Systemic alterations with spaceflight associated health risks originating from both circulating miRNAs and mitochondrial biology

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"...genomics, transcriptomics, proteomics, and metabolomics offer an immense opportunity to understand the effects of spaceflight on biological systems..."

"...Such techniques generate considerable amounts of data that can be mined and analyzed for information by multiple researchers..."
This is truly an exciting time for cellular and molecular biology, omics and biomedicine research on ISS with these amazing additions to the suite of ISS Laboratory capabilities.

Omics Acquisition in Space is Now a Reality

- Sample Preparation Module
- Oxford Nanopore MinION Gene Sequencer
- Cepheid Smart Cycler qRT-PCR
- Reaction tube containing lyophilized chemical assay bead (proprietary)
- Mini-PCR
• Sequencing on ISS is still limited in the amount of data generated
  – Most of the work needs to happen on earth
• Measurements on human cannot be too invasive and limited in numbers
  – Usage of animals

GeneLab ecosystem: maximizing knowledge by bringing experiments together as a system

Identify Shared Processes/ Molecular Signatures
  • Hypoxic Response ?
  • Oxidative Stress
  • Common Tissue (e.g. muscle, liver, heart, eyes, brain,...)

For Spaceflight
  • High "n" number – statistically significant data
  • Genetically identical animals
  • Low resource requirements
  • Short life cycle - multiple generations
  • Measure response of a whole multicellular animal
  • Flies used as a model for humans for innate immunity, circadian rhythm, oxidative stress, neurobehavior, development, genetics, GWAS, “omics” studies etc.
GeneLab Data Democratization

**Community**
- Citizen scientists
- Biologists
- Data scientists

**Data type**
- Disease states
- Pathway changes
- Differential expression
- Mapped reads
- Raw data

**Missions/Experiments**

**GeneLab Sample Processing**

**Open Data**
- GeneLab
  - ANL MG-RAST
  - EBI PRIDE
  - NCBI GEO

**Visualization Portal**
- New Knowledge
- Analysis Working Groups
  - Web Portal
  - Analysis Toolshed
  - User Data Workspace

**Data Federation**
October 2019 will be the release for GeneLab’s Visualization Portal! Analyze unique genomics data from spaceflight.
GeneLab Database: >200 data sets

A) 
- fish: 23.75%
- fruit flies: 10.63%
- human: 32.5%
- invertebrate: 5.63%
- microbes: 20.63%
- plant: 1.88%
- rodents: 5%

B) 
- Ground: 46.94%
- Spaceflight: 53.06%

C) 
- microarray: 76.69%
- RNA-seq: 23.31%

Beheshti et al., Radiation Research 2018
Space Biology Interest for NASA

**Biological Systems**
- Model Organisms
- Cell and Microbial Biology
- Biomolecules

**Human Health**
- Mammalian Cells
- Model Organisms

**Human Exploration**
- Exploration Subsystems
- Bioregenerative Life Support

From the bench to exploration medicine: NASA life sciences translational research for human exploration and habitation missions.

[SLPSRA Logo]

[Human Exploration Image]
Space Environment

![Diagram showing the space environment with labels for distance from Earth, gravity fields, radiation, and hostile/closed environments.](https://www.nasa.gov/mission_pages/sunearth/new/radiationbelts.html)

**2½ Years, 2,600 X-Rays**

Americans on average absorb the radiation equivalent of at least 7 chest X-rays each year.

Space missions, outside of Earth’s protective atmosphere and magnetic field, expose astronauts to many times more.

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**Credits:** NASA
Space Health Risks On Astronauts

Select health effects due to space radiation exposures.
Circulating miRNA Signature Predicts Health Risks Associated with Radiation and Microgravity

Multi-Omics Analysis using GeneLab database recognizes Mitochondrial Dysfunction as a mediator of spaceflight health risks
What are miRNAs and why study miRNAs

- A single miRNA has been estimated to regulate up to 500 mRNAs.
- miRNAs are ~22nt
- Due to the size and stability of the miRNAs, it can float freely in the blood.
- miRNAs are now known to be involved in all aspects of diseases.
- miRNA are not only found in mammals, but everything else living: plants, microbes, fish, C. Elegans, fruit flies, insects, etc...
- miRNAs play a big role in radiation response (which also relates to space radiation).

