

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

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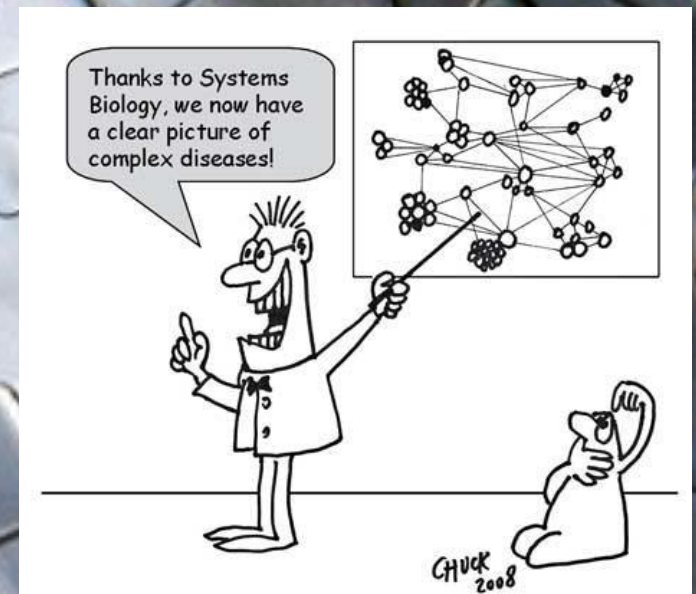
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What is Systems Biology?

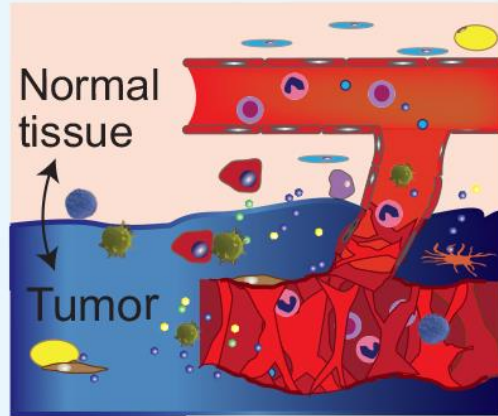
- Systems biology attempts to understand biological organisms or systems as a whole rather than researching their individual components in isolation from one another.
- NIH defines Systems Biology as: “Systems biology is an approach in biomedical research to understanding the larger picture—be it at 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.”



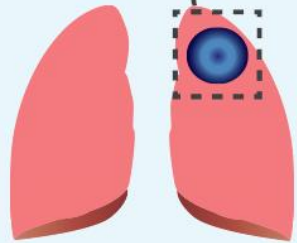
General Approach to Studying a Systematic Response in the Host

An example for cancer research

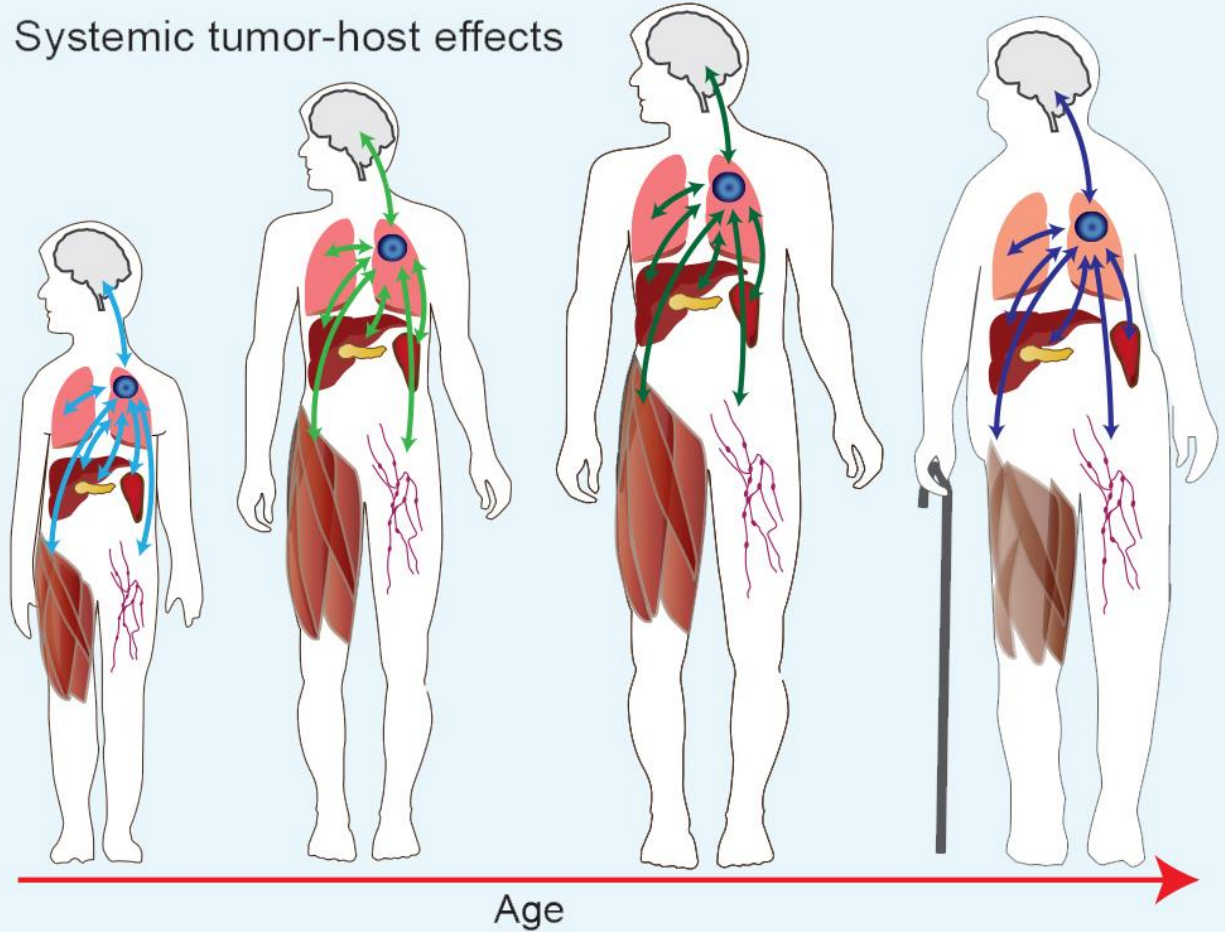
Local tumor-host effects



Lung tumor

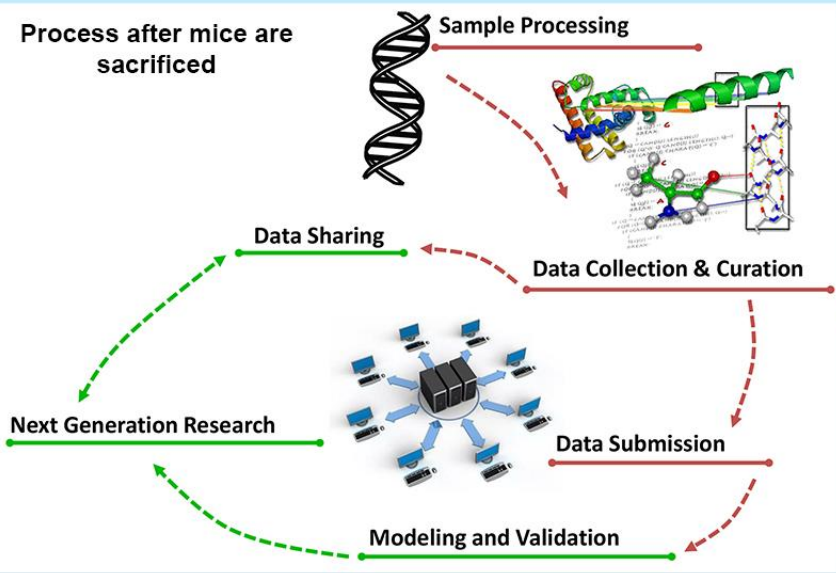


Systemic tumor-host effects



GeneLab Data Used to Generate Results

Process after mice are sacrificed

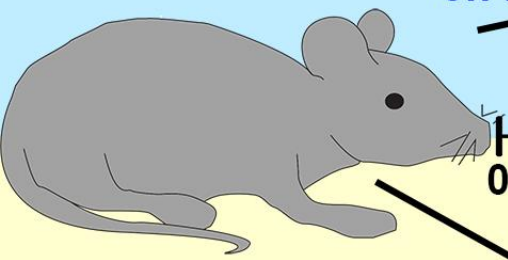


- Extensor Digitorum Longus Muscle
- Soleus Muscle
- Gastrocnemius Muscle
- Quadriceps
- Tibialis Anterior Muscle
- Adrenal Glands
- Kidney
- Liver

