Systemic Microgravity Response: Utilizing GeneLab (genelab.nasa.gov) to Develop Hypotheses for Spaceflight Risks

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What is Systems Biology?

• 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.”
General Approach to Studying a Systematic Response in the Host

An example for cancer research

Local tumor-host effects

Systemic tumor-host effects

Age
GeneLab Data Used to Generate Results

Process after mice are sacrificed

Sample Processing

Data Sharing

Data Collection & Curation

Next Generation Research

Data Submission

Modeling and Validation

International Space Station (ISS) Missions

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

Mice Sacrificed on ISS

Mice flown on STS and Sacrificed after Re-entry

Liver
Skeletal Muscle
Mammary Gland
Thymus
Soleus Muscle
Extensor Digitorum Longus Muscle

Time in Space for Mice (days)

Space Shuttle (STS) Missions

Skin
Number of Significant Genes from Each Dataset

<table>
<thead>
<tr>
<th>Dataset</th>
<th># of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADR</td>
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<td>1000</td>
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<td>Ski M</td>
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<tr>
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</tr>
</tbody>
</table>

Pathway/Functional Predictions:
- Ingenuity Pathway Analysis (IPA)
- Gene Set Enrichment Analysis (GSEA)

Fold-Change ≥ 1.2
Predicted Master Regulators

A) Upstream Regulators
- Age
- Sex
- Tissue Type
- Time in Flight
- Flight Condition
- Data Set
- Effected Pathways
  - PPARα Activates Pathways
  - Circadian Clock
  - Glycolytic Process
  - Circadian Clock
  - PPARα Activates Pathways
  - White Adipocyte Differentiation
- Signaling by Interleukins
- Immune System
- Signal Transduction
- TGFβ Signaling
- Cytokine Signaling in Immune System
- EGFR Activity
- Immune System
- Glycogen Catabolic Process
- DNA Hypomethylation
- PI3K/AKT activation
- Interleukin-6 Family Signaling
- Cell Cycle
- Cellular Responses to Stress
- Signaling by ERBB4
- Signaling by Interleukins
- Cell Cycle
- Signal Transduction
- p53 Regulation
- Transcription
- Insulin Receptor Activity

B) Canonical Pathways
- Age
- Sex
- Tissue Type
- Time in Flight
- Flight Condition
- Data Set
- PPAR Signaling
- RhodGDI Signaling
- Protein Kinase A Signaling
- cAMPS-mediated signaling
- STAT3 Pathway
- Gioma Signaling
- 14–3–3-mediated Signaling
- CREB Signaling in Neurons
- P2Y Purinergic Receptor Signaling Pathway
- Cardiac Hypertrophy Signaling
- GnRH Signaling
- Phospholipase C Signaling
- Tec Kinase Signaling
- IL-8 Signaling
- Colorectal Cancer Metastasis Signaling
- Signaling by Rho Family GTPases
- Akt/CTK/Beta Catenin Signaling
- Integrin Signaling
- PI3K/AKT Signaling
- Mouse Embryonic Stem Cell Pluripotency
- p38 MAPK Signaling
- NF-κB Signaling
- Production of Nitric Oxide and Reactive Oxygen Species in Macrophages

C) Toxicity Functions
- Age
- Sex
- Tissue Type
- Time in Flight
- Flight Condition
- Data Set
- Apoptosis of liver cells
- Increased Levels of Red Blood Cells
- Liver tumor
- Liver cancer
- Nephritis
- Cell death of cardiomyocytes
- Congenital heart disease

D) Z-scores
- TP53
- PPARα
- TGFβ1
- Flight Conditions
- Time (days)
- Z-scores

Z-scores: -3.68, 0.16, 5.44
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
General Approach to Studying a Systematic Response in the Host

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.
Impact of Circulating microRNAs

- Circulating miRNAs can carry signals from organs to other various parts of the body through the blood stream.
- The miRNAs can be transported in Exosomes, microparticles, lipoproteins, and outside any type of packaging.
- Our preliminary data shows that a miRNA signature is carried over from the spleen to the tumor with age.
Systems Biology View of miRNAs

- Tumor Suppressor miRNAs
- OncomiRNAs

- Only looking at a single miRNA
  - Tumors Inhibited

- Looking at a pair of miRNAs
  - No Change in Tumors

- Systems Biology Approach: Looking at how the entire system impacts the most important miRNAs
  - Tumors Inhibited
  - Tumors Promoted
Predicted miRNAs Involved with Microgravity Effects

- miRNAs predicted from interaction from all key genes

A) Top 10 predicted miRNAs from p-values

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

miR-125b-5p
miR-145-5p
miR-146

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 (RWW) bioreactor. Our results show that 42 miRNAs were differentially expressed in M500 exposed PBLs compared to controls. Among these, miR-9-5p, miR-9-3p, miR-155-5p, miR-150-5p, and miR-17-3p 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-regulated 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-150-5p, and miR-17-3p expression with that of genes involved in immune/inflammatory response (e.g., HNG and IL17), apoptosis (e.g., PDCD4 and PTEN), and cell proliferation (e.g., NKX3-1 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.
Predicted miRNAs Involved with Microgravity Effects

Health Risk Due to miRNAs

HRS = Health Risk Score

Biological Health Risk Increased

Predicted Activation
Predicted Inhibition
Negative Impact on Health
Positive Impact on Health
Both Positive and Negative Impact

HRS = -12.79
Overall Summary of All Data

- Systems biology approach allows for systemic understanding of the impact of Microgravity.
- Circulating miRNAs can influence overall progression of health risk to the host.
- miRNAs can potentially be used for novel minimally invasive therapeutics and countermeasures
- GeneLab (genelab.nasa.gov) is a powerful tool to generate hypotheses and direct future space research
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genelab.nasa.gov
Thanks to Systems Biology, we now have a clear picture of complex diseases!