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.

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**Distance from Earth**

- **Low-Earth Orbit (LEO)**: International Space Station (230 miles)
- **Geostationary Orbit (GSO)**: NASA’s Solar Dynamics Observatory (22,000 miles)
- **GPS Satellites**: 12,500 miles
- **Outer Belt**: 12,000 – 25,000 miles
- **Van Allen Probes**: A and B
- **Trip to and from Mars (1 year)**: 80,000 miles
- **Mars (1.5 years)**: 30,000 miles
- **From Sclar Flare**: 20,000 miles

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**Isolation/Confinement**

- **Hostile/closed environments**

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**Space Radiation**

- **Gravity Fields**

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**Credits:** NASA

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**Source:** Brookhaven National Laboratory, U.S. Department of Energy
Space Health Risks On Astronauts

Select health effects due to space radiation exposures.
A microRNA signature and TGF-$\beta$1 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-$\beta$1 being the most common regulator. We hypothesized the space environment leads to the release of biomolecules circulating inside the bloodstream. Through datamining we identified 13 candidate microRNAs (miRNA) which are common in all studies and directly interact with TGF-$\beta$1 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

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 innovative systems biology approach. We harnessed a novel murine model of spontaneous DLBCL initiation.

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

Young Mice
(2 months old)

Smurf2 T/T

C57BL/6 Wild-Type
miRNAs Associated with DLBCL Development: in Humans

A) miRNA signature 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

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).

Cancer Impact

Tumor Suppressor

oncomiR

Map

Go Pathways

Color Key

Manuscript Accepted
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 programs 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 analyses of transcriptomic data from seven different inbred rat datasets reveal 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 in the bloodstream. Through data mining 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 GeneLab data repository to aid in the process of performing novel hypothesis-based 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

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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 1g incubated ones. Among these, miR-9-5p, miR-155-5p, miR-150-5p, and miR-378-5p 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-9-5p, miR-21-5p, miR-150-5p, and miR-378-5p with that of genes involved in immune/inflammatory response (e.g., STING and IL1F7), 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.
Technique to Quantify miRNAs

Hindlimb Unloading

- Collect 200µl of Serum
- Isolate miRNA from Serum
- Convert RNA to cDNA

Mix cDNA, EvaGreen Master mix & primers and generate droplets

Process & Analyze ddPCR data

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

miRNA from Controls

miRNA from Experimental Condition

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 $^{56}$Fe (1 Gy and 2 Gy)
  - 150 MeV Proton (1Gy)
  - ‘1Gy Mix’ (0.5Gy $^{56}$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

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

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

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

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