Using OpenTarget to generate potential countermeasures for long-term space exposure from data available on GeneLab

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What is GeneLab?

• Currently, GeneLab is a (1) data repository that hosts space biology datasets, (2) a collaborative workspace for users to share files and access data analysis tools, and (3) workspace to do metadata curation.
What is GeneLab?

- Currently, GeneLab is a (1) data repository that hosts space biology datasets, (2) a collaborative workspace for users to share files and access data analysis tools, and (3) workspace to do metadata curation.
Ultimately, GeneLab aims to provide a single platform where users can **capture**, **curate**, **store**, **search**, **share**, **transfer**, **analyze**, and **visualize** spaceflight datasets.
Overall, the goal of GeneLab is to allow for better understanding of the impact of spaceflight on biology!
What Already Exists on GeneLab Database: 154 data sets

Data Growth Since 2014

Distribution by organism type

Majority is spaceflight samples

Distribution by assay type
Overall Goal of GeneLab

- Overall goal is to allow for better understanding of the impact of spaceflight on biology using publicly available omics data
  - Generate **Hypothesis** to direct future experimental research
  - Determine acceptable health risks for long-term space missions
  - Develop potential countermeasures against

- A rich resource for both the scientific and non-scientific community to explore questions they have on space biology.
- A platform which can be used by both advanced and basic users to explore all omics data.
Systems biology attempts to understand biological organisms or systems as a whole rather than researching their individual components in isolation from one another.

NIH defines Systems Biology as: “Systems biology is an approach in biomedical research to understanding the larger picture—be it at the level of the organism, tissue, or cell—by putting its pieces together. It’s in stark contrast to decades of reductionist biology, which involves taking the pieces apart.”
Hypothesis generation from GeneLab Data

International Space Station (ISS) Missions

Mice Sacrificed on ISS

Mice flown on STS and Sacrificed after Re-entry

GeneLab
Open Science for Exploration

Process after mice are sacrificed
Sample Processing
Data Sharing
Data Collection & Curation
Next Generation Research
Modeling and Validation

Data Submission
Extensor Digitorum Longus Muscle
Soleus Muscle
Gastrocnemius Muscle
Quadiceps
Tibialis Anterior Muscle
Adrenal Glands
Kidney
Liver

Space Shuttle (STS) Missions
Skin

Time in Space for Mice (days)
Number of Significant Genes from Multiple Datasets

Number of Significant Genes

- ADR
- Kidney
- Liver
- EDL
- GST
- Quad
- SLS
- TA
- Skin
- Thymus
- Liver
- MG
- Skl
- M
- SLS
- EDL

Flight Duration
- 9 days
- 12 days
- 13 days
- 30 days
- 37 days
- 91 days

Species
- Mouse
- Rat

p-value < 0.05
FDR < 0.05

# of genes
Predicted Master Regulators

- p53 found in all tissues
  - p53 is a transcription factor and in response to genotoxic stress, DNA damage, oncogene activation, and hypoxia, it is recruited to sites in chromatin, thus promoting transcription of apoptosis related genes.
Determination of Key Genes/Drivers

Key Genes and the Connections

A) Direct Connections for Key Genes for Flight vs AEM

B) Connections Between all Key Genes for all Datasets (Flight vs AEM): Radial Plot with the most Connected Gene in the Middle
Key Genes and the Connections

- **TGFβ1 found to be central regulator of key genes**
  - TGFβ is known to play a context specific role in sustaining tissue homeostasis predominantly via transcriptional regulation of genes involved in differentiation, cell motility, proliferation, cell survival along with regulating immune responses during homeostasis and infection.
  - Previous Studies found reduction in gravitational force to diminish TGF-β expression and apoptosis with higher carcinoembryonic antigen expression in 3D human colorectal carcinoma cells, as compared to 3D cultures in unit gravity.
  - In another study, differential regulation of blood vessel growth using basic fibroblast growth factor was identified in modeled microgravity with induction early and late apoptosis, extracellular matrix proteins, endothelin-1 and TGFβ1 expression.
Generating a Hypothesis from GeneLab Analysis

Systemic tumor-host effects
Generating a Hypothesis from GeneLab Analysis

Circulating miRNAs

Systemic tumor-host effects
A single miRNA has been estimated to regulate up to 500 mRNAs.

miRNAs are single-stranded RNA sequences, of about 22 nucleotides in length, processed from longer transcripts.

