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

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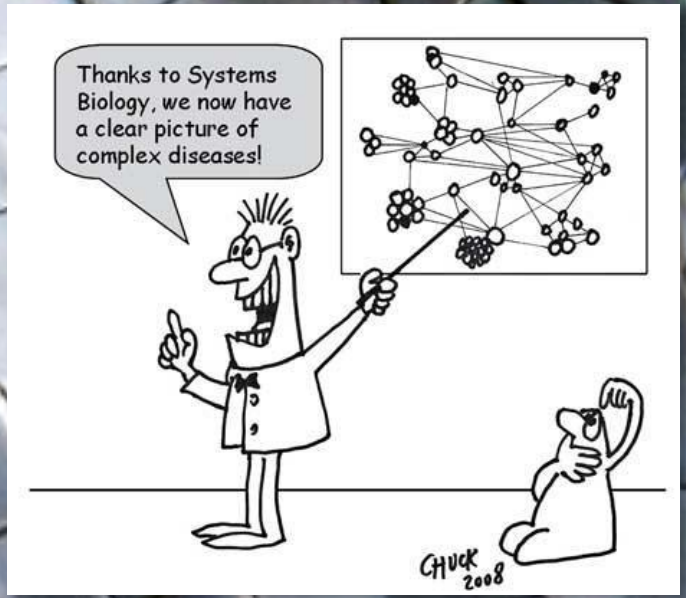
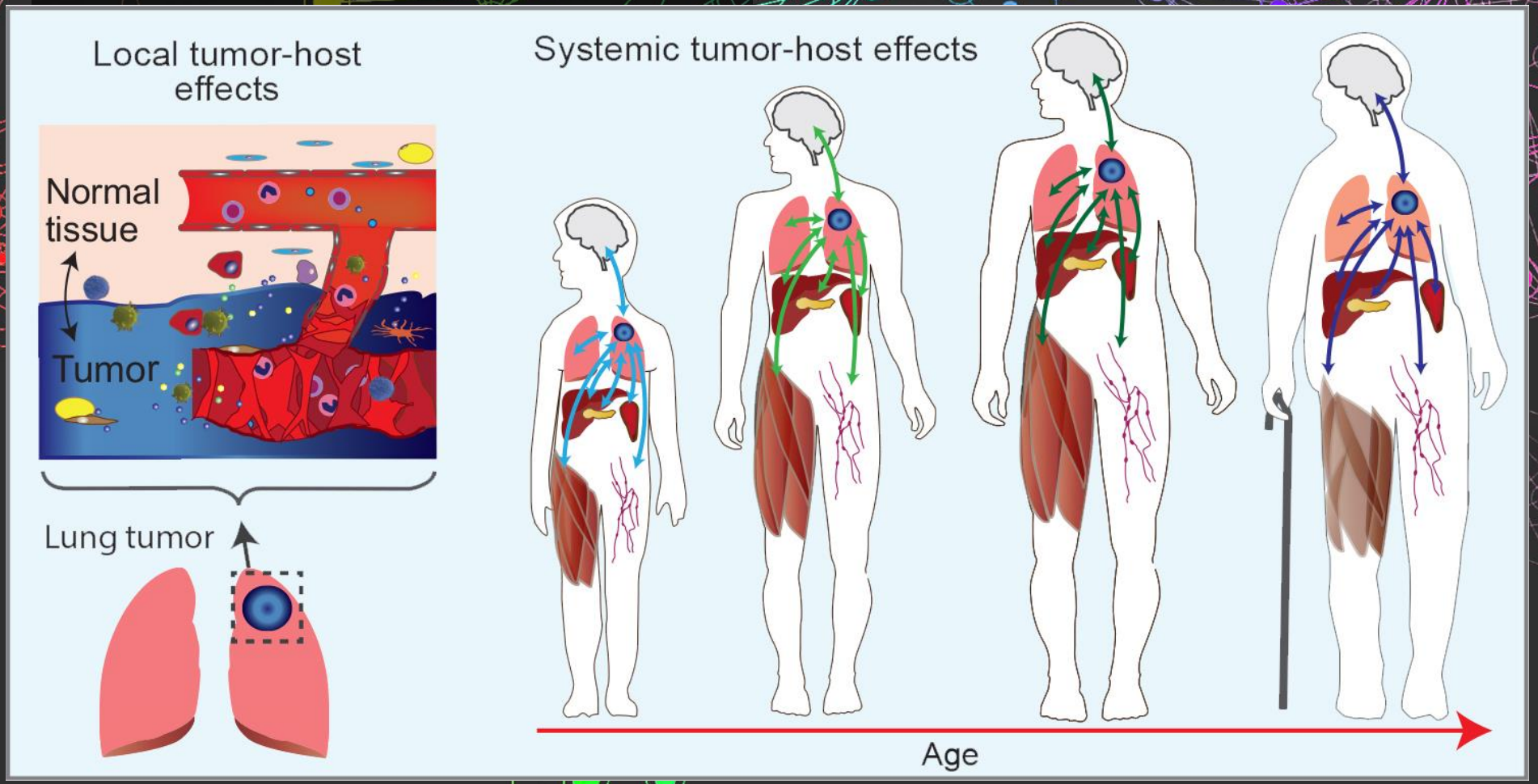
What is Systems Biology?

- 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."

