

Lipidomic biomarkers of ethanol induced hepatotoxicity in human HepaRG liver cells

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Intro

- Lack of sensitive and specific early biomarkers for alcohol-associated liver disease
- Lipidomics offers an interesting approach for biomarker elucidation since early stages are characterized by steatosis and steatohepatitis
- In vivo* studies require a large sample size, *in vitro* studies enable the use of fewer replicates due to lower inter-sample variability

Objective

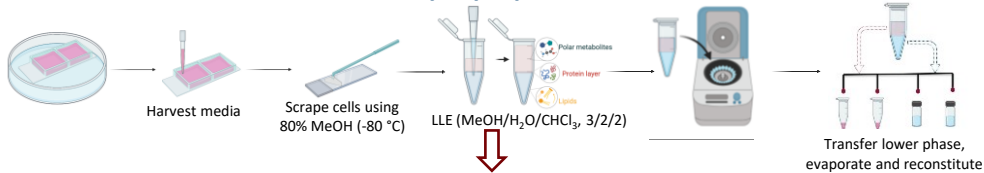
Elucidation of biomarkers of ethanol exposure in HepaRG cells using LC-MS based lipidomics



Exposure conditions

- Determination of IC10 (24h) using neutral red uptake assay
- Evaluation of ethanol cross-contamination using GC-FID
- Exposure to high (IC10, n=6) and low concentration (1/10 IC10, n=6) vs. no ethanol (n=6)

Sample preparation



LC-MS based lipidomics

Liquid chromatography

- UPLC BEH C18
- A: ACN/buffer* (30/70)
- B: buffer*/ACN/IPA (2/10/88)
- *5 mM NH4Ac (+0.1% HAc ESI+)

Mass spectrometry

- ESI – QToF – HRMS
- Agilent 6560
- MS1 (samples) + MS2 (QC)
- DDA iterative exclusion

Worklist

- System suitability
- Conditioning (+DDA)
- Randomized injection order
- Pooled QC regular intervals

Data processing

Pretreatment

Preprocessing

- Peak picking & alignment
- Deisotoping, duplicate removal & drift correction
- Filtering (e.g. mRSD < 30%)

- Random forest imputation
- Log transformation
- PQN normalization
- Pareto scaling

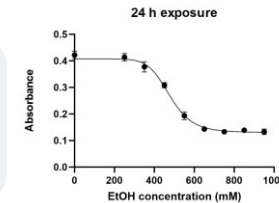
Statistics

- Mann-Whitney U – Welch T
- $p < 0.05$ & $FC > 10$ | < 0.1
- PCA – PLS-DA
- VIP > 1

Results and Discussions

Exposure conditions

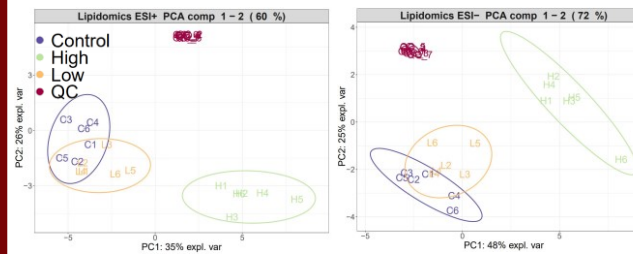
- IC10 = 368 mM
- Cross-contamination of ethanol to negative controls = 1.3%



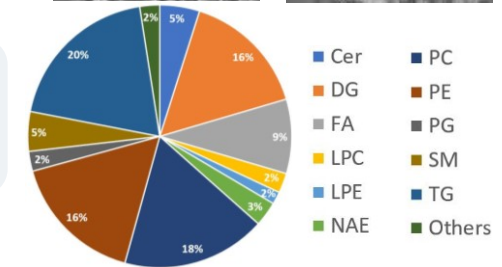
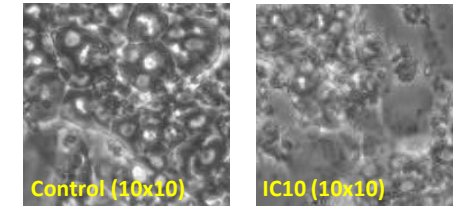
HepaRG lipidomic profiling

- 328 annotated lipid species
- MS-DIAL & LipidMatch for MSMS matching
- Manual confirmation using mirror plots
- RT following equivalent carbon number model

Signature of ethanol exposure



Species	High dose	Low dose
Carnitines		
Ceramides		
Cardiolipins		
Prenol lipids		
Diaclyglycerols		
Lysophosphatidylcholines		
Phosphatidylcholines (< 3 DB)		
Phosphatidylcholines (≥ 3 DB)		
Phosphatidylethanolamines		
Phosphatidylserines		
Sphingomyelins		
Triacylglycerols		



- Upregulation of di- and triacylglycerols
 - Steatosis
- Up- or downregulation of phosphatidylcholines
 - No. of double bonds
- Downregulation of cardiolipins at high exposure concentration
 - Sign of decreased respiratory chain capacity
- Downregulation of phosphatidylethanolamines

- Ethanol exposure clearly disturbs lipid metabolism
- Need for validation experiment

Color	Green	Light Green	White	Light Orange	Orange	Dark Orange
Number of species	5-10	1-4	0	1-4	5-10	>10
Abundance	Lower	Lower	NA	Higher	Higher	Higher