miRNAs are important regulators that repress the translation of mRNA transcripts.
A) Top 10 predicted miRNAs from p-values

B) All miRNAs with Z-scores > 2 or < -2

- miR-125b-5p
- miR-145-5p
- miR-34
- miR-116-5p

Legend:
- Red: Upregulated
- Green: Downregulated
- Orange: Predicted Activation
- Blue: Predicted Inhibition
- Orange: Leads to activation
- Blue: Leads to inhibition
- Yellow: Findings inconsistent with state of downstream molecule
- Black: Effect not predicted
Predicted miRNAs Involved with Microgravity Effects

A) Top 10 predicted miRNAs from p-values

B) All miRNAs with Z-scores > 2 or < -2

Upregulation of miR-223 in the rat liver inhibits proliferation of hepatocytes under simulated microgravity

Yongjie Chen1,2,4, Ji Xu3,4, Chao Yang2, Hongyu Zhang3, Feng Wu2, Jian Chen2, Kai Li2, Haialong Wang3, Yu Li1,2, Yinghui Li1,2 and Zhongqian Dai3

Long-term spaceflight affects numerous organ systems in the body, including metabolic dysfunction. Recently, ample evidence has demonstrated that the liver is a vulnerable organ during spaceflight. However, the changes in hepatocyte proliferation and cell cycle control under microgravity remain largely unexplored. In the present study, we first confirmed that the serum levels of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase, biochemical markers of liver function, were altered in rats under tail suspension (TS) conditions to simulate microgravity, as shown in previous reports. Next, we demonstrated that the cell proliferation activity, determined by Ki67, PCNA and PH3, was significantly decreased at the different TS time points (TS for 14, 28 and 42 days) compared with that in the control group. Consistently, the positive cell cycle regulators Ccna2, Ccnd1, Cdk1, Cdk2 and cyclin D3 were also significantly decreased in the TS groups as shown by quantitative real-time PCR and western blotting analysis. Subsequent analysis revealed that the aberrant hepatocyte proliferation inhibition under simulated microgravity was associated with the upregulation of miR-223 in the liver. We further found that miR-223 inhibited the proliferation of Hepa1-6 cells and identified CDK2 and CUL1 as its direct targets. In addition, the decreased expression of CDK2 and CUL1 was negatively correlated with the level of p27 in vitro and in vivo, which may have been responsible for retarding hepatocyte proliferation. Collectively, these data indicate that upregulation of miR-223 was associated with the inhibition of liver cell growth and reveal the role of miR-223 in rat hepatocyte proliferation disorders and the pathophysiological process under simulated microgravity.

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Predicted miRNAs Involved with Microgravity Effects

A) Top 10 predicted miRNAs from p-values

B) All miRNAs with Z-scores > 2 or < -2

Spaceflight alters expression of microRNA during T-cell activation

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ABSTRACT

Altered immune function has been demonstrated in astronauts during spaceflight dating back to Apollo and Skylab; this could be a major barrier to long-term space exploration. We tested the hypothesis that spaceflight causes changes in miRNA (miRNA) expression. Human leukocytes were stimulated with mitogens on board the International Space Station using an onboard normal gravity control. Bioinformatics showed that miR-21 was significantly up-regulated 2-fold during early T-cell activation in normal gravity, and gene expression was suppressed under microgravity. This was confirmed using quantitative real-time PCR (n = 4). This is the first report that spaceflight regulates miRNA expression. Global microarray analysis showed significant (P < 0.05) suppression of 85 genes under microgravity conditions compared to normal gravity samples. ES30, FASLG, B2G1, SPIP2, and TCF7L2 are biologically confirmed targets and are co-up-regulated with miR-21. These genes share common promoter regions with miR-21, and it is possible that regulation of these genes may affect T-cell function. These data suggest that spaceflight regulates T-cell activation not only by transcriptional promotion but also by blocking translation via noncoding RNA mechanisms. Moreover, this study suggests that T-cell activation itself may induce a sequence of gene expressions that is self-limited by miR-21.