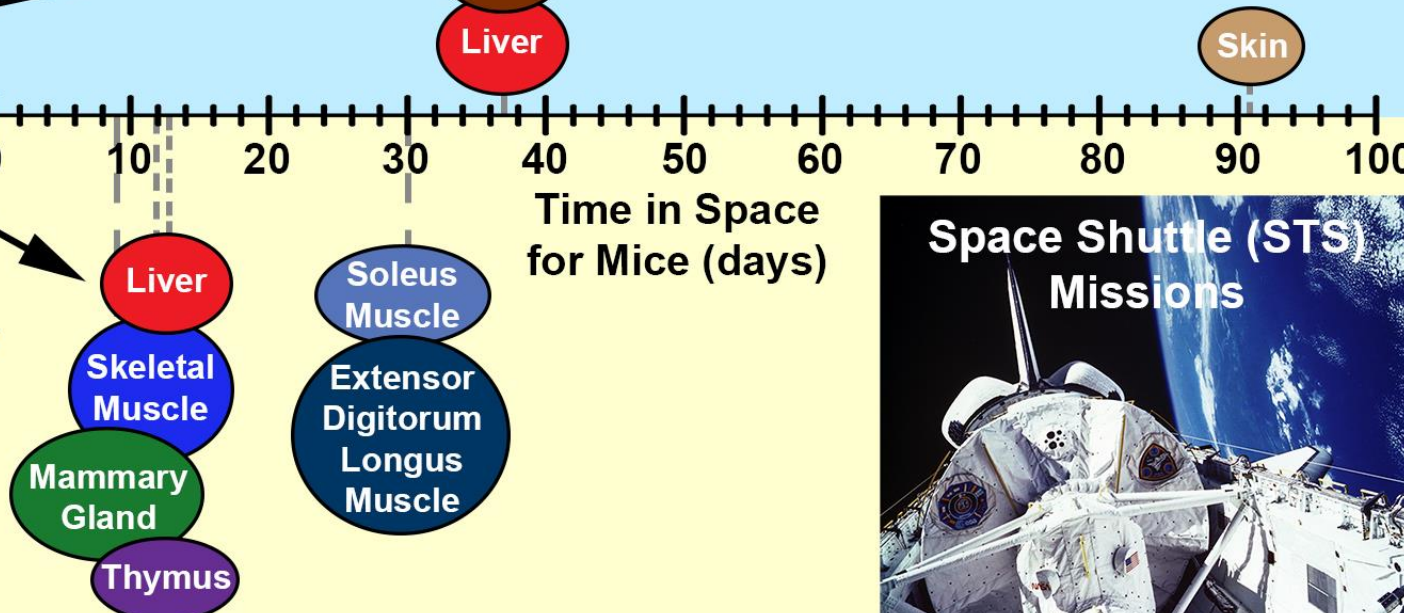


International Space Station (ISS) Missions

Mice Sacrificed on ISS

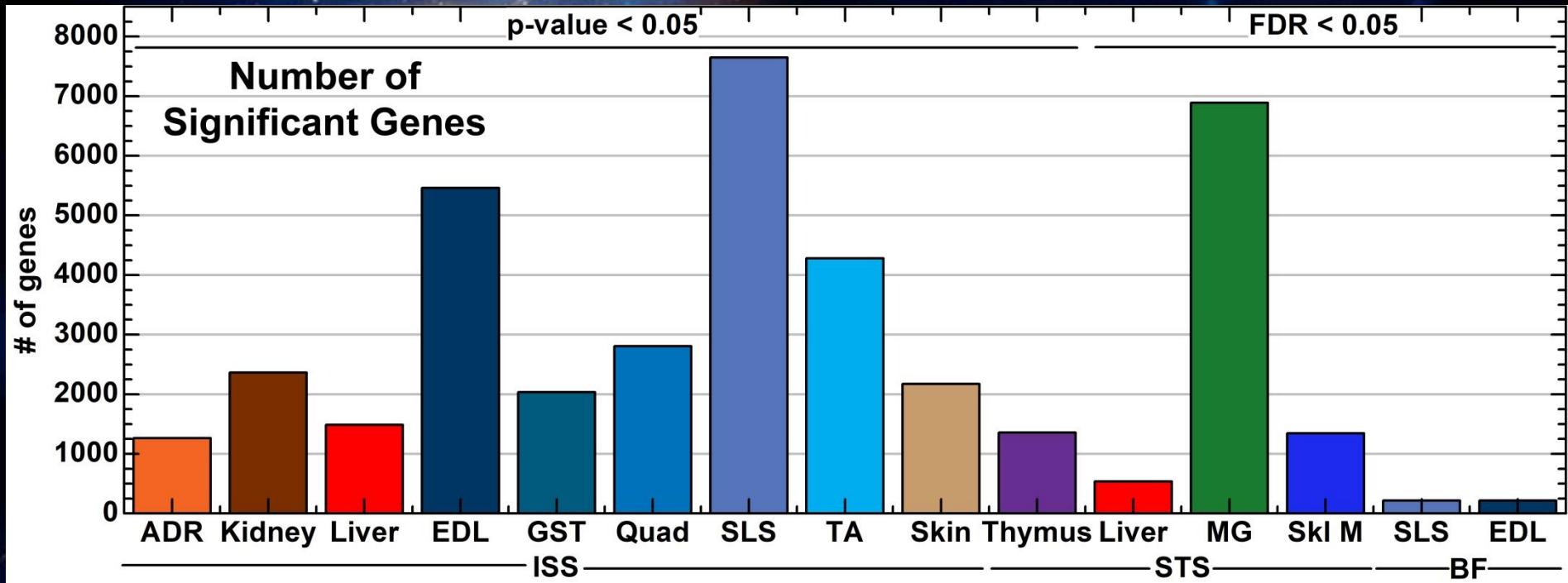


Mice flown on STS and Sacrificed after Re-entry



Space Shuttle (STS) Missions

