Validating The Gene Expression of Clinically Relevant CYP450 Enzymes and Transporters in Human upcyte® Hepatocytes to Develop an *in vitro* Predictive Tool for DDI

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Introduction

Drug-drug interactions (DDI) in the liver can have a major impact on the efficacy, potency, and safety of a drug. In particular, a drug's interaction with any of the xenobiotic-sensing nuclear receptors will likely affect the expression of genes encoding drug metabolizing enzymes and transporters. The physiological consequences can be significant, including altered kinetics of drug absorption, metabolism, and elimination, and the potential for altered drug activity/specificity through chemical transformation.

Human hepatocytes are the current standard *in vitro* model system for assessing drug metabolism and safety. However, their limited supply from any one donor and the inability to propagate differentiated cells pose challenges to their routine use for iterative, comparative drug screening. Immortalized human hepatoma (i.e. HepG2) and hepatocarcinoma cell lines have been used due to their unlimited proliferative potential. A serious drawback to transformed cell lines is their decreased expression of hepatocyte differentiation markers, including nuclear receptors and their target genes, that are necessary to address drug-drug interactions.

upcyte® hepatocytes are human donor-derived hepatocytes established by upcyte® technologies GmbH to confer limited proliferative ability while maintaining their native constitutive and inducible xenobiotic metabolizing enzyme activities. Human upcyte® hepatocytes combine the characteristics of normal human hepatocytes with the practical advantage of a cell line. Herein we demonstrate the utility of upcyte® hepatocytes as a model system for assessing potential drug-drug interactions.

Aims and Objectives

This study assessed the utility of upcyte® hepatocytes to:

- conduct rapid *in vitro* assessments of a drug's impact on the expression of genes involved in drug metabolism, predictive of DDI, *and*
- screen drugs for agonist or antagonist activities against nuclear receptors, resulting in altered expression of the clinically-relevant drug metabolizing enzymes and transporters that they regulate.

Human upcyte® Hepatocytes: Morphology and Basal Expression of Xenobiotic receptors, CYP450 Enzymes, and Transporters

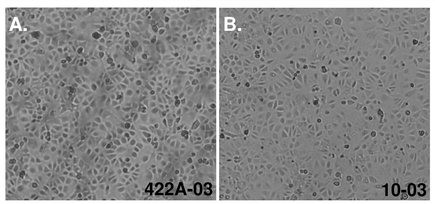
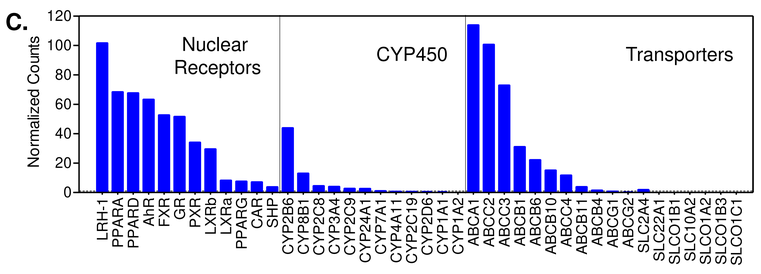


Figure 1: Human upcyte® hepatocytes from (**A**) newborn (422A-03) and (**B**) adult (10-03) donors. (**C**) Normalized basal mRNA expression of selected xenobiotic and nuclear receptors, CYP450 enzymes and transporters in vehicle-treated upcyte® hepatocytes (#10-03), measured by Next Generation Sequencing.



Nuclear Receptor-Specific Induction of Hepatic Drug Metabolizing Enzymes and Transporters

