The rat microRNA body atlas: miRNA identification and characterization

A Project of the HESI Genomics Committee

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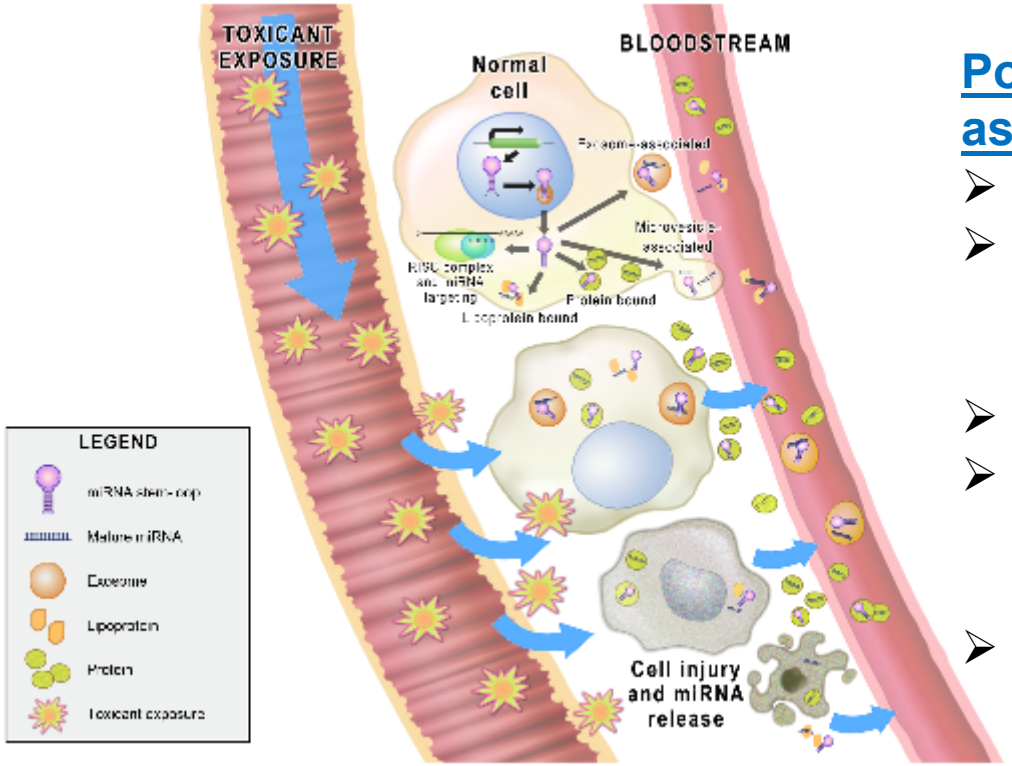
MicroRNAs: Tissue Specific Biomarkers

miRNA function: RNA silencing and post-transcriptional regulation



Potential advantages of miRNAs as biomarkers:

- **Sensitivity and specificity**.
- Can **easily measure,** utilizing a small volume of serum in a qPCR based format.
- **Stable** in serum and plasma.
- **Conservation across species** and non-Ab detection enables ready translational use.
- Identification of **isomiRs** may yield **additional candidate biomarkers.**

