Circulating miRNA Signature Predicts Health Risks Associated with Radiation and Microgravity

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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).

Space Environment

**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.

Source: Brookhaven National Laboratory, U.S. Department of Energy

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**Isolation/Confinement**

**Hostile/closed environments**

**Distance from Earth**

**Gravity Fields**

**Space Radiation**

Credits: NASA
Space Health Risks On Astronauts

Select health effects due to space radiation exposures.

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 risk from space flights has been an ongoing challenge. We propose to use NASA GeneLab database to gain new knowledge on potential systemic responses to space. Unbiased systems biology analysis of transcriptomic data from seven different rodent datasets reveals for the first time the existence of potential “master regulators” coordinating a systemic response to microgravity and/or space radiation with TGF-β1 being the most common regulator. We hypothesized the space environment leads to the release of biomolecules circulating inside the bloodstream. Through datamining we identified 13 candidate microRNAs (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.

**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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Determining miRNA signature associated with diseases: Lymphoma

RESEARCH ARTICLE
A Circulating microRNA Signature Predicts Age-Based Development of Lymphoma
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Abstract
Extensive epidemiological data have demonstrated an exponential rise in the incidence of non-Hodgkin lymphoma (NHL) that is associated with increasing age. The molecular etiology of this remains largely unknown, which impacts the effectiveness of treatment for patients. We proposed that age-dependent circulating microRNA (miRNA) signatures in the host influence diffuse large B cell lymphoma (DLBCL) development. Our objective was to examine tumor development in an age-based DLBCL system using an inventive systems biology approach. We harnessed a novel murine model of spontaneous DLBCL initiation.

Serum from Patients Isolate miRNA with oligo(dT)• Hybridan
Healthy Remission Relapsed Unreated
Collect 200μl of Serum
Isolate miRNA From Serum
Convert RNA to cDNA

Through ddPCR we are able to get exact counts of circulating miRNA in the serum

Every single blue point on the plots represents one copy of miRNA (see arrows).

10 significant miRNAs that overlap and are regulated in same direction compared to controls
miRNAs Associated with DLBCL Development: in Humans

A) miRNA signature in serum with 9 miRNAs

B) ROC Curves for Comparisons

C) miRNA signature with 5 most significant miRNAs

D) miRNA Signature in Serum of Humans

A) KEGG Pathways

B) GO Pathways

C) Color Key

D) ddPCR data

Through ddPCR we are able to get exact counts of circulating miRNA in the serum.
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 risk assessment has been an ongoing challenge. We propose to use NASA Genelab database to gain new knowledge on potential systemic responses to space. Unbiased systems biology analysis of transcriptomic data from seven different rodent datasets reveals for the first time the existence of potential "master regulators" coordinating a systemic response to microgravity and space radiation with TGF-β1 being the most common regulator. We hypothesized the space environment leads to the release of biomolecules circulating inside the blood stream. Through data mining we identified 13 candidate microRNAs (miRNAs) 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 GeneLab data repository to aid in the process of performing novel hypothesis-based research.

https://genelab.nasa.gov/
Predicted miRNAs Involved with Spaceflight

A) Top 10 predicted miRNAs from p-values

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

Health Risk Due to miRNAs

- Predicted Activation
- Predicted Inhibition

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

Response of miRNAs to spaceflight

Biological Health Risk Increased

Correlation of miRNA-mRNA pairs

miR-9-3p
miR-155-3p
miR-150-5p
miR-378-3p

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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 MMC-incubated PBLs compared with Ig incubated ones. Among these, miR-9-3p, miR-155-3p, miR-150-5p, 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-155-3p, miR-150-5p, and miR-378-3p with that of genes involved in immune/inflammatory response (e.g., IFNG and IL17F), 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.


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Technique to Quantify miRNAs

**Hindlimb Unloading**

1. **Collect** 200µl of Serum
2. **Isolate** miRNA From Serum
3. **Convert** RNA to cDNA
   - Mix cDNA, EvaGreen Master mix & primers and generate droplets
4. **Mix** cDNA, EvaGreen Master mix & primers and generate droplets
5. **Process & Analyze** ddPCR data

Through ddPCR we are able to get exact counts of circulating miRNA in the serum.

Every single blue point on the plots represents one copy of miRNA (see arrows).
Presence of miRNA signature in Serum of Mice in Simulated Space Environment

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

Significance compared to serum from Sham NL (Time Post IR)
* $p$-value < 0.05
** $p$-value < 0.01
*** $p$-value < 0.001
Confirmation exists in the miRNAs from the NASA Twin Study!!!
miRNA Research will Further Assist with NASA's Future Missions

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

PROVING GROUND
MISSION: 1 TO 12 MONTHS
RETURN TO EARTH: DAYS
Expanding capabilities by visiting an asteroid redirected to a lunar distant retrograde orbit

MARS READY
MISSION: 2 TO 3 YEARS
RETURN TO EARTH: MONTHS
The next step: traveling beyond low-Earth orbit with the Space Launch System rocket and Orion spacecraft

U.S. companies provide access to low-Earth orbit

Developing planetary independence by exploring Mars, its moons and other deep space destinations

www.nasa.gov

NASA 2018 Strategic Plan Framework

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<td>EXPAND HUMAN KNOWLEDGE THROUGH NEW SCIENTIFIC DISCOVERIES.</td>
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<td>1.1: Understand the Sun, Earth, Solar System, and Universe.</td>
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<td>EXPLORE</td>
<td>EXTEND HUMAN PRESENCE DEEPER INTO SPACE AND TO THE MOON FOR SUSTAINABLE LONG-</td>
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<td>TERM EXPLORE AND UTILIZATION.</td>
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<td>2.1: Lay the Foundation for America to Maintain a Constant Human Presence in</td>
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<td>Low Earth Orbit Enabled by a Commercial Market.</td>
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<td>2.2: Conduct Exploration in Deep Space, Including to the Surface of the Moon.</td>
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<td>DEVELOP</td>
<td>ADDRESS NATIONAL CHALLENGES AND CATALYZE ECONOMIC GROWTH.</td>
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<td>3.1: Develop and Transfer Revolutionary Technologies to Enable Exploration</td>
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<td>Capabilities for NASA and the Nation.</td>
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<td>3.2: Transition Aviation Through Revolutionary Technology Research, Development,</td>
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<td>ENABLE</td>
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<td>4.1: Engage in Partnership Strategies.</td>
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EXPAND HUMAN KNOWLEDGE THROUGH NEW SCIENTIFIC DISCOVERIES.
Acknowledgments for miRNA Studies

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Appendix G: Solicitation of Proposals for Flight and Ground Space Biology Research

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