Systems Biology View of miRNAs

A) Only looking at single miRNA

Decreased Health Risk

B) Only looking at a pair of miRNAs

No Change for Health Risk

C) Decreased Health Risk

Increased Health Risk

Systems Biology Approach: Looking at how the most important miRNAs impact the entire system

miRNAs Associated with Decreased Health Risk

miRNAs Associated with Increased Health Risk

Abstract

Translating fundamental biological discoveries from NASA’s Space Biology program into health risk from space flight has been an ongoing challenge. We propose to use NASA GeneLab database to gain new knowledge on potential systemic responses to space. Uncovered systems biology analysis of transcriptomic data from several different cohorts revealed for the first time the existence of putative ‘master regulators’ coordinating a systemic response to microgravity and/or space radiation with TGF-β1 being the most common regulator. We hypothesize the space environment leads to the release of biomolecular circulations within the blood stream. Through determining we identified 13 candidate miRNAs (miRNA) which are common in all studies and directly interact with TGF-β1 that can be potential circulating factors impacting space biology. This study exemplifies the utility of the International Journal of Molecular Sciences.

GeneLab Database Analyses Suggest Long-Term Impact of Space Radiation on the Cardiovascular System by the Activation of FYN Through Reactive Oxygen Species

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miRNA Signature Prediction Associated with Space Flight

A microRNA signature and TGF-β1 response were identified as the key master regulators for spaceflight response.

Abstract

Translating fundamental biological discoveries from NASA Space Biology program into health and fitness for spaceflight has been an ongoing challenge. We propose to use the GeneLab database to gain new knowledge on potential systemic responses to space. An integrative systems biology analysis of transcriptomic data from eleven different rat models reveals for the first time the existence of potential "master regulators" conferring a systemic response not only in space but also in response to TGF-β1 being one of the most common regulators. We hypothesize that a space environment leads to the release of biomarkers circulating in the bloodstream. Through data mining, we analyzed a large database (miRBase) which contains all known microRNAs for murine species, looking for potential circulating factors including space biology. This study exemplifies the utility of the GeneLab database to aid in the process of performing novel hypothesis-driven research.
Predicted miRNAs Involved with Spaceflight

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

C. Girardi, E. De Pitska, S. Casara, E. Gabara, C. Ronsardli, L. Cermoli, and M. Magnoli

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We analyzed miRNA and mRNA expression profiles in human peripheral blood lymphocytes (PHA-stimulated) in microgravity conditions, stimulated by a ground-based rotating wall vessel (RWW) bioreactor. Our results show that miRNAs were differently expressed in space (simulated) compared with the control sample (phase: miR-1-5p, miR-9-5p, miR-10-5p, miR-150-5p, and miR-21-5p) were the most dysregulated. To improve the detection of functional miRNA-mRNA pairs, we performed gene expression profiling on the same samples analyzed 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/anti-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-6-5p, miR-106-5p, miR-146a, and miR-126-5p with genes involved in immune/anti-inflammatory response (e.g., IFNG and IL1B), apoptosis (e.g., PP1D2 and EGFL7), and cell proliferation (e.g., KITLG and LEF1). Experimentally, overexpression of miR-126-5p caused a decrease in cells viability and apoptosis, whereas overexpression of miR-126-5p caused an increase in cell viability and an increase in apoptosis.

Health Risk Due to miRNAs
- Predicted Activation
- Predicted Inhibition

Response of the body to spaceflight

Both Positive and Negative Impact

Negative Impact on Health
Positive Impact on Health
Both Positive and Negative Impact

NASA

Beheshti, et al., PLOS One, 2018
Predict miRNAs with Space Radiation Cardiovascular Risk

International Journal of Molecular Sciences

Article

GeneLab Database Analyses Suggest Long-Term Impact of Space Radiation on the Cardiovascular System by the Activation of FYN Through Reactive Oxygen Species

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HUVECs flown on the ISS

1.544 mGy

Mice irradiated on Earth with Protons and 56Fe. Cardiomyocytes isolated at the time points above.
Circulating miRNAs predicted to be involved in general with spaceflight from Beheshti et al. PLOS One 2018

