Analysis of liver and heart spheroids in drug-induced hepatotoxicity and cardiotoxicity

N. Selevsek[1], B. Roschitzki[1], O. R. Clayton[3], C. Fortes[1], L. Kunz[1], J. Grossmann[1], W. Wolski[1], P. Nanni[1], A. B. Roth[3], J. Kelm[2] , R. Schlapbach[1]

[1]*Functional Genomics Center Zurich, UZH/ETHZ, Zurich, Switzerland;* [2]*InSphero AG, Schlieren, Switzerland,* [3]*Roche Innovation Center Basel, Basel, Switzerland*

Overview

Aiming at developing integrative *in silico* tools for predicting human liver and heart toxicity*, computational chemistry and systems toxicology are combined to model toxic perturbations in liver and heart across multiple scales. Models are populated with data generated using advances Omics technologies applied to in vitro 3D liver and heart spheroids that are challenged with prototypical hepato- or cardiotoxicants.

Introduction

Drug-induced cardiotoxicity and hepatotoxicity are currently the main reasons for market withdrawal of drugs and exclusion of drugs in clinical phases. Recently, 3D cell spheroids have shown to be suitable for testing chronic exposure toxicity compared to 2D cells, due to their longer lifespans and greater stability. Besides, their 3D architecture display more organ-like function than conventional monolayer cell cultures.

To demonstrate the relevance of the spheroids for investigating toxicity of drug compounds at the proteome level, we analyzed by shotgun proteomics protein digests extracted from 3-D hepatocytes and cardiomyocytes to deliver a first view in sample complexity and protein dynamic range of the human liver and cardiac proteome (1). In parallel, we applied label-free quantitative approach for the profiling of protein abundances from 3D liver spheroids treated with different cardiac and liver toxicants.

Workflow

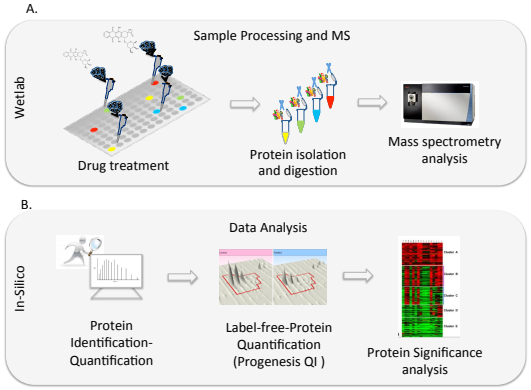


Figure 1: Highly sensitive analytical workflow for the proteomics analysis of drug-treated human spheroids. A. Protein extraction from drug-treated human spheroids and mass spectrometric analysis of digested proteins. B. Proteome identification/quantitation and statistical analysis using Mascot/Scaffold-Progenesis QI.

Methods

Cardiac and liver spheroids were incubated with multiple toxicants and harvested at different time points. Proteins were extracted from spheroids using freeze-thaw cycles. After cell lysis, protein extracts were subjected to tryptic digestion. Peptides were analyzed on an Orbitrap Fusion instrument in data dependent mode. MS/MS spectra were searched using Mascot and validated using Scaffold. Protein quantification was performed using Progenesis QI.

Results I

Figure 2

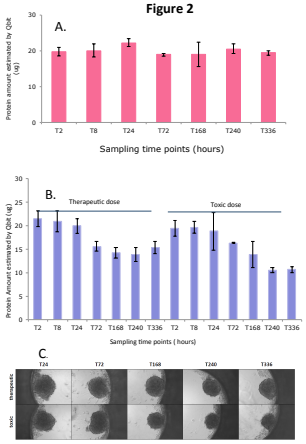


Figure 2:
Protein amount extracted from 12 cardiac spheroids treated **A**. DMSO and with **B**. compound. Samples were processed in biological replicates using the freeze/thaw method . The protein amount (i.e ug of proteins) was estimated by fluorometric assays.

