The human hepatocyte TXG-MAPr: gene co-expression network modules to support mechanism-based risk assessment

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INTRODUCTION

Liver is a primary target organ for drugs and chemical toxicants due to its role in metabolism and disposition. Hepatotoxicity can be regarded as a multistep, multicellular disease process, where an initial molecular stress is followed by key events to couple to an apical endpoint. Some of these steps can be recapitulated in non-animal-based testing systems and could be used to inform about the emergence of the adverse outcome. For these reasons, approaches that quantitatively reveal mechanistic details of preserved responses between animals and human are needed to advance risk assessment practice. We applied gene co-expression analysis to the Primary Human Hepatocytes (PHH) toxicogenomic dataset TG-GATEs and deployed it in an R-Shiny analysis framework accessible via an interactive website, the PHH TXG-MAPr (https://txg-mapr.eu/WGCNA_PHH/TGGATEs_PHH/) to identify co-expressed gene sets that can be mapped to key events of cellular response to stress. Their responses can be quantitatively measured, as well as their level of preservation in animal-based testing systems, therefore providing mechanistic information on the cellular processes involved in adaptation and progression.

METHODOLOGY

TG-GATEs datasets (https://dbarchive.biosciencedbc.jp/en/open-tggates/download.html) were jointly normalized (RMA), probes annotated with BrainArray chip description file (CDF) and log2FC calculated with the limma R package. Additional human hepatocytes microarray datasets downloaded from Gene Expression Omnibus were analysed similarly. TempO-Seq data of PHHs derived from 50 individuals exposed to a wide concentration range of tunicamycin were analysed at BioSpyder® using the TempO-Seq technology in combination with the S1500+ gene set. Raw counts were normalized with DESeq2 normalization, log2 transformed and analysed with BMD express. We applied the WGCNA R package to the TG-GATEs PHH dataset (unsigned modules, softpower threshold of 5), obtaining 398 modules containing 10275 genes. For each module, we calculated the eigengene score (EGs,

summary of the constituent genes log2 fold changes), annotated them with Over Representation Analysis (ORA) via ConsensusPathDB, TF enrichment (Dorothea DB), and calculated preservation towards rat TG-GATEs datasets. The user interface application of the TXG-MAPr tool has been implemented using the R-shiny package.

RESULTS

We present the TXG-MAPr webtool , an R-Shiny-based implementation of Weighted Gene Co-regulated Network Analysis (WGCNA) on the PHH TG-GATEs dataset. The 398 gene co-expression networks (modules) were annotated with functional information (pathway enrichment & transcription factor) to reveal their mechanistic interpretation. Several well-known stress response pathways were captured in the modules, were perturbed by specific stressors and showed preservation in rat systems, with the exception of DNA damage and oxidative stress responses. A subset of 87 well-annotated and preserved modules was used to evaluate mechanisms of toxicity of endoplasmic reticulum stress and oxidative stress inducers, including cyclosporine A, tunicamycin and acetaminophen. Additionally, module responses were calculated from external datasets obtained with different hepatocyte cells and platforms, including targeted RNA-seq, therefore imputing biological responses from a limited gene set. As another application, donors' sensitivity towards tunicamycin was investigated, identifying higher basal level of intrinsic immune response in donors with pre-existing liver pathology.

DISCUSSION

In conclusion, the TXG-MAPr takes advantage of the modular nature of gene co-expression networks to achieve mechanistically relevant, cross-species and cross-platform evaluation of toxicogenomic data. Toxicogenomic data can be acquired for candidate compounds, even in a high-throughput platform. A summarised profile of preserved and highly interpretable co-expression module responses can be extracted to determine the prevalent mode of action and to compare with public and internal data. Overall, we demonstrated that gene co-expression analysis coupled to a facile visualisation environment, the PHH TXG-MAPr, is a promising approach to analyse in vitro human transcriptomic data and derive mechanistic interpretation, and therefore a substantial step towards the integration of transcriptomic data in mechanistic risk assessment.

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