Toxicogenomic studies reveal the trans-sulfuration pathway as a possible mechanism for liver toxicity

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Outline

1. Introduction: Drug-induced liver injury focusing on glutathione depletion
2. A metabonomic study with 10 hepatotoxicants
3. A meta-analysis of microarray data for 7 hepatotoxicants
4. Discussion: Identification of potential biomarkers for hepatotoxicity
Drug-induced Liver Injury is Threatening American Health

Drug-induced liver injury is now the leading cause of acute liver failure (ALF) in USA, exceeding all other causes combined.
Drug-induced Liver Injury Hinders Drug Development

1. The **most frequent single cause** of safety-related drug withdrawn from market for the past 50 years

2. **Limits the use of many drugs**, including aceminophen, labetalol, trovafloxacin, and felbamate

Broaden the understanding of drug-induced liver injury has been identified as an opportunity in the FDA Critical Path Initiative
Glutathione is an important factor in phase II drug metabolism, and plays a key role in the liver detoxification. Glutathione depletion is often observed in drug-induced liver injury. Can we identify biomarkers/pathways of the hepatotoxicity related to glutathione depletion?
NMR-Metabonomic Study Design

- NMR data of rat urine were collected at 24, 48, 72, 96, 120, 144 and 168hr post-dose, with 7~10 biological replicates
  - 7 compounds plus control in training set
    Acetaminophen; 1,1-Dichloroethylene; Indomethacin; Microcystin-LR; Rotenone; Phenyl diisothiocyanate; Phenyl isothiocyanate
  - 3 compounds plus control in test set
    Allyl alcohol; Thioacetamide; Galactosamine

* All the compounds were reported to cause hepatotoxicity and glutathione depletion.
Principal Components Plot of Time-course Trajectory

- Maximal toxicity at 24 & 48 hr
- 48 hr post-dose data used to build model

Sun, J. Chrom B, in press
Modeling Work Flow

Table 1. Summary of modeling results

<table>
<thead>
<tr>
<th></th>
<th>Accuracy</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training set</td>
<td>86.3%</td>
<td>98.6%</td>
<td>75.4%</td>
</tr>
<tr>
<td>Test set</td>
<td>80.0%</td>
<td>100.0%</td>
<td>71.4%</td>
</tr>
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</table>
Hepatotoxic Markers Identified in Metabonomic Study

Is the decreased level of N-Methylnicotinate (the product of SAM methylation) caused by the decreasing SAM?

<table>
<thead>
<tr>
<th>Integral bins</th>
<th>Metabolite</th>
<th>P-value</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>[9.0870 .. 9.1391]</td>
<td>N-Methylnicotinate</td>
<td>1.01E-11</td>
<td>-2.00</td>
</tr>
<tr>
<td>[8.8132 .. 8.8685]</td>
<td>N-Methylnicotinate</td>
<td>3.13E-11</td>
<td>-1.86</td>
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<td>[6.5543 .. 6.6139]</td>
<td>trans-aconitate</td>
<td>8.48E-10</td>
<td>-1.93</td>
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<tr>
<td>[7.6038 .. 7.6635]</td>
<td>Hippurate</td>
<td>1.95E-10</td>
<td>-1.64</td>
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<tr>
<td>[6.6367 .. 6.6672]</td>
<td>Unidentified</td>
<td>7.67E-05</td>
<td>-1.31</td>
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<tr>
<td>[2.3996 .. 2.4243]</td>
<td>Succinate</td>
<td>1.14E-07</td>
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<tr>
<td>[7.8568 .. 7.8942]</td>
<td>Unidentified</td>
<td>1.07E-07</td>
<td>1.49</td>
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<tr>
<td>[7.9913 .. 8.0417]</td>
<td>Unidentified</td>
<td>1.16E-07</td>
<td>1.63</td>
</tr>
</tbody>
</table>

Abbreviation:
- SAM, S-adenosylmethionine
- SAH, S-adenosylhomomethionine

Diagram:
- Methionine is converted to N-Methylnicotinate, which is increased.
- Homocysteine and Glutathione are decreased, indicating altered metabolism.
- SAM and SAH are shown with their possible roles in methylation processes.
SAM Decreases When Treated with Acetaminophen

SAM level in rat urine decreases at 24 and 48 hr post-dose, which suggests that the transsulfuration pathway from methionine to gutathione is altered.

