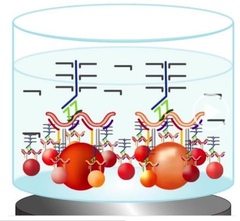
thermoscientific invitrogen

QuantiGene Plex Analysis of ADME Gene Expression in Primary Human Hepatic 3D Spheroid Culture



Sujoy Lahiri[1], Jason H. Stafford[2], Julia Tritapoe[1], Michael F. Millett[1], Deborah K. Tieberg[1], and David T. Kuninger[1]

[1]Thermo Fisher Scientific, 7300 Governors Way, Frederick, MD, 21704. [2]Thermo Fisher Scientific, 3380 Central Expy, Santa Clara, CA 95051

INTRODUCTION

Identifying the induction of drug-metabolizing enzymes in the liver is a key step along the drug discovery pipeline. Primary Human Hepatocytes (PHH) are considered the gold standard *in vitro* model for studying hepatic biology, liver function, and drug induced hepatotoxicity. However, PHH grown in traditional two-dimensional (2D) monolayer cultures rapidly de-differentiate and lose the hepatic-specific functions within a week. Recently developed 3-dimensional (3D) spheroid cultures, on the other hand, mimic the hepatic microenvironment and maintain hepatic function for at least 5 weeks. Thus, 3D *in vitro* models have been shown to more accurately reflect *in vivo* liver biology.[1]

In this study, we used QuantiGene Plex to probe 42 genes related to drug absorption, distribution, metabolism and excretion (ADME) in 3D spheroids cultured from PHH samples. 3 key markers for 3 major nuclear receptor pathways are often used to identify hepatic induction: 1) CYP1A2 for aryl hydrocarbon receptor (AhR) activation, 2) CYP2B6 for constitutive androstane receptor (CAR), and 3) CYP3A4 for pregnane X receptor (PXR). However, a broader assessment of ADME gene signaling pathways has the potential for improved characterization of drug-drug interactions and prediction of clinical outcomes.[2]

MATERIALS AND METHODS

3D spheroid culture

Hepatic spheroids were formed using Gibco™ cryopreserved spheroid–qualified human hepatocytes (Cat. No. HMCPSQ) following the user guide.[3] Each well contained 3000 PHHs. The spheroids formed within 5 days of cell seeding. Starting on day 5, half of the plating medium was changed every 48–72 hours. On Day 5 of the 3D culture 2D hepatic cultures were initiated in collagen I coated 24-well plates using the same lots of PHH following user manual.[4]

Prototypical ligands were used to induce the 3 major nuclear receptor pathways commonly associated with drug metabolism in the liver. 2D and 3D cultures were treated with either 50 µM omeprazole (AhR ligand), 1 mM phenobarbital (CAR ligand), 10 µM rifampicin (PXR ligand) or DMSO (vehicle control). 2D cultures were treated on Days 2 and 3 whereas 3D cultures were treated on Days 6 and 7 (Fig 2).

QuantiGene Plex

The QuantiGene Plex Assay was used to quantitate a custom-designed panel of 57 genes including 42 ADME genes, 7 apoptosis genes, and 8 housekeeping genes (Table 1). Target-specific capture extenders and label extenders were incubated overnight at 54 ºC with the cell lysates and Luminex® MagPlex beads. The beads are coated with capture probes specific to the capture extenders, thus hybridizing each target gene to a specific bead (Fig 1). After overnight incubation, the branched DNA signal amplification "tree" was built through a series of 3 sequential 1 hour hybridizations at 50 ºC with single-stranded DNA oligos called PreAmplifier, Amplifier and Label Probe. The beads were washed prior to each hybridization. The Label Probe oligos are biotinylated and, after washing, the beads were incubated at room temp for 30 minutes with the detection reagent streptavidin phycoerythrin (SAPE). Finally, the beads were washed, resuspended in SAPE buffer and read on a FlexMAP 3D Luminex® instrument.

