Profiles of circulating mRs as non-invasive liquid biopsies for investigating hepatotoxicity in human subjects

Jiri Aubrecht, PharmD, PhD
Translational Biomarker Research
jiri.aubrecht@takeda.com
miR122 - potential biomarker of Liver Injury

Correlation of miRNA122 vs. ALT

Healthy Subjects
Liver Transplant Subjects

APAP Overdose Subjects

N = 72
29 Healthy + 43 Liver Injury
Hypothesis

- Profiles (signatures) of circulating miRs reflect mechanistic information about toxicity, disease
- miR signatures might be useful for:
  - understanding tox effect
  - Diagnosis of disease
  - Susceptible populations
  - Patient stratification
Proof of concept studies

1. miR signature of APAP overdose

   Application of High-Throughput Sequencing to Circulating microRNAs Reveals Novel Biomarkers for Drug-Induced Liver Injury

   Julian Krauskopf*1, Florian Caiment*, Sandra M. Claessen*, Kent J. Johnson†, Roscoe L. Warner†, Shelli J. Schomaker‡, Deborah A. Burt‡, Jiri Aubrecht‡, and Jos C. Kleinjans*

   *Department of Toxicogenomics, Maastricht University, Maastricht 6200 MD, The Netherlands, †Pathology Department, University of Michigan, Ann Arbor, Michigan 48109 and ‡Drug Safety Research and Development, Pfizer, Inc., Groton, Connecticut 06340

2. miR signatures of liver diseases

   Serum microRNA signatures as “liquid biopsies” for interrogating hepatotoxic mechanisms and liver pathogenesis in humans.

   Julian Krauskopf1*, Theo M. de Kok1, Shelli J. Schomaker2, Mark Gosink2, Deborah A. Burt2, Patricia Chandler3, Roscoe L. Warner4, Kent J. Johnson4, Florian Caiment1, Jos C. Kleinjans1, Jiri Aubrecht2

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3. Differentiate outcome of lethal APAP poisoning
1. miR signatures of APAP Overdose – Study design

24 samples

6 APAP Overdose

Patient1

6 samples

Patient2

2 samples

Patient3

2 samples

Patient4

2 samples

Patient5

2 samples

Patient6

4 samples

6 Normal
Circulating miR profiles differentiate APAP-induced liver injury
miR's time course patterns cluster with conventional biomarkers

- miRNAs with a similar pattern of response as GLDH
- miRNAs with a similar pattern of response as Tbil
- miRNAs with a similar pattern of response as ALT and AST
- miRNAs with different pattern
- Biomarkers not associated with liver injury

Hierarchical Clustering Based on Spearman Distance
Biological significance of observed miR5s

- Liver-specific processes indicated by miRs are consistent with molecular mechanism of APAP toxicity
2. miR signatures of liver impairments

Hypothesis:
miR “signatures” in serum can differentiate among variety of liver impairments including providing insights into pathophysiology of disease
Study design

- 54 subjects
  - 9 APAP (DILI)
  - 9 Liver cirrhosis (LC)
  - 7 Hepatitis (HBV)
  - 7 Diabetes (T2DM)
  - 22 healthy (Control)

- Age and gender matched groups of subjects selected based on adjudication of medical records
- NextGen sequencing analysis of serum samples
- Bioinformatic analysis
miR profiles differentiate among variety of liver impairments
miR signatures reveal relevant pathways and mechanisms

<table>
<thead>
<tr>
<th>Table 1. Comparison of pathways derived from literature analysis with pathways derived from miRNA target genes.</th>
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<tbody>
<tr>
<td><strong>Cirrhosis Pathways</strong></td>
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<tr>
<td>Bladder Cancer Signaling</td>
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<tr>
<td>Regulation of the Epithelial-Mesenchymal Transition Pathway</td>
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<td>Agranulocyte Adhesion and Diapedesis</td>
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<td>STAT3 Pathway</td>
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<td>Antiproliferative Role of TOB in T Cell Signaling</td>
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<td><strong>DILI Pathways</strong></td>
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<td>NRF2-mediated Oxidative Stress Response</td>
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<td>Apoptosis Signaling</td>
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<td>Acetone Degradation I (to Methylglyoxal)</td>
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<td><strong>Hepatitis B Pathways</strong></td>
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<td>Antigen Presentation Pathway</td>
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<td>Interferon Signaling</td>
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<td><strong>Type-2 Diabetes Pathways</strong></td>
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<tr>
<td>AMPK Signaling</td>
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<tr>
<td>Insulin Receptor Signaling</td>
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<tr>
<td>TR/RXR Activation</td>
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<td>eNOS Signaling</td>
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<td>Type II Diabetes Mellitus Signaling</td>
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<tr>
<td>Leptin Signaling in Obesity</td>
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<td>Role of NFAT in Cardiac Hypertrophy</td>
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</tbody>
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Significance values for pathways predicted to be the most specific for each individual disease state were calculated based on published literature or known miRNA target genes. Pathways in the table are arranged by relative specificity determined from the literature for individual disease state.
3. Differentiate outcome of lethal case of APAP poisoning

Female, 46 years, admitted for APAP overdose, progressed to liver failure and death
miR signatures predict clinical outcome of APAP overdose

Cellular stress, autophagy....

Prothrombin activation pathway, fibrosis....
Conclusions

• miR signatures have a potential to provide a fundamental advancement (paradigm shift) as a non-invasive tool for studying molecular mechanisms with impact on:

  - Understanding of disease process, efficacy and safety of new therapies
  - Stratification of subjects
  - Reverse translation

• miR based approach has a potential to translate across species and in vitro models to clinic – future direction
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