Comprehensive proteomic analysis of 3D human liver spheroids for drug toxicity investigation

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Overview
To demonstrate the relevance of the spheroids for investigating toxicity of drug compounds at the proteome level, we analyzed by shotgun proteomics in depth-fractionated protein digests isolated from 3D human liver spheroids to deliver a first view in sample complexity and protein dynamic range of the human liver proteome. In parallel, we applied a label-free quantitative approach for the profiling of protein abundances from 3D liver spheroids treated with different concentrations of acetaminophen. We obtained high reproducibility in protein abundances between biochemical replicates and across all tested acetaminophen (APAP) concentrations. Thus we generated quantitative profiles for thousands of proteins in response to acetaminophen treatment, revealing dozens of proteins affected by that drug.

Introduction
Drug-induced hepatotoxicity is currently one of the main reasons for market withdrawal of drugs and exclusion of drugs in clinical phases. Better models than in vitro 2D cells and animal subjects are needed for testing new drugs before entering human clinical trials. Recently, 3D cells or spheroids have shown to be suitable for testing chronic exposure toxicity compared to 2D cells, due to their longer lifespans and greater stability. Several fabrication methods are used to create spheroids such as the hanging drop approach that consists in forming cell aggregates in drops hanging from a surface (1).

Results I
To investigate the minimum number of liver spheroids required to perform reproducible protein extraction for proteomics analysis, several protein extraction and digestions protocols were tested. Two approaches namely the freeze/thaw method - combined with in-solution digestion and the high intensity focused ultrasound (HIFU) - combined with filter-aided sample preparation (FASP) (2) were evaluated in terms of reproducibility, sensitivity and robustness. In general, both approaches enabled high reproducibility in protein extraction from 12 spheroids (i.e 12000 cells), however a higher protein yield was obtained with the HIFU-FASP protocol.

Results II: In order to increase the coverage of the liver proteome by mass spectrometry, the peptide fractionation by hydrophilic interaction chromatography (HILIC) (3) is an excellent approach to decrease sample complexity and also allows low amount of starting material (i.e 30000 cells),

Proteins were mapped on the representative metabolic and regulatory protein networks available for the KEGG human pathway database and revealed higher coverage of the metabolic and regulatory pathways for the HILIC fractionated samples (red) compared the non-fractionated samples (blue).

Results III: To demonstrate the relevance of the spheroids for investigating toxicity of drug compounds at the proteome level, we applied label-free quantitative shotgun proteomics for the generation of protein abundance profiles from 3D liver spheroids treated with different concentrations of acetaminophen.

Protein identification and label-free quantification were assessed with Mascot-Scaffold and Progenesis QI, respectively. Overall, 4000 proteins were identified and quantified at least with 2 unique peptides per protein over the complete data sets (Protein FDR 1%). Overall hundred proteins were found significantly regulated with more than 2 fold and with ANOVA p value less than 0.05. Proteins were also grouped by their expression patterns using correlation analysis and unsupervised hierarchical clustering. Thus different groups of proteins were identified ranging from proteins regulated at very low doses to very high doses of APAP.

Conclusion
The results demonstrated that proteomic analysis of 3D human liver microsomes are suitable for investigating drug toxicity and can be applied to other 3D tissue models such as cardiac spheroids.
Such quantitative data sets will allow the identification of potential protein networks involved in drug toxicity and will be further integrated in existing systems toxicity models together with other “omics” and functional data sets.

References

www.fgcz.ethz.ch/applications/proteomics