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Project Title:	Counteracting Space Radiation by Targeting Neurogenesis in a Human Brain Organoid Model		
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Comments:			
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No. of Master's Candidates:		No. of Bachelor's Degrees:	
No. of Bachelor's Candidates:		Monitoring Center:	TRISH
Contact Monitor:		Contact Phone:	
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Key Personnel Changes/Previous PI:			
COI Name (Institution):	Grosshans, David M.D., Ph.D. (The University of Texas M.D. Anderson Cancer Center) Gaber, Mostafa Ph.D. (Baylor College of Medicine) Young, Damian Ph.D. (Baylor College of Medicine) Liu, Zhandong Ph.D. (Baylor College of Medicine)		
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Task Description:

Neurogenesis, the generation of new neurons throughout life, is essential for formation of new spatial memory and mood control in the hippocampus. New neurons are formed from neural stem cells, which are very sensitive to all forms of radiation. If exposed, they die and therefore, neurogenesis declines leading to decline in learning and memory as well as depression. Therefore, understanding this phenomenon in the context of space radiation is of utmost importance if we are to avoid at least some of the cognitive and mental health pathologies during spaceflight.

Herein, we propose to examine neurogenesis in the human brain organoid models exposed to Linear Energy Transfer (LET) proton beam to mimic Galactic Cosmic Rays (GCR). These models are ideal not only to examine the effects of radiation on neural stem cells but also the effects of drug compounds, because they provide a high-throughput system with multiple neurogenic sites (rosettes) and diverse cell types including both neurons and astrocytes. We will use two complementary cerebral organoid models and will expose them to the proton beam at the MD Anderson Proton Center at different time points and different frequency of exposure and will examine molecular, metabolic, cellular, and physiological properties of the variety of cell types that are part of the neurogenic niche. Furthermore, we will take advantage of our new small molecules that promote neural stem cell self-renewal and neurogenesis in animal models in vivo. Based on their mechanism of action, they may also decrease microglial inflammation; thus, targeting multiple elements of the neurogenic niche. In addition, we will use transient bursts of electrical stimulation as a non-pharmacological countermeasure to GCR radiation, as increased neuronal activity promotes neurogenesis.

Overall, our proposal is responsive to all requirements of this solicitation and stands to deliver new data relevant to the effects of space radiation on multiple human cell types that are part of the neurogenic niche and the efficacy of countermeasures on molecular, metabolic, cellular, and physiological properties of these cells. Our work focuses on neurogenesis as this is the only natural mechanism to regenerate lost brain tissue in vivo in the center for learning and memory and mood control. Thus, the relevance to this solicitation and NASA objective in general is high.

Rationale for HRP Directed Research:

This proposal arises from our pioneering studies on adult neurogenesis. We have long known that low levels of neurogenesis are associated with cognitive decline and depression in humans and animal models. However, because no one could measure this process in the human brain in vivo, discovery in this critical area has been stagnant. Our team was the first to demonstrate that neural stem cells (NSCs) that generate newborn neurons are abundant in mono-unsaturated fatty acids (MUFAs). These fatty acids are detectable both in vitro and in vivo, including in the human brain. We also showed that 18:1?9 MUFAs (oleic acid) bind to tailless receptor (TLX) (a nuclear receptor critical for NSC proliferation and neurogenesis) and that this binding increases NSC proliferation and neurogenesis. We now use this knowledge to counteract the detrimental space radiation effects on NSCs and neurogenesis. To accomplish this goal and, eventually, develop efficient clinical interventions, we use the most innovative chemistry, biophysics, molecular imaging, and complex human brain organoid models available to determine the cellular, molecular, metabolic, and neuroimmune effects of space-like radiation on NSCs and neurogenesis and then counteract with potent stimulators of neurogenesis.

To stimulate NSCs and neurogenesis, we engaged novel synthetic chemistry to identify small molecules that can modulate the function of TLX and stimulate the generation of newborn neurons, creating a paradigm shift in our quest to achieve cell-based repair and brain regeneration in the future. To this end, we will employ a highly innovative platform developed in Dr. Young's lab to generate potent and selective drugs based on Fragment-Based Drug Discovery (FBDD). As an alternative strategy to high-throughput screening (HTS) of large screening collections, FBDD focuses on the development of optimal ligands. FBDD originated in the early 1990s when Jencks found that low molecular weight (MW) and weak-binding ligands to protein targets are actually highly efficient binders. A decade later, medicinal chemists began to appreciate that these "fragments" were valuable starting points for developing lead-like compounds. Fragments are generally defined as small molecules with a MW<300Da that bear fewer than 20 heavy atoms. Although fragments bind to their targets with low affinity, their ligand efficiency (defined as the free energy of binding per heavy atom) is high. Given this thermodynamic efficiency metric, fragment hits—which are ideally contoured to the small area in which they bind to a given protein—are nearly optimal binders. We can convert the weak-binding fragment into a more potent modulator by using synthetic organic chemistry to opportunistically place substituents or "grow" the fragment to increase productive interactions within the binding pocket. Fragments' smaller size also allows us to explore the chemical space they occupy more efficiently. Accordingly, a library of several thousand appropriately chosen fragments is usually sufficient to yield high-quality binders for a particular target rather than the millions of compounds required for HTS. Fragment-screening is generally achieved using highly sensitive biophysical experiments capable of detecting weak fragment-target binders. With FBDD, however, we need fewer compounds and can use biophysical approaches to develop versatile screening platforms, enabling us to produce results in a more efficient, cost-effective way. The Young lab has synthetically constructed a highly 3D-diverse fragment library toward the goal of modulating a wide swath of biological targets. These fragments have already shown to be promising starting points for developing novel TLX ligands, and thus, stimulating NSCs to produce new neurons when needed.