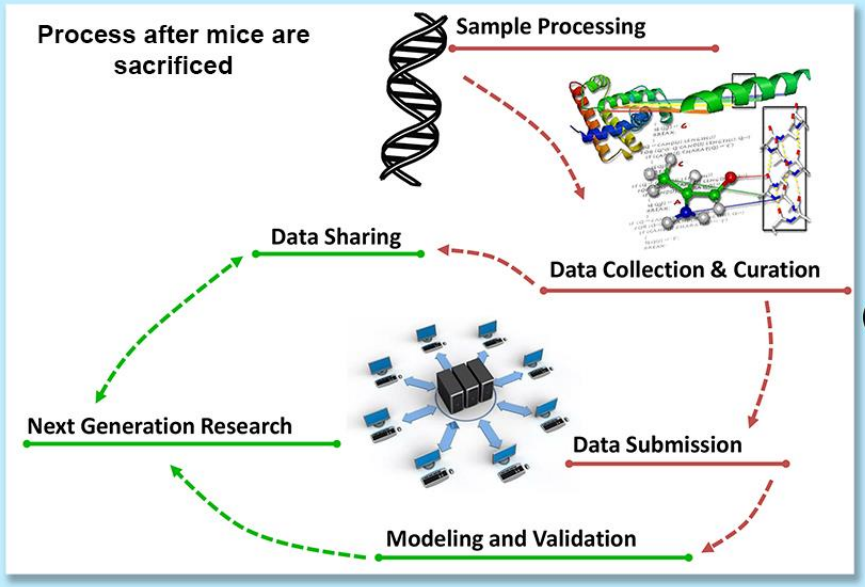


General Approach to Studying a Systematic Response in the Host

An example for cancer research

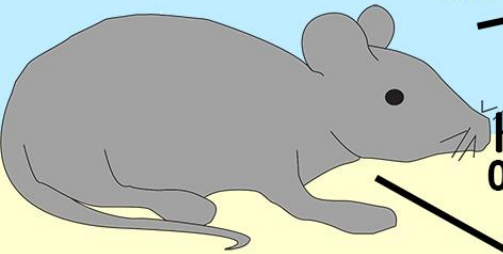


GeneLab Data Used to Generate Results



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

Mice Sacrificed on ISS



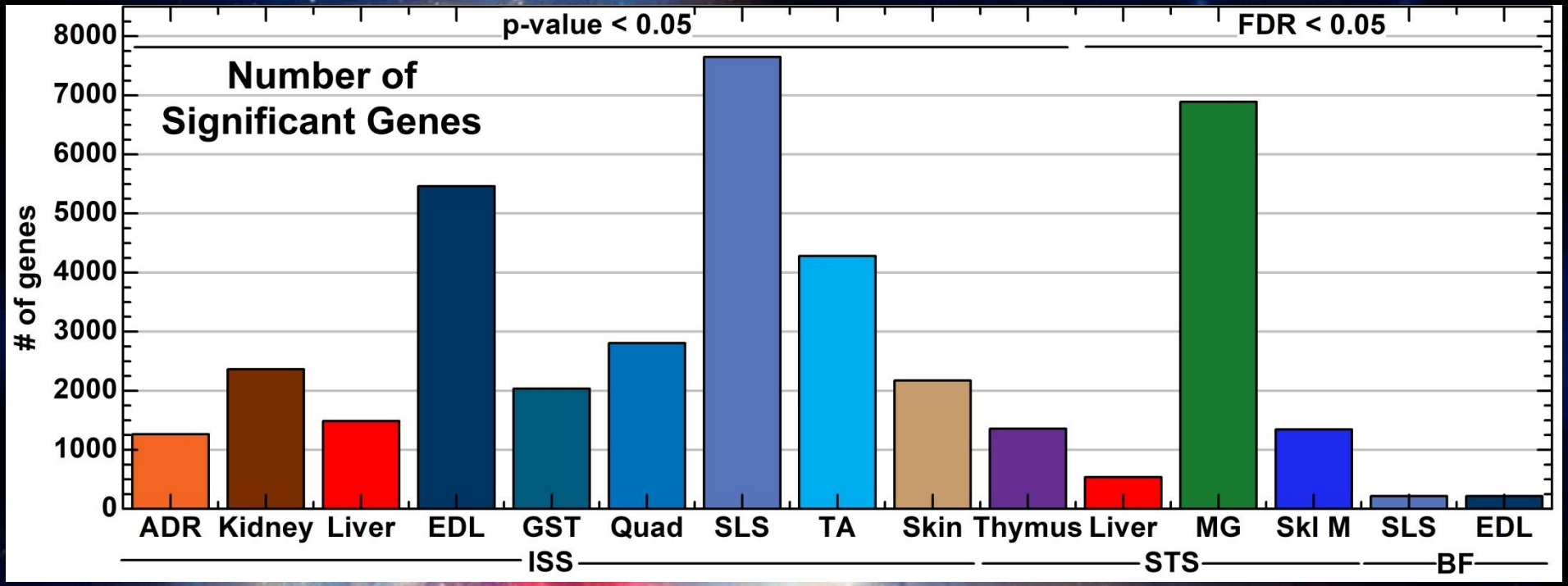
0 10 20 30 40 50 60 70 80 90 100

Time in Space for Mice (days)

Mice flown on STS and Sacrificed after Re-entry

Liver
Skeletal Muscle
Mammary Gland
Thymus
Soleus Muscle
Extensor Digitorum Longus Muscle

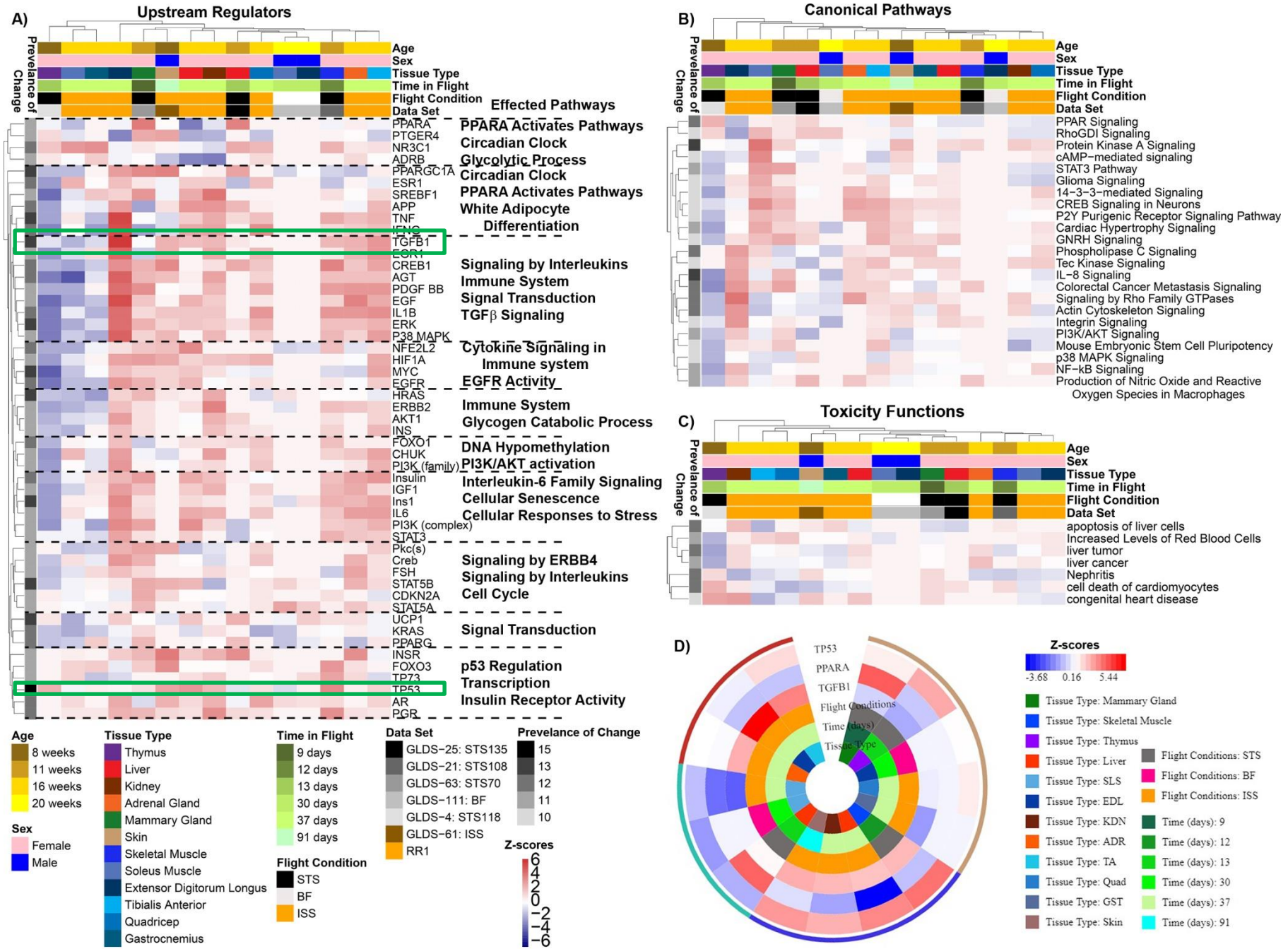
Number of Significant Genes from Each Dataset



