Regulation of Steatosis in upcyte® Hepatocytes by Nuclear Receptor Agonists

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Introduction

- Steatosis, typified by excessive accumulation of intracellular lipids, is a common liver disease triggered by various factors, including exposure to certain drugs and environmental chemicals, such as cyclosporine A (CsA), tetracycline and amiodarone.
- Nuclear receptors (NR) are important transcriptional regulators with roles in hepatic lipid metabolism and homeostasis. Several nuclear receptors such as AhR, CAR, FXR, LXR, PPAR, and PXR are involved in regulating genes involved in steatosis.
- Human upcyte® hepatocytes, established by upcyte® technologies GmbH, has recently emerged as a good alternative to primary hepatocytes to examine liver diseases *in vitro*, including steatosis.
- Herein we demonstrate the utility of upcyte® hepatocytes as a model system for assessing drug induced steatosis and provide insight into the potential use of nuclear receptors as therapeutic targets to treat liver disease.

Induction of Steatosis phenotype in upcyte® Hepatocytes and Comprehensive Gene analysis by NGS

upcyte® Hepatocytes (donor 10-03) → Treat with CsA (48 hr) → Oil Red O staining & NGS

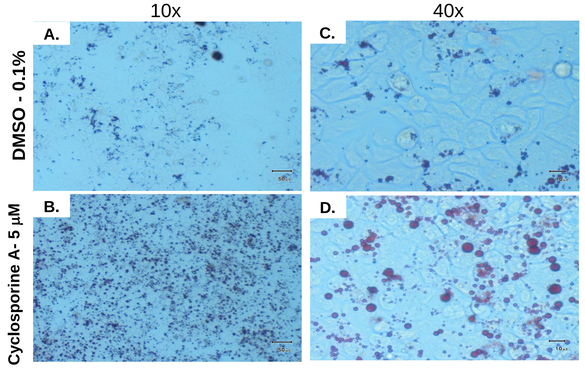

Figure 1: Oil Red O staining of lipid droplets. Adult human upcyte® hepatocytes (donor 10-03) were treated with vehicle control, 0.1% DMSO (A&C) or CsA, 5 µM (B&D) for 48h. Lipid accumulation was assessed by staining of lipid droplets with Oil Red O. Left panels=10X; right panels =40X magnification.

Table 1. Summary of significantly regulated genes by AmpliSeq® Transcriptome Analysis

Compound	P<0.05, FC all	P<0.05, 2-fold	P<0.05 , 3-fold	Top regulated genes
Cyclosporine (10 µM)	3555	1546	573	ROM1, MLLT11, ALDH1L2, PIF1, COL1A2, IFITM1

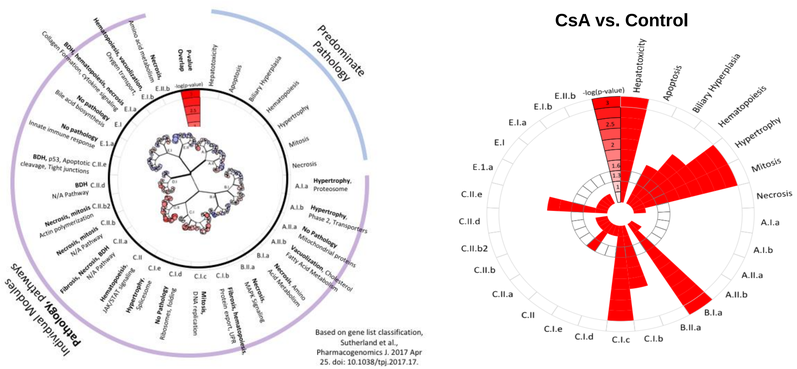

Figure 2: Hepatotoxic module association. Genes associated with hepatotoxicity as described by Sutherland *et al.,* were compared to the list of significantly regulated genes (See Table 1). IBIPlots™ were used to visualize the p–value of overlap for each gene list. -log(p-value)>1.3 is a statistically significant overlap.

Evaluation of the role of NR in the regulation of steatosis gene markers

upcyte® Hepatocytes (donor 10-03) → Co-treat for 48h with CsA (5 µM) +/- prototypical ligands of NR → Gene Expression of steatotic markers by qPCR

