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Task Description:	Overall hypothesis: Low-dose radiation induces molecular manifestations of a pro-inflammatory response as a function of radiation type, radiation doses, doses rates, LET (linear energy transfer) value, and time. An oral available anti-inflammatory countermeasure, already in human clinical trials with a good safety profile, will significantly reduce proton and HZE-ion exposure associated tumor initiation and progression. Although biological mechanisms of normal tissue radiation injury are not completely understood, the roles of specific pathways in some cell types are becoming elucidated. While cell death is generally believed to be one the main causes of tissue injury from exposure to higher doses of low and high LET radiation, the dose and dose rates likely to be encountered by an astronaut on long-term missions into deep space are unlikely to cause massive cell death. Pathological manifestations after low-dose space radiation should be strongly influenced by non-cytotoxic radiation effects, resulting in incremental small changes in cell function, immune (micro-environmental) altered responses, and changes in metabolism. To more fully understand the tissue effects of exposure to space radiation compared to background cancer on Earth, it will require a more integrated "omics" and biological end point analysis as is proposed in this focused proposal using mouse models to help form the basis of a new description of radiation quality effects and cancer risk. Our published data (Clin Cancer Research, 2014) led us to the hypothesis that protracted/fractionated high LET irradiation can have long-term effects by changing the microenvironment in tissues leading to a pro-inflammatory cancer progressing phenotype. Importantly, the microarray signatures in these published studies on the K-ras lung cancer susceptible mouse model of lung cancer were shown to be applicable to overall survival in humans with lung and breast cancer. Thus, the studies proposed are likely to be applicable to human risks. In the current proposal we will test this hypothesis rigorously with normal mice, mice susceptible to lung cancer (LA1-Kras), and a colon cancer susceptible mouse model (CPC:APC) by incorporating the countermeasure arm in already approved studies. We have already established dose responses for tumor incidence in the K-ras and CPC;APC mouse. We will conduct experiments with these mouse models of cancer susceptibility and WT mice using a GCR (galactic cosmic ray) simulation that involve fast switching of three ion Si, He, and protons using low doses (total 30cGy) and dose rates (0.5 cGy/min). We will also test if an oral deliverable countermeasure, CDDO (with a known mechanism of action) using mission relevant irradiation doses can significantly decrease tumor incidence. We will focus on intermediate/persistent effects (14-100 days post-IR) including some long-term effects (~150-250 days). We will conduct tissue micro-dissections and “omics” analyses of normal tissues, precancerous lesions, malignant lesions, and cleared margins surrounding the precancerous lesions in mice with and without being provided the medical radioprotector, CDDO. We propose that using a variety of radiation qualities and biological models, we will be able to dissect the important difference between space radiation and terrestrial radiation. This will lead to improved risk quantification and development of new systems biology risk modeling approaches that can be extrapolated to human cancer risks.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Although radiation therapy is commonly used for treatment of many human diseases, including cancer, ionizing radiation produces reactive oxygen species that can damage both cancer and healthy cells in tissues. Our NASA supported studies using the biological countermeasure, CDDO, an anti-oxidant, anti-inflammatory modulator with a known mechanism of action, reduces both cancer initiation and progression in mouse models after exposure to either terrestrial or space radiation. We demonstrated that CDDO can be used as a radioprotector in normal non cancerous human lung and breast epithelial cells exposed to space and terrestrial irradiations while cancer cells were not protected. This suggests the use of this oral available, non-toxic class of drug can protect non-cancerous healthy during radiotherapy, resulting in better outcomes with less toxicity for patients. CDDO is currently in a phase 3 clinical trial for patients with pulmonary arterial hypertension. In the future, CDDO may be used to protect astronauts on long-term mission to Mars as well as patients receiving radiotherapy on Earth. This radioprotector may also have utility in protecting first responders to nuclear accidents.