Number of Significant Genes from Each Dataset

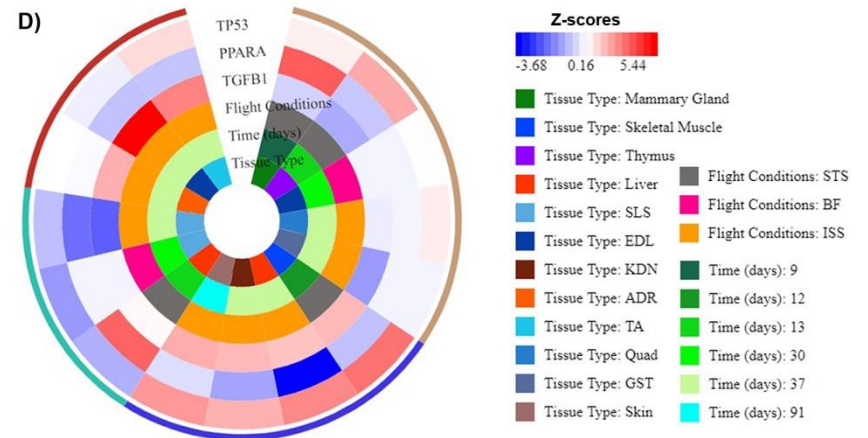
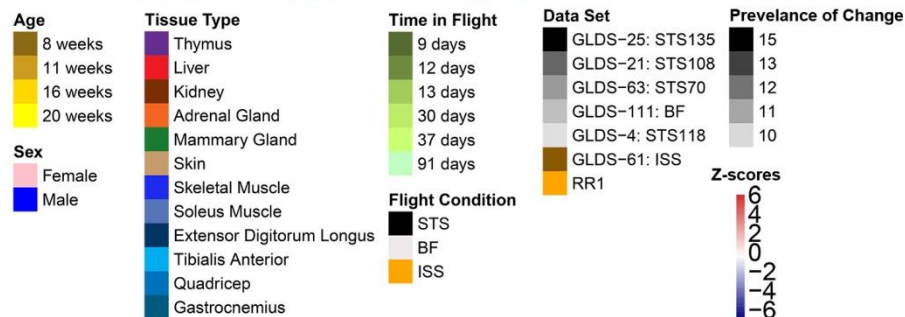
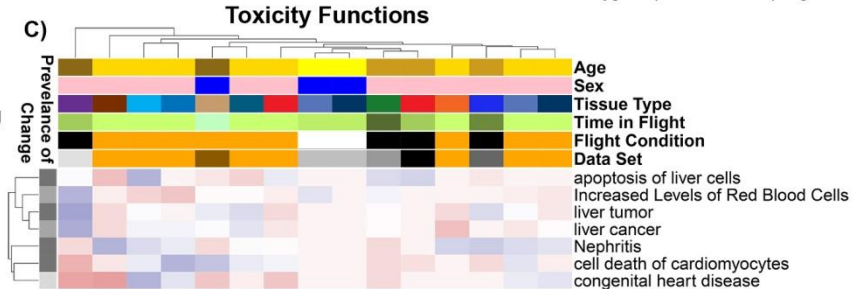
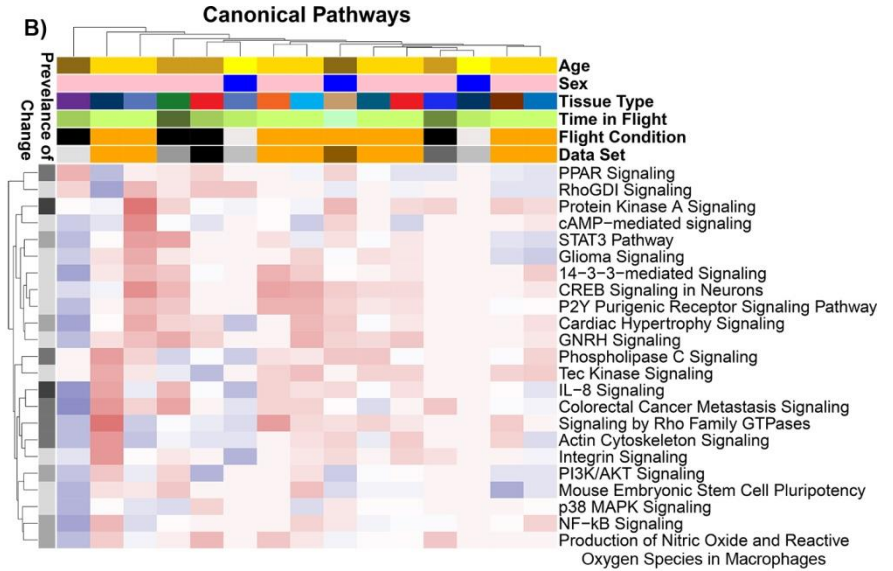
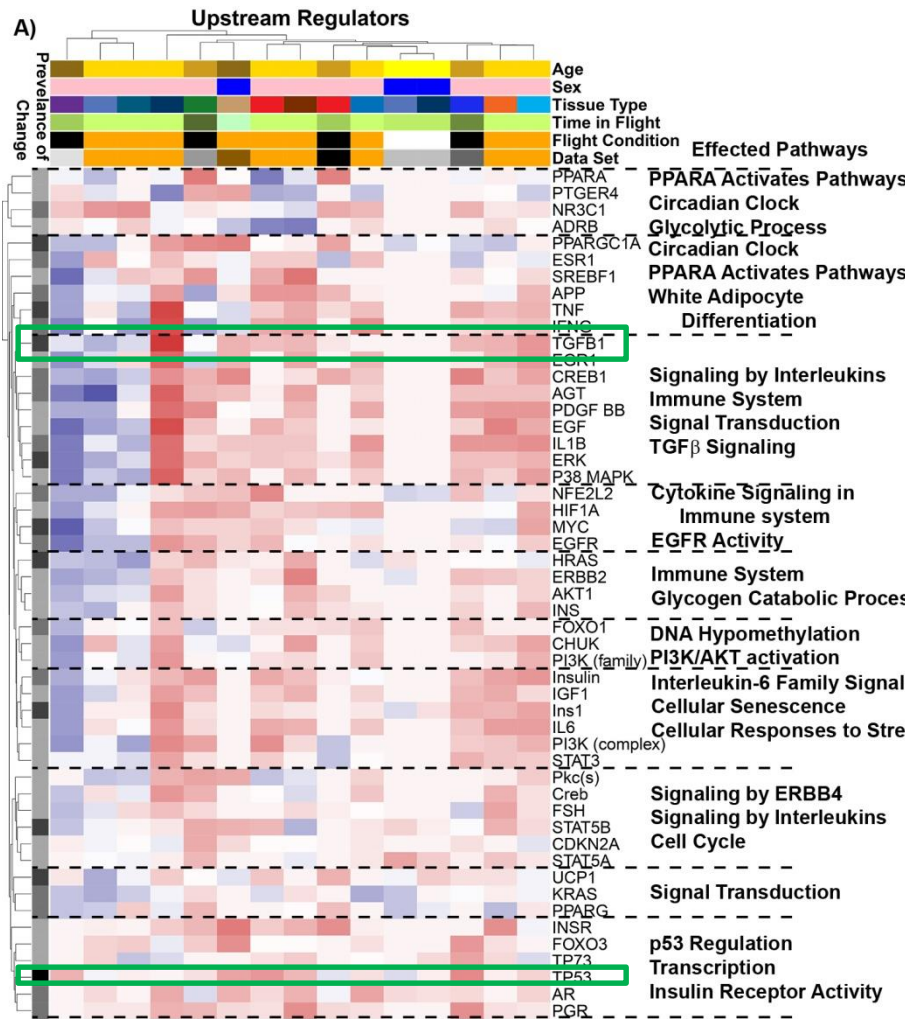