Harrill et al. Tox Sci. In Press

Fill gaps for tissues where no reliable injury biomarkers exist

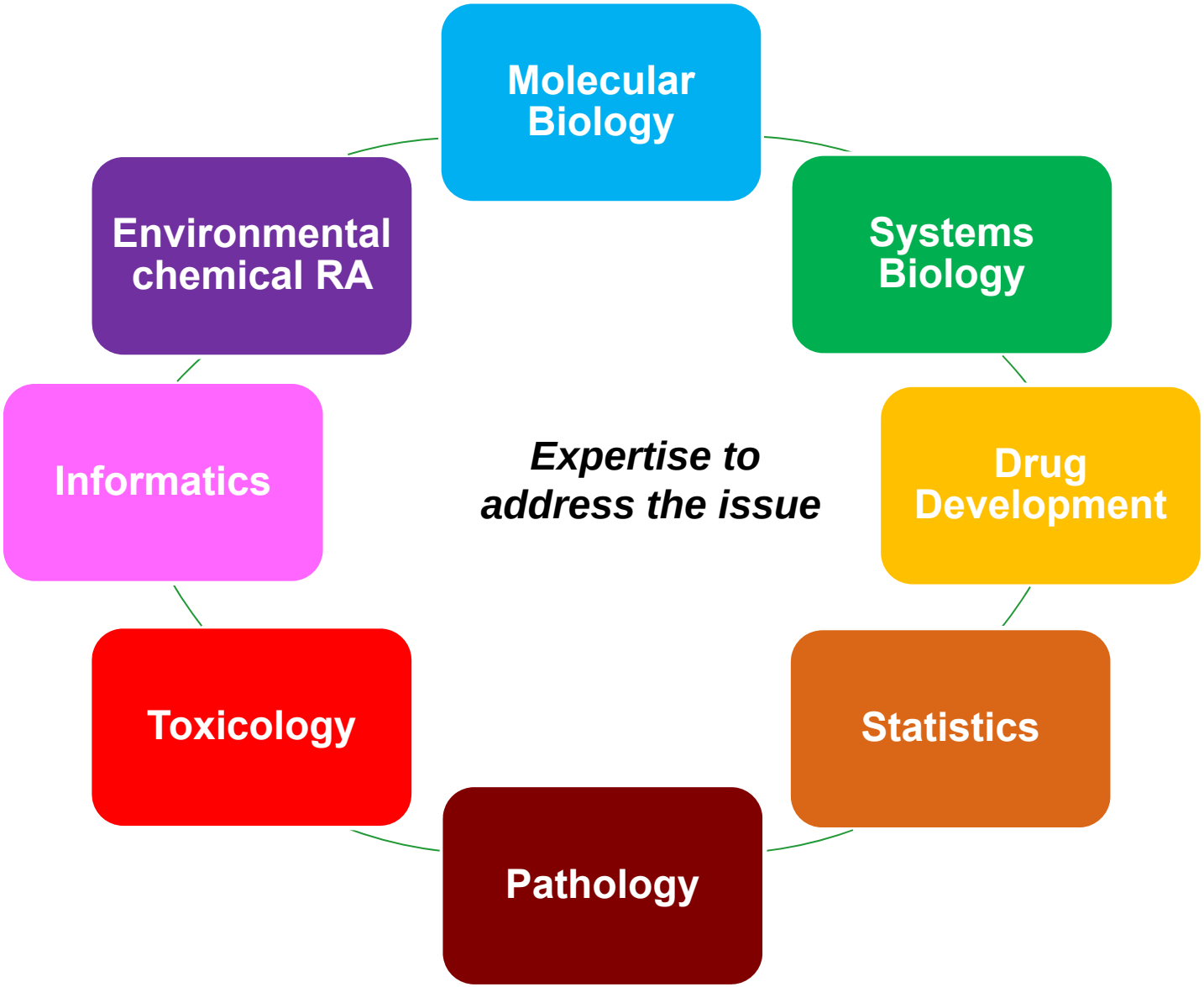
Decrease time and cost of drug development

Opportunities for miRNA Biomarkers in Chemical/Drug Safety Assessments

Enhance clinical translation & clinical monitoring

Improved sensitivity and specificity over existing biomarkers

Applying the Genomics Committee Model to miRNA Biomarker Discovery



Rat miRNA Body Atlas Phase I – Identification of Tissue Specific miRNA

23 Tissues Assessed

Kidney, whole
Medulla
Cortex
Liver
Heart
Pancreas
Adrenal
Glandular stomach
Non glandular stomach
Jejunum
Ileum
Duodenum
Cerebrum
Cerebellum
Hippocampus
Brain stem
Dorsal root ganglion
Soleus
Biceps femoris
Whole blood
Testis
Ovary
Uterus

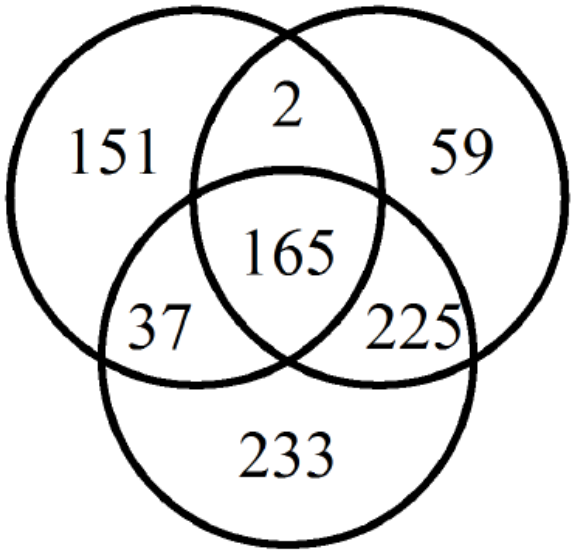
RNA

Tissues were extracted from 5 male and 5 female SD rats and RNAs were subjected to Illumina deep sequencing.

