Gene and microRNA expression profile changes in ISS crewmembers' blood samples

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INTRODUCTION

In space, living organisms are exposed to multiple stress factors including microgravity and space radiation. For humans, these harmful environmental factors have been known to cause negative health impacts such as immune dysfunction. Understanding the mechanisms by which spaceflight impacts human health at the molecular level is critical not only for accurately assessing the risks associated with spaceflight, but also for developing effective countermeasures. This study is part of the Functional Immune Project, intended to determine alterations in crewmember immunobiology before, during, and after spaceflight. It emphasizes the study of DNA damage in the ISS crewmembers' peripheral blood mononuclear cells (PBMCs), expression patterns of damage-response and inflammatory process genes, and changes in latent virus reactivation biomarkers.

METHODOLOGY

For this project, blood samples were collected at two pre-flight time points of 180 days (L-180) and 45 days (L-45) before launch. During flight, blood was drawn around the midpoint (mid-flight, MF) of the mission, and shortly before egress from the ISS (late-flight, LF). Post-flight blood samples were collected within 24 hours after landing (R+0), and at two other time points 30 (R+30) and 90 days (R+90) after landing. For every crewmember, blood was also drawn from a matching test subject on the ground at the corresponding time point. Density gradient centrifugation was performed to isolate the peripheral blood mononuclear cells (PBMCs) from the whole blood, washed with PBS and lysed with lysis buffer. Lysates were frozen at -80ºC until several crewmembers completed all sampling timepoints. RNA was isolated from lysates using a Qiagen AllPrep kit.

The RNA samples were sent to the University of Wisconsin-Madison Gene Expression Center for RNA sequencing analysis. Using next-generation sequencing (NGS) data, we present preliminary results of expression patterns in transcriptomic (mRNA) and microRNA (miRNA) targets in the crewmembers' PBMCs during and following an ISS mission.

The differentially expressed genes were analyzed for associated pathways using the Ingenuity Pathway Analysis (IPA) tool.

RESULTS

After mRNA and miRNA were quantified with RNA-seq, expression changes in the astronauts at the pre- in- and post-flight time points were determined by comparing to the ground matching controls.

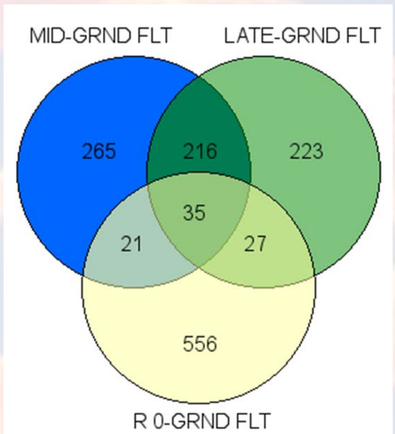

Figure 1. Venn diagram of mRNA expression changes at MF, LF and R+0 in the crewmembers in comparison to the ground controls. About half of the genes are common between MF and LF, but only a small fraction of the genes are common between MF/LF and R+0.

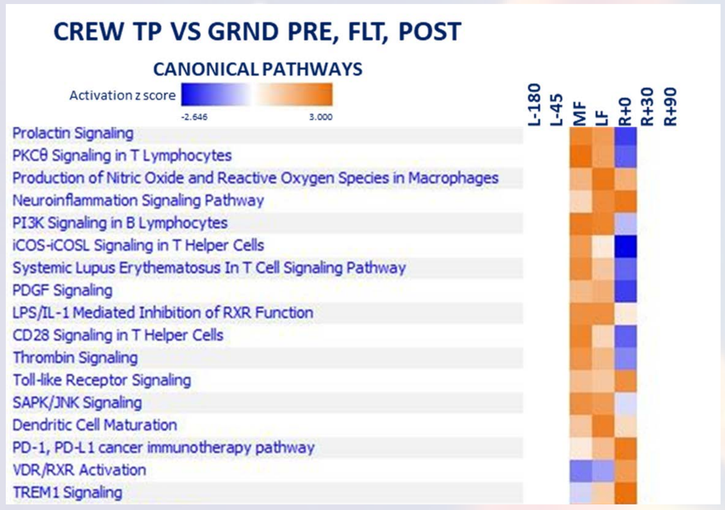

Figure 2. Pathway analysis of the differentially expressed genes at MF, LF and R+0 using IPA. Oxidative stress and inflammation signaling pathways appear to be common at these three time points.

microRNA	Status
hsa-miR-873-5p	DOWN
hsa-miR-125a-5p	DOWN
hsa-miR-139-5p	UP
hsa-miR-409-5p	DOWN
hsa-miR-654-3p	DOWN
hsa-miR-376c-3p	DOWN
hsa-miR-181a-2-3p	DOWN
hsa-miR-369-5p	DOWN
hsa-miR-323b-3p	DOWN
hsa-miR-1307-3p	UP
hsa-miR-369-3p	DOWN
hsa-miR-485-3p	DOWN
hsa-miR-134-5p	DOWN
hsa-miR-382-5p	DOWN
hsa-miR-1911-5p	UP
hsa-miR-409-3p	DOWN
hsa-miR-411-5p	DOWN
hsa-miR-127-3p	DOWN
hsa-miR-539-3p	DOWN
hsa-miR-10a-5p	DOWN
hsa-miR-154-3p	DOWN
hsa-miR-10399-3p	UP
hsa-miR-494-3p	DOWN

Table 1. Some of the differentially expressed miRNA at MF and LF in comparison to the ground matching controls.

DISCUSSION

- Results of the preliminary analysis indicate that most of the gene expression changes occurred at MF, LF and R+0, whereas most of these expression levels returned to baseline by R+30.
- Network analysis of the RNA data suggests a spaceflight effect on pathways not only in the immune system, but also on others involving neuroinflammation and oxidative stress.
- A number of miRNAs were differentially expressed in space. Some of the miRNAs, such as miR-139, are known to be associated with microgravity exposures.

Acknowledgement

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