Fold-Change $\geq |1.2|$

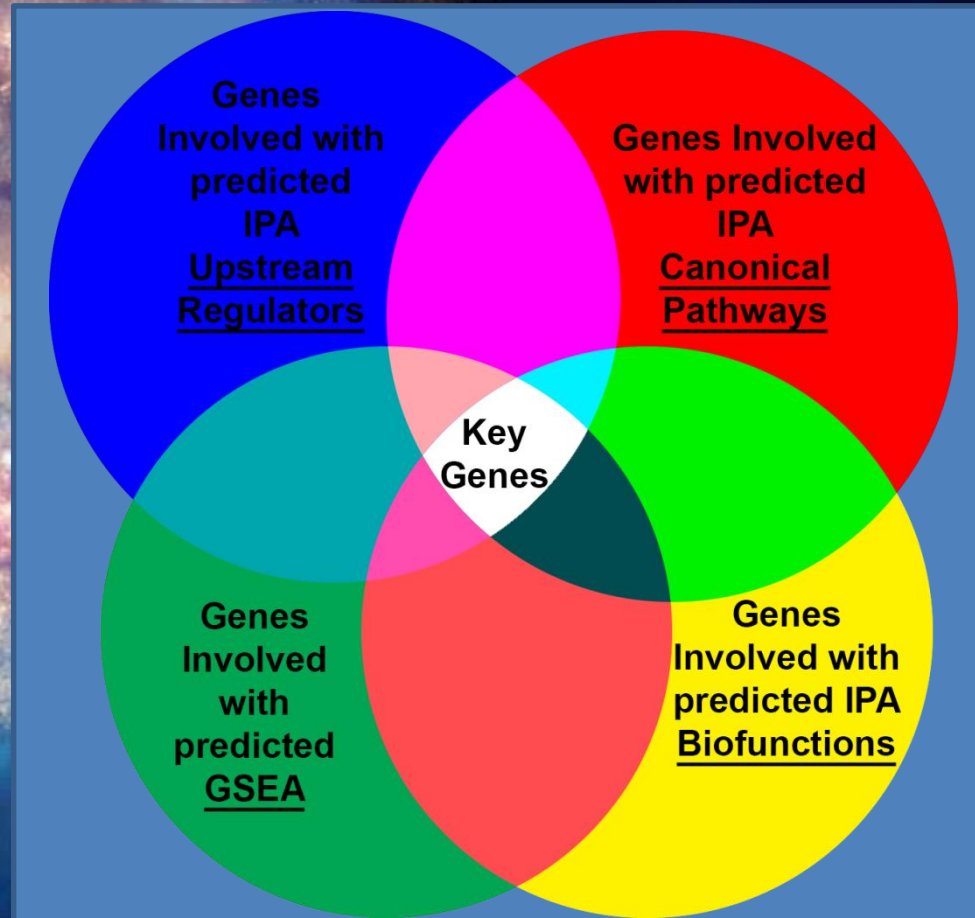


Pathway/Functional Predictions:
Ingenuity Pathway Analysis (IPA)
Gene Set Enrichment Analysis (GSEA)

Predicted Master Regulators



Determination of Key Genes/Drivers



Beheshti, et al. Cancer Research 2015

Beheshti, et al. Oncotarget 2015

Beheshti, et al. Cancer Informatics 2015

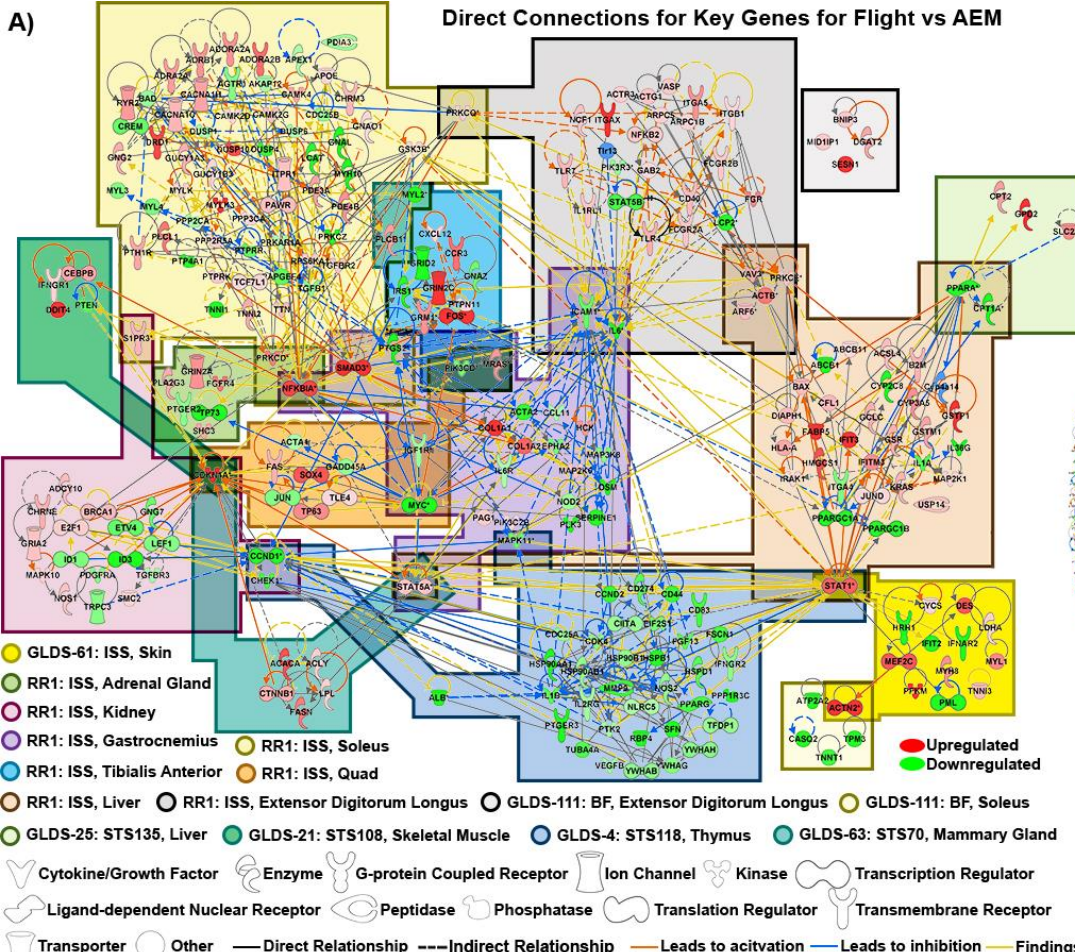
Beheshti, et al, Radiat Res. 2014 & J. Radiat Res. 2015.

Ravi, Beheshti, et al. Cancer Research 2016

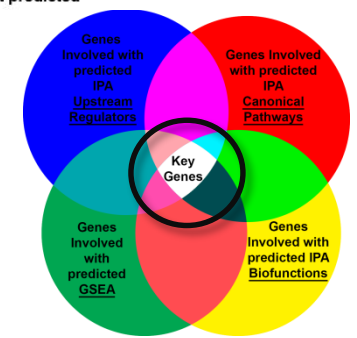
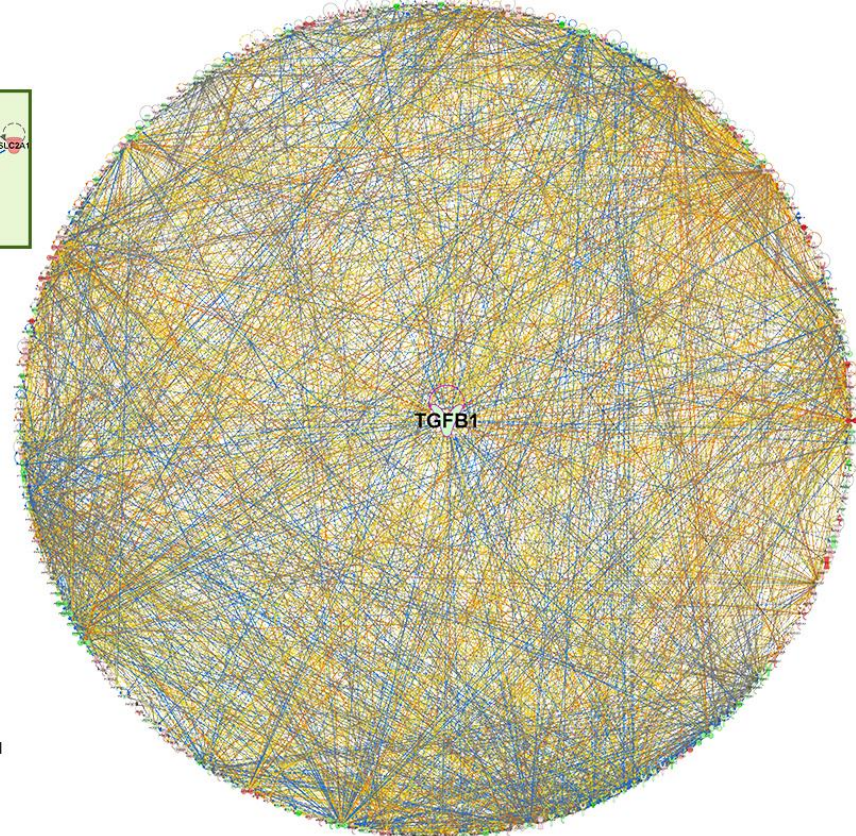
Key Genes and the Connections