RNA quality was assessed and found to be of good quality (pancreas was an exception with a lower than average quality not unusual for that tissue)

Various Analysis Methods Were Utilized to Determine Tissue miRNA Abundance

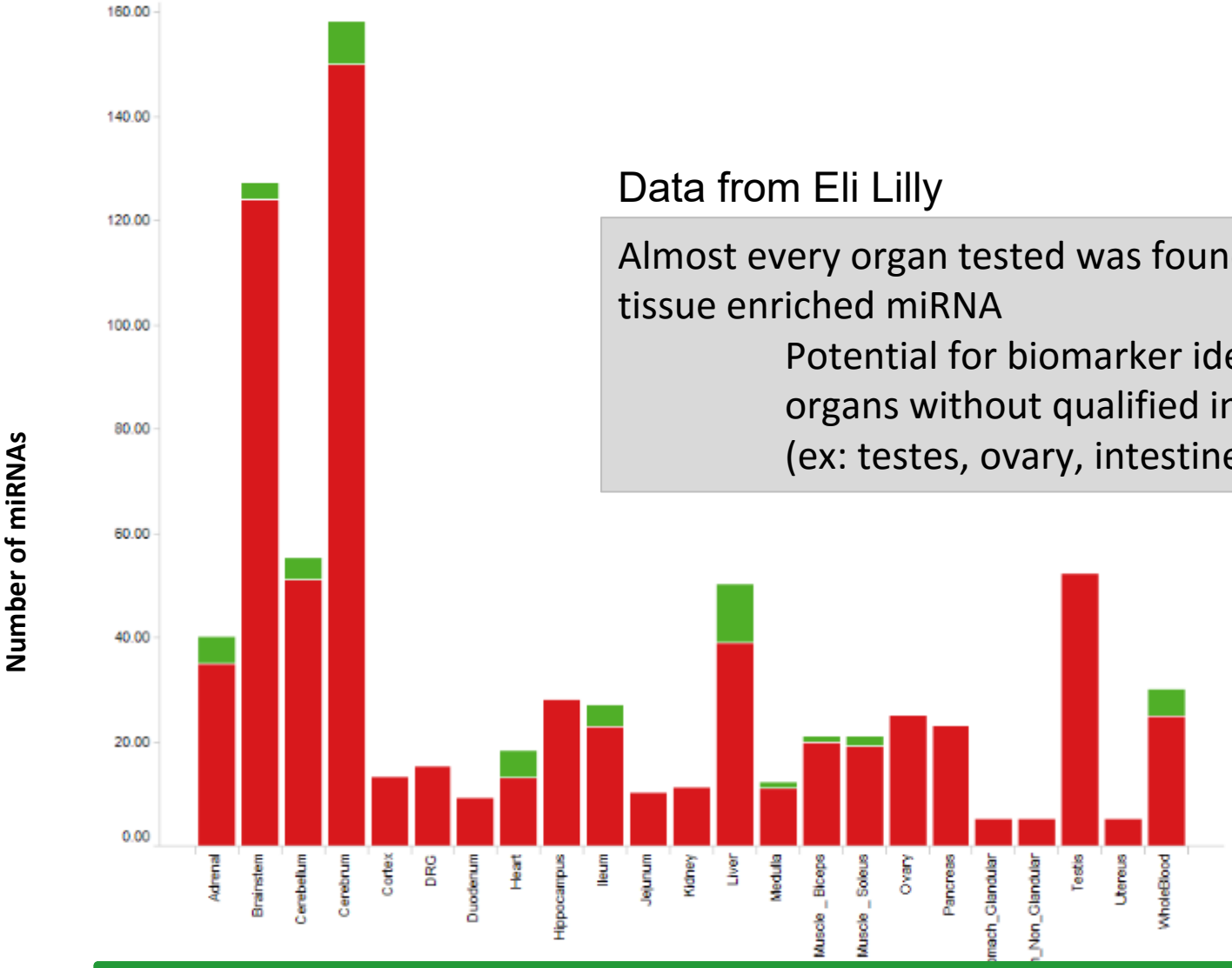
Each institution used a different statistical method for the RNA-Seq data