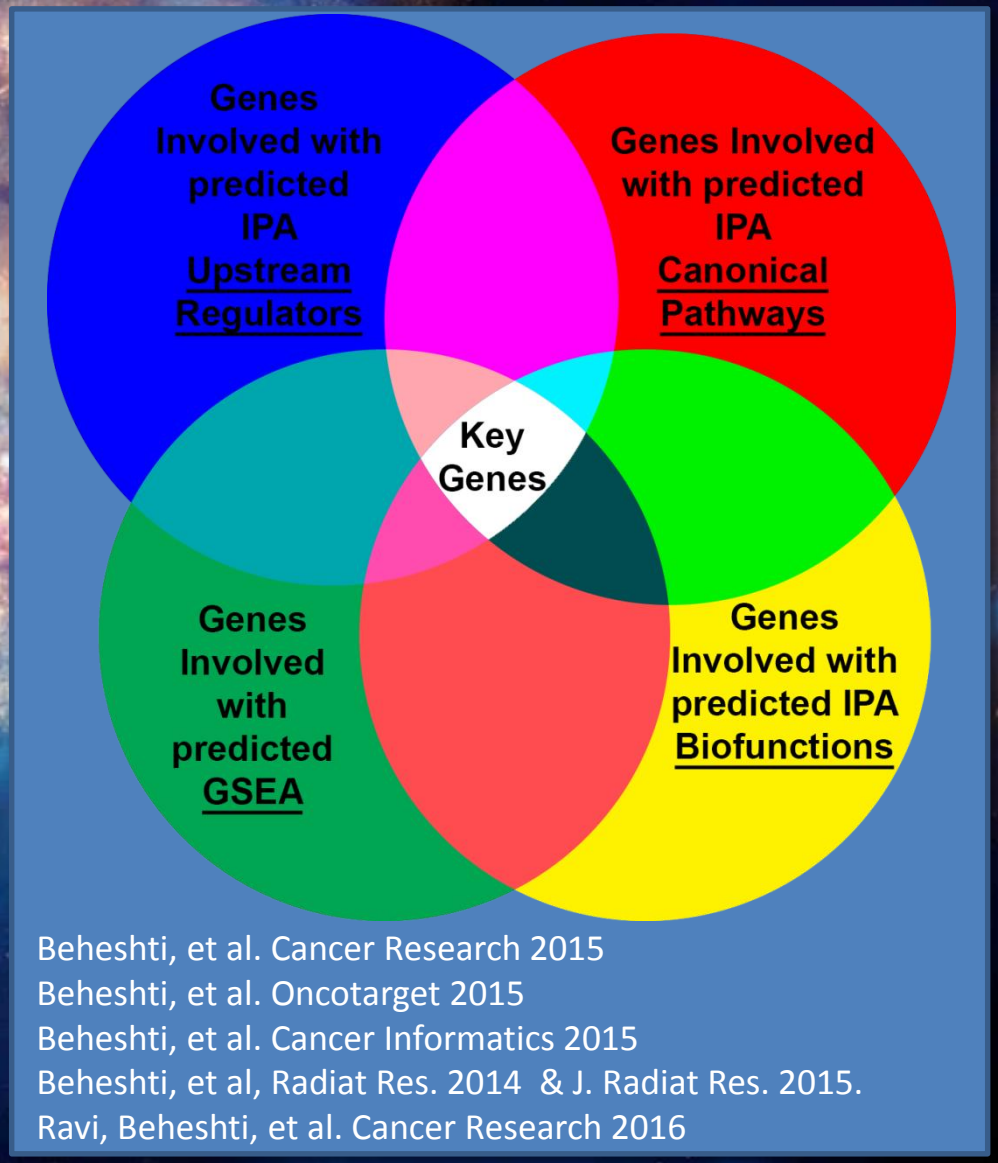
Fold-Change ≥ |1.2|

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

Predicted Master Regulators

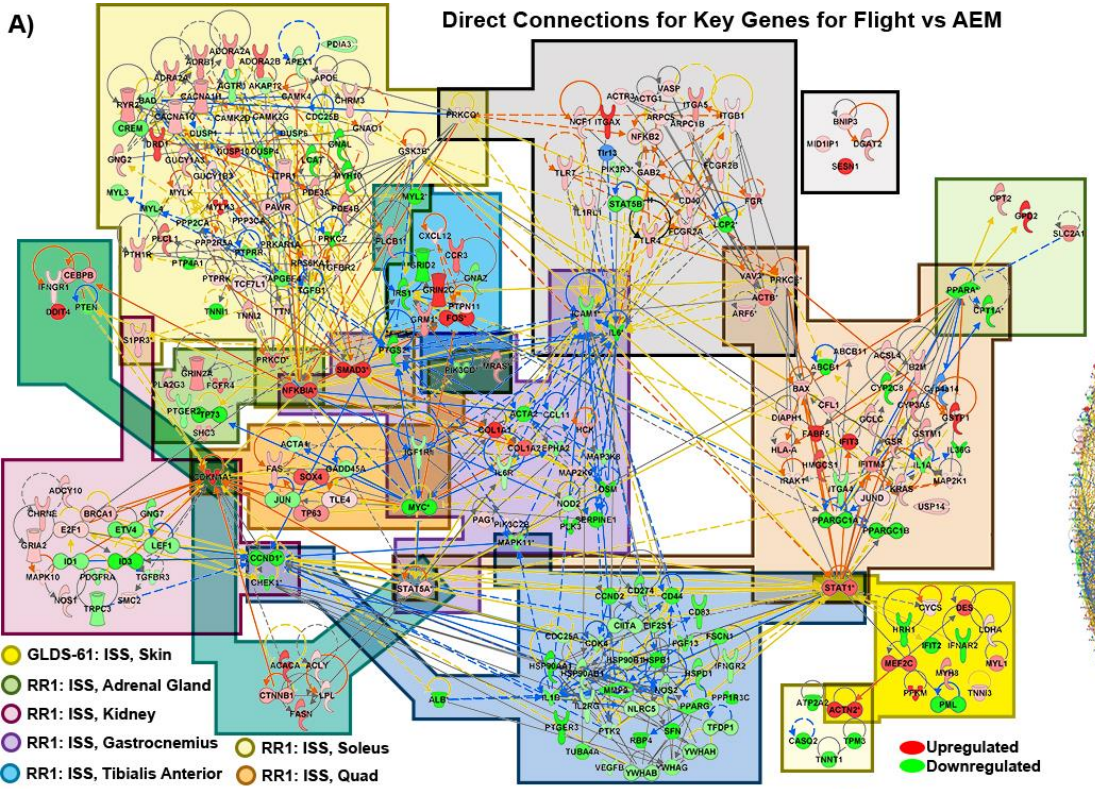


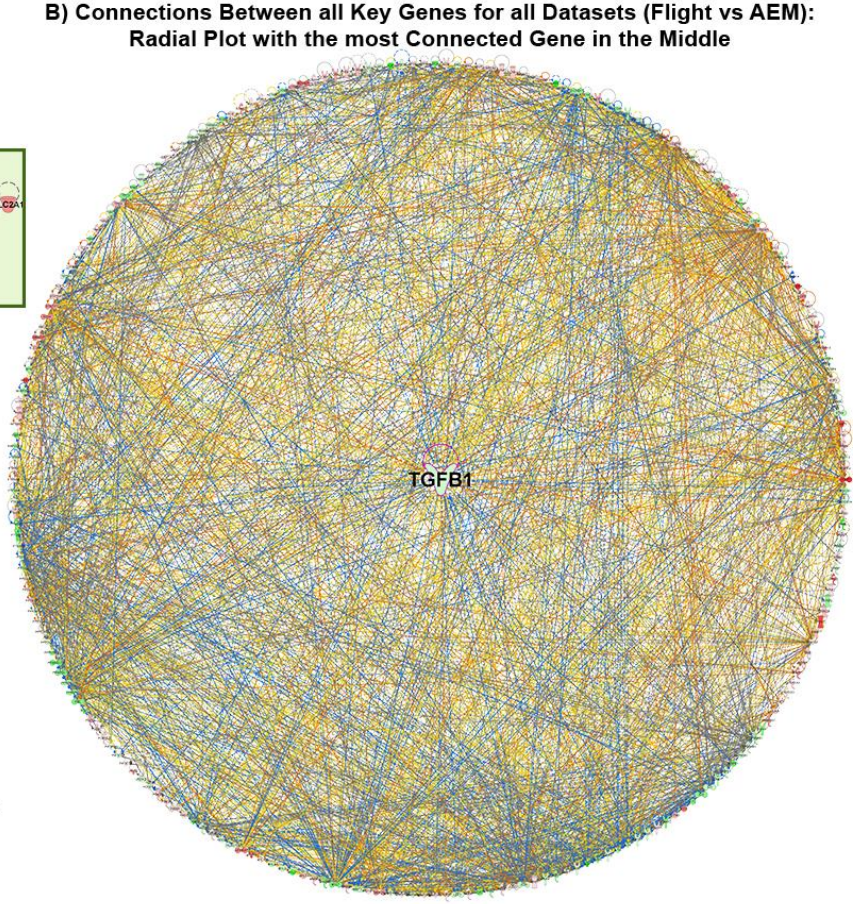
Determination of Key Genes/Drivers

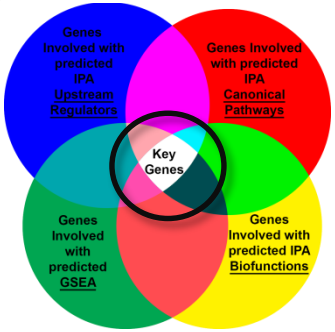


Beheshti, et al. Cancer Research 2015
Beheshti, et al. Oncotarget 2015
Beheshti, et al. Cancer Informatics 2015
Beheshti, et al, Radiat Res. 2014 & J. Radiat Res. 2015.
Ravi, Beheshti, et al. Cancer Research 2016