	Introduction/Background: Experiments conducted at the NASA Space Radiation Laboratory (Brookhaven, NY) demonstrate that HZE ion components of the GCR result in persistent inflammatory signaling, increased mutations, and higher rates of cancer initiation and progression compared to that seen with terrestrial radiation. While physical shielding may reduce some of the risks of space radiation, there is mounting evidence that biological countermeasures will be required to ensure that the established limits of increased lifetime fatal cancer risks are not exceeded. CDDO (also termed Bardoxolone Methyl) is an oral available anti-inflammatory/anti-oxidant modulator that has been tested in humans in a variety of clinical trials and is currently in a Phase 3 trial for patients with pulmonary arterial hypertension (Reatapharma.com). Radioprotector Previous Studies: We have previous demonstrated that CDDO is also a potent radioprotector in vitro and in vivo with a known molecular mechanism of action. CDDO activates Nrf2, a key transcription factor that when translocated to the nucleus binds to anti-oxidant response elements increasing cytoprotective and DNA repair kinetics. Using wild type mice we observed CDDO provided in lab chow prior to a lethal dose of whole-body irradiation protected mice from acute gastrointestinal toxicity with enhanced DNA damage repair resulting in improved overall survival. Using lung (LA-1) and colon cancer (CPC;Apc) susceptible mouse models, we examined the effects of providing CDDO for up to 100 days on the spontaneous initiation and progression of tumorigenesis. While the spontaneous rate of premalignant (hyperplasias, adenomas) lesions is 100% in these mice, the LA-1 model develops 9-10% invasive cancers while the CPC;Apc mouse model develops 6-8% invasive cancers. We demonstrated CDDO dramatically prevented the development of spontaneous invasive cancers in un-irradiated cancer susceptible mice. When these mice are exposed to x-rays, protons, or GCR ions, the spontaneously rate of invasive cancer increases 2-4 fold depending on ion, doses, and dose rates used. We next tested if the LA-1 and CPC;Apc mice fed CDDO diet only 2-3 days prior to x-rays or protons provided as a single dose or as a solar particle event simulation would lead to a lower incidence of invasive carcinomas. The results were that CDDO provided as a biological countermeasure prior to irradiation reduced the percent of invasive cancers 2-3 fold. Similar results were observed when mice were irradiated with GCR ions. These results document that exposure to space radiation increases the risk of invasive cancers in cancer susceptible mouse models, and that radioprotectors such as CDDO may reduce the overall risk of fatal cancers without affecting normal cells. Simulated Solar Particle Events (SPE) Promotes Senescence-Associated Inflammatory Responses in Colorectal Cancer Susceptible Mouse Model: While protons and high charge and energy (HZE) particles are considered to be major risk factors for humans during space missions, the mechanism underlying the biological effects of protons and HZE particles still remain to be more fully characterized. In our recent studies we simulated solar particle events (SPE) at the Brookhaven NASA Space Radiation Laboratory to characterize the biological effects of low dose rate protons in vivo.

Task Progress:	Using the colorectal cancer susceptible (CPC;Apc) mouse model, we studied colonic tumorigenesis after whole-body exposure to a simulated SPE with varying energy (50-150 MeV/n) using a total dose of 2 Gy over a 2 hour period (at an average dose rate of 1.67 cGy/min). We also exposed mice to 2 Gy of acute (50 MeV/n) proton or X-ray (250 kVp, 1mA, 1.65 mm Al filter) at a dose rate of 20 cGy/min as a reference radiation. We observed that whole-body irradiation with simulated SPE is more effective in inducing invasive adenocarcinoma incidence (4-fold increase compared to un-irradiated controls) followed by induced senescence-associated inflammatory responses (SIR), which are involved in colon cancer initiation and progression. After irradiation to SPE simulation, a subset of SIR genes (Troy, Sox17, Opg, Faim2, Lpo, Tlr2, and Ptges) and a gene known to be involved in invasiveness (Plat), along with the senescence-associated gene (P19Arf) are markedly increased. Following these changes, p53 mutations are increased compared with the same doses of acute proton or x-ray irradiation. Pretreatment with the oral available countermeasure, CDDO reduced SPE-associated SIR gene expression and tumorigenesis. Thus, exposure to SPE irradiation elicits significant changes in colorectal cancer initiation and progression that can be protected by CDDO-EA pretreatment. Investigating Lung Cancer Risk to Solar Particle Event (SPE) Simulations: SPEs are comprised of varying energies and doses over a period of time (protracted dose of radiation), and occurrences are difficult to predict. On a mission to Mars and back it is predicted that up to 7 SPEs will occur and while shielding may partially protect astronauts, it cannot block all irradiation exposures. It is predicted that SPEs have high carcinogenic effects compared to equivalent low energy terrestrial radiation (e.g., X-rays). However, data are still required to determine more exactly the increased risk of invasive cancer with low dose rates and varying energies