Research Impact/Earth Benefits:

Neurogenesis, the generation of new neurons throughout life, is essential for formation of new spatial memory and mood control. New neurons are formed from neural stem cells, which are very sensitive to all forms of radiation. If exposed, they die; and therefore, neurogenesis declines, leading to decline in learning and memory as well as depression. Thus, understanding this phenomenon in the context of space radiation is of utmost importance if we are to avoid at least some of the cognitive and mental health pathologies during long-term spaceflights.

In our proposal, we aim to examine neurogenesis in the human brain organoid models exposed to proton beam to mimic Galactic Cosmic Ray (GCR) irradiation. These models are ideal not only to examine the effects of radiation on neural stem cells but also the effects of countermeasures because they provide a high-throughput system with multiple neurogenic sites (rosettes) and diverse cell types, including both neurons and astrocytes. To ensure model-independent, robust findings, we use two complementary cerebral organoid models exposed to the proton beam at the MD Anderson Proton Center at different timepoints and different frequencies of exposure. To examine the effects of GCR-like irradiation, in Aim 1 we examine molecular, metabolic, cellular, and physiological properties of the variety of cell types that are part of the neurogenic niche. In Aim 2, we test the effects of our new small molecules that target TLX, a nuclear receptor that promotes neural stem cell self-renewal and neurogenesis in animal models in vivo. Based on their mechanism of action, these small molecules may also decrease microglial inflammation, thus targeting multiple elements of the neurogenic niche. In addition, we will use transient bursts of electrical stimulation as a non-pharmacological countermeasure to GCR radiation, as increased neuronal activity promotes neurogenesis.

Task Progress:	<p>Over the course of the past year, we have made major accomplishments in our proposed experiments. First, we have developed, tested, and validated a proton beam delivery platform that enables irradiation of numerous organoids in a single setup, across doses and Linear Energy Transfers (LETs). This platform enables testing of the proton beam GCR-like radiation on multiple organoids at the time, highly affecting the feasibility of our proposed experiments. Following testing of several dosages and LETs, we have established that 0.5Gy low LET proton beam is sufficient to cause apoptosis in the human brain organoid models. We examined the effects on apoptosis at different timepoints, from 2-30 days following irradiation, using immunostaining, polymerase chain reaction (PCR), flow cytometry, and lactate dehydrogenase assay. We observed increased apoptosis very early on (2 and 4 days following irradiation), and the effect remained very robust 10 days thereafter, when irradiated organoids were significantly smaller and the expression of apoptotic genes was high compared to non-irradiated ones. Flow cytometry indicated that increase in apoptosis occurs up to 26 days following irradiation, but to a lesser degree compared to early timepoints. We are currently completing a new set of data to examine the reproducibility of our findings and to determine which cell types and pathways are particularly sensitive to 0.5Gy low LET proton beam. Further, we have developed a set of synthetic molecules using fragment-based drug discovery to modify an FDA-approved parent drug, NSI-189, which we discovered was a weak TLX agonist. Out of about 30 generated compounds, we determined the two that had the most robust effect on neurogenesis in healthy organoids. Finally, we have completed a new analytical algorithm, developed to data mine longitudinal metabolomic datasets. We have identified key metabolic points critical for proper differentiation of neurons in healthy conditions (paper in preparation). We will further develop this tool within this grant to apply it to other datasets we generate, because it can be easily modified to input any type of data queried by multivariate approaches—particularly data acquired in the longitudinal studies as we proposed here.</p> <p>In Year 2 of the grant, we will continue to delve deeper into the mechanistic aspect of the radiation-induced phenotype in our human brain organoids using single cell RNA sequencing and cell-type specific analyses. Further, we will test the two proposed countermeasures: 1. the new synthetic TLX agonists, which we will examine in the context of irradiation, and 2. the electrical stimulation, which we just started to optimize. These data will move us closer to the ultimate goal—to take advantage of our natural capacity to repair and regenerate the brain, which is particularly relevant for space travel. If successful, at the end of this grant period, we might have a new therapeutic modality to accelerate pre-clinical trials for enhancing neurogenesis in vivo.</p>
Bibliography Type:	Description: (Last Updated: 10/20/2022)
Articles in Peer-reviewed Journals	<p>Bokhari RS, Beheshti A, Blutt SE, Bowles DE, Brenner D, Britton R, Bronk L, Cao X, Chatterjee A, Clay DE, Courtney C, Fox DT, Gaber MW, Gerecht S, Grabham P, Grosshans D, Guan F, Jezuit EA, Kirsch DG, Liu Z, Maletic-Savatic M, Miller KM, Montague RA, Nagpal P, Osenberg S, Parkitny L, Pierce NA, Porada C, Rosenberg SM, Sargunas P, Sharma S, Spangler J, Tavakol DN, Thomas D, Vunjak-Novakovic G, Wang C, Whitcomb L, Young DW, Donoviel D. "Looking on the horizon; potential and unique approaches to developing radiation countermeasures for deep space travel." Life Sci Space Res. 2022 Aug 7. https://doi.org/10.1016/j.lssr.2022.08.003 , Aug-2022</p>
Articles in Peer-reviewed Journals	<p>McNerlin C, Guan F, Bronk L, Lei K, Grosshans D, Young DW, Gaber MW, Maletic-Savatic M. "Targeting hippocampal neurogenesis to protect astronauts' cognition and mood from decline due to space radiation effects." Life Sci Space Res. 2022 Jul 29. https://doi.org/10.1016/j.lssr.2022.07.007 , Jul-2022</p>