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HMGB1 Variants Determine if DILI is Benign or Dangerous

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Development of new DILI biomarkers

Unmet need:

- Biomarkers with improved hepatic specificity
- Enhanced mechanistic basis (translational)
- Earlier detection of DILI
- Patient response
  - Outcome / prognosis
  - Benign vs serious ALT elevations

Figure courtesy of Ina Schuppe-Koistinen
Biomarkers for mechanisms – HMGB1

High Mobility Group Box-1 (HMGB1)

- 25 kDa chromatin binding protein, 3 domains (A, B, C-tail)
- Regulates transcription (DNA binding)
- DAMP – ligand for TLR4, RAGE, CXCR4
- Necrosis – passive release
- Active immune cell secretion (requires NLS acetylation)
- Redox regulation

Figure courtesy of Ulf Andersson, Karolinska
Biomarkers for mechanisms – HMGB1

- Tracking HMGB1 from hepatocyte to blood

**H&E**

**anti-HMGB1**

**Normal**

**APAP (10h)**

**HMG1 [ng/ml]**

- Un
- 6h
- 12h
- 24h

- **p=0.059**

**PT**

**CV**

**Bar Graph**

- **APAP**
Biomarkers for mechanisms – HMGB1

Hyper-acetylated HMGB1

Hypo-acetylated HMGB1

D.J. Antoine et al. 2009 Toxicol Sci
Translational mechanisms

**Of mice.......................**

**and men**

DJ Antoine et al 2012 J Hepatol
Inflammation in clinical APAP toxicity (deleterious in vivo)? Can Acetyl-HMGB1 predict outcome?

Outcome biomarkers based on mechanism

- Acetylated HMGB1
- ALT

Died / Liver transplant
Survivors

Acetylated HMGB1 (ng/ml)

Healthy volunteers, APAP -No ALI, APAP -ALI

Sensitivity

1 - Specificity

QTRAP 5500

K182-185 (Acetyl)
K177+180 (Acetyl)
K173 (Acetyl)

Cytokine domain

Nuclear Localization sequence

DJ Antoine et al 2012 J Hepatol
Biomarkers = Therapeutics?

HMGB1 – functional role in DILI

**Percentage survival**

Time (h)

APAP

APAP + anti-HMGB1

**ALT activity (U/l)**

IgY

Anti-HMGB1

APAP

APAP + Anti-HMGB1

IgY

**Necrosis and inflammation**

DJ Antoine et al 2010 Mol Med
HMGB1 – mechanistic role in DILI

- Novel mouse model development – Conditional \( \text{Hmg}b1^{\Delta \text{HEP}} \)

\[
\begin{align*}
\text{hmg}b1 & \quad 5' \quad \text{loxP} \quad 2 \quad 3 \quad 4 \quad \text{loxP} \quad 3' \\
\text{hmg}b1^{\text{fl/fl}} \quad \times \quad \text{ALB-Cre} \quad \Downarrow \\
\text{hmg}b1^{\text{WT}} \quad \text{hmg}b1^{\Delta \text{HEP}}
\end{align*}
\]

- Novel hepatocyte specific KO, whole body embryonic lethal

X Ge & DJ Antoine et al 2014 J Biol Chem

P Huebner et al 2015 J Clin Invest
Conditional *Hmgb1*\(^{ΔHep}\) are protected from APAP hepatotoxicity.
HMGB1 – mechanistic role in DILI

- Conditional *Hmgb1\textsuperscript{\DeltaHep}*
  protected from APAP hepatotoxicity
- Reduced neutrophil, but not macrophage, infiltration into liver

P Huebner et al 2015 J Clin Invest
HMGB1 – mechanistic role in DILI

- Adenoviral HMGB1 gene delivery restores hepatic inflammation and injury after APAP treatment

P Huebner et al 2015 J Clin Invest
HMGB1 in liver injury and disease

Non-APAP

ALD (AASLD SIG Jan 2015)

High Mobility Group Box-1 (HMGB1) Participates in the Pathogenesis of Alcoholic Liver Disease (ALD)

Biomarkers Distinguish Apoptotic and Necrotic Cell Death During Hepatic Ischemia/Reperfusion Injury in Mice

I/R Injury

Cholestasis
Prediction of serious DILI

- Transient elevations in ALT often seen during clinical trials
- Hy’s Law
  - Most widely used predictor of serious DILI
  - Only regulatory endorsed model
  - Drug-induced hepatocellular jaundice
  - Some mortality (10 – 50%)
  - Temporal observation and differential medical diagnosis
  - ALT 3x ULN and TBL 2x ULN
Chronic therapeutic APAP administration – DILI, but not serious?