Predict miRNAs with Space Radiation Cardiovascular Risk
Presence of miRNA signature in Serum of Mice in Simulated Space Environment

miRNA Signature in Serum

Hindlimb Unloading

Irradiation Type of Loading
Sham
2Gy Gamma

Type of Loading
Normal Loading
Hindlimb Unloading

Fold-Change (Log_{2})

HU vs Sham

miR-25-5p
miR-217-5p
miR-223-3p
miR-146a-5p
miR-92a-3p
let-7a-5p
let-7c-5p
miR-34a-5p
miR-21-5p
miR-145-5p
miR-17-5p
miR-16-5p
miR-24-3p
miR-125b-5p
miR-26a-5p
Preliminary data on miRNA signature Presence with Space Radiation

- HU for an initial three days followed by IR and continuation of HU for another 1 or 11 days
- Radiation exposure: Total body irradiation, conscious mice, 600 MeV/n $^{56}$Fe (1 Gy and 2 Gy), 150 MeV Proton (1Gy) or ‘1Gy Mix’ (0.5Gy $^{56}$Fe and 0.5Gy Proton)

Increasing Dose and Ions causes increase miRNAs in serum

Significance compared to serum from Sham NL (Time Post IR)
- * p-value < 0.05
- ** p-value < 0.01
- *** p-value < 0.001
Impact of the Space Biology miRNA Signature on Functions and potential use for a novel Countermeasure
Acknowledgments for miRNA Studies

This work is supported by:
16-ROSBFP GL-0005: NNH16ZTT001N-FG
Appendix G: Solicitation of Proposals for Flight and Ground Space Biology Research

The Translational Research Institute through NASA Cooperative Agreement NNX16AO69A (T-0404)
Multi-Omics Analysis using GeneLab database recognizes Mitochondrial Dysfunction as a mediator of spaceflight health risks

• Paper being written
• Plan to submit paper by end of September
• Target journal to submit is New England Journal of Medicine
  • Contacted the editor of the journal and he was interested in the paper and encouraged submission to their journal.
AWG Members Involved

Kathleen Fisch  Brin Rosenthal  Deanne Taylor  Hossein Fazelinia  Komal Rathi  Douglas Wallace  Larry Singh

Kathleen Fisch  Children's Hospital of Philadelphia  Perelman School of Medicine

Helio Costa  Kathryn Grabek  J. Tyson McDonald  Gary Hardiman  Willian da Silveira  Jeffrey Scott Willey

Stanford University  Hampton University  Queen's University Belfast  Wake Forest University
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<th>Cem Meydan</th>
<th>Jonathan Foox</th>
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<th>Evagelia C. Laiakis</th>
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<td>Georgetown University Medical Center</td>
<td>Morehouse School of Medicine</td>
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<th>Susana Zanello</th>
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<td>NASA</td>
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<td>UCSF University of California San Francisco</td>
<td>NASA</td>
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<td>Ames Research Center</td>
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Health Risks On Astronauts in Space

One thing is always missing: **LIVER** and **Mitochondrial** as factors of interest for spaceflight related health risks!!

Select health effects due to space radiation exposures.
The Mitochondrial Stress Response

ATP
Energy
Growth/adaptation
Biosynthesis
Protein modification
Mitochondrial-nuclear communication

Inflammation
mtDNA or peptides
ROS

Cell death
mPTP opening
Cytochrome c release
Energy deprivation

Thermogenesis

Functional
Dysfunctional

Ca²⁺ transport
Metabolic stimulation
Stress response
Ca²⁺ homeostasis

ROS
Oxidative stress
Redox regulation
Cell signaling

Adipocytokine
Apoptosis

Mitochondrial Dysfunction

Electron Transport Chain
Respiration
Ca²⁺
β-Oxidation

Accumulation of Lipids

Altered Insulin Signaling
Altered adipocytokine levels

Insulin resistance / Type-2 Diabetes

Blood glucose level↑

Release of free fatty acids from adipocytes

Hepatic glucose production↑
Glucose utilization↓

Metabolic Syndrome

Neurodegeneration

Cell Death

Nutrients in Clinical & Translational Research 4 (2020) 18–196

Contents lists available at ScienceDirect
Biochimica et Biophysica Acta

Linking mitochondrial dysfunction, metabolic syndrome and stress signaling in Neurodegeneration®