of proton. During our studies, we used the K-rasLA1 mouse model which mimics the human adenocarcinoma non-small cell lung cancer progression by spontaneous activation of mutant K-ras lesions. Using K-rasLA1, we studied survival and the progression of lung cancer after total body exposure to a simulated SPE with varying energies (50 – 150 MeV/n) using a total dose 0.5 Gy, 1.0 Gy, and 2.0 Gy (at an average dose rate of 1.67 cGy/min). We also exposed mice to 2 Gy of monoenergetic (50 MeV/n) proton or X-ray (250 kVp) at a dose rate of 20 cGy/min as a reference radiation exposure. The SPEs simulation, and monoenergetic proton radiation resulted in increases in invasive carcinoma as compared to the X-rays. K-rasLA1 mice exposed to 2.0 Gy of sSPE radiation and 2.0 Gy of monoenergetic acute proton (50 MeV/n or 150 MeV/n) exhibited a significant decrease in median survival compared to un-irradiated control cancer susceptible mice. We also observed there was significant increase in the average number of tumor lesions in SPEs simulated animals as compared to monoenergetic proton radiation and X-rays. To evaluate the underlying mechanistic details involved in radiation-mediated tumorigenesis in our lung cancer model, the phosphorylation status (activation) of various targets important to the process of tumorigenesis were investigated. We found K-rasLA1 mice exposed to energetic protons exhibited altered growth factor signaling compared to un-irradiated controls. We are testing if alterations in growth factors are due to the chronic oxidative stress caused by the SPEs leading to increase in invasive cancer. Further studies are in progress to understand the SPEs biological effect including DNA sequencing of candidate genes such as p53 (which we found was increased in the colon cancer susceptible mouse model). Significant biological and mechanistically data obtained from these studies may help in risk assessment of space travel and provide insights into molecular mechanisms which could be applicable in mitigating or preventing cancer initiation and progress during long-duration space travel. Ongoing Experiments and Future Directions: Most accelerator-based space radiation experiments have been performed with single ion beams at fixed energies. However, the space radiation environment consists of a wide variety of ion species with a continuous range of energies. Due to recent developments in fast beam switching technology implemented at the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory (BNL), it is now possible to rapidly switch ion species and energies, allowing for the possibility to more realistically simulate the actual radiation environment found in space. We were recently approved to conduct galactic cosmic ray (GCR) simulations at NSRL, to determine if there is an increase in cancer initiation or progression following ~30cGy total exposure of three sequential beams that are based on discussions with John Norbury and the Virtual Systems Biology – Cancer Risk working group over the last year. On a trip to Mars and back, every human cell will be traversed by a proton and most by helium and very rarely by a HZE particle such as silicon. We cannot mimic the dose protraction that occur in deep space and we recognize that there are issues of scaling from a mouse to human. However, we have already initiated our first experiments at the NSRL 16C run. We are using a simplified GCR simulation consisting of protons, helium, and silicon using a dose rate of 0.5cGy/min (to keep within the beam time approved). We will use 20cGy of protons (120 MeV/n), 5cGy of helium (250 MeV/n), and 5cGy of silicon (300 MeV/n). We have run over 200 mice with two main variables in 16C: 1) protons, then helium, then silicon (plus or minus CDDO) and 2) silicon then protons, then helium. The first group of mice were irradiated without any problems at the NSRL. These experiments are to test the hypothesis that GCR simulations even at very low doses and dose rates may increase carcinogenesis in cancer susceptible mice and that a biological countermeasure will reduce the increases in more lethal cancers. We will also conduct molecular analyses on these and wild type mice exposed to GCR simulations at various times points. If we observe an increase in tumor formation with GCR simulations, we will conduct whole genome sequencing and a variety of other molecular studies to determine and further understand the molecular mechanism involved. We will determine if cancer susceptible and wild type mice have molecular changes that may indicate an increased risk of cancer. Finally, to more closely mimic the space environments, we will reduce the dose fractions and repeat the GCR simulation using 3 sequential cycles in the Spring 17A NSRL run. The total dose will not exceed 30cGy only the dose in each cycle. We have been told we can conduct fast switching up to 10 times but that will require more beam time and are not justified at this stage. We will conduct some single and two ion experiments going forward if we observe an increase in carcinogenesis from the 16C run in order to dissect molecular mechanisms (e.g., silicon alone, or silicon plus helium, or silicon plus protons). We are also planning mitigation experiments in the future (e.g., provide CDDO after irradiation instead of prior to irradiation).
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