- PB Watkins et al., 2006. JAMA
- 30-40% volunteers develop transient ALT increase
- Significant apoptotic component

Aminotransferase Elevations in Healthy Adults Receiving 4 Grams of Acetaminophen Daily
A Randomized Controlled Trial

Context: During a clinical trial of a novel hydrocodone/acetaminophen combination, a high incidence of serum alanine aminotransferase (ALT) elevations was observed.

Objective: To characterize the incidence and magnitude of ALT elevations in healthy participants receiving 4 g of acetaminophen daily, either alone or in combination with selected opioids, as compared with participants treated with placebo.


- P Thulin et al 2013 Liver Int
Chronic therapeutic APAP administration – DILI, but not serious?

- PB Watkins et al., 2006. JAMA
- 30-40% volunteers develop transient ALT increase
- Elevated circulating DAMPs (HMGB1)
- *Why do they not develop serious DILI?*
Cysteine redox chemistry

Disulphide bond

- Sulphenic (usually labile)
- Sulphinic (only reduced enzymatically)
- Sulphonic (irreversible)
# HMGB1 Redox Regulates Function and Mode of Action

## Schematic Molecular Overview

<table>
<thead>
<tr>
<th>Molecule / Cysteine Redox Level</th>
<th>Schematic Molecular Overview</th>
<th>Cytokine-Inducing Activity</th>
<th>Chemoattractant Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-thiol HMGB1</td>
<td><img src="image" alt="C33-C45-C106" /></td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>CySH</td>
<td></td>
<td></td>
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<tr>
<td>Disulfide-containing HMGB1</td>
<td><img src="image" alt="S-S" /></td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>CyS-SyC</td>
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<tr>
<td>HMGB1 terminally oxidized by ROS</td>
<td><img src="image" alt="C33-C45-C106" /></td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

## References

- DJ Antoine et al 2014 Mol Med
- B Lu et al 2012 Nature
- B Lu et al 2014 PNAS
- S Nystrom et al 2013 EMBO J
- H Yang et al 2012 Mol Med
Cell death dependent HMGB1 isoform release and outcome in vivo

FED – APAP (apoptosis/necrosis)

FAST – APAP (necrosis)

FED APAP+Z-VAD.fmk (necrosis)

H&E

5hr

Active caspase-3

24hr

Regeneration vs Necrosis & Inflammation

SO$_3$H \[ \begin{array}{c|c|c}
\text{C} \_23 & \text{C} \_45 & \text{C} \_106
\end{array} \]

SO$_3$H \[ \begin{array}{c|c|c}
\text{C} \_23 & \text{C} \_45 & \text{C} \_106
\end{array} \]

SO$_3$H \[ \begin{array}{c|c|c}
\text{C} \_23 & \text{C} \_45 & \text{C} \_106
\end{array} \]
HMGB1 redox: Mechanistic biomarker of serious DILI

**Serious liver injury**  
*(Overdose)*

- Apoptosis (5-15%), Necrosis & Inflammation

**Transient liver injury**  
*(chronic therapeutic)*

- Apoptosis (40-70%) & Necrosis

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**Whole Protein ESI-LC-MS**
**Outcome** and **insult** dependent HMGB1 redox profiles

<table>
<thead>
<tr>
<th>HMGB1 isoform (%)</th>
<th>Sulfonyl</th>
<th>Disulfide</th>
<th>Fully Reduced</th>
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</thead>
<tbody>
<tr>
<td>APAP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Therapeutic</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>OD (Survive)</td>
<td>58</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>OD (Died/LT)</td>
<td>8</td>
<td>32</td>
<td>60</td>
</tr>
</tbody>
</table>

Functionally distinct HMGB1 isoforms determine if APAP liver injury is serious or benign.
HMGB1 redox – Apoptotic index

- Tool to determine if DILI is serious or benign based on mechanism

Graph showing the relationship between HMGB1 redox ratio ($SO_xH / SH$) and apoptotic ratio (peak M30 / peak M65). The graph includes data points for APAP Therapeutic (N=15), APAP OD Survive (N=47), and APAP OD Died/LT (N=31).
Development of new DILI biomarkers

Summary:

- HMGB1
  - Mechanistic biomarker in experimental and clinical liver injury (APAP, ALD, IR, Cholestasis)
  - Functional role in mechanism of pathology (Novel KO mice)
- Patient response
  - Outcome / prognosis (acetyl-HMGB1)
  - Benign vs serious ALT elevations (HMGB1 redox)

Figure courtesy of Ina Schuppe-Koistinen
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