Gaurab Kumar Jha,1 Nisaj Kumar Jha,1 Divyaj Kumar, Rahul K. Ambasta, Pramod Kumar,2

1Researcher, Department of Biotechnology, National Institute of Technology, Jodhpur, Rajasthan, India.
2Researcher, Department of Biotechnology, Banaras Hindu University, Varanasi, Uttar Pradesh, India.
The Mitochondrial Stress Response

Respiratory Chain Dysfunction resulting in halt of translation

Cell arrest in response to oxidative stress

Mitochondrial stress as a trigger innate immune responses

**Trends in Cell Biology**

Mitochondrial dysfunction as a trigger of innate immune responses and inflammation

Do Zhou, Peng Yan

Published: August 20, 2012

DOI: https://doi.org/10.1016/j.tcb.2012.08.002

**Journal of Cell Science**

Mitochondrial stress-dependent regulation of cellular protein synthesis

Ukran Tof, Barbara Szczeklik-Nowakowska, Agnieszka Drozdowska


**SLPSRA**

**JCI** The Journal of Clinical Investigation

Mitochondrial dysfunction in pathophysiology of heart failure

Do Zhou, Peng Yan
The Mitochondrial Stress Response

Mitochondrial Dysfunction Impacts Many Organs

- Heart
  - Conduction disorder
  - Wolf-Parkinson-White syndrome
  - Cardiomyopathy
- Eye
  - Optic neuropathy
  - Ophthalmoplegia
  - Retinopathy
- Liver
  - Hepatopathy
- Skeletal muscle
  - Weakness
  - Fatigue
  - Myopathy
  - Neuropathy
- Brain
  - Seizures
  - Myoclonus
  - Ataxia
  - Stroke
  - Dementia
  - Migraine
- Kidney
  - Fanconi’s syndrome
  - Glomerulopathy
- Pancreas
  - Diabetes mellitus
- Blood
  - Pearson’s syndrome
- Inner ear
  - Sensorineural hearing loss
- Nuclear DNA
- Subunits
- Oxidative phosphorylation
- Mitochondrial DNA
- Colon
  - Pseudo-obstruction

Mitochondrial Dysfunction May Differ Between Organs

- Neurodegeneration
- Strokes
- Demyelination
- Epilepsy
- Ataxia
- Parkinsonism
- Migraines
- Cognitive decline
- Psychiatric symptoms
- Liver disease
- Kidney dysfunction
- Diabetes
- Malabsorption
- Diarrhoea
- Infertility
- Premature menopause
- Anaemia
- Immunological defects
- Lactacidemia

Figure 1 | The variability of mitochondrial disease manifestations. Mitochondrial diseases can manifest both in children and in adults, and can present in various organs, including in multiple organs that may have no apparent functional links to each other. Some manifestations may affect one tissue, such as the heart or the optic nerve. Children may recover from one phenotype and later develop another—for example, in Pearson syndrome, the primary manifestation is exocrine pancreatic dysfunction and megaloblastic anaemia, and the survivors may later develop brain disease. Typically, these disorders are progressive.

Multi-Omics Analysis using GeneLab database recognizes Mitochondrial Dysfunction as a mediator of spaceflight health risks
Quantitative Response to Spaceflight Global view of the data

A

B

C

D

Sylvain Costes
Deanne Taylor
Hossein Fazelinia
Komal Rathi

Chris Mason
Cem Meydan
Jonathan Foon

Sylvain Costes
In Vitro Human Dataset Analyses Reveals Conserved Mitochondrial Response to Spaceflight
In Vitro Human Dataset Analyses Reveals Conserved Mitochondrial Response to Spaceflight

Gene Set Enrichment Analysis (GSEA) of human microarray datasets GLDS-13, GLDS-52, GLDS-114, and GLDS-174 comparing flight to ground treatments. (A,B) Venn diagrams of statistically significant GSEA (A) Gene Ontology (GO) and (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) gene sets with FDR < 10%. (C,D) Cytoscape enrichment maps of (C) GO sets with FDR <10% in at least two GLDS datasets and (D) KEGG gene sets with FDR < 10% in at least one GLDS dataset. Green Highlights indicate all pathways involved with