A)

Direct Connections for Key Genes for Flight vs AEM

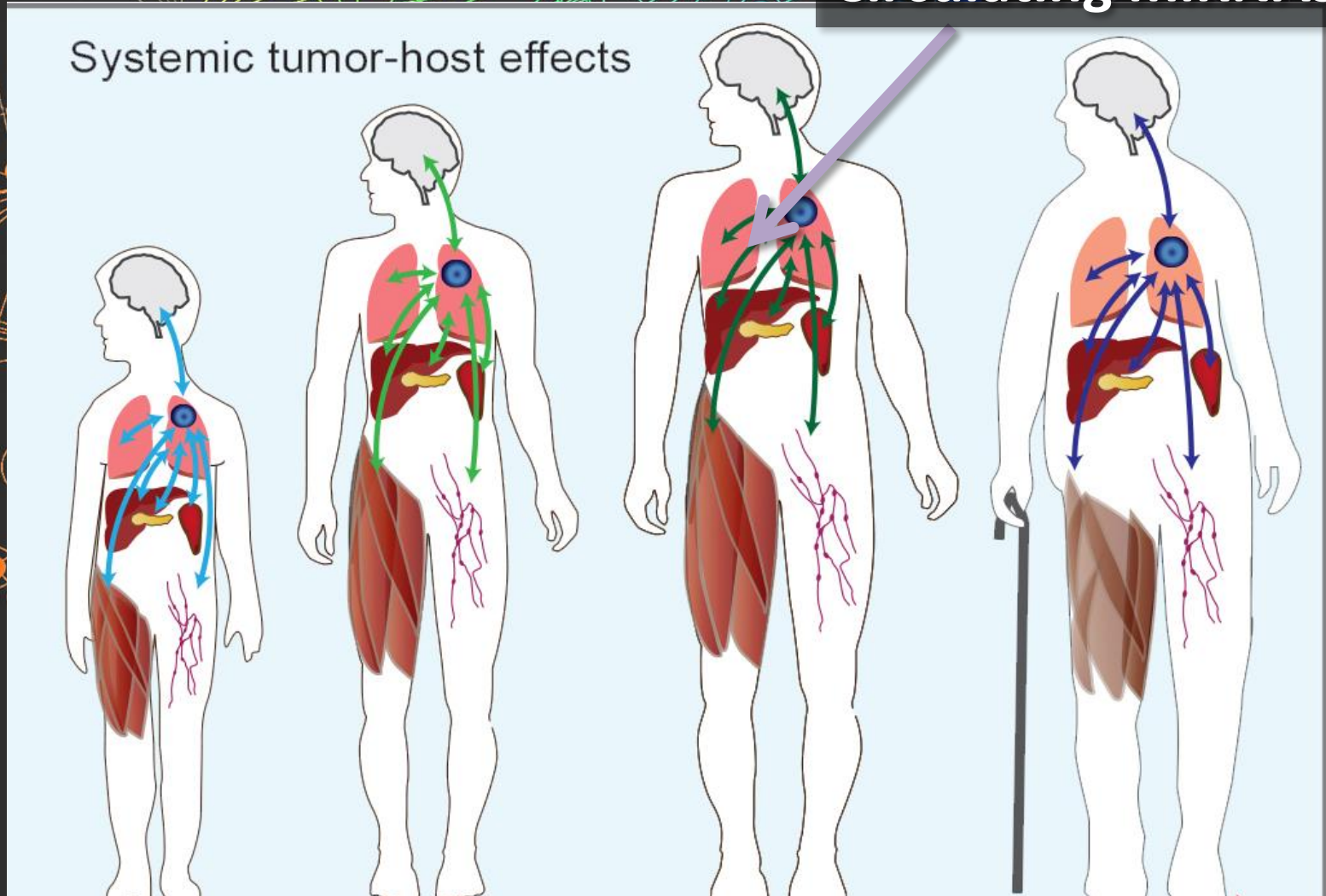


B) Connections Between all Key Genes for all Datasets (Flight vs AEM): Radial Plot with the most Connected Gene in the Middle

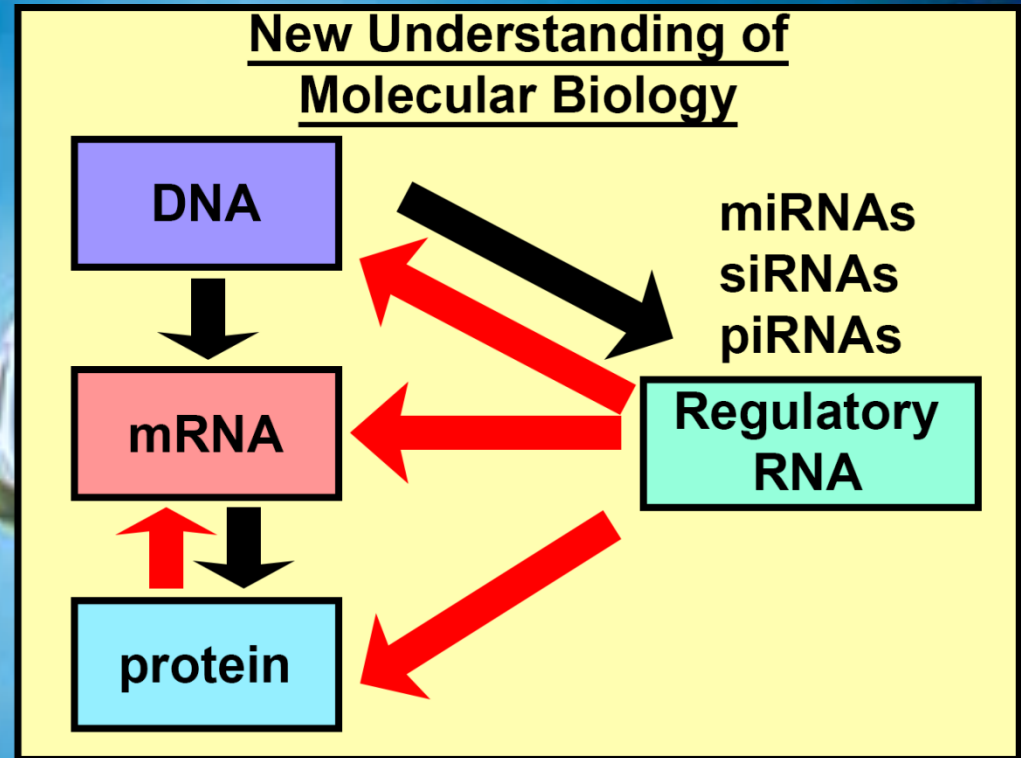
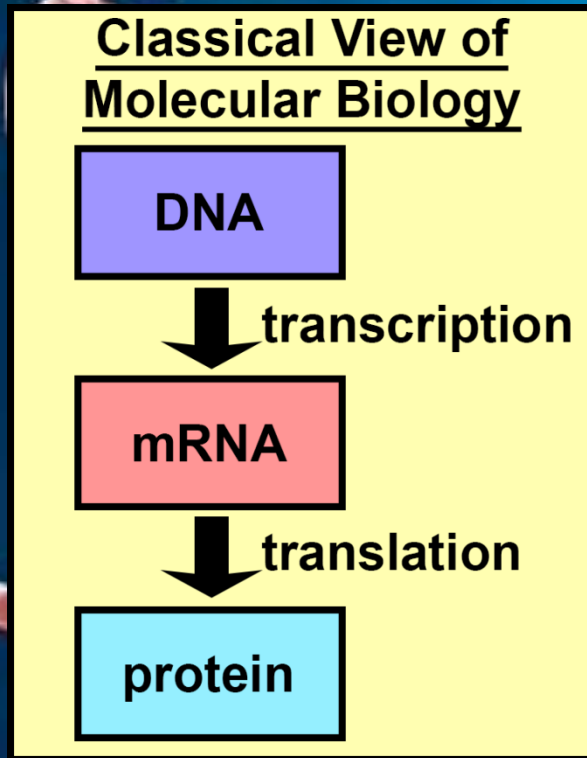


