EXPLORING THE ROLE OF PIWI/piRNA PATHWAY IN EPIGENETIC DYSREGULATION IN LOW DOSE RADIATION INDUCED CARDIOVASCULAR DISEASE. O. A. Jejelowo and M.A. Tariq. Global Progressive Network, LLC., 3410 Park Springs Lane, Kingwood, Texas 77345. gracedtf@gmail.com.

Introduction: Human explorers will experience similar flux of interplanetary solar and cosmic radiation in a cislunar space as on a trip to Mars. There is a need to ensure healthy crews operating at peak performance during long-duration space exploration to cislunar and beyond. Exposure to ionizing radiation constitute a change from the normal cell environment and can result in dysfunctions to almost all organs of the body depending on the total dose and site of irradiation.

Opportunity: For example, exposure to radiation hazards could cause cardiovascular disease and other degenerative tissue effects (Degen) [1]. To identify and understand the risks of Degen due to radiation exposure and design countermeasures to mitigate these risks, we must understand the underlying cellular responses. We need to identify biomarkers specific to space radiation, investigate the regulatory switches of inflammation and identify cross-risk biomarkers. Information will aid development of countermeasures to regulate the low dose radiation inflammatory response in astronauts. The cumulative biological effects of low dose space radiation responsible for atherosclerosis and heart attack include chronic inflammation and endothelial cell alterations. Both hypo- and hyper- methylations occur in major inflammatory biomarkers of radiation. We seek to further understand the mechanism for epigenetic switches and modifiable risk factors. The Piwi-interacting RNAs (piRNAs) a class of 26- to 32- nt non-coding RNAs are thought to play a role in the inflammatory processes. The piRNAs typically form RNA-protein complexes, by partnering with PIWI proteins. These RNA-protein complexes then silence the transposable elements (TEs). The Piwi-piRNA pathway is also linked to somatic functions such as, genome rearrangement, epigenetic programming, stem cell function, whole-body regeneration, memory and possibly cancer. The epigenetic programming function could involve transcriptional repressions, via establishment of a repressive chromatin state; or DNA methylation of promoter region of the target gene. Methylation is the major regulatory mechanism in most inflammatory response genes and chronic inflammation is important in many diseases, including neurodegenerative diseases, cancer, autoimmunity and infections. To understand the regulatory switches of inflammation, identify cross-risk biomarkers and design countermeasures to mitigate these risks, we will study freshly irradiated and archived irradiated tissue samples provided by NASA. Specifically, we will study the effects of radiation on epigenome and targeted proteome of inflammatory pathways relative to CVD at the cell, tissue and organismal levels. We will identify cross-risk biomarkers for cardiovascular disease and other degenerative tissue effects caused by radiation exposure as well as surrogate endpoints that could be used to access the efficacy of radio-protective countermeasures. Characterizing epigenetic and genome wide epigenomic profiles provides the capability to modulate epigenetic factors. Thus, they represent novel powerful paradigms for identifying and monitoring the development of cardiovascular disease in real time, while providing opportunities for halting, preventing and possibly reversing the condition. Given that CVDs constitute the number one cause of death globally, information on applicable gene-regulatory functions is valuable to humans during space exploration missions and on earth.

Our discussion will focus on four main Thrusts:

I. **Epigenetic changes:** Identify entire set of differentially expressed piRNAs, DNA methylation and histone modification patterns of irradiated murine blood vessel and heart tissues.
II. **Biomarkers of inflammation:** Identify target proteins for dysregulated piRNAs by informatics.
III. **Signaling molecules and pathways:** Elucidate and characterize signaling molecules and pathways regulating inflammation by utilizing piRNAs as epigenetic switch in cardiovascular disease using primary endothelial cells culture system.
IV. **Countermeasures**: Identify countermeasures to reverse epigenetic effects.