Common significant dysregulation of the gene ontology genes sets for:
- mitochondrial ATP synthesis
- mitochondrial electron transport
- oxidative phosphorylation
- hydrogen ion transmembrane transportation
Multi-Omics Analysis on mice flown to ISS reveals Mitochondrial driven response stemming from the liver

RR1 mice: Female C57BL/6, 32 weeks old at launch
RR3 mice: Female BALB/C, 18 weeks old at launch
RR9 mice: Male C57BL/6, 9 weeks old at launch
Multi-Omics Analysis on mice flown to ISS reveals Mitochondrial driven response stemming from the liver

A) Mitochondrial Activity

B) Ribosome and Translation

C) Cell Cycle

D) Lipid Metabolism

E) Immune Response

F) Photoreceptor Activity / Circadian Rhythm

Kathleen Fisch       Brin Rosenthal
Gary Hardiman   Willian da Silveira

Gary Hardiman   William da Silveira
Cross-tissue, cross-omics pathway analysis reveals a convergence on key dysregulated processes

Supplementary Figure in Paper showing the entire pathway multi-omic pathway analysis
Metabolomics on muscles reveal mitochondrial factors as top biological factors being regulated by spaceflight

Panel A - Volcano plots and heatmaps from analysis of habitat ground control versus flight samples. Panel B – Enrichment pathway analysis based on the subset of putative metabolites from Supplementary Table 2. Blue letters highlight pathways with mitochondrial involvement.

- RR1 mice: Female C57BL/6, 32 weeks old at launch
- RR3 mice: Female BALB/C, 18 weeks old at launch
- RR9 mice: Male C57BL/6, 9 weeks old at launch
Metabolomics Related to Proteomic and Transcriptomic data

- C) Mitochondrial Activity
- D) Ribosome and Translation
- E) Proteasome
- F) TNF/IL1 Response
- G) Sympathetic and Feeding Behavior
- H) Metabolism and ECM
- I) Interferon Response

Kathleen Fisch       Brin Rosenthal
Gary Hardiman   Willian da Silveira
“Fatty acids are transported via carnitine into mitochondria for their subsequent oxidation to generate ATP. Studies have also shown that carnitine has a protective effect both on mitochondria and in whole cells by inhibiting free fatty acid-induced mitochondrial membrane damage and/or its secondary effects”

From: PMID: 20648231

Supplementary Figure 2: Panel A – GEDI self organizing maps showing global metabolomic shifts due to the effects of spaceflight. Panel B - Carnitine and malate levels with their theoretical fragmentation spectra from Progenesis QI. Levels are depicted as mean ± standard error of the mean. * p<0.05, ** p<0.01.
Astronaut Physiological Factors Confirm Omics *in vitro* and *in vivo* analysis!
Astronaut Physiological Factors Confirm Omics in vitro and in vivo analysis!

Can cause bone loss
Mitochondrial Driven Factors Might be Key to Systemic Spaceflight Associated Increase in Health Risk

- GeneLab was instrumental to determine this universal response!
- No other possible way to piece the puzzle together without the power of GeneLab
- The large collaborative nature of the AWG was essential to drive this work!!
Many Space Biology Questions and Challenges Still Need to Addressed!

**HUMAN EXPLORATION**  
*NASA’s Path to Mars*

**EARTH RELIANT**  
MISSION: 6 TO 12 MONTHS  
RETURN TO EARTH: HOURS

- Mastering fundamentals aboard the International Space Station
- U.S. companies provide access to low-Earth orbit

**PROVING GROUND**  
MISSION: 1 TO 12 MONTHS  
RETURN TO EARTH: DAYS

- Expanding capabilities by visiting an asteroid redirected to a lunar distant retrograde orbit
- The next step: traveling beyond low-Earth orbit with the Space Launch System rocket and Orion spacecraft

**MARS READY**  
MISSION: 2 TO 3 YEARS  
RETURN TO EARTH: MONTHS

- Developing planetary independence by exploring Mars, its moons and other deep space destinations
-www.nasa.gov
Acknowledgements

https://genelab.nasa.gov/