General Approach to Studying a Systematic Response in the Host

Circulating miRNAs



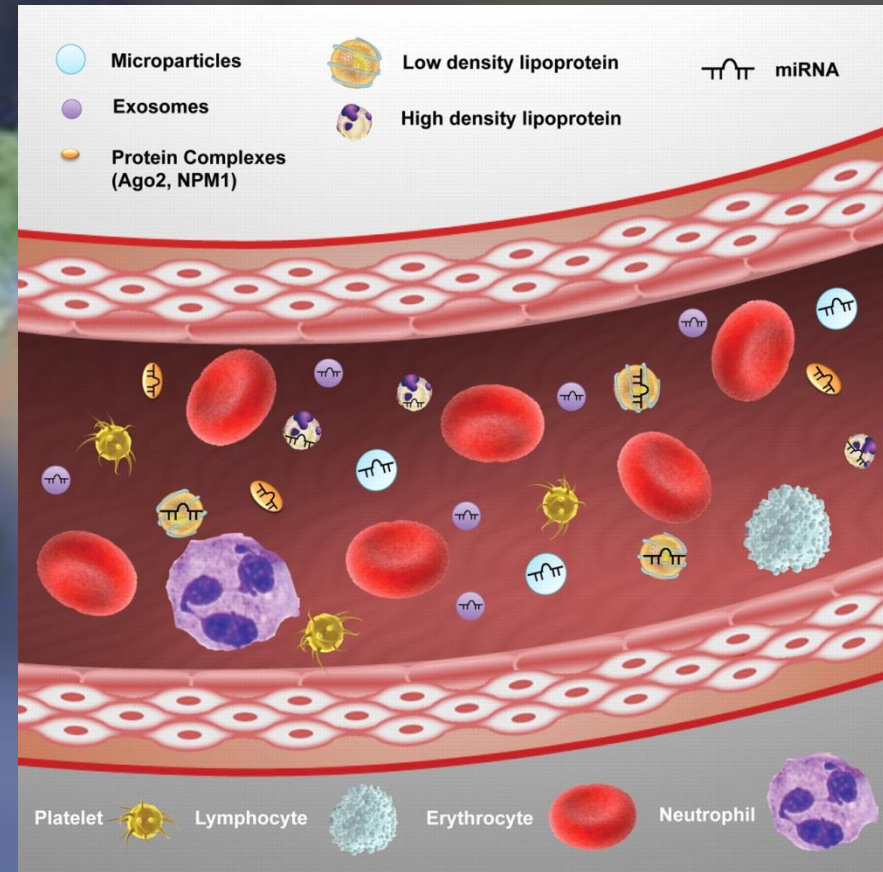
Revised View of Molecular Biology



- 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.
 - Beheshti, et al. *PLoS ONE* 2017

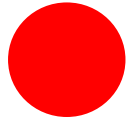


Profiling of circulating microRNAs: from single biomarkers to re-wired networks Anna Zampetaki, Peter Willeit, Ignat Drozdov, Stefan Kiechl, Manuel Mayr. *Cardiovascular Research*, 2011.

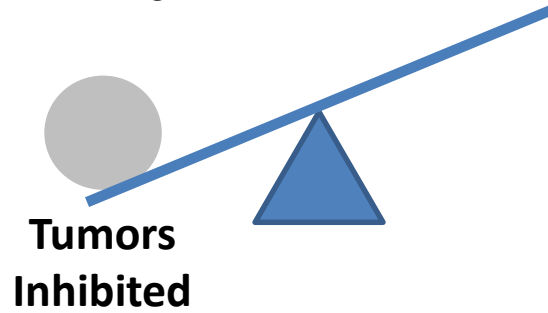
Systems Biology View of miRNAs



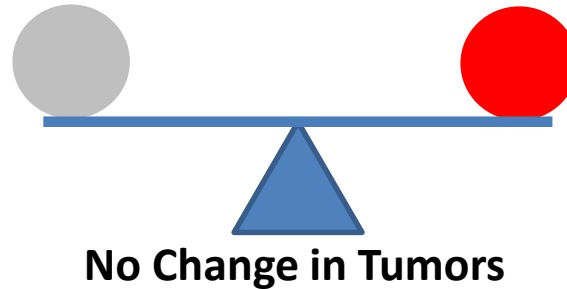
Tumor Suppressor miRNAs



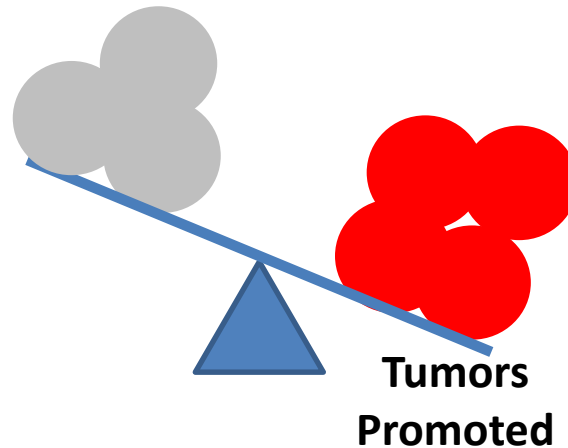
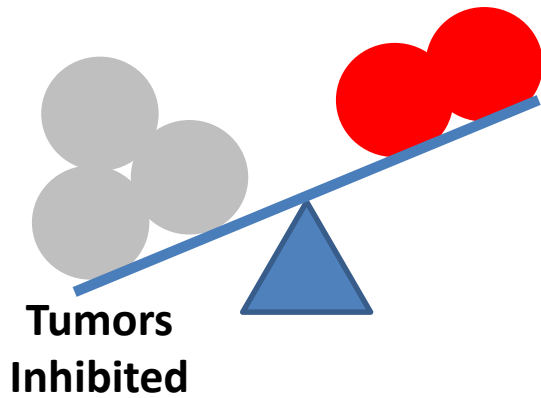
OncomiRNAs