Conclusions:

- Analysis method is important, 165 miRNA in agreement across methods
- A single analysis method would not have found as many miRNA with high confidence as potential biomarker candidates

Tissue Specific (TS) and Tissue Enriched (TE) miRNAs



Data from Eli Lilly

Almost every organ tested was found to have some tissue enriched miRNA

Potential for biomarker identification for organs without qualified injury biomarkers (ex: testes, ovary, intestine, brain)

- **Rat body atlas paper submitted**
- **Rat miRNA body atlas to be made available to other HESI committees for use in future studies**

WHAT LIES AHEAD?

- Pool of Tissue Specific & Tissue Enriched miRNAs identified
  - Methods to further characterize putative biomarker candidates?
  - The committee is scoping a 2nd phase of work…

Rat miRNA Body Atlas Phase II

1st Round Scoping Assessment

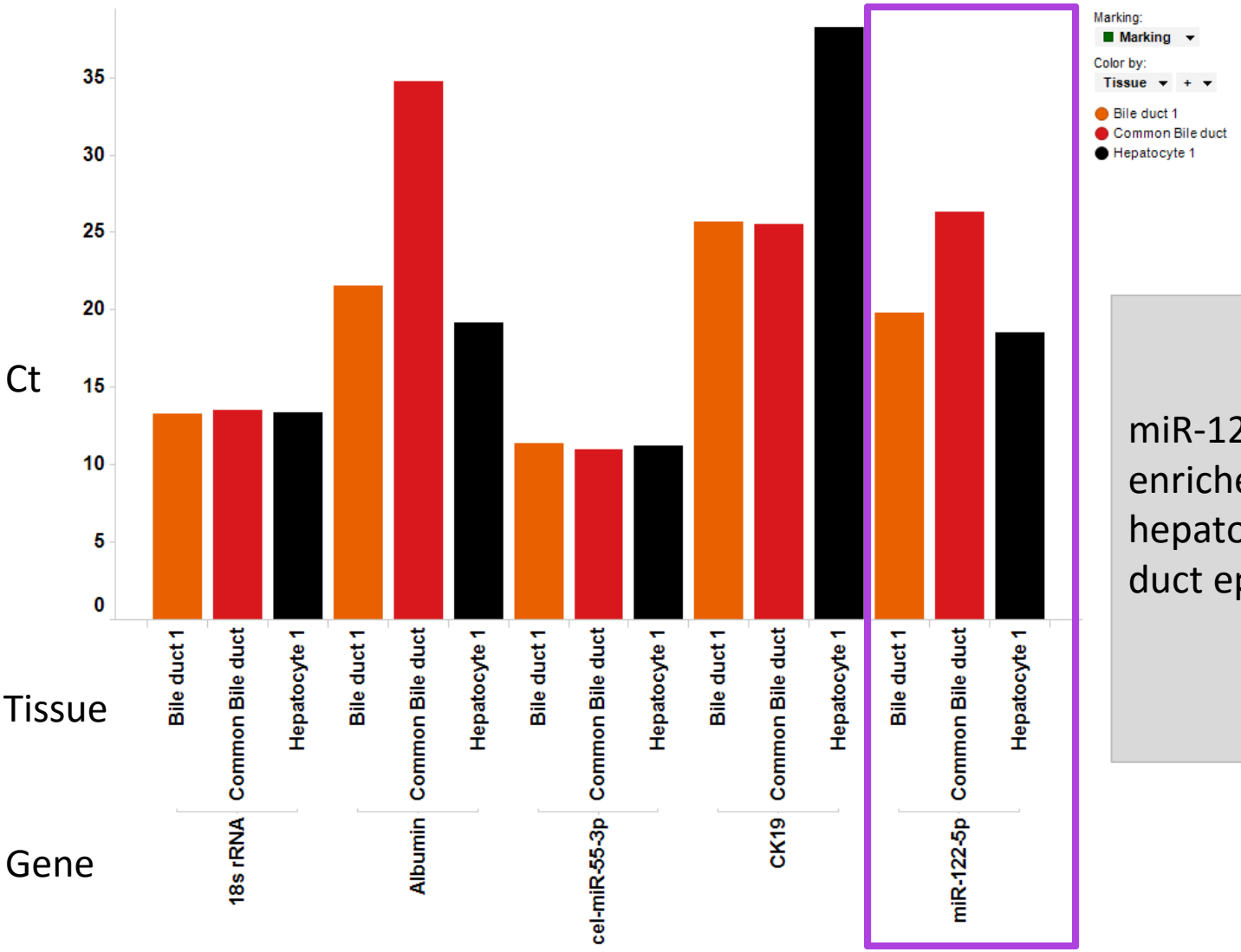
Characterizing 'information content' of miRNA profiles and integrating other endpoints, longitudinal analyses, adding mechanistic value, and tying to the underlying biology