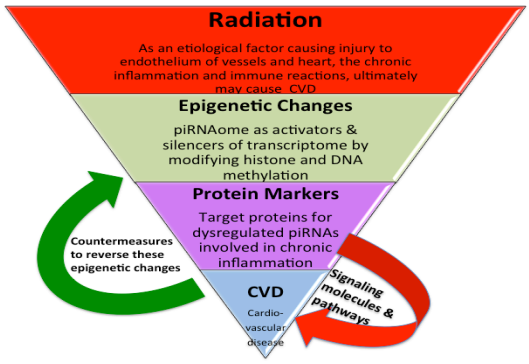

Figure 1: Research Areas.

Methods:

Epigenetics: Radiation exposures present serious risks to astronauts. There is a need to understand the pathophysiology of CVD and other degenerative diseases due to space radiation exposure..

Objective 1.1. To Investigate differential Expression of piRNA and transcriptome by Next Generation Sequencing (NGS). Cells and murines subjected to radiation will be monitored for altered piRNA and whole transcriptome epigenetic changes.

Objective 1.2. ***To Study Differential Expression of DNA and Histone Methylation Patterns.*** We will examine cells and murines subjected to radiation for epigenetic changes including DNA and histone methylation patterns, and accumulation of DNA damage.

Biomarkers: Biomarkers are important assessment tools for prediction, diagnosis and monitoring of diseases.

Objective 2.1. ***Use informatics to identify the proteins targets which dysregulated piRNAs use in epigenetic regulation.*** We will use existing statistical methods and when applicable develop new methods for modeling data that enable identification of protein targets of dysregulated piRNAs.

Objective 2.2. ***Use informatics to identify the proteins targets which dysregulated piRNAs use in epigenetic regulation.*** Statistics will also be used to obtain epigenetic regulation signatures of piRNA protein targets.

Objective 2.3. ***To establish theoretical basis for selecting biomarkers of inflammation during radiation exposures***. Chemical and biochemical protocols for detecting and quantitatively evaluating radiation stress damage and inflammation will be integrated. We will use genomics, transcriptomics and proteomics tools to select biomarkers of inflammation for *in vitro/in vivo* testing. The theoretical basis for inferences will also be explained using available literature.

Signaling Molecules and Pathways: High energy particle radiation causes cellular damage, and the resulting inflammation is thought to be involved in the pathophysiology and prognosis of CVD. However, we have limited knowledge of the mechanisms involved**.**

Methods (Research Design)

Objective 3.1. To examine expression of target proteins of piRNAs in vitro. We will examine differential expression of target piRNAs in Human Umbilical Vein Endothelial cells (HUVECs) model. We will then perform mimics and antagomir assays to examine accuracy of predicted target proteins for respective piRNAs from Biomarkers Thrust above.

Objective 3.2. To identify epigenetic changes due to methylation in vitro. We will identify changes in promoter methylation status of target genes, using methylation-specific polymerase chain reaction.

Objective 4. To develop countermeasures for the effects of radiation.

We will test a variety of compounds, including antixodants, curcumin, resveratrol, and various other natural products in an attempt to prevent the damage caused by radiation,. The assays developed in Objective 1 to 3 above will be utilized to determine if these treatments prevent such damage.

Conclusion: Cells inherently respond to changes in their environment by working to maintain genomic integrity through initiation of complex responses that rely on changes in gene expression. Advances in genomics, has opened windows into inner workings of the cell, how cells interact with other cells and respond to changing environments. Additional single 'omic fields are advancing rapidly, and mechanistic studies reveal high integration at both intra and inter-cellular levels. We are learning more about cellular functions through transcriptomics, proteomics, metabolomics and the processes that regulate them, including methylation, acetylations, phosphorylation, ubuiquitination, miRNAs, piRNAs and other non-coding RNAs as well as diet and physiological processes within the human body. It is imperative that we take advantage of these advances to identify robust biomarkers and utilize knowledge to understand diseases, adverse environmental effects and develop countermeasures. Information obtained will enhance development of capabilities to monitor health in real time and for mitigation of risks. Our efforts will not only enhance productive human space exploration, but will also improve the quality of life here on earth.

References:

1. https://humanresearchroadmap.nasa.gov/Risks/risk.aspx?i=98