Only looking at a single miRNA



looking at a pair of miRNAs

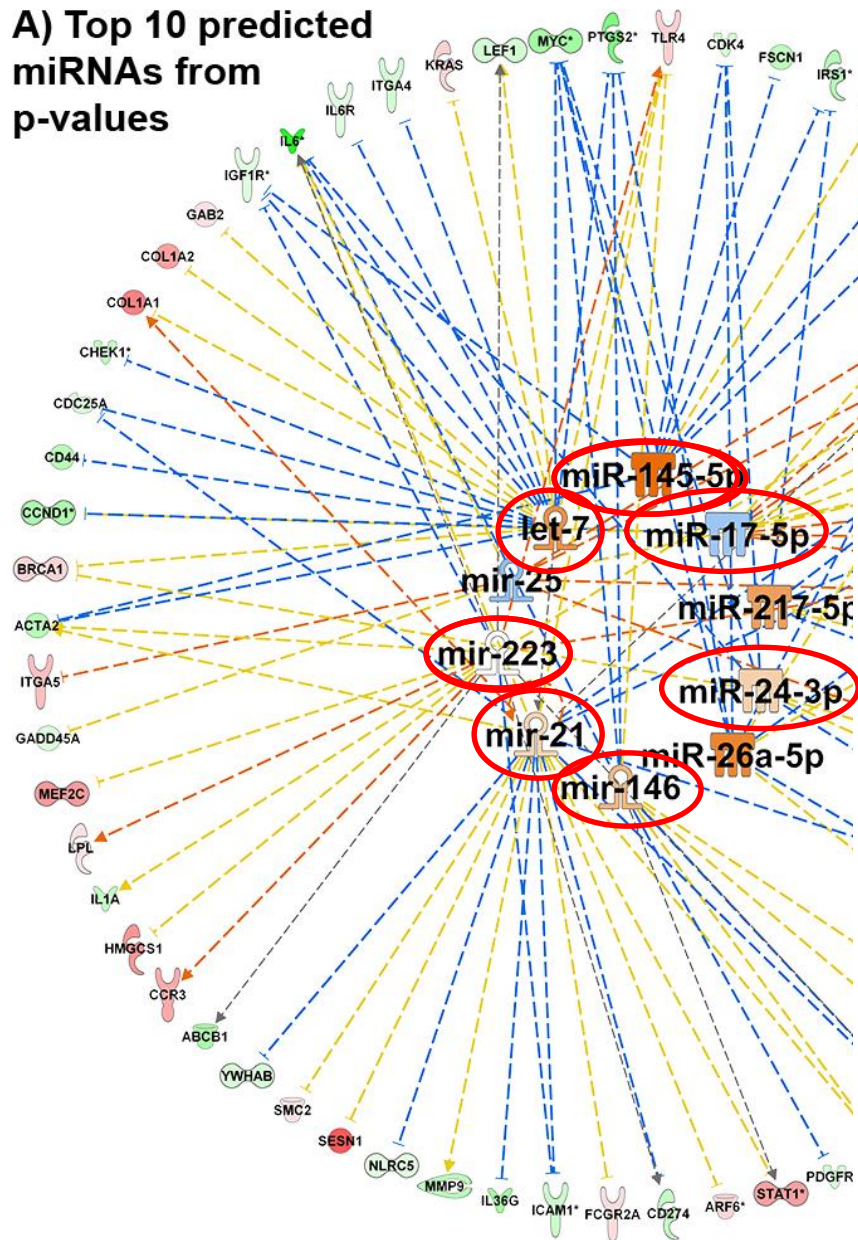


Systems Biology Approach:
Looking at how the entire system impacts the most Important miRNAs

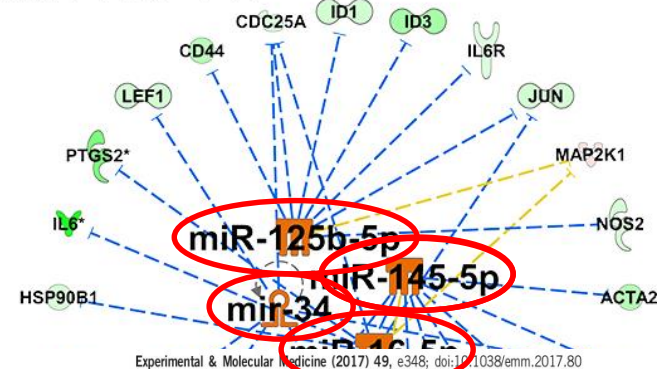
Predicted miRNAs Involved with Microgravity Effects

- 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



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Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 296747, 16 pages
<http://dx.doi.org/10.1155/2014/296747>

Experimental & Molecular Medicine (2017) 49, e348; doi:10.1038/emm.2017.80



Research Article

Integration Analysis of MicroRNA and mRNA Expression Profiles in Human Peripheral Blood Lymphocytes Cultured in Modeled Microgravity

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Received 16 April 2014; Revised 22 May 2014; Accepted 22 May 2014; Published 23 June 2014

Academic Editor: Mariano Bizzarri

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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 MMG-incubated PBLs compared with 1g incubated ones. Among these, miR-9-5p, miR-9-3p, miR-155-5p, miR-150-3p, 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-155-5p, miR-150-3p, and miR-378-3p expression 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., 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.

