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COI Name (Institution):	Eisenberg, Carol Ph.D. (New York Medical College) Rota, Marcello Ph.D. (New York Medical College)		
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Task Description:	These projects seek to study the consequences of galactic cosmic radiation (GCR) exposure. Space travel increases solar and cosmic particle radiation exposure, which is significantly elevated once travel moves beyond low Earth orbit. This includes a combination of high-energy protons and heavy ions such as Fe56, Si28, and O16. Low dose radiation induced damage is observed months or years after exposure. Our preliminary findings observed that GCR induced degradation of cardiac function with a phenotype that was similar to that observed following doxorubicin treatment. Although there are significant differences from GCR, survivors of cancer that have undergone low-LET (linear energy transfer) radiotherapy are also at risk for several adverse health outcomes including abnormal pulmonary function, endocrine disorders, neurocognitive impairment, and heart failure. All these organ systems are characterized by a low turnover of cells and it is possible that an accelerated cell death and/or the failure of regeneration by progenitor cells may be the underlying cause of organ failure. Although this project initially focused on protection from cardiomyopathies, our findings have implications across all organ systems. These projects have focused on developing countermeasures to GCR using small molecules from a FDA (Food & Drug Administration) approved library, as well as additional molecules identified by NASA personnel as high priority compounds. These drugs are part of other ongoing investigations and their inclusion will be useful in making comparisons across platforms. With regard to the Map to Human Research, this project primarily addresses two Risks. All are designated as High LxC for longer endurance missions or long-term health and wellbeing. Countermeasures fall into three categories; radio protectors are given prophylactically or concurrently to prevent damage. Radiation therapeutics are those that stimulate repair or regeneration processes. Radionuclide eliminators discorporate or block absorption of internalized radionuclides. This project will focus on radio protectors and radiation therapeutics with the hope of developing protocols that will diminish the need for radiation therapeutics.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	The focus of this NASA funded research project is to develop countermeasures to cosmic radiation exposure with the goal of protecting flight crews on long duration missions. However, the findings of this project will also benefit those with more Earth-bound problems. We know that airline pilots and flight attendants have a small but significantly higher risk of cancer that is directly attributable to the chronic exposure to cosmic radiation during their careers. Radiation therapy has been used for the treatment of cancer for many years, and it has long been known that these survivors are at risk for other illnesses related to their treatment. Proton Therapy is an increasingly popular radiation protocol for cancer treatments. This protocol generates similar types of radiation and energy levels that are part of the solar radiation spectrum. And unfortunately, we live in an age when terrorists might eventually gain access to weapons that will generate very high radiation exposures. Hopefully this won't happen but the lessons learned from the present investigation will have overlap to the nuclear countermeasures that others are studying. The results from the current project will hopefully contribute to the knowledge base that other fields will find useful.
	Our approach has utilized a high throughput screen of small molecules from an FDA approved drug library. We have as a partial bias, degradation of mitochondrial function as one readout. Several studies using RNA-seq and other "omics" analyses have repeated identified mitochondrial dysfunction as participating in the response to high-LET radiation [4-6]. Mitochondrial dysfunction is also one antecedent event in the transition from a healthy to cancerous cell and in part underlies the Warburg effect, a hallmark of cancer cells [7-10]. Related to this, radiation-induced cardiac dysfunction leading to heart failure remains a significant clinical problem. The heart is almost completely reliant on aerobic metabolism and healthy mitochondria are critical for cardiomyocyte function. Some of our preliminary studies have demonstrated degradation of cardiac function and mtDNA integrity as a delayed consequence of low dose 56Fe (50 cGy) radiation exposure. We have participated in several campaigns at the NASA Space Radiation Laboratory (NSRL). All protocols to date have use cultured cells including H9c2 (myoblasts), RBL-2H3 (mast cell), Hy926 (endothelial), and ES-D3 (stem/pluripotent cell). For all campaigns, cells were exposed to a total of 75 cGy using the simplified 5-ion GCR protocol developed by NSRL. Our initial studies used a follow-on paradigm, where the drugs were introduced shortly after GCR exposure. Of 725 drugs, more than 500 showed degradation of cellular function or were ineffective for managing exposure to GCR. Within the160 drugs observed to be useful, 33 are known anti-inflammatories, while some other were also thought to have a lesser capacity as anti-inflammatory. The library included 54 COX inhibitors and of the 7 deemed effective all were COX-2 specific. Meta-analysis of COX-2 inhibition found that the anti-inflammatory effect reduced metastases following primary cancer [11]. In the context of our studies, it is important to understand that the H9c2 cell line contains the proinflammatory TLR4/NFkappaB/TNFalpha pathways which may underlie its radiation-induced increases in IL-1ß and TNFalpha and other cytokines [12-15]. 12 of 25 angiotensin converting enzyme inhibitors or AT1 antagonists were observed to be effective, while no AT2 antagonists appeared useful. The library contained 20 drugs that bound to adrenergic receptors of which 5 were deemed effective, although given their direct impact on blood pressure they are not likely to be useful as countermeasures. A common theme among the effective drugs was that they are mediated by GPCR/G coupled proteins, a class of signaling proteins that mediate and control cellular function. Distinct from those described, 5-HT3 antagonists appear to be useful. Those found not to be useful were non-selective or had high affinity for other 5-HT receptor isoforms (i.e., 5-HT2a). Unlike other 5-HT receptors which are GPCR/G coupled proteins, the 5-HT3 receptors are Ca+2 activated small K+ ion channel receptors. Others have reported that 5-HT3 antagonism may ameliorate the effects of damaging radiation and may serve to protect against radiation-induced bystander effects [16, 17]. Clinically the 5-HT3 antagonists are used for the treatment of nausea and vomiting and are currently serving in that capacity aboard the International Space Station (ISS). The follow-on paradigm examined the restorative capability of a candidate drug when presented shortly following exposure to GCR and a short list of useful leads was developed. To determine if the delayed presentation of protective drugs impacted on recovery, cells exposed to GCRsim or control conditions were maintained in culture for one month before treatment was undertaken. Although most drugs were without effect, a few were still protective even when treatment was delayed. This included metformin and sulfacarbamide, both antidiabetic therapeutics as well as dolasetron, a 5-HT3 antagonist. Using a pretreatment paradigm in which drugs were added the day prior to GCR exposure and then the cells were maintained in culture for a week, we have found that metformin, sulfacarbamide, and olmesartan offered the highest level of protection. Several others including bilastine, ramelteon, or granisetron also demonstrated some protective

Task Progress:

efficacy.

