Fiscal Year:	FY 2011	**Task Last Updated:**	FY 09/07/2011
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Project Title:	A role for homologous recombination in complex DSB repair after HZE particles		
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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
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No. of Bachelor's Candidates:	3	**Monitoring Center:**	NASA JSC
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Flight Program:			
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Task Description:	We have studied whether or not homologous recombination (HR) is an important pathway for the repair of spatially correlated complex DSBs such as introduced by 1 GeV/n Fe ions. Our investigation was initiated by several published reports that approached the question indirectly. First, non-homologous end-joining (NHEJ) was progressively inhibited by an increase in the structural complexity of DSBs in vitro, suggesting that secondary lesions, proximal to the DSB end and within the footprint of the eukaryotic NHEJ complex, would interfere with the end-joining process. Second, high LET radiation was shown to stimulate HR in CHO cells, and third, a defect in the ATR signaling and checkpoint protein, that decreased the efficiency of HR, was shown to increase the sensitivity of human cells to 1 GeV/n Fe ions. Interestingly, more recent studies, all carried out in rodent cells, now describe a direct role for HR in DNA repair after high LET radiation, including 1 GeV/n Fe ions. These studies suggest that bi-stranded clustered lesions are left unrepaired in G1 phase, to be processed by HR in S phase through restart of broken replication forks by strand invasion. We also had hypothesized that, for a fraction of spatially correlated breaks induced by high-energy iron ions, extensive resection of the DSB ends to generate protruding 3'-overhangs for HR would be a more feasible way to repair these lesions than NHEJ. To test the contribution of homologous recombinational repair (HR) in repairing DNA damaged sites induced by high-energy Fe ions, we used: 1) HR-deficient rodent cells carrying a deletion in the RAD51D gene, 2) syngeneic human cells impaired for HR by RAD51D or RAD51 knockdown using RNA interference. We show that in response to Fe ions, HR is essential for cell survival in rodent cells, and that HR-deficiency abrogates RAD51 foci formation. Complementation of the HR defect by human RAD51D rescues both enhanced cytotoxicity and RAD51 foci formation. For human cells irradiated with Fe ions, cell survival is decreased, and, in p53 mutant cells, the levels of mutagenesis are increased when HR is impaired. Human cells synchronized in S phase exhibit more pronounced resistance to Fe ions as compared with cells in G1 phase, and this increase in radioresistance is diminished by RAD51 knockdown. These results implicate a role for RAD51-mediated DNA repair (i.e. HR by strand invasion) in removing a fraction of clustered DNA lesions induced by charged particle irradiation. Our results are the first to directly show the requirement for an intact HR pathway in human cells in ensuring DNA repair and cell survival in response to high-energy high LET radiation.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Our research has led to a better understanding of the mechanisms of DNA double-strand break repair in response to low and high LET ionizing radiation. Our results have shown that, in p53 wild type human cells, homologous recombination (HR) is essential for protecting from both spontaneous and radiation-induced DNA damage. Furthermore, our results show that, in p53 wild type human cells, HR cannot be easily substituted for by other, more mutagenic DNA double-strand break repair pathways. Conversely, in permissive p53 mutant human cells deficient in HR, mutagenic DSB repair pathways can substitute for HR, promoting mutagenesis and carcinogenesis. Interestingly, the HR gene (i.e. RAD51D) which we inactivated in hamster and in human cells in this investigation, in its inactivated state, has recently been associated with a cancer predisposition syndrome in humans.
Task Progress:	We have shown that DNA double-strand break (DSB) repair by homologous recombination (HR) is an important pathway for the repair of spatially correlated complex DSBs such as introduced by 1 GeV/n Fe ions. Our investigation was initiated by several published reports that approached the question indirectly. First, non-homologous end-joining (NHEJ) was found to be progressively inhibited by an increase in the structural complexity of DSBs in vitro, suggesting that secondary lesions, proximal to the DSB end and within the footprint of the eukaryotic NHEJ complex, would interfere with the end-joining process. Second, high LET radiation was shown to stimulate HR in CHO cells, and third, a defect in the ATR signaling and checkpoint protein, that decreased the efficiency of HR, was shown to increase the sensitivity of human cells to 1 GeV/n Fe ions. Interestingly, more recent studies, all carried out in rodent cells, now describe a direct role for HR in DNA repair after high LET radiation, including 1 GeV/n Fe ions. These studies suggest that bi-stranded clustered lesions are left unrepaired in G1 phase, to be processed by HR in S phase through restart of broken replication forks by strand invasion. We also had hypothesized that, for a fraction of spatially correlated breaks induced by high-energy iron ions, extensive resection of the DSB ends to generate protruding 3'-overhangs for HR would be a more feasible way to repair these lesions than NHEJ. Nonetheless, our results also show that NHEJ is important for the repair of DSBs introduced by 1 GeV/n Fe ions (see below). To test the contribution of homologous recombinational repair (HR) in repairing DNA damaged sites induced by high-energy Fe ions, we used: 1) HR-deficient rodent cells carrying a deletion in both alleles of the RAD51D gene, and 2) syngeneic human cells impaired in HR by RAD51D or RAD51 knockdown using RNA interference. We show that, in response to Fe ions, HR is essential for cell survival in rodent cells, and that HR (i.e. RAD51D)-deficiency abrogates RAD51 focus formation in response to Fe ions. Complementation of the HR defect by human RAD51D in rad51d-deficient CHO cells rescues both enhanced cytotoxicity and loss of RAD51 focus formation. In these experiments we chose a RAD51D-complemented CHO clone whose protein expression level for human RAD51D was very similar to that of endogenous hamster RAD51D expressed in AA8 wild-type CHO cells. Our results showing full complementation for cell survival in response to graded dosed of X-rays also support a role for human RAD51D in the response to low LET ionizing radiation. We obtained RBE values for Fe ions of 2.4 and 2.0 for wild-type and rad51d-deficient cells, respectively. From our data we infer that HR is active in rodent cells after exposure to 1 GeV/n Fe ions and that proper function of this high-fidelity DNA DSB repair pathway is required to fully protect CHO cells from the cytotoxic effects of Fe ions. For human cells irradiated with Fe ions, cell survival is decreased, and, in p53 mutant cells, the levels of mutagenesis are increased when HR is impaired. The effects of RAD51D protein knockdown on cell survival are more pronounced in p53 wild-type TK6 cells than in p53 mutant WTK1 cells. The reason for this difference is unclear at this point, but it is possible that mutagenic DNA DSB repair pathways (i.e. NHEJ, single-strand annealing (SSA) or microhomology-mediated end-joining (MMEJ)) may substitute at higher levels in p53 mutant cells, rescuing cytotoxicity in the absence of fully functional HR. Our results also suggest that, after exposure to 1 GeV/n Fe ions, p53 wild-type human cells rely more heavily on faithful HR and do not tolerate unfaithful DSB repair mechanisms in substitution for HR. Our results for high and low LET radiation-induced mutagenesis are in support of this, because very different effects of RAD51D knockdown on Fe ion-induced mutagenesis are observed in TK6 and WTK1 cells. When we reduced the expression of RAD51D protein by RNA interference, TK mutant fractions were increased significantly in Fe ion- and X-irradiated WTK1 cells which express mutant p53. In these cells, approximately twice as many TK mutants were recovered from RAD51D-depleted cells than from control-transfected cells at all Fe ion and X-ray doses investigated. These results suggest that in the absence of faithful HR, unfaithful DNA repair pathways take over, leading to elevated

