Gene expression changes in peripheral blood mononuclear cells of ISS crewmembers suggest impacts of spaceflight on cell death

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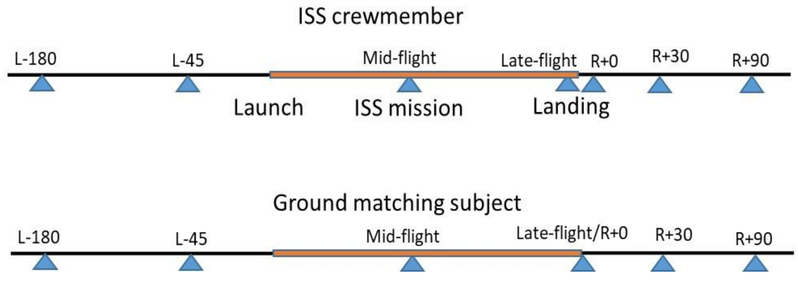
Abstract

In space, living organisms are exposed to numerous 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 crewmembers` immunobiology before, during, and after spaceflight. For this project, blood samples were collected from International Space Station (ISS) crewmembers at the following time points: i) at two pre-flight time points of 180 days (L180) and 45 days (L45) before launch. ii) During flight, blood was drawn at approximately the midpoint (mid-flight, MF) of the mission, and shortly before egress from the ISS (late-flight, LF). iii) Post-flight blood samples were collected within 24 hrs (R0), 30 days (R30) and 90 days (R90) after landing. For each crewmember, blood was also drawn from a matching test subject on the ground at the corresponding time point. For both the ISS crewmembers and the ground control subjects, total RNA was isolated from peripheral blood mononuclear cells (PBMC) and mRNA was analysed using next generation RNA-sequencing (NGS). Differentially expressed genes were determined by performing contrast analysis. Using the data from all of the time points from the ground control subjects as a control, a number of dysregulated genes were identified in astronauts at MF, LF and R0, including downregulations of several cell cycle related genes including CDKN1A and VEGFA at MF and LF. Pathway analysis of these differentially expressed genes indicated that, in space, pathways associated with autophagy and senescence were affected. Our analysis also indicated that the genes related to metabolisms were downregulated in the microgravity environment. Taken together, we hypothesize that PBMC in the ISS crewmembers may be starved, resulting in autophagy and delayed senescence in space. Such findings are in agreement with delayed cell death in PBMC under simulated microgravity conditions on the ground and offer an explanation for telomere lengthening that has been reported among the ISS astronauts in flight.

Methods

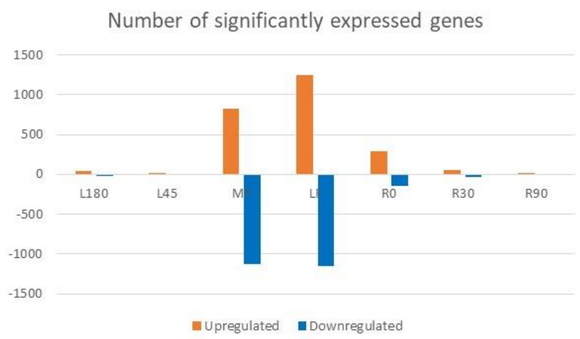
- Blood drawn in flight was transported to JSC at ambient temperature.
- For crewmembers, the R0 samples were drawn immediately after returning to JSC, or in the case of international astronauts, after returning to DLR .
- Blood samples drawn from crewmembers and from ground matching subjects at JSC were aged.
- Data from 8 crewmembers are presented here.

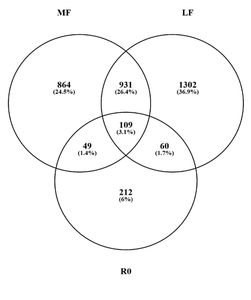
Blood collection schedule (Functional Immune)



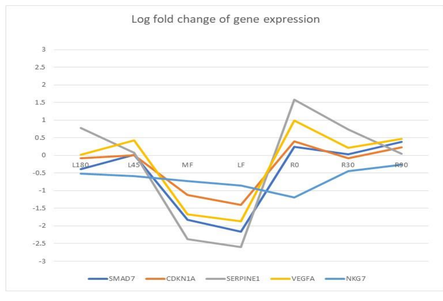
- RNA was isolated from peripheral blood mononuclear cells (PBMC).
- Transcriptomics analysis was performed using RNA-seq.
- Differentially expressed genes were determined by performing contrast analysis, using all time points of the ground control subjects combined as a control.
- The fold change threshold is 1.5 and the FDR < 0.05.