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contracted Pseudomonas auruginosa and experienced intense chills and fever (1). P. aeruginosa is an opportunistic pathogen that rarely causes disease unless the person is immunosuppressed. As a result, the U.S. National Aeronautics and Space Administration (NASA) implemented a peptidoglycan program that subsequent reduced the number of reported infections in a single Apollo mission (1). To this day, the peptidoglycan program is still actively used in both the U.S. and Russian programs. Even with the precautions, autapses in various Skylab (2), Shuttle (3, 4), International Space Station (ISS) (5), and Soyuz (5) showed signs of immune function and depression in immune function as compared to levels before spaceflight. Experiments from Skylab and Shuttle have confirmed that T cells have a suppressed immune response in vivo and in vitro with lower T-cell proliferation, activation, lower IL-2 synthesis and severely reduced IL-2R expression (RNA and protein) (6, 7). More recently, these studies have demonstrated that microgravity has a direct effect on immune responses, as well as affecting the T-cell function in space. The current study demonstrates that microgravity alters the expression of miRNAs involved in T-cell activation and proliferation, which may provide insights into the mechanisms underlying immune suppression during spaceflight. The results suggest that microgravity-induced changes in miRNA expression may play a role in the suppression of T-cell proliferation and activation, potentially contributing to the observed immune suppression observed during long-duration spaceflights (180 days) in terms of viral gene expression that resembled activation patterns seen in healthy controls, while astronauts on board the ISS for longer-duration spaceflights (180 days) had lower and hypervarial gene expression that resembled activation patterns seen in healthy controls.
Predicted miRNAs Involved with Microgravity Effects

A) Top 10 predicted miRNAs from p-values

B) All miRNAs with Z-scores > 2 or < -2

Research Article
Integration Analysis of MicroRNA and mRNA Expression Profiles in Human Peripheral Blood Lymphocytes Cultured in Modeled Microgravity

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We analyzed miRNA and mRNA expression profiles in human peripheral blood lymphocytes (PBLs) incubated in microgravity condition, simulated by a ground-based rotating wall vessel (RWV) bioreactor. Our results show that 42 miRNAs were differentially expressed in MMG-incubated PBLs compared with Ig incubated ones. Among these, miR-9-3p, miR-9-5p, miR-155-5p, miR-150-3p, and miR-378-3p were the most dysregulated. To improve the detection of functional miRNA-mRNA pairs, we performed gene expression profiles on the same samples assayed for miRNA profiling and we integrated miRNA and mRNA expression data. The functional classification of miRNA-correlated genes evidenced significant enrichment in the biological processes of immune/inflammatory response, signal transduction, regulation of response to stress, regulation of programmed cell death, and regulation of cell proliferation. We identified the correlation of miR-9-3p, miR-155-5p, miR-150-3p, and miR-378-3p expression with that of genes involved in immune/inflammatory response (e.g., IFNG and IL10), apoptosis (e.g., PDCD4 and PTEN), and cell proliferation (e.g., NFKB1 and GADD45A). Experimental assays of cell viability and apoptosis induction validated the results obtained by bioinformatics analyses demonstrating that in human PBLs the exposure to reduced gravitational force increases the frequency of apoptosis and decreases cell proliferation.
A) Top 10 predicted miRNAs from p-values

B) All miRNAs with Z-scores > 2 or <-2

Analysis of miRNA and mRNA Expression Profiles Highlights Alterations in Ionizing Radiation Response of Human Lymphocytes under Modeled Microgravity

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Abstract

Background: Ionizing radiation (IR) can be extremely harmful for human cells since an improper DNA-damage response (DDR) to IR can contribute to carcinogenesis initiation. Perturbations in DDR pathways can originate from alteration in the functionality of the microRNA-mediated gene regulation, being microRNAs (miRNA) small noncoding RNA that act as post-transcriptional regulators of gene expression. In this study we gained insight into the role of miRNAs in the regulation of DDR to IR under microgravity, a condition of weightlessness experienced by astronauts during space missions, which could have a synergistic action on cells, increasing the risk of radiation exposure.

Methodology/Principal Findings: We analyzed miRNA expression profile of human peripheral blood lymphocytes (PBL) incubated for 4 and 24 h in normal gravity (1 g) and in modeled microgravity (MMG) during the repair time after irradiation with 0.2 and 2 Gy of γ-rays. Our results show that MMG alters miRNA expression signature of irradiated PBL by decreasing the number of radio-responsive miRNAs. Moreover, let-7p, miR-7, miR-7-1*, miR-27a, miR-146, miR-200a, miR-958, miR-650 are deregulated by the combined action of radiation and MMG. Integrated analyses of miRNA and mRNA expression profiles, carried out on PBL of the same donors, identified significant miRNA-mRNA anti-correlations of DDR pathway. Gene Ontology analysis reports that the biological category of "Response to DNA damage" is enriched when PBL are incubated in 1 g but not in MMG. Moreover, some anti-correlated genes of p53-pathway show a different expression level between 1 g and MMG. Functional validation assays using luciferase reporter constructs confirmed miRNA-mRNA interactions derived from target prediction analyses.