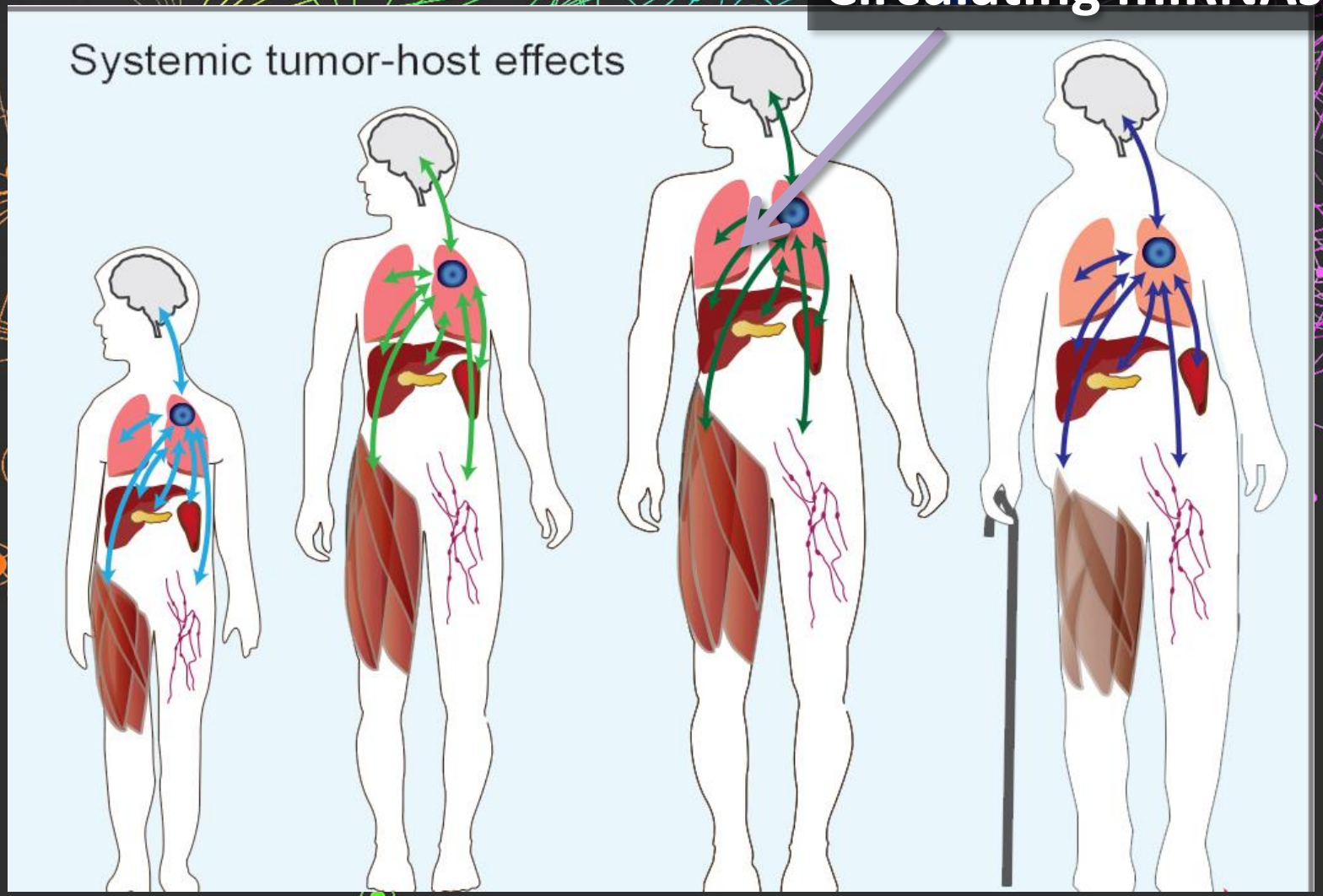
Key Genes and the Connections



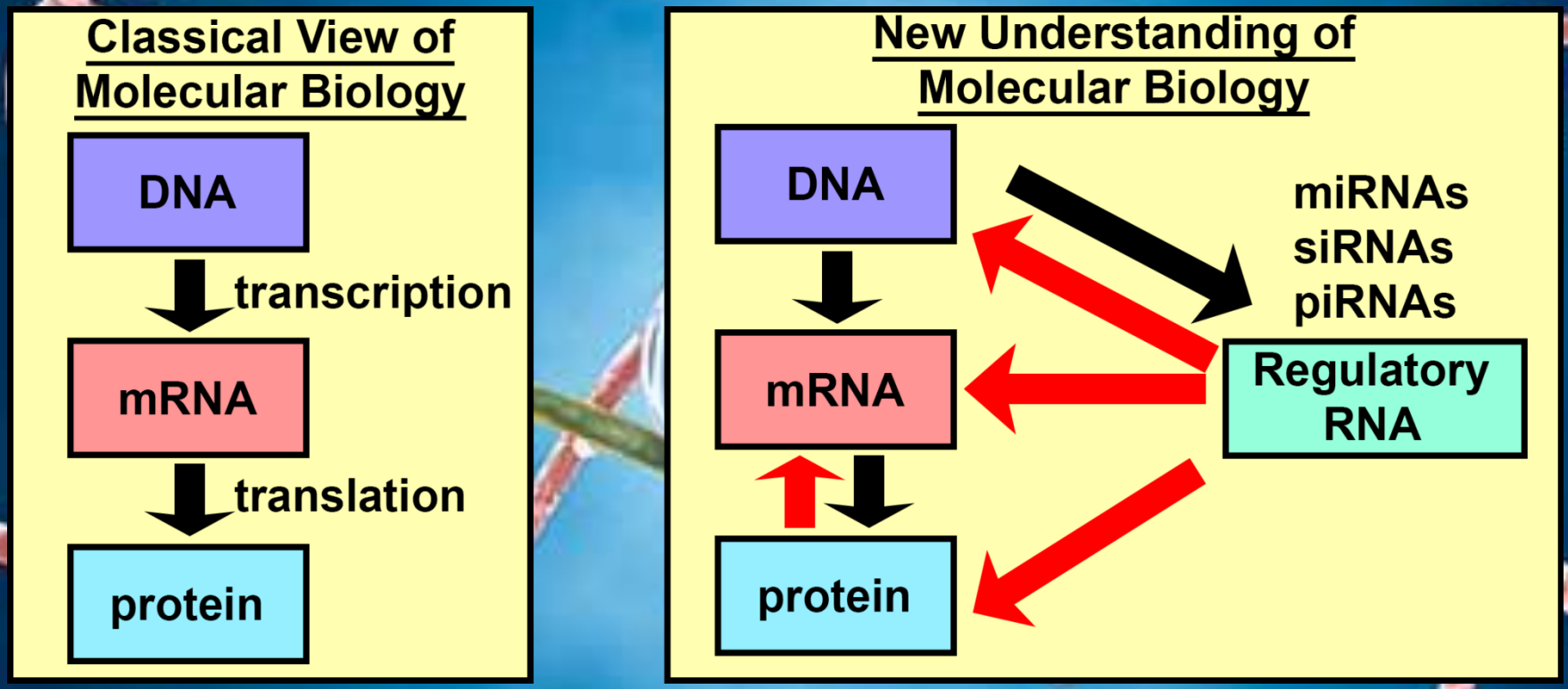




General Approach to Studying a Systematic Response in the Host



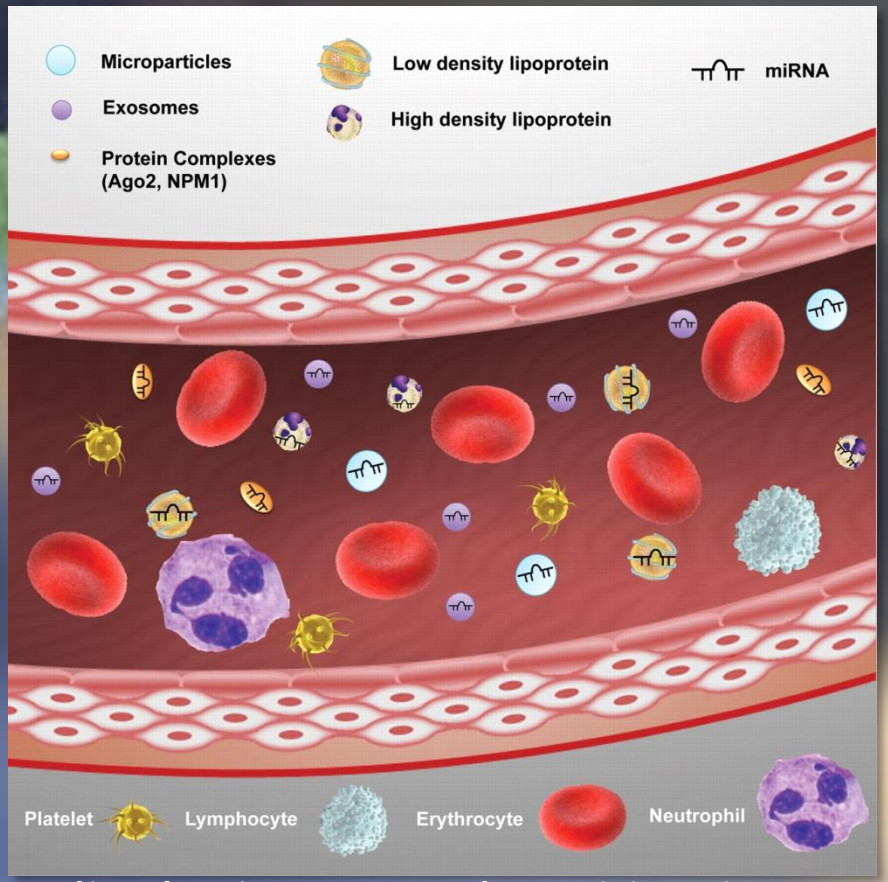
Revised View of Molecular Biology



- 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*

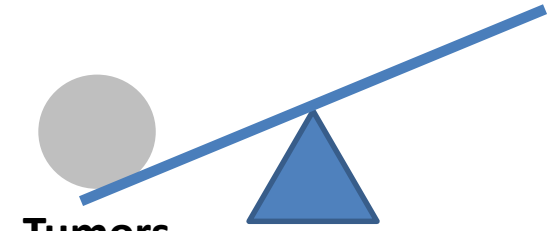


Profiling of circulating microRNAs: from single biomarkers to re-wired networks Anna Zampetaki, Peter Willeit, Ignat Drozdov, Stefan Kiechl, Manuel Mayr. Cardiovascular Research , 2011.