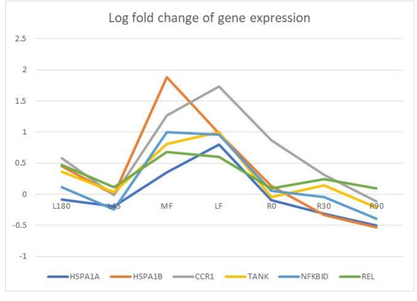
Results





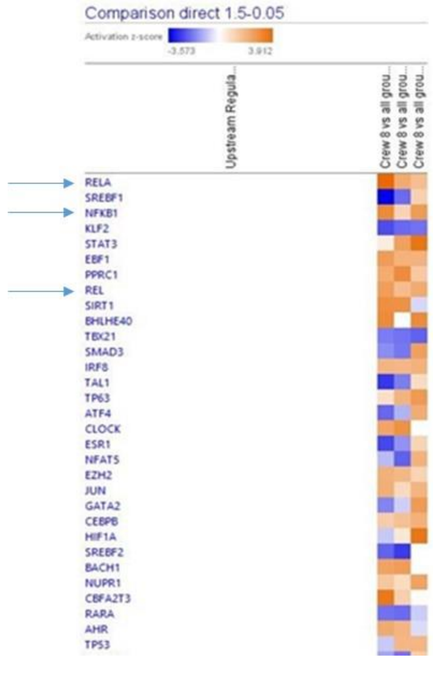
Selected differentially expressed genes



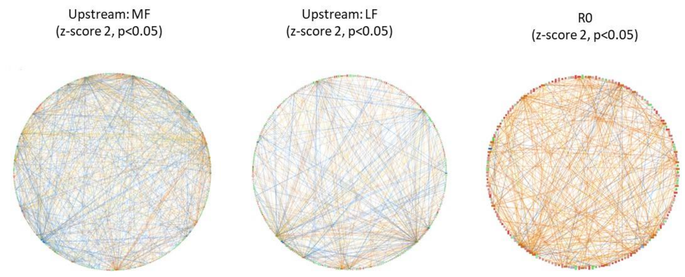


- CDKN1A (p21) was downregulated in space.
- Several NFkB and NFkB inhibitor genes were upregulated in space.

Upstream regulators
(Ingenuity Pathway Analysis, IPA)

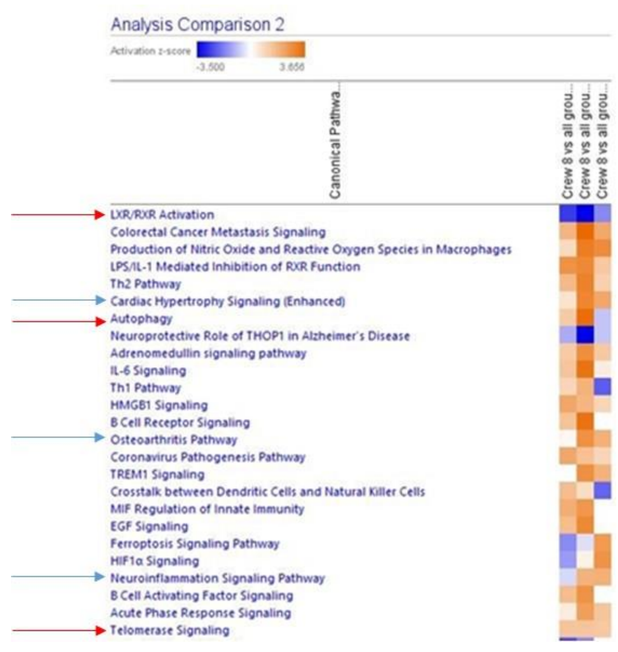


- Upstream regulators were determined using z>2.0 and p<0.05 as thresholds.
- No significant upstream regulators were identified for other pre- and post-flight time points.
- NFkB genes (NFKB1, REL and RELA) were identified as upstream regulators.



- Most of the regulation relationships were activation at R0.
- Does the data indicate adaptation at LF?

Canonical pathways
(Ingenuity Pathway Analysis, IPA)



- The impacted canonical pathways are related to metabolisms, as well as to cardiac hypertrophy, osteoarthritis and neuroinflammation (Z>2, p<0.05). These pathways are activated more at LF.

Conclusions

- Results of the study indicate that most of the gene expression changes occurred at MF, LF and R+0, with most of these expression levels returning to baseline by R+30.
- Our data suggests chronic activation of the NFkB pathway during flight.
- A number of cell cycle related genes such as p21 and VEGF were downregulated in space. Our data suggests delayed cell cycle progression of PBMC in the microgravity environment. The pathways associated with autophagy and senescence were affected. The impact on the cell growth/cell death may be related to the decreased metabolic activities in space.
- There appears to be an adaptation to the space environment at LF time point.
- 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.

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