Spheroids were resuspended in lysis buffer containing 8 M Urea, 1 mM dithiothreitol and 50 mM Tris/HCl (pH8). In total, four freeze-thaw cycles were performed by freezing the cell suspension in a liquid nitrogen bath and thawing the material at room temperature. After centrifugation, cells debris were discarded and the supernatant was used to estimate the protein concentration by fluorometric assay.

C. Microscopic view of the cardiac spheroids (ie 4000 cardiomyocytes) during drug treatments at different time points.

Figure 3

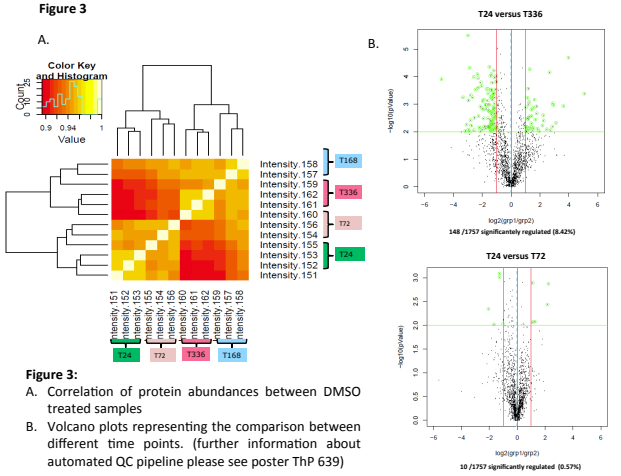


Figure 3:
A. Correlation of protein abundances between DMSO treated samples
B. Volcano plots representing the comparison between different time points. (further information about automated QC pipeline please see poster ThP 639)

Results II

Figure 4:
Comparison of Gene Ontology annotations for cellular component from pooled spheroids processed in biological triplicates. Bars shows the percentage of proteins with the indicated Gene Ontology terms for three replicates.

Gene Ontology analysis was performed in order to classify the proteins according to their cellular component and their biological process based on the information available in the GOA database. The proteins were classified as followed for the cellular component: cytoplasm associated 58%, membrane associated 28%, nucleus associated 24%, mitochondrion associated 16% and cytosol associated 23%.

Figure 4

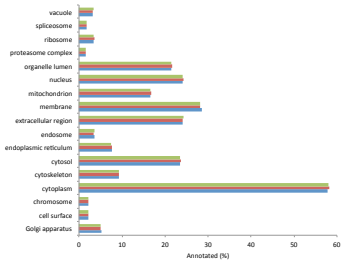
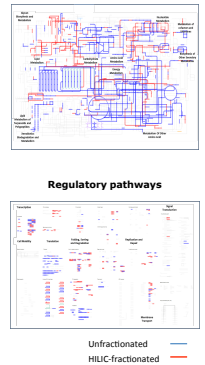


Figure 5:
In order to increase the detection of proteins from subcellular cell compartments, the peptide fractionation by hydrophilic interaction (HILIC) chromatography is an excellent approach to decrease sample complexity, and also required very low amount of starting material.

Proteins were isolated from pooled microtissue samples, homogenized be high intensity focused ultrasound, and digested using the FASP approach (2). The resulting peptides were then subjected to HILIC chromatography . In total eight fractions were collected and analysed twice by LC-MS/MS. The different protein pathways covered by both approaches (i.e non fractionated and HILIC-fractionated samples) are displayed in figure 4.

Figure 5



Conclusion-Outlook

Using novel 3D cell culture systems in combination with sensitive and accurate MS-based technologies and sophisticated bioinformatics methods, the comprehensive analysis of drug mechanisms and toxicity is feasible at the proteome scale and can be applied to various types of 3D tissue model systems in the future. Together with data generated at the epigenome and the metabolome level, the resulting proteomics data will allow for a comprehensive mapping of molecular regulation and the precise description of cellular and tissue behavior.

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References:
1. Selevsek and Schlapbach, 2015, Chimia
2. Wiśniewski et al., 2009, Nat.Methods