Systems Biology View of miRNAs

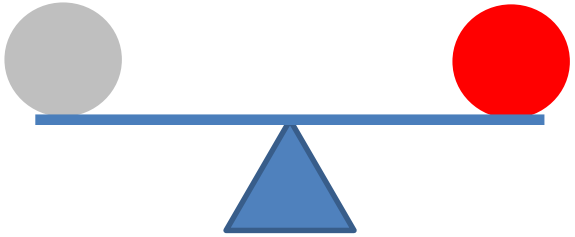
Tumor Suppressor miRNAs

OncomiRNAs



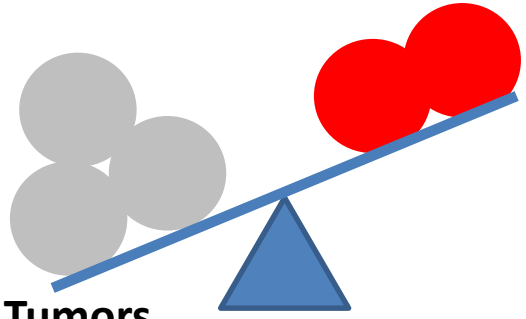
Tumors Inhibited

Only looking at a single miRNA



No Change in Tumors

looking at a pair of miRNAs



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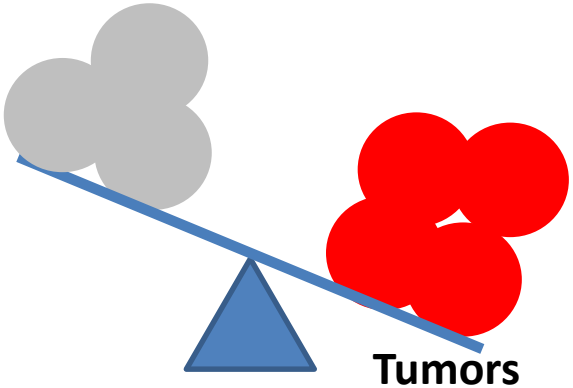
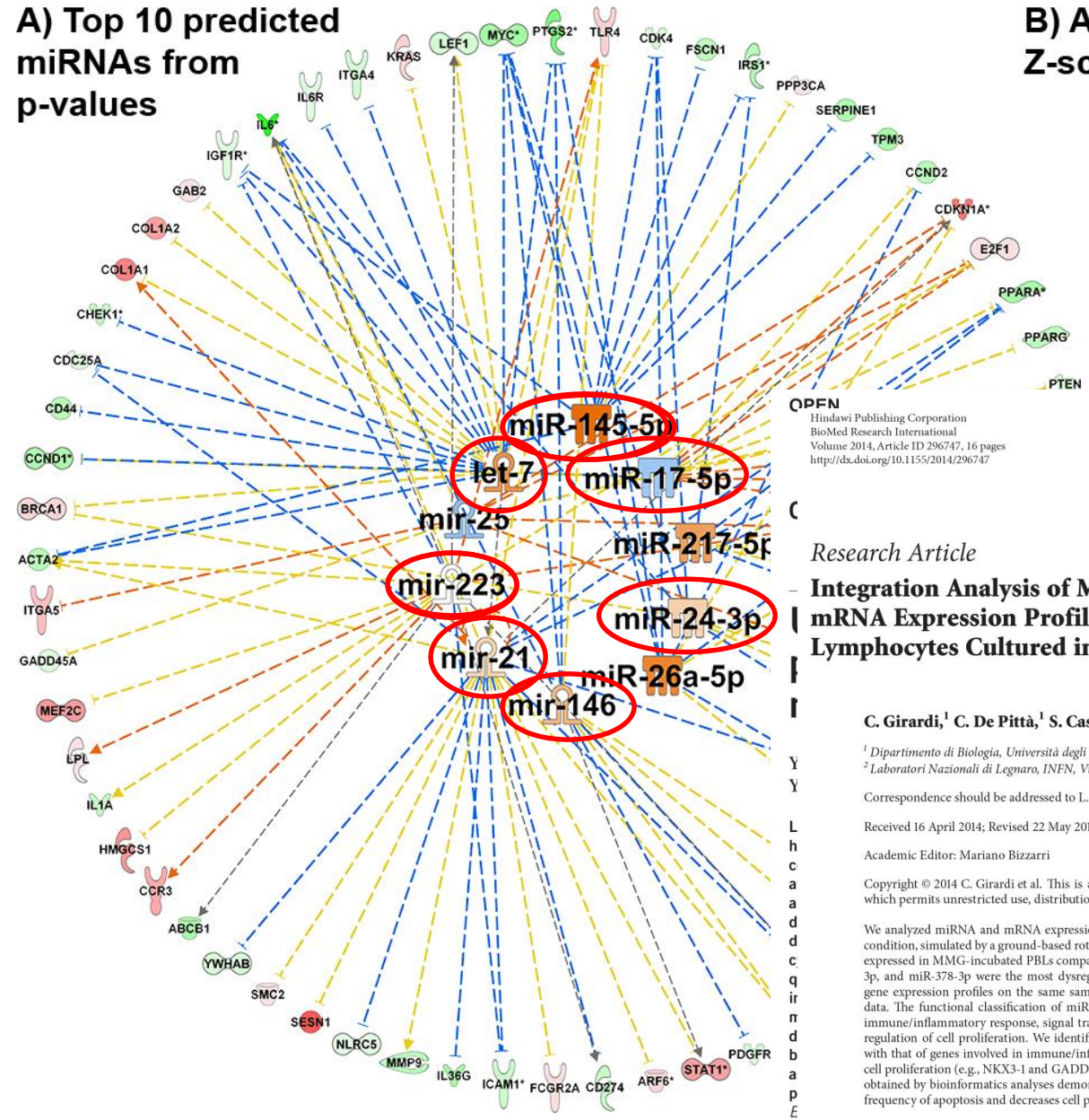


