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microRNA-122: Uses and Applications

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Currently used markers of liver injury

- Alanine aminotransferase (ALT) is not specific for the type of injury
- Aspartate aminotransferase (AST) is not specific for the type of injury

- ALT/AST are not specific:
  - Poor correlation with progression of liver disease.
  - Cannot distinguish between hepatic injury and inflammation.
  - Drug induced liver injury (DILI) from other causes of liver injury.

There is a need for sensitive, specific and stable biomarkers.

Circulating miRNAs, exosomes?
Circulating miRNAs as biomarkers

- Regulate genes both at the post-transcriptional and transcriptional levels.
- Cell-free body fluids including serum, plasma, urine, amniotic fluid, milk etc.
- Stable at extreme conditions (high and low pH, freeze-thaw, RNase A and HCl treatments etc).
- Found in vesicles (exosomes, microvesicles, apoptotic bodies) and lipoproteins (HDL, LDL).
- Complexes with proteins (Ago's).
- The stability of circulating miRNAs make them **attractive new non-invasive biomarkers.**
- Circulating miRNAs signatures in various diseases.
- Cell-cell communication

miRNA-122, a liver specific miRNA

- miR-122 accounts for ~70% of the total miRNA population in hepatocytes (Chang et al., RNA Biology, 2004).

- miR-122 regulates genes involved in cholesterol biosynthesis and facilitates HCV replication (Catherine et al., Science 2005).

- Increased plasma/serum levels of miR-122 in humans:
  - Drug-induced liver injury (Starkey et al., Hepatology, 2012).
  - Chronic hepatitis C infection or non-alcoholic fatty-liver disease or HCV-induced fibrosis (Cermelli et al., Plos one, 2011, Trebicka et al., J Hepatol: 2013).
  - Hepatocellular carcinoma (Xu, Mol Carci, 2011).
miR-122 is an early plasma marker of APAP-induced liver injury

C57BL/6 mice received saline or acetaminophen (500mg/kg).  

Early increase of serum miR-122 during onset of fulminant hepatitis

Wilson Disease (Rat model) Ramsi et al., Hepatol Int 2012
The extent of miR-122 increase varies and correlates with ALT in different liver injury models

**ALD model**

**APAP model**

**CpG+LPS model**

Bala et al, Hepatology 2012
J Translational Medicine 10:151 (2012)
Increased serum and decreased liver miR-122 in a diet-induced model of non-alcoholic steatohepatitis

Liver International
MAP3K3 is regulated by miR-122 in hepatocytes

B

MAP3K3 mRNA fold change

3 6 8 weeks
Total liver

6 weeks
Hepatocytes

MCS MCS MCD MCD

C

Hepatocytes

pre-miR-122 pre-miR-control anti-miR-control anti-miR-122

p=0.002 p=0.03

D

MAP3K3 protein in liver

MAP3K3 protein in hepatocytes

3 weeks 6 weeks 8 weeks

MCS MCD MCS MCD MCS MCD

MCS MCD

p=0.052

Liver International
Increased NF-κB activation in NASH

A  
NFκB nuclear binding in liver

B  
NFκB nuclear binding in hepatocytes

C  
NFκB nuclear binding in normal hepatocytes
Expression of the miR-122 targets, HIF-1, lysil-oxidase and vimentin are increased in NASH
microRNA-122 regulates hypoxia-inducible factor-1 and vimentin in hepatocytes in diet-induced steatohepatitis

Liver International
Exosomes

- Exosomes are small extracellular membrane vesicles (50-100 nm in diameter) produced by most cell types into the extracellular space or into biological fluids.

- Exosomes mainly function in mediating cell-to-cell communication with both beneficial (physiological) and harmful (pathological) potential outcomes.

- Hepatocytes are exosome-releasing cells as well as targets for exosomes.
Exosome Biogenesis

A. Diagram showing the process of exosome biogenesis, including endocytosis, invagination, fusion, and degradation. The diagram also shows the involvement of cytosolic proteins, miRNAs, and mRNAs.

B. Diagram illustrating the flow of exosomes through the apical membrane and their potential role in intracellular signaling pathways.

C. Images showing electron micrographs of exosomes and their distribution in various cellular compartments.

Masyuk et al., J Hepatol, 2013; Masyuk et al., Am J Physiol Gastrointest Liver Physiol, 2010
# Exosomes as biomarkers of liver diseases

<table>
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<tr>
<th>Liver Disease</th>
<th>Source of exosomes</th>
<th>Exosomal cargo</th>
<th>Observation</th>
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<td>Liver injury induced by DGAL</td>
<td>Urine</td>
<td>CD10, CD26, CD81</td>
<td>Decreased number of exosomes</td>
<td>Conde-Vancells et al., 2010</td>
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<tr>
<td>Liver injury associated with steatosis, Fibrosis, HCC</td>
<td>Urine</td>
<td>CD10</td>
<td>Increased number of exosomes</td>
<td>Conde-Vancells et al., 2010</td>
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<td>HCV</td>
<td>Serum</td>
<td>CD81</td>
<td>Increased number of exosomes</td>
<td>Welker et al., 2012</td>
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<tr>
<td>Liver injury induced by DGAL/APAP</td>
<td>Serum</td>
<td>Liver-specific mRNAs</td>
<td>Increased number of exosomes</td>
<td>Wetmore et al., 2010</td>
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<tr>
<td>Liver injury/rejection</td>
<td>Serum</td>
<td>miRNA-122, miRNA-148a, miRNA-194</td>
<td>Increased number of exosomes</td>
<td>Farid et al., 2012</td>
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<tr>
<td>Alcoholic, drug-induced, inflammatory liver diseases</td>
<td>Serum, Plasma</td>
<td>miRNA-122, miRNA-155</td>
<td>Increased number of exosomes</td>
<td>Bala et al., 2012</td>
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<tr>
<td>HCC</td>
<td>Plasma</td>
<td>miRNA-92a</td>
<td>Decreased number of exosomes</td>
<td>Ohno et al., 2012</td>
</tr>
</tbody>
</table>

Masyuk et al., J Hepatol, 2013
Could exosomes serve as therapeutic vehicles?

Stimulation of B cells (IL-4 & CD40)

3 days culture

Condensation of media+ Isolation of exosomes with CD63 immunomagnetic beads

Characterization of exosomes (Western blot, Nanoparticle tracking analysis & TEM)

Loading exosomes with miRNA-155 mimic and miRNA-155 inhibitor

Re-isolation of loaded exosomes

Functional delivery of miRNA-155 inhibitor loaded exosomes to RAW macrophages

Delivery of miRNA-155 mimic to mouse primary hepatocytes

In vivo delivery of miRNA-155 to miRNA-155 knockout mice

Momenheravi et al. Nanomedicine in press
Exosome delivery of a miR-155 inhibitor attenuates TNFα production in macrophages

Momenheravi et al. Nanomedicine in press
Exosomes are effective in delivery of a miR-155 mimic both in vitro hepatocytes and in vivo in the liver.

Momenheravi et al. Nanomedicine in press
Exosomes as therapeutic vehicles

Momenheravi et al. Nanomedicine in press
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