We have as a partial bias, degradation of mitochondrial function as a central readout. Several studies using RNA-seq and other “omics” analyses have repeatedly identified mitochondrial dysfunction as participating in the response to high-LET radiation. Mitochondrial dysfunction is one antecedent event in the transition from a healthy to cancerous cell.

Separate from examining mitochondrial and cellular dysfunction as readouts, we have developed a novel cell line using an Afp-tdTomato construct, as a biomarker for the transition to a cancerous cell. The construct is analogous to the Afp-mCherry construct described by M. Weil for the Carcinogenesis NASA Specialized Center of Research (NSCOR) [19]. Validation protocols using either ethidium bromide (0.4 µg/ml) treatment or UVC light (4 j/m2) exposure significantly increased Afp-tdTomato expression. GCRsim (75 cGy) significantly increased tdTomato expression compared to the No-Radiation control cells. Using the follow-on protocols, to date only a select group of drugs have been tested. Of those dimethyl fumarate, sulfacarbamide, and pargyline all appear to be promising leads.

We have developed a Afp-tdTomato expressing cell line that is responsive to GCR as well as different carcinogenic reagents. We have also demonstrated that some of the lead candidates will at least partially rescue the cells from GCR induced Afp driven expression. We are currently evaluating the efficacy of other biomarkers thought to beleading indicators for carcinogenesis: neuron specific enolase (SCLC, neuroblastoma), tyrosinase (breast), EGFR (NSCLC), HER2 (breast, ovarian, pancreatic, & g-i), LDH-A (lymphoma, leukemia, Warburg). We are currently evaluating the impact of GCR on the expression levels of these biomarkers in the Hy926 and H9c2 cell lines. Those results should will allow us to identify which biomarkers are more responsive to GCR. The use of the tdTomato expression vectors will allow for screening protocols to identify the transformation processes towards carcinogenesis.

The Comet DNA Assay, gammaH2Ax levels, and micronuclei formation have all been used to measure DNA damage in response to stress environments. These measures are important since they focus on one major pathway leading to carcinogenesis. Colony formation or the clonogenic assay has long been used as a measure of the cell’s proliferative ability [20]. Successful cellular proliferation incorporates many different aspects of cell well-being including DNA integrity as well as cellular and mitochondrial function. We have adapted the cell doubling time protocols to examine the impact of GCR on cell function. Similar to the clonogenic assay this approach also examines overall cell well-being. Unlike the clonogenic assay that typically has an incubation time of 2-3 weeks, our protocol incubates the cells for only 2-3 days. We have previously adapted this protocol to demonstrate that a single exposure to doxorubicin had an early and persistent effect to increase cell doubling time reflective of continued cell stress1. Recently we have been able to test this potential approach. We observed that with GCR (75 cGy GCRsim) treatment, the irradiated cells had a significant increase in cell doubling time. Of the select group of candidates, we observed that dimethyl fumarate or olmesartan significantly decreased cell doubling time of the GCR treated cells. The use of the flow cytometer to analyze these cells confers a novel advantage in that we are able to measure forward scatter and side scatter of all cells. Forward scatter is an estimator for cell size, while side scatter reflects intracellular complexity. We have used this approach in the past to differentiate mature from immature mast cells [1]. The Forward*Side Scatter Index is the product using the geometric means for forward and side scatter from each sample. To some extent it is analogous to the ColonyArea function available from Image J for the clonogenic assay [21]. Although somewhat preliminary we have observed that GCR treatment significantly depressed the Forward*Side Scatter Index.

Cancer development from a single cell undergoing transformation progresses through the steps of initiation, promotion, proliferation, to metastasis. It is not likely that the cell doubling time protocol or the colony formation assay alone could differentiate between healthy or malignant growth. The Forward*Side Scatter Index may be useful. The proliferative phase of cancer development is characterized by accelerated growth but potentially unlikely that these cells would develop the same level of complexity or size of a normal cell. In conjunction with lactate release or lactate dehydrogenases release assays could potentially differentiate between these different forms of proliferation, which would be useful in a countermeasure screen.

The focus of these projects has been to perform a high throughput screen to identify reasonable countermeasures to GCR for long duration missions. Starting from a FDA approved drug library we have taken the candidates from several hundred down to a select group nearing twenty. The complexity of the problem is that the response to space radiation is more than just ion species, time, or dose dependent. It is also dependent on the specific risk factor that is being ameliorated--that different organ systems may have different sensitivities. However, there are common threads, that include [2] chronic inflammation, chronic elevated oxidant stress, and direct DNA damage. These are all relevant whether one is focused on cardiomyopathy, carcinogenesis, or a decline in cognitive function. All are partially mediated through mitochondrial dysfunction which warrants the attention paid to it thus far. Going forward, more closely examining the common signaling pathways associated with the initiation of carcinogenesis or cardiomyopathy to identify the best leading indicators as biomarkers may offer the best path for countermeasure development. This will be followed by development of the tdTomato expression vectors that should facilitate future screens.

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