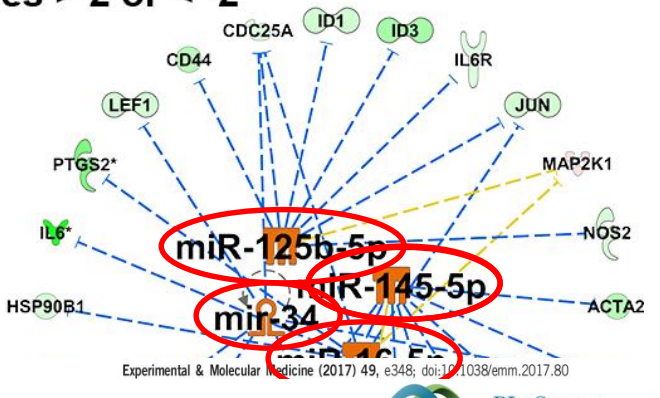
Tumors Promoted

<u>Systems Biology Approach:</u> 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





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

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PLoS one

Research Article

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

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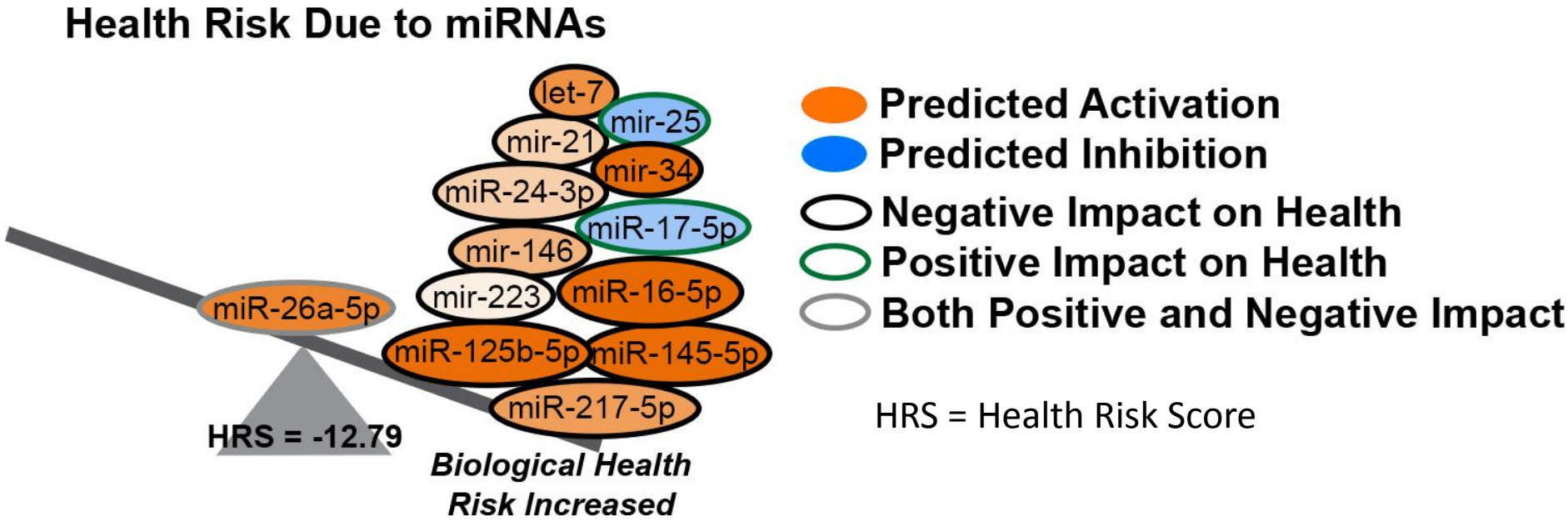
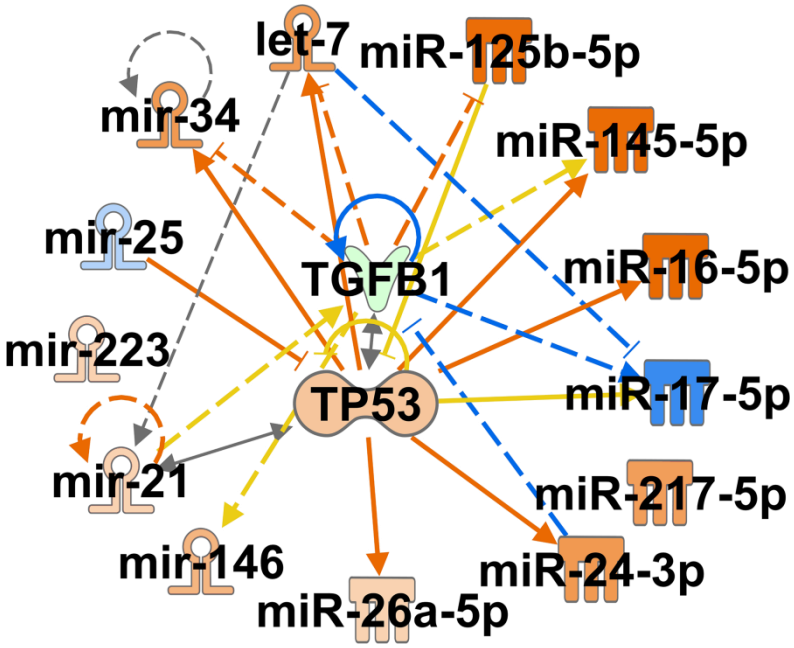
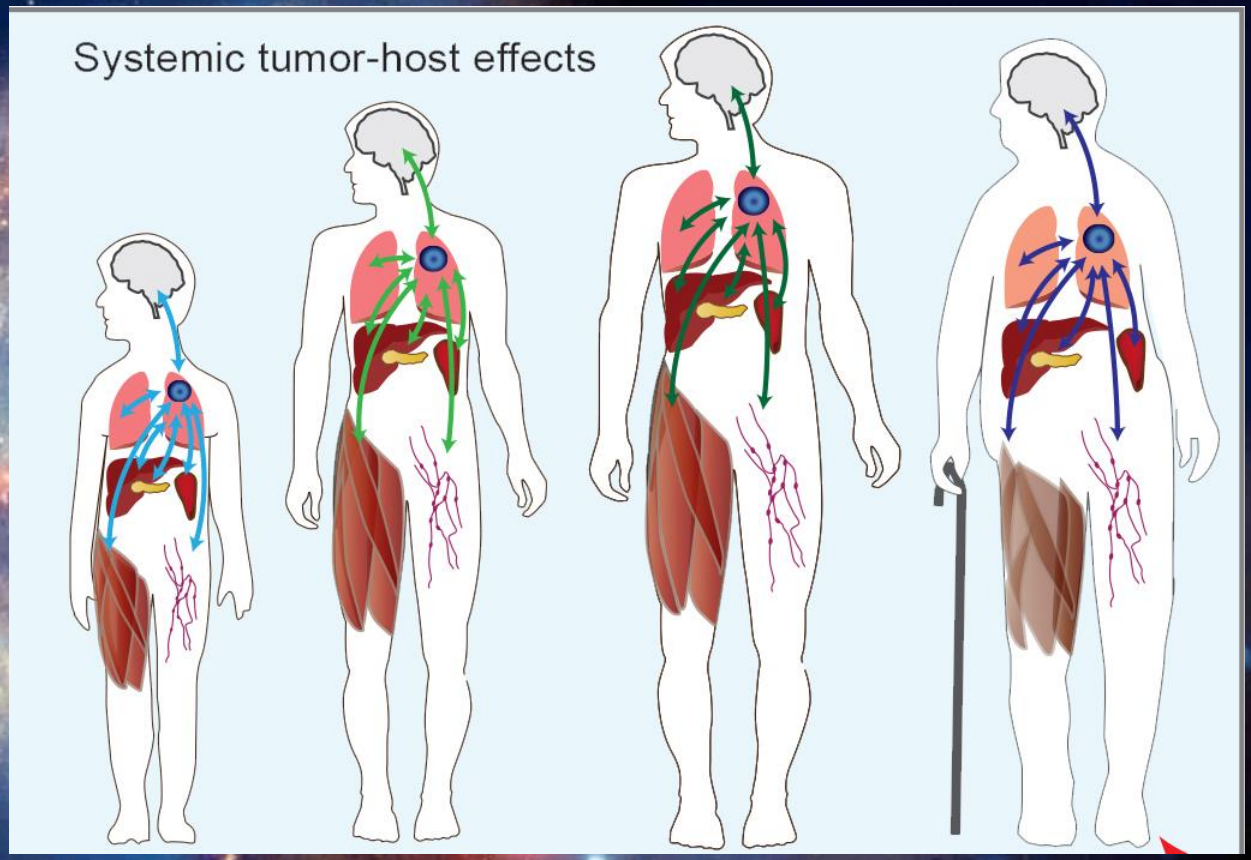
We analyzed miRNA and mRNA expression profiles in human peripheral blood lymphocytes (PBLs) incubated in microgravity condition, simulated by a ground-based rotating wall vessel (RWV) bioreactor. Our results show that 42 miRNAs were differentially expressed in MMG-incubated PBLs compared with 1 g incubated 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.

