to investigate, we have concentrated on cell cytotoxicity and colony formation assays to date. We have successfully developed an approach to deplete essential HRR proteins in the human lymphoblastoid TK6 cell line that expresses wild type p53. Moreover, we have assessed these cells for their ability to carry out homologous recombinational repair (i.e. gene conversion). Using the plasmid-based system for expression of shRNAs we now are able to significantly reduce the levels of XRCC3, RAD51 or RAD51AP1 (three different proteins

	required for HRR) in p53 wild type TK6 cells. In TK6 cells, depletion of either XRCC3 or RAD51 results in suppression of HRR (3- to 4-fold; determined by monitoring the frequency of gene conversion at a site-specific DSB within an integrated recombinational reporter construct). These findings are directly relevant to our Research Project since the demonstration that the expression of shRNAs targeting either XRCC3 or RAD51 indeed confers a defect in HRR is a prerequisite for the proposed study. We have assessed the radiosensitivity of TK6 cells depleted for XRCC3 or RAD51AP1 by exposing these cells to increasing doses of sparsely ionizing radiation and determining the fractions of cells surviving. For control purposes, we have used TK6 cells transfected with the plasmid encoding the mutated hairpin, expression of which does not result in protein depletion. As expected and compared to control cells (transfected with plasmid encoding a non-depleting hairpin), asynchronous cultures of TK6 cells depleted for either XRCC3 or RAD51AP1 are not sensitized to the cytotoxic effects of low LET ionizing radiation. Studies are underway to also perform similar experiments with RAD51-depleted TK6 cells, but more importantly, in the attempt to synchronize TK6 cells, we have initiated experiments to perform centrifugal elutriation with XRCC3-depleted TK6 cells. In preliminary studies, but using HeLa cells, we have synchronized RAD51AP1-depleted cells and found that RAD51AP1-depleted S-phase cells are more sensitive to X-rays than are control cells. We have generated lentiviral vectors that encode shRNAs to deplete XRCC3, RAD51 or RAD51AP1 for our analyses in near-normal (i.e. hTERT-immortalized) and finite lifespan (i.e. normal) human cells. These lentiviral vectors have considerable advantages over normal plasmid expression vectors, in that they allow for higher efficiency of transduction, as compared to plasmid transfection. In addition, the lentiviral constructs are very efficiently integrated into the genome, enabling studies in normal human cells directed towards obtaining results relevant to the radiation risk estimate for normal human tissues. Although our progress on the original three aims is slower than anticipated, this is largely due to the suggested (i.e. by the reviewers) and acknowledged (i.e. by us) necessity to perform our Research Project in p53 wild type human cells. We have presented evidence that we are now fully capable of performing the proposed study in TK6 cells, using the plasmid-based shRNA expression system. Moreover, we have spent considerable time and effort in generating retroviral shRNA-expressing constructs that are likely to work for many different human cell types, making our investigation more practical for human space travel risk assessment.
Bibliography Type:	Description: (Last Updated: 04/11/2018)
Articles in Peer-reviewed Journals	Wiese C, Hinz JM, Tebbs RS, Nham PB, Urbin SS, Collins DW, Thompson LH, Schild D. "Disparate requirements for the Walker A and B ATPase motifs of human RAD51D in homologous recombination." Nucleic Acids Res. 2006 May 22;34(9):2833-43. PMID: 16717288 , May-2006