2nd Round Scoping Assessment – Under Consideration

Investigating cell or tissue region specific localization of miRNAs
-Which tissue or cell type?

Exploring kinetics of miRNA level changes in tissue and circulation (potentially including transient vs. long-term miRNA changes)
-When is the optimal time to measure a miRNA biomarker?

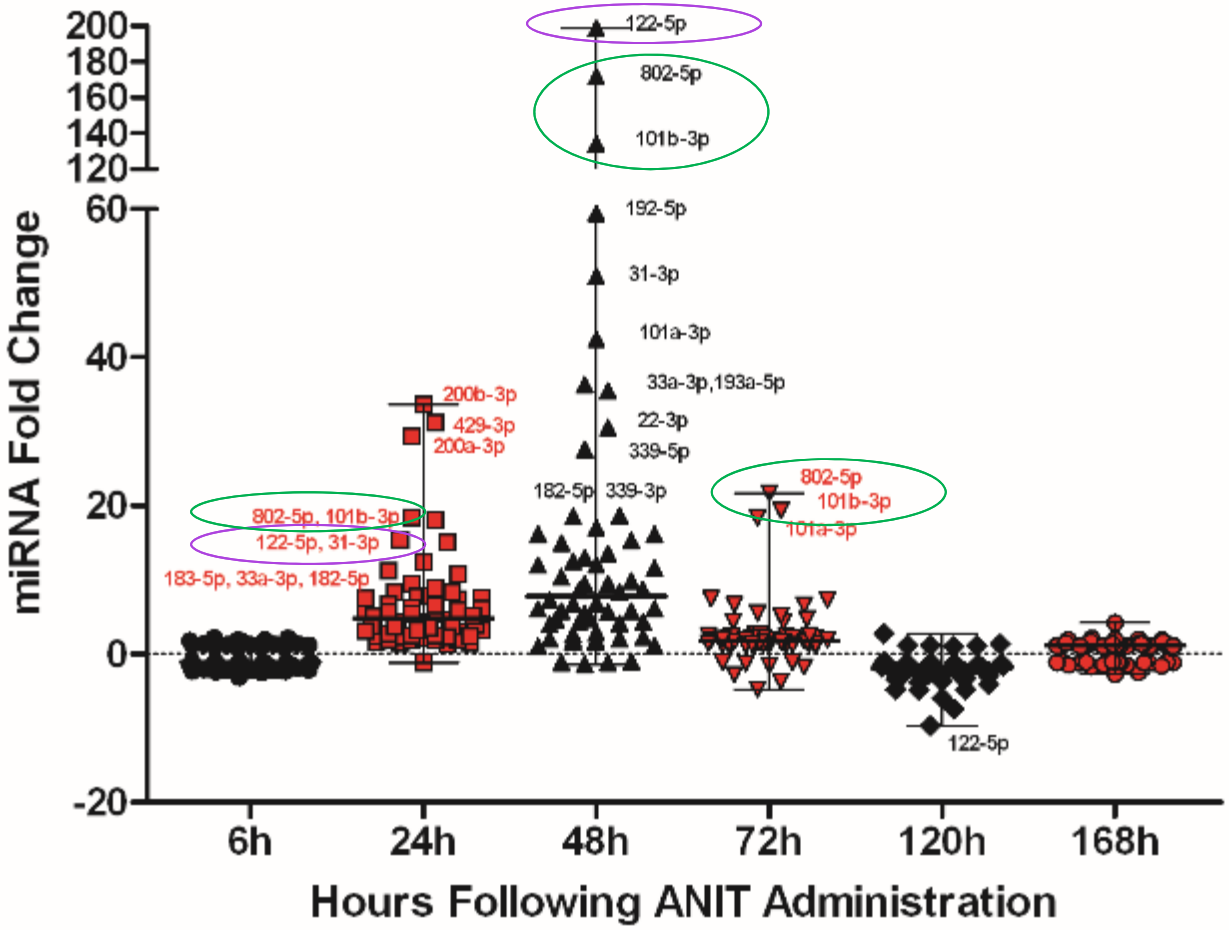
Cell Type Specificity: Example Data with miRNA-122