Predicted miRNAs Involved with Microgravity Effects



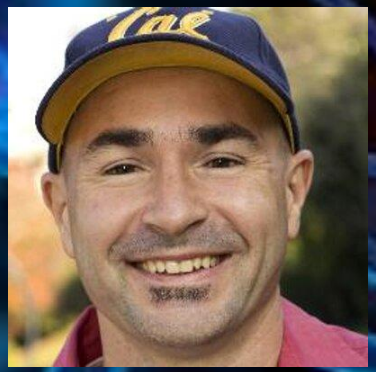


Overall Summary of All Data



- 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



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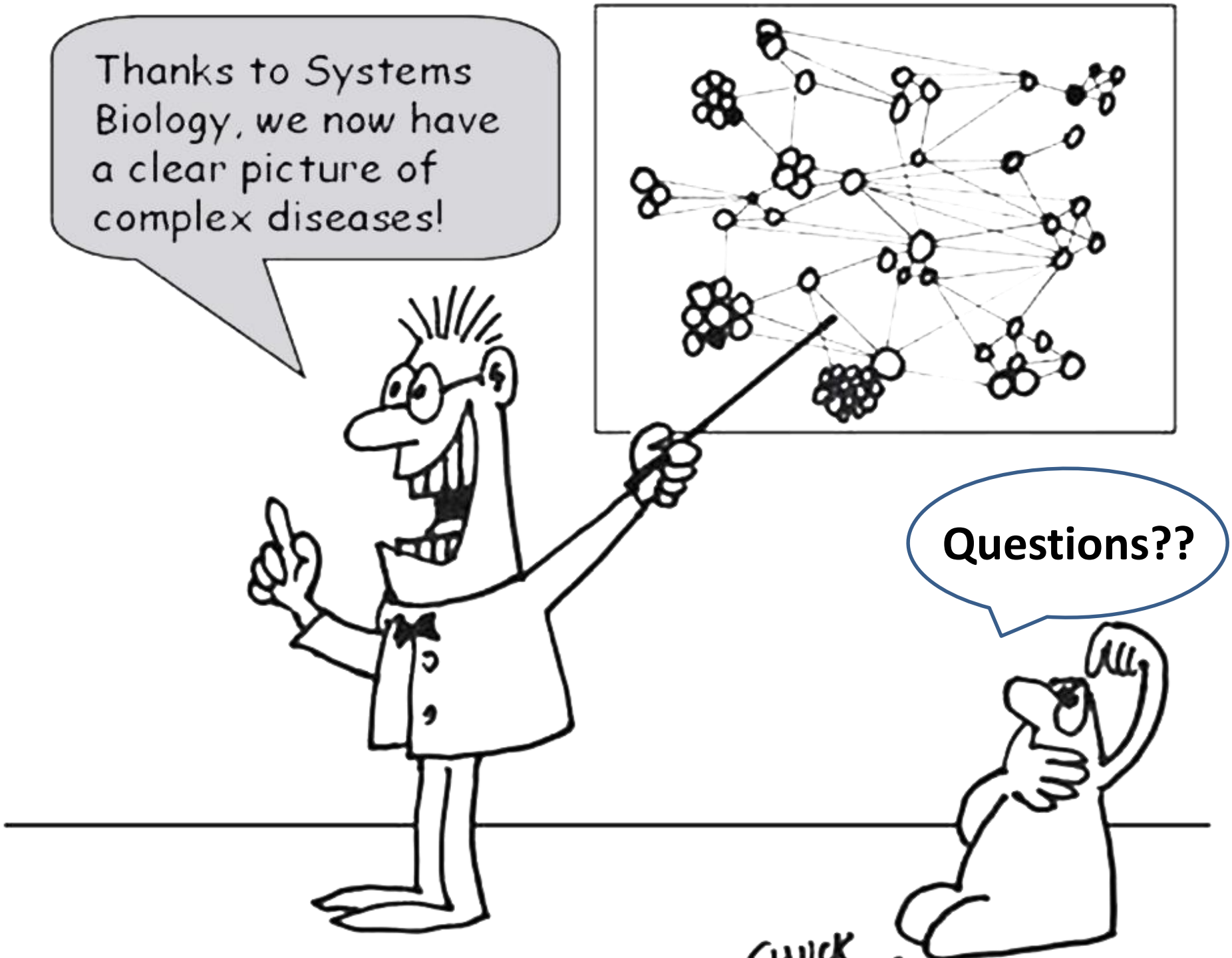
Thanks to Systems Biology, we now have a clear picture of complex diseases!
Questions??