Serum microRNA biomarkers for human DILI

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Serum microRNA biomarkers for human DILI

- Circulating microRNAs as translational biomarkers for safety, efficacy and disease states
- Liver-enriched microRNAs as promising serum DILI biomarkers
  - Evidence from preclinical models
  - Specificity for acute liver injury in humans
  - Correlation with existing biomarkers of liver injury & patient outcomes
  - Importance of normalisation
  - Investigating mechanisms & kinetics of liver microRNA release into biofluids

- Perspectives
microRNAs

Biological functions & biomarker potential

- small regulatory RNAs ~22nt
- Function: post-transcriptional regulation
- microRNAs are tissue-specific
  - miR-122 is enriched in liver
- Shown to play role in:
  - Embryogenesis & differentiation; Cancer; Cardiotoxicity; Viral infections
- Present in a range of biofluids:
  - Blood, urine, cerebrospinal fluid…

Circulating microRNAs as disease state biomarkers:
  - oncology, cardiovascular, musculoskeletal, autoimmune…

Circulating microRNAs as novel tissue-injury biomarkers:
  - liver, pancreas, muscle, kidney, heart, brain…
Circulating microRNAs as translational biomarkers

**TRANSLATIONAL BIOMARKERS:**
- Safety, Efficacy & Disease States

Extracellular signalling functions?
(protein-bound, vesicles, exosomes & HDL-cargo)

Mechanistic Insight via Integrated molecular profiling + pathology (mRNA + microRNA + epigenomics)

Preclinical Tissues

Clinical Biopsies

Preclinical body fluids

Clinical body fluids
Paracetamol-induced Liver Injury

- Paracetamol (acetaminophen, APAP) is a widely used, over-the-counter analgesic and antipyretic drug
- Very safe at therapeutic doses (4g/day) but leads to potentially fatal, hepatocellular necrosis and acute liver failure in overdose
- The most common cause of drug-induced liver injury in UK ¹ & US ² (~ 500 deaths per annum)
- n-acetylcysteine treatment most effective <8 hrs of ingestion ³. In some cases (e.g. in late presentation), liver transplantation is the only therapeutic option
- New biomarkers of DILI are urgently sought. Ideal biomarkers would be sensitive, specific and predict clinical outcome ⁴

Blood-based microRNA biomarkers for DILI

**Evidence from preclinical models**

- miR-122 / miR-192 DILI biomarkers
  - Liver tissue specific
  - Translatable to human
  - Earlier detection than ALT; greater sensitivity; less variability.

*US patent application, 2011, 111976 A1*

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**Graphs**

- **CCl₃Br/CCl₄/rat**
  - Sensitivity vs. 1-Specificity

- **APAP/mouse**
  - 1 Hour After Exposure
  - 3 Hour After Exposure
  - Fold change in miRNA concentration compared to control

*Wang et al, PNAS, 2009*
Blood-based microRNA biomarkers for DILI

**Potential advantages**

- **Diagnostic / prognostic liver injury biomarker potential ?**
  - Extract from recent US patent application (2011/111976 A1)
    
    If miR-122A detected in blood *above a threshold level*:
    
    - then the subject is classified as having suffered damage to the liver
    
    - then the subject is identified as being at risk of suffered tissue injury to the liver and exposure to the agent should be altered, i.e. Stopped or the exposure lowered
    
    - then the agent is identified as having a risk of causing injury to the liver

- **Improved specificity versus ALT ?**

Serum microRNAs as human DILI biomarkers

Specificity of miR-122 for liver injury

ALI: acute liver injury
CKD: chronic kidney disease
APAP: acetaminophen
non-APAP ALI:
- autoimmune
- HBV
- HCV
- Clarithromycin DILI

Starkey-Lewis et al., (2011)
Hepatology 54:1767
Serum microRNAs as human DILI biomarkers

miR-ALT correlations

Starkey-Lewis et al., (2011)
Hepatology 54:1767
Serum microRNAs as human DILI biomarkers

Functional marker-miR correlations

Starkey-Lewis et al., (2011)
Hepatology 54:1767
Serum microRNAs as human DILI biomarkers

Distinct biomarker dynamics for miR vs ALT

Circulating half-life ALT > miR-122

Turnover/half-life of miR-122 in human serum?