miR-122-5p is enriched ~64 fold in hepatocytes vs. bile duct epithelial cells

Albumin= Hepatocyte marker
CK19= Bile duct marker
Data generated by Aaron Smith, Eli Lilly

miRNA Time Course Profiles: Plasma Following Liver Toxicity



miR-122-5p is released only during peak liver injury

miR-802-5p and miR-101b-3p offer advantage of increases during peak injury AND repair phases

Timing of measurement is important

Church et al. Tox Sci 2016

Impact of miRNA Body Atlas

- Development of “liquid biopsies” – inform underlying pathology
- Translation of miRNA biomarkers across species
- Clinical translation for organ injuries under a variety of disease states and contexts (not exclusive to toxicology)
- Foundational research for others to interrogate their own tissue-specific questions
  - Collaboration with other HESI committees, consortia, academic institutions

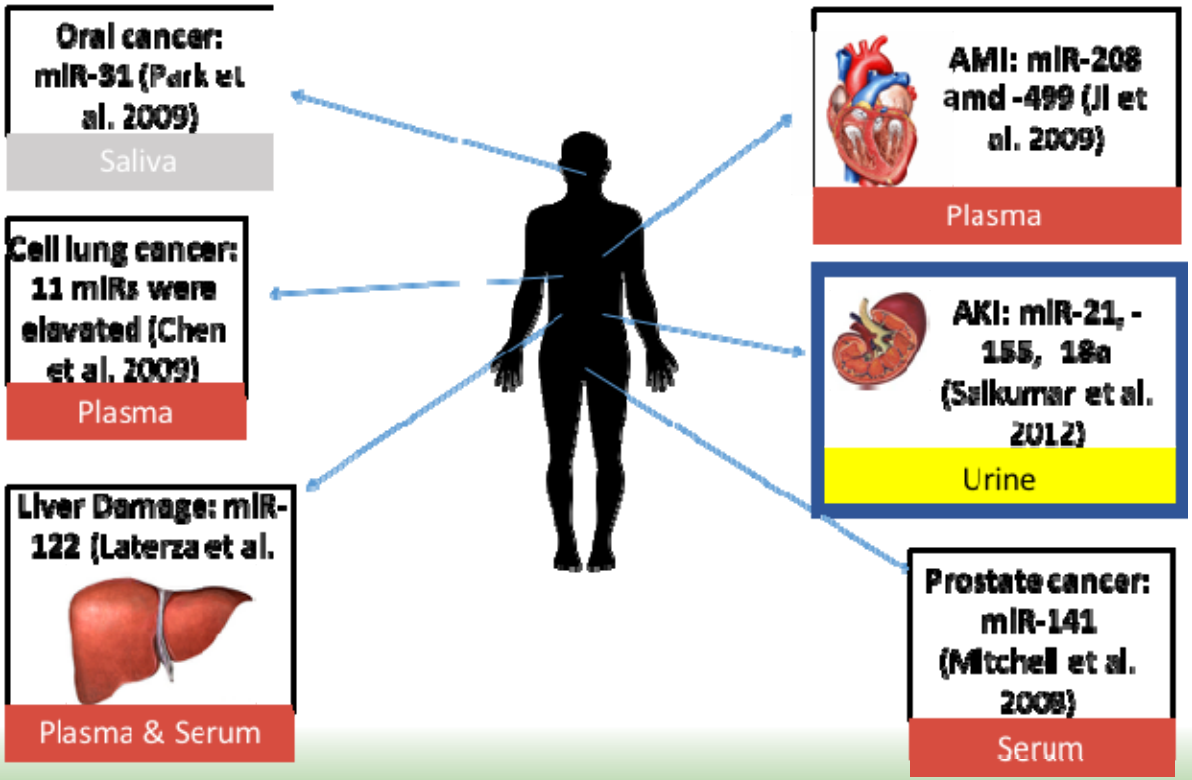


Image: Julia Tobacyk and Alison Harrill

Acknowledgements

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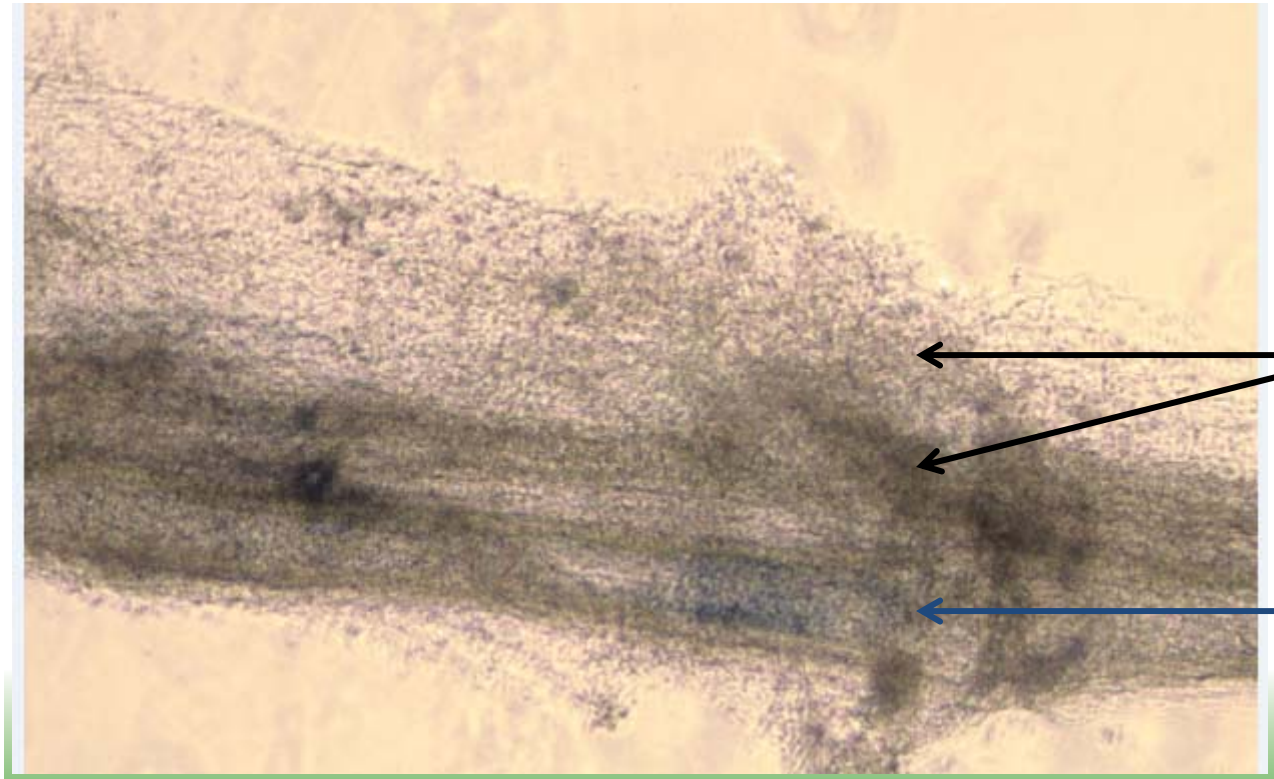
BACKUP SLIDES

miR-122-5p localization in hepatocytes vs bile ducts

1. Rat liver was collagenase digested and the common bile duct (CBD)was cannulated.
2. Blue agar was injected into the CBD and was visibly detectable in the bile ducts within the liver.
3. The CBD was placed in Trizol and the hepatocytes were separated from micro-disected liver.
4. RNA was isolated from CBD, hepatocytes and micro-dissected bile ducts.



Micro-dissected bile duct with likely hepatocyte contamination

Data generated by Aaron Smith, Eli Lilly