levels of mutagenesis in permissive (i.e. p53 mutant) human cells. Conversely, no such increase in mutagenesis was observed in HR-compromised p53 wild-type cells, suggesting that precise HR is tightly regulated in TK6 cells, does not lead to the induction of TK mutations, and cannot be substituted for by error-prone DNA damage repair pathways. However, downregulation of the NHEJ gene XRCC4 in p53 wild-type cells limited mutagenesis after both Fe ion- and X-irradiation, demonstrating that NHEJ is a mutagenic DNA DSB repair pathway that is active in p53 wild-type cells after exposure to graded does of Fe ions and X-rays. Unexpectedly, this was not observed for XRCC4-depleted WTK1 cells, for which the levels of Fe ion- and X-ray-induced TK mutant fractions remained at those of control-transfected cells. These results indicate that NHEJ does not contribute to mutagenesis in WTK1 cells under wild-type repair conditions. These findings also suggest that error-prone homology-mediated events such as SSA, MMEJ or PARP-dependent alternative end-joining may account for radiation-induced mutagenesis in these p53 mutant cells.

Furthermore, we find that human U2OS cells synchronized in S phase exhibit more pronounced resistance to Fe ions as compared to cells irradiated in G1 phase, and that this increase in radioresistance is diminished by RAD51 knockdown. To the best of our knowledge, our results for U2OS cells are the first to show S-phase-dependent radioresistance for human cells after exposure to high LET Fe ions. To our surprise, the difference in radioresistance between U2OS cells irradiated in G1 or S phase was almost as pronounced after exposure to Fe ions as after X-irradiation (sensitivity factors (D10) are 1.6 and 1.8, respectively), suggesting that S-phase-specific DNA repair pathways (i.e. HR and SSA) are as important after Fe ion exposure as after X-irradiation. Notably, whereas knockdown of RAD51 in S-phase U2OS cells reduced X-ray resistance to the levels of G1 phase cells, a significantly smaller decrease in radioresistance was observed for Fe ion-exposed RAD51-depleted cells in S-phase. We propose that SSA, a mutagenic sub-pathway of HR, that is restricted to S/G2 cells and that requires DSB end resection and is independent of RAD51, operates at higher levels after high LET radiation than after low LET radiation. It is also possible, that in Fe ion-irradiated S-phase cells a higher fraction of the induced DSBs are repaired by NHEJ. Compared to X-rays, HR in S-phase would than be less important after exposure to 1 GeV/n Fe ions. In summary, proper HR is required to fully protect human and rodent cells from the cytotoxic effects of Fe ions. Furthermore, in permissive human cells (i.e. cells with a gain-of-function mutant p53), a reduced level of HR leads to a significant increase in Fe ion-induced mutant fractions. No such increase in mutagenesis is observed in HR-compromised p53 wild type human cells. While HR is important for some lesion repair (i.e. helps limit cytotoxicity after Fe ions), recombinational repair events in p53 wild-type cells appear to be highly controlled, are mostly faithful, and cannot easily be substituted for by other DSB repair pathways. In addition to HR, our results support a role for other S-phase-specific DSB repair pathways after high LET radiation. These pathways are currently the subject of intense investigation in rodent cells, and should also be investigated in human cells to better understand the cancer risks for astronauts after exposure to space radiation.

Bibliography Type:	Description: (Last Updated: 04/11/2018)
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