upcyte® Hepatocytes → 48h drug treatment → qPCR

Induction of Target Genes encoding Hepatic Drug Metabolizing enzymes

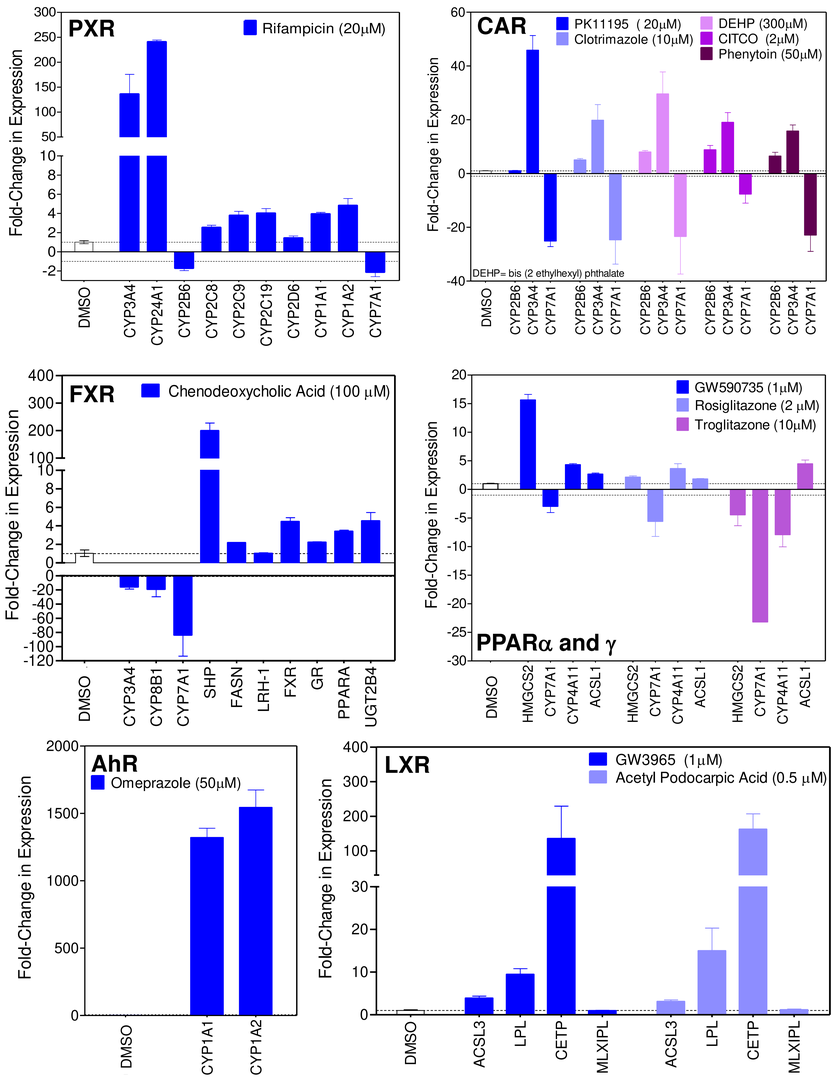


Figure 2: Validation of upcyte® hepatocytes to screen drugs for Nuclear Receptor-mediated induction of genes encoding drug metabolism enzymes.

Induction of Target Genes encoding Hepatic Transporters

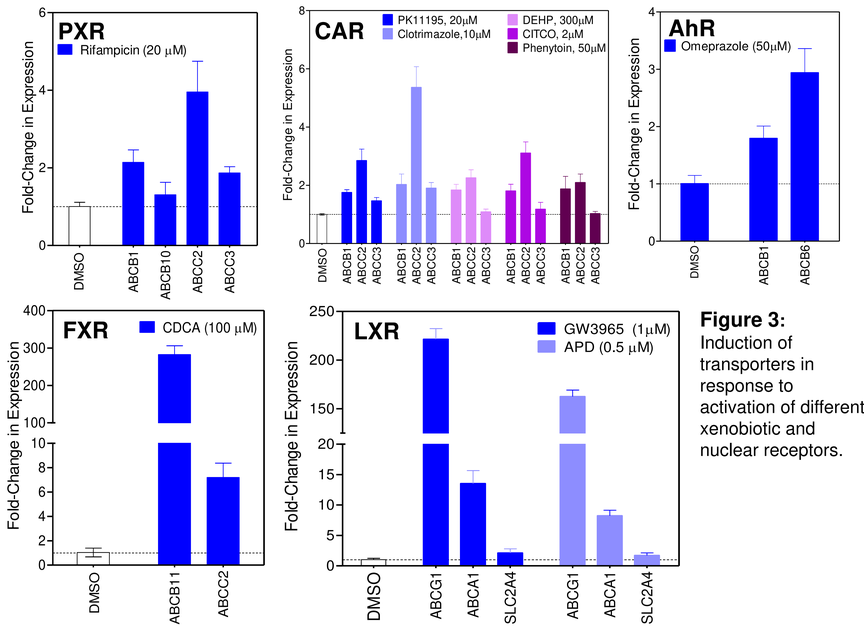


Figure 3: Induction of transporters in response to activation of different xenobiotic and nuclear receptors.

Antagonism of xenobiotic receptors in Human upcyte® Hepatocytes

upcyte® Hepatocytes → 24h