Conclusions/Significance: On the whole, by integrating the transcriptome and microRNAome, we provide evidence that modeled microgravity can affects the DNA-damage response to IR in human PBL.
Predicted miRNAs Involved with Microgravity Effects

Health Risk Due to miRNAs

miR-26a-5p

miR-24-3p

miR-17-5p

miR-145-5p

miR-125b-5p

miR-16-5p

miR-217-5p

let-7

mir-25

mir-34

mir-146

mir-223

HRS = -12.79

Predicted Activation

Predicted Inhibition

Negative Impact on Health

Positive Impact on Health

Both Positive and Negative Impact

Biological Health Risk Increased

HRS = Health Risk Score
A recent report showed that inactivation of p53 altered TGF-β signaling, which ironically displayed both tumor-suppressive and pro-oncogenic functions. p53 functions to integrate crosstalk between Ras/MAPK and TGF-β signaling via binding to Smad3, dislocating the Smad3/Smad4 complex formation and differentially regulating subsets of TGF-β target genes.
Using OpenTarget to Generate Countermeasures

Key Genes for individual tissues
Output of OpenTarget

Summary page for 13 targets

Gene Ontology

Drugs

Pathways

Interactions between targets
Steps to Determine Potential Drugs for Countermeasures

1. Determine drugs targets for each tissue from “key genes”


2. Find common drugs targets that exist between all the tissues
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Two possible drug candidates for countermeasures against Spaceflight.
Also known as: 7-hydroxystaurosporine

Inhibits many phosphokinases, including the serine/threonine kinase AKT, calcium-dependent protein kinase C, and cyclin-dependent kinases
- arrests tumor cells in the G1/S of the cell cycle and prevents nucleotide excision repair by inhibiting the G2 checkpoint kinase chk1, resulting in apoptosis
- Phase 1 and 2 clinical trails with drug for various cancers (information available on ClinicalTrials.gov)
  - Pancreatic Cancer: Completed, No results listed
  - Small Cell Lung Cancer: Completed, Response rate: 2/19 patients with CR or PR and 9/19 with Stable Disease
  - Melanoma: Terminated, early termination for discouraging results
  - Lymphoma, Terminated, due to low accrual and cost
  - Kidney Cancer: Completed, No results listed
  - Advanced Solid Tumors: Completed, No results listed
(sold under the name Rydapt) is a multi-targeted protein kinase inhibitor that has been investigated for the treatment of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and advanced systemic mastocytosis.

It is a semi-synthetic derivative of staurosporine, an alkaloid from the bacterium Streptomyces staurosporeus.

a multikinase inhibitor for oral use

a small molecule that inhibits multiple receptor tyrosine kinases

– inhibit the activity of wild type FLT3, FLT3 mutant kinases (ITD and TKD), KIT (wild type and D816V mutant), PDGFRα/β, VEGFR2, as well as members of the serine/threonine kinase PKC (protein kinase C) family.

– Midostaurin demonstrated the ability to inhibit FLT3 receptor signaling and cell proliferation, and it induced apoptosis in leukemic cells expressing ITD and TKD mutant FLT3 receptors or overexpressing wild type FLT3 and PDGF receptors

Approved FDA drug (2017)
Both UCN-01 and midostaurin inhibit cytosolic PKC
  - Jirousek & Goekjian, Expert Opin Investig Drugs. 2001 Dec;10(12):2117-40

Can these anti-cancer drugs be applied to the negative health effects associated with long term space missions?
  - Midostaurin has been shown to improve Bone Loss effects
    • Inhibiting FLT3 (inhibited by Midostaurin) can prevent immune related effects due to spaceflight

Inhibiting PKC (by Midostaurin and UCN-01) has beneficial effects with space related health risks
  - Can impact diabetic complications, heart failure, myocardial infarction, pain and bipolar disease

There are possible examples of how these two drugs can be adapted as a potential countermeasure
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Questions and Discussion???