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

Lung tumor

Age
GeneLab Data Used to Generate Results

International Space Station (ISS) Missions

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
Gastrocnemius Muscle Quadriceps
Tibialis Anterior Muscle
Adrenal Glands
Kidney
Liver
Skin

Time in Space for Mice (days)

Space Shuttle (STS) Missions
Number of Significant Genes from Each Dataset

- **Fold-change ≥ |1.2|**

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

A) Upstream Regulators
- Age
- Sex
- Tissue Type
- Time in Flight
- Flight Condition
- Data Set

Effected Pathways
- PPARK Activates Pathways
- Circadian Clock
- Glycolytic Process
- Circadian Clock
- PPARK 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
- Cellular Senescence
- Cellular Responses to Stress

Signaling by ERBB4
- Signal Transduction
- p38 MAPK Signaling

Transcription
- AR
- IKK
- FOXO3
- TP53

B) Canonical Pathways
- Age
- Sex
- Tissue Type
- Time in Flight
- Flight Condition
- Data Set

Toxicity Functions
- Age
- Sex
- Tissue Type
- Time in Flight
- Flight Condition
- Data Set

- Cell death of cardiomyocytes
- Congenital heart disease
Determination of Key Genes/Drivers

Key Genes and the Connections
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
- No Change in Tumors

Looking at a pair of miRNAs:
- Tumors Inhibited
- Tumors Promoted

**Systems Biology Approach:**
Looking at how the entire system impacts the most important miRNAs
Predicted miRNAs Involved with Microgravity Effects

- miRNAs predicted from interaction with all key genes

A) Top 10 predicted miRNAs from p-values
- mir-145-5p
- let-7
- mir-17-5p
- mir-223
- mir-21
- mir-26a-5p
- mir-146

B) All miRNAs with Z-scores > 2 or < -2
- mir-125b-5p
- mir-145-5p

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 conditions, simulated by a ground-based rotating wall vessel (RWW) bioreactor. Our results show that 47 miRNAs were differentially expressed in MDMG incubated PBLs compared with 1g 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-miRNA 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., HING and IL17), apoptosis (e.g., PDCD4 and PTEN), and cell proliferation (e.g., NRXN1 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
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!

Questions??