Sun, J. Chrom B, in press
Comparison of N-methylnicotinate with ALT, AST Biomarker’s Potential using ROC Curve

AUC of N-methylnicotinate (0.86) is larger than ALT (0.74) and AST (0.64) suggesting N-methylnicotinate as a potential biomarker candidate for hepatotoxicity.
Meta-analysis of Microarray Data from 3 Independent Studies

7 compounds

- GSE 2303: Valproic acid, DEHP
- GSE 2187: Indomethacin, DEC
- GSE 5509: ANIT, DMN, NMF

**Abbreviation**
- DMN: dimethylnitrosamine
- NMF: N-methylformamide
- ANIT: alpha-Naphthylisothiocyanate
- DHEP: diethylhexylphthalate
- DEC: 1,1-Dichloroethylene.

- All were reported to cause hepatotoxicity and glutathione depletion
- Data were obtained from GEO (http://www.ncbi.nlm.nih.gov/geo/)
Time-course trajectory of principal components analysis using ebTrack/ArrayTrack

- Maximal effect at 24 & 48 hr
- 24 or 48 hr post-dose data used for meta-analysis

Microarray data of Valproic acid
Work Flow of Meta-analysis using ebTrack/ArrayTrack

- Meta-profiling
  - compound
  - gene

- Identify most enriched genes in meta-profiles
  - **gene** | **count**
    - Npm1: 7
    - Dpys: 6
    - .....: 6

- Chance assessment of the enriched genes using permutation analysis
- Define ‘meta-signatures’ if gene significantly enriched (FDR<threshold)
- Functional analysis of ‘meta-signatures’

- GO
- KEGG
- PubMed

(FDR: False discovery rate)
Identified Meta-signatures Related to Hepatotoxicity with Glutathione Depletion
Gene Ontology Analysis Reveals Altered Trans-sulfuration Pathway using ebTrack/ArrayTrack

Some biological processes involved in trans-sulfuration pathway

<table>
<thead>
<tr>
<th>GeneName</th>
<th>locusId</th>
<th>VPA</th>
<th>NMF</th>
<th>IND</th>
<th>DMN</th>
<th>DEHP</th>
<th>DEC</th>
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<tr>
<td>L-cysteine catabolic process to taurine (GO:001945, P=0.000003, level=10.02)</td>
<td></td>
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<tr>
<td>Cdo1</td>
<td>81718</td>
<td>-1.60</td>
<td>-1.62</td>
<td>Non-sig</td>
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<td>-2.49</td>
<td>-2.82</td>
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<td>S-Adenosylmethionine metabolic process (GO:0046500, P=0.00190, level=5.33)</td>
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<tr>
<td>Gnmt</td>
<td>25134</td>
<td>-1.36</td>
<td>-2.13</td>
<td>-2.63</td>
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<td>-1.39</td>
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<tr>
<td>Gamt</td>
<td>25257</td>
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<td>-1.39</td>
<td>-2.16</td>
<td>Non-sig</td>
<td>-1.68</td>
<td>-2.31</td>
<td>-1.81</td>
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<tr>
<td>ATP catabolic process (GO:0006200, P=0.000159, level=9.76)</td>
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<tr>
<td>Abcc6</td>
<td>81642</td>
<td>-1.70</td>
<td>-2.09</td>
<td>-2.90</td>
<td>-1.70</td>
<td>-1.35</td>
<td>-1.92</td>
<td>-1.59</td>
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<tr>
<td>Acly</td>
<td>24159</td>
<td>-5.21</td>
<td>-3.06</td>
<td>-3.52</td>
<td>-2.60</td>
<td>-2.63</td>
<td>-2.53</td>
<td>Non-sig</td>
</tr>
</tbody>
</table>
Metabonomic and Genomic Studies Reveal Trans-sulfuration Pathway Altered
Summary

1. Both metabonomic and genomic studies reveal disturbance in the trans-sulfuration pathway

2. Urinary level of N-methylnicotinate could be a non-invasive potential biomarker of hepatotoxicity related to glutathione depletion

3. Systematic integrated studies (e.g. metabonomic and genomic) can help to understand complex toxicity mechanisms
   • Systems such as the ebTrack/ArrayTrack can facilitate such integrated analysis
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