Starkey-Lewis et al., (2011)
Hepatology 54:1767
Serum microRNAs as human DILI biomarkers

*miR-122* is suggestive but not predictive of patient outcome

Starkey-Lewis et al., (2011)
Hepatology 54:1767
Serum microRNAs as human DILI biomarkers

Extracellular miR states & importance of normalisation

- Serum miR-122 is mainly carried by argonaute 2 protein complexes

- Options for normalisation of serum microRNAs:
  - Total volume/amount of biofluid RNAs
  - Endogenous snRNAs (e.g. U6 snRNA; ubiquitously expressed in tissue)
  - Endogenous miRs
  - Exogenous miRs

- Is circulating U6 snRNA a reliable internal normaliser?
Serum microRNAs as human DILI biomarkers

Abundance & variation of candidate normalisers

**hsa-let-7d-5p**
(endogenous)

**U6 snRNA**
(endogenous)

**hsa-miR-374a-3p**
(endogenous)

**cel-lin-4-5p**
(exogenous)
Increased serum miR-122 during APAP-induced liver injury (alternate normalisers)

hsa-let-7d-5p (endogenous)

hsa-miR-374a-3p (endogenous)

U6 snRNA (endogenous)

cel-lin-4-5p (exogenous)
Increased serum miR-122 during APAP-induced liver injury (without normaliser)

hsa-miR-122 (no normaliser)
Investigating mechanisms & kinetics of liver microRNA release into biofluids

**Schematic Model**

- **Mechanism-based DILI biomarkers:**
  - discrimination between transient damage/repair vs. progressive liver injury?

- **Origin of serum miR-122:**
  - sporadic diffuse damaged liver cells? focal cellular damage? zonal progression?

- **Sequence of early tissue injury events leading to miR-122 release?**

- **Distinct mechanisms for release of liver miR122 vs. ALT/CK18/HMGB1 etc.?**

**Apoptosis**
- Cytokeratin 18 abundant in hepatocytes
- Is cleared by caspases
- Fragment released into plasma

**Necrosis**
- HMGB1 - released by necrotic cells
- But NOT by apoptotic cells

**ACETYL HMGB1**
- Provides inter-cellular signalling
- INNATE IMMUNE SYSTEM

Hepatocytes

- GSH depletion
- Adduct formation
- Ca²⁺ protein damage

Cytoplasm

- Macrophages
- Kupffer cells

Kinetics of liver microRNA release into biofluids

In situ localisation of miR122 (APAP/mouse)

1 h  2 h  4 h  8 h

Control

APAP

Progressive loss of miR-122 staining
Concomitant serum miR-122 increase
Kinetics of liver microRNA release into biofluids

In situ localisation of miR122 (APAP/mouse)

Control

1 h 2 h 4 h 8 h

Progressive loss of miR-122 staining
Concomitant serum miR-122 increase

APAP
Serum miR-122 as a human DILI biomarker

Current status & knowledge gaps

• miR-122 is at least as informative as ALT for DILI (e.g. APAP)

• miR-122 tissue-specificity provides advantages over ALT

• Combine miR-122 + ALT to more accurately detect liver injury?

• Urine as a potential source of microRNA hepatotoxicity biomarkers
  - Yang et al., 2012 Toxicological Sciences 125:335

• miR-122 not an ideal biomarker - does not distinguish benign ALT elevations (e.g. heparin) versus serious liver injury potential
  - Harrill et al., DILI meeting March 2011

Lack of baseline and dynamic range data in normal & disease states

Opportunities to investigate specificity and dynamics of miR-122 vs. clinical ALT signals: IMI SAFE-T; longitudinal/prospective studies
<table>
<thead>
<tr>
<th>Serum or Plasma Marker</th>
<th>Assays</th>
<th>Liver specificity</th>
<th>Human data</th>
<th>Pathology</th>
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<tr>
<td>Albumin mRNA</td>
<td>RT-PCR</td>
<td>✓</td>
<td>yes</td>
<td>hepatocellular damage</td>
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<td>Microglobulin precursor (Ambp) mRNA</td>
<td>RT-PCR</td>
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<tr>
<td>Micro RNA 122</td>
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<td>Conjugated/unconjugated bile acids</td>
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<td>Purine nucleoside phosphorylase (PNP)</td>
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✓ SAFE-T has already developed an assay for singleplex measurement
* ELISA commercially available
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