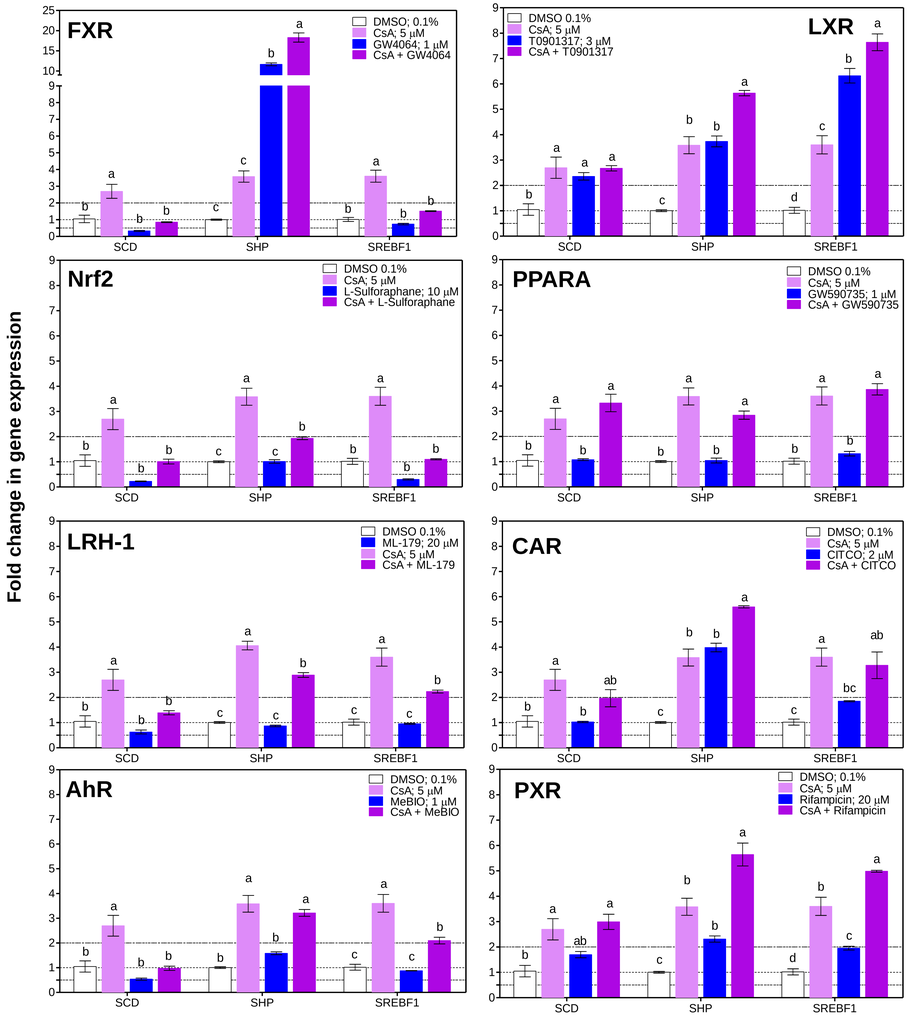

Figure 3: Evaluation of the role of NR in Steatosis by qPCR. Fold of change in gene expression of 3 (out of 12) steatosis markers that were evaluated by qPCR using pre-validated primers (IDT, Illinois, USA) and the $2^{-\Delta\Delta CT}$ method. β-Actin was used as a reference gene. Statistical analysis was done using ANOVA and Tukey test for multiple comparisons (n=3). Columns with same alphabet letter indicate treatments that are not statistically different. Columns with different alphabet letters indicate treatments that are significantly different (p<0.05). Gene abbreviations: Stearoyl-CoA desaturase (SCD), sterol regulatory element-binding transcription factor 1 (SREBF1), and small heterodimer partner (SHP).

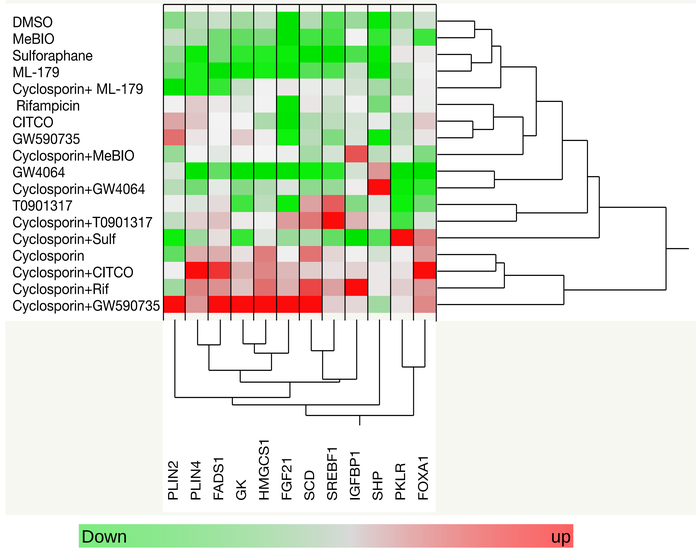

Figure 4: Summary of qPCR gene expression of 12 steatotic gene markers. The modulation of gene expression of 12 steatotic gene markers in response to cyclosporine treatment in the presence and absence of prototypical activators of different nuclear receptors. Gene abbreviations: perilipin 2 (PLIN2), perilipin 4 (PLIN4), pyruvate kinase isozymes R/L (PKLR), forkhead box protein A1 (FOXA1), fatty acid desaturase 1 (FADS1), Stearoyl-CoA desaturase (SCD), glycerol kinase (GK), hydroxymethylglutaryl-CoA synthase (HMGCS1), insulin-like growth factor binding protein1 (IGFBP1), sterol regulatory element-binding transcription factor 1 (SREBF1), fibroblast growth factor 21 (FGF21), and small heterodimer partner (SHP). β-Actin was used as a reference gene.

Results and Conclusions

- upcyte® human hepatocytes exhibit microvesicular steatosis upon treatment with a known hepatotoxicant, Cyclosporine A. The pattern of altered gene expression, as determined by NGS, is indicative of a steatotic response.
- The ability of several NR ligands to reverse steatosis caused by cyclosporine A was explored. The LXR agonist T0901317 exasperated the steatotic pattern of gene expression caused by cyclosporine A. In contrast, the FXR agonist GW4064 and the Nrf2 agonist L-sulforaphane reversed the cyclosporine-dependent induction of SCD and SREBF1
- These results confirm the utility of upcyte® hepatocytes in assessing drug-induced steatosis and introduce a platform for discovering new modes of treatment.