Data exported from the FlexMAP 3D instrument was analyzed with QuantiGene Analysis software (apps.thermofisher.com/apps/quantigene) integrated with Transcriptome Analysis Console (TAC) 4.0.2

Figure 1. QuantiGene Plex Workflow

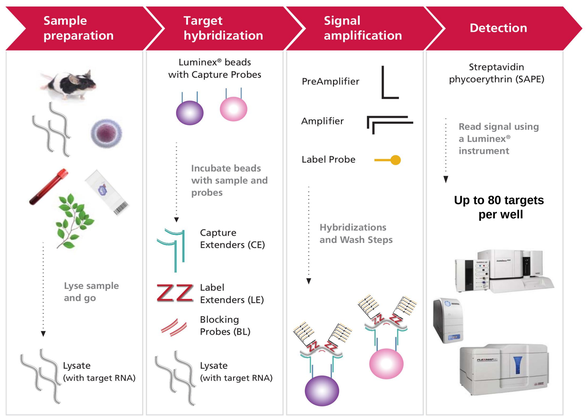


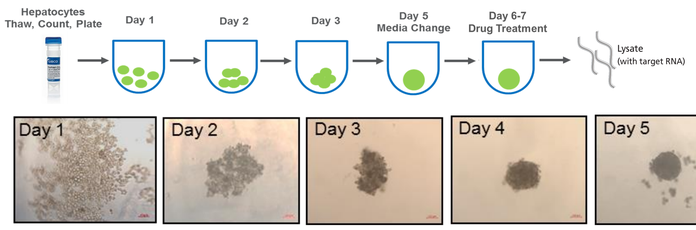
Table 1. Custom ADME 57-plex QuantiGene Panel

CYP1A1	*CYP3A4*	*CYP1A2*	*CYP2C19*	*ABCB11*	*ACTB*	*SLCO1B1*	*ABCB1*	*AOX1*	*CYP3A5*
MAOB	*CYP2C9*	*CYP2D6*	*UGT1A9*	*SULT2B1*	*SLC22A1*	*ABCG2*	*UGT1A4*	*HPRT1*	*TBP*
SLC10A1	*SULT2A1*	*CYP2J2*	*UGT1A1*	*XDH*	*UGT1A6*	*GAPDH*	*UGT2B7*	*CYP2A6*	*SULT1A1*
FMO3	*GUSB*	*POLR2A*	*CYP2E1*	*UGT1A3*	*SLC3A1*	*HMGB1*	*SLC2A1*	*SLCO2B1*	*CYP2C8*
PGK1	*ABCC2*	*FMO1*	*ABCA1*	*CASP9*	*FMO5*	*PPIB*	*SLCO1B3*	*MAOA*	*CYP2B6*
ABCC1	*UGT2B15*	*RIPK1*	*BAX*	*TP53*	*BCL2*	*CASP3*			