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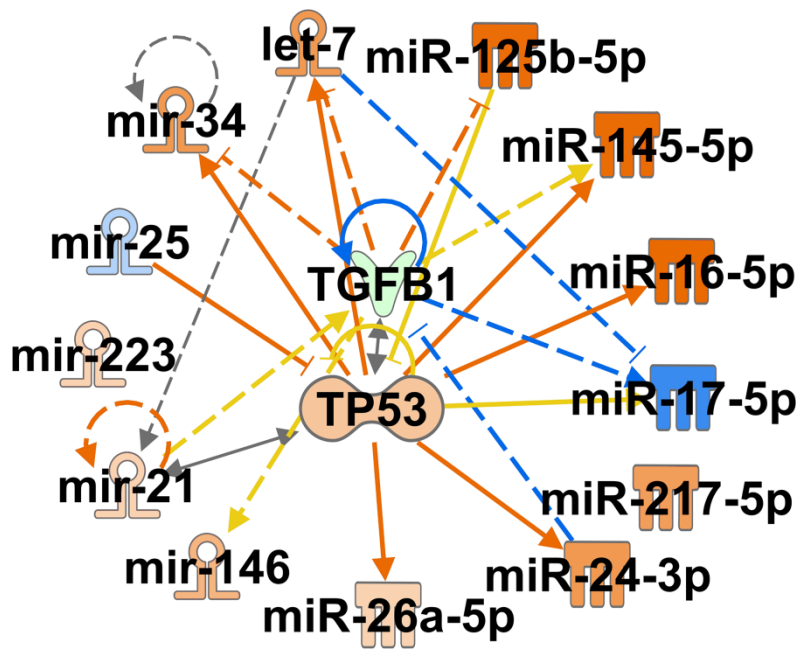
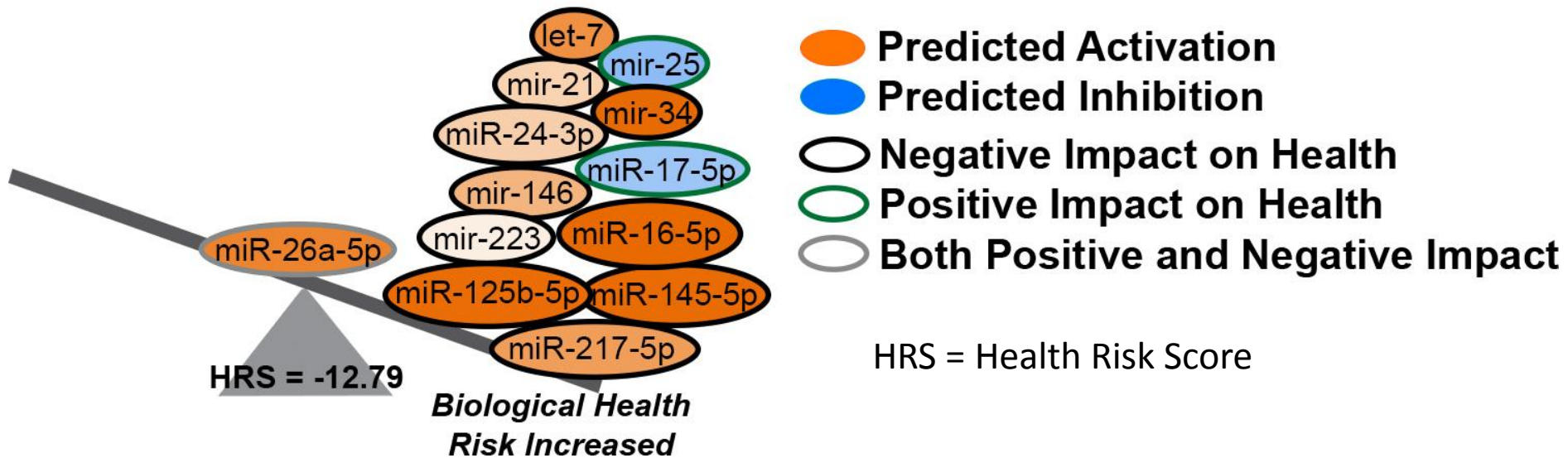
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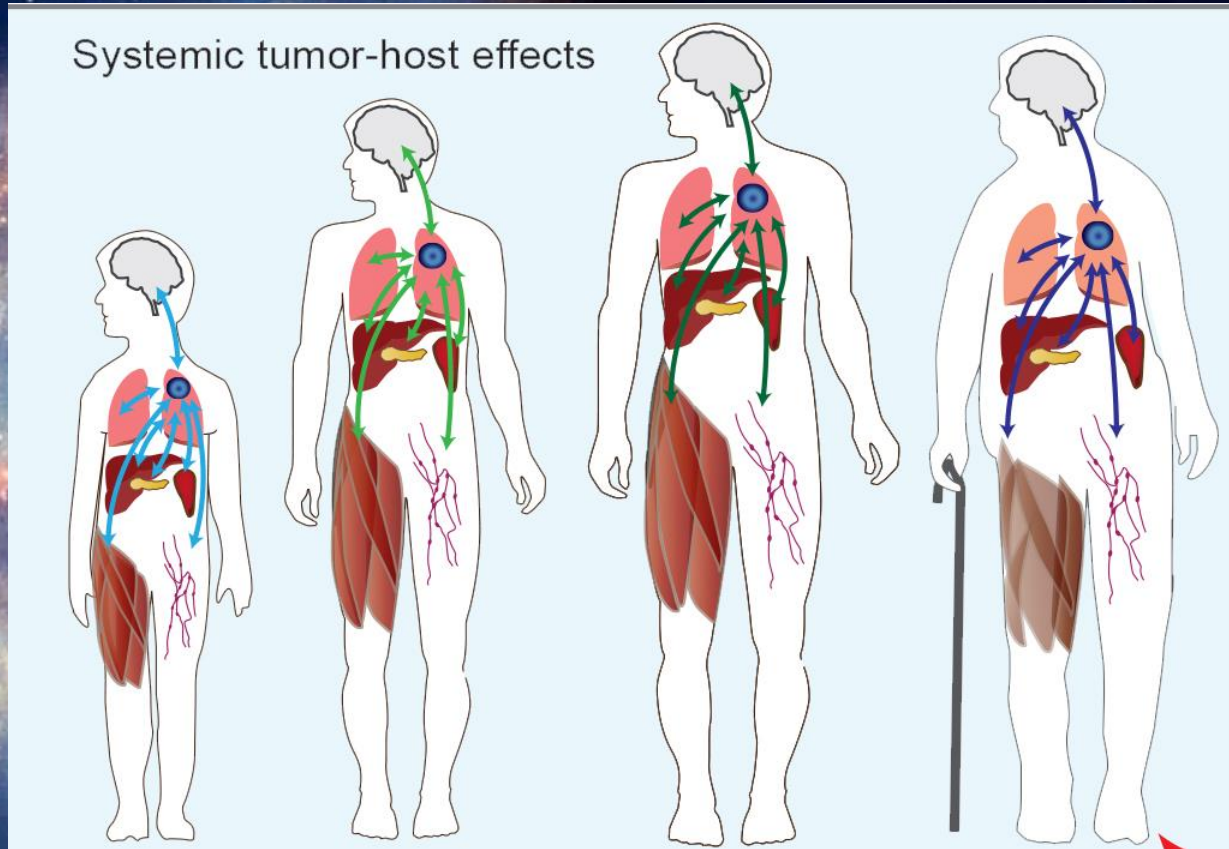
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Predicted miRNAs Involved with Microgravity Effects

Health Risk Due to miRNAs

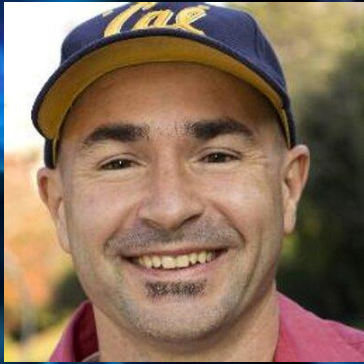


Overall Summary of All Data



- **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**

Acknowledgements



Sylvain Costes, PhD
GeneLab
Project Manager



Homer Fogle, PhD



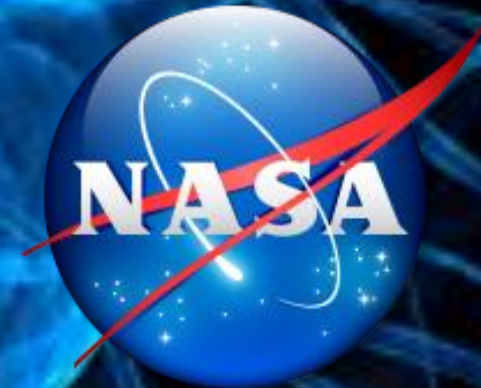
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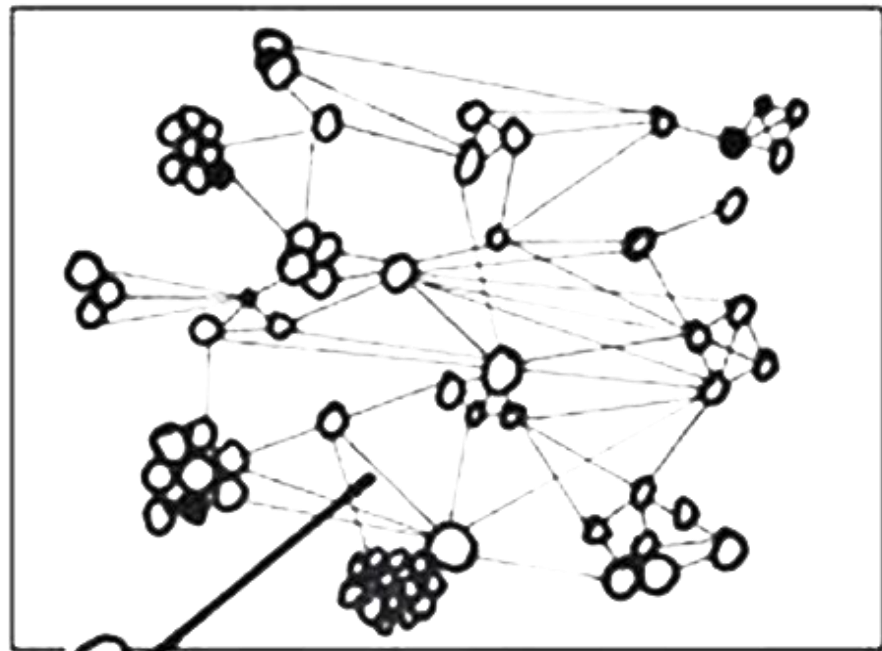
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Thanks to Systems Biology, we now have a clear picture of complex diseases!



Questions??



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