ADME genes = Green, Apoptosis genes = Blue, HSK genes = Yellow

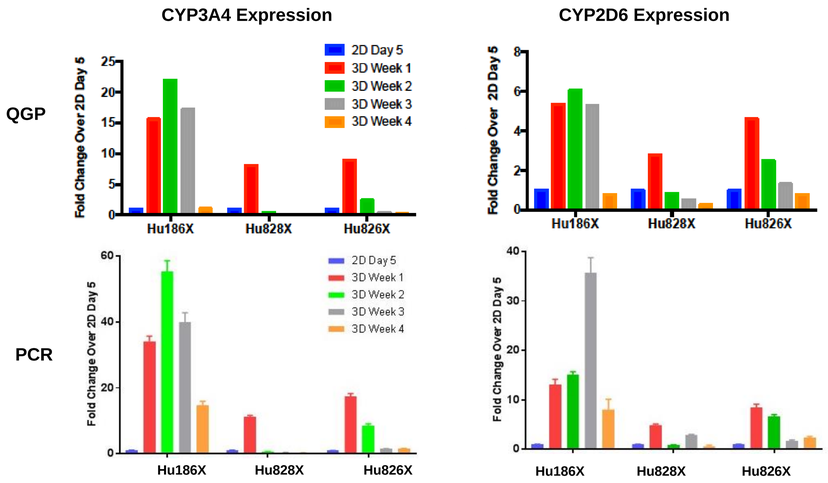
RESULTS

Figure 2. Primary Human Hepatocytes Self-Assemble Into 3D-Spheroids after 5 Days



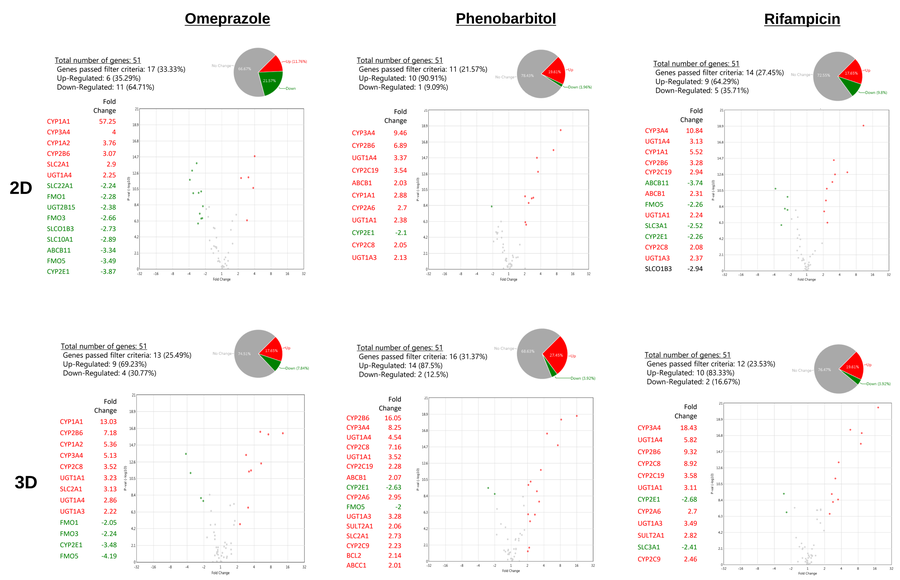
Gibco™ Human Spheroid-Qualified Hepatocytes (Cat. No. HMCPSQ)are seeded in Nunclon™ Sphera™ low attachment U-bottom 96-well Microplates (Cat. No.174925), where they self -assembled into 3D-spheroids by day 5. Spheroids were treated with various Cytochrome P450 (CYP) inducers on days 6-7 and lysates were prepared on day 8 using a QuantiGene sample processing kit (Cat. No. QS0100).

Figure 3. Elevated Gene Expression in 3D PHH Cultures Detected by QuantiGene Plex and PCR



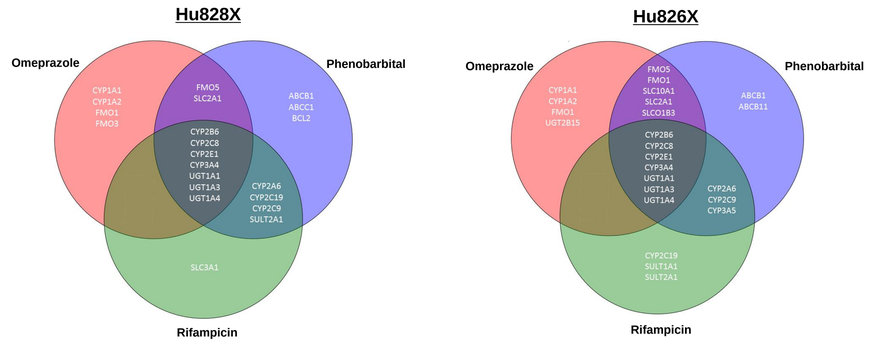
QuantiGene Plex (QGP) and real-time quantitative PCR analysis of CYP3A4 and CYP2D6 mRNA levels in 3 individual sample lots of PHH (Hu186X, Hu828X, and Hu826X). Target gene expression was normalized to the geometric mean of the housekeeping genes. Fold change (Δ) in expression levels of 3D cultures was calculated relative to day 5 of 2D culture. QGP and PCR data demonstrated good correlation detecting increased transcript expression in all 3D PHH cultures compared to their 2D counterparts.

Figure 4. ADME Gene Expression in 2D v. 3D PHH Cultures Treated with Prototypical Inducers



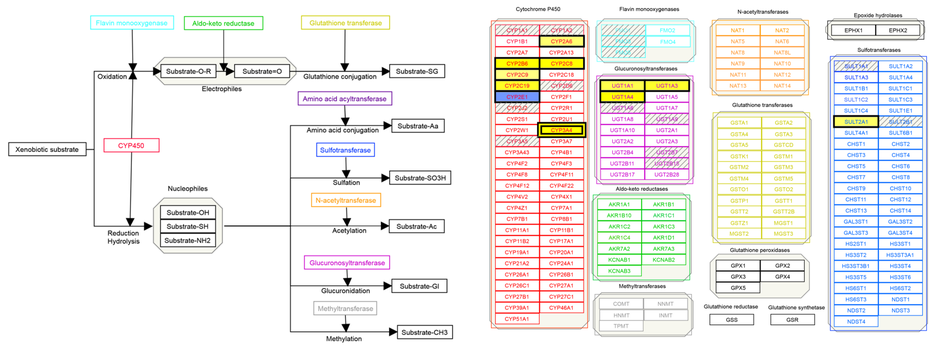
Volcano plots show Log2 fold change in gene expression in Hu828X PHH cultures treated with omeprazole, phenobarbital and rifampicin compared to DMSO controls (p value <0.05, One-way ANOVA). Genes up-regulated >2-fold are highlighted in red and genes down-regulated >2-fold are highlighted in green. Gene expression profiles of 2D and 3D PHH cultures differed significantly following induction with all 3 ligands.

Figure 5. Differentially Expressed ADME Genes Specific to Each Inducer



Venn diagram showing shared (circle overlap) and treatment-specific (no overlap) differentially expressed genes across the different inducers in 3D spheroids cultured from Hu828X and Hu826X PHH samples. Genes listed demonstrated Log2 fold change >2 (p value <0.05, One-way ANOVA). CYP1A2, known to be a marker of AhR activation was specifically up-regulated by the AhR ligand omeprazole. However, CYP2BP and CYP3A4, markers used for CAR and PXR activation respectively, were found to be up-regulated by all 3 ligands.

Figure 6. Metapathway Biotransformation Phase I and II



QuantiGene analysis software allows export of .chp files that are compatible with Transcriptome Analysis Console (TAC) software. The TAC software allows more than simple identification of differential expression by providing powerful, interactive visualizations. TAC searches the WikiPathway database and allows you to visualize data on the pathway diagrams and calculate pathway metrics. Metapathway biotransformation Phase I and Phase II was identified as the primary pathway induced by all 3 ligands. Genes up-regulated by rifampicin are highlighted in yellow.

CONCLUSIONS

- Gibco™ Primary Human Hepatocytes (HMCPSQ) can easily be assembled into a 3D culture in 5 days using either Gibco™ Hepatic Spheroid Kit (A41390) or Nunclon™ Sphera™ low attachment U-bottom 96-well Microplates, Gibco™ plating media supplements.
- QuantiGene Plex data showed increased CYP3A and CYP2D expression in 3D spheroids, which corroborates gene expression data obtained by real-time quantitative PCR analysis.
- Changes in ADME gene expression following induction with prototypical ligands differed significantly between 2D and 3D PHH cultures.
- ADME signaling pathway analysis and the identification of secondary markers and/or specific gene signatures may provide a more comprehensive characterization of drug-drug interactions in the liver.

FUTURE DIRECTIONS

- QuantiGene Plex analysis of *in vitro* liver model established by co-culturing PHH and non-parenchymal cells
- Using QuantiGene Plex to profile PHH 3D spheroids isolated from diseased livers such as NAFLD, NASH and hepatic fibrosis.

REFERENCES

1. Bell et al. Scientific Reports 2015 6:25187 DOI: 10.1038/srep25187
2. Moeller et al. Thermo Fisher Application Note: Transcript regulation of 18 ADME genes by prototypical inducers in human hepatocytes
3. Thermo Fisher Scientific. User Guide: Cryopreserved 3D-Spheroid Qualified Human Hepatocytes. Pub. No. MAN0018280.
4. Thermo Fisher Scientific. User Bulletin: Thawing and Use of Plateable and Suspension Cryopreserved Hepatocytes. Pub. No. MAN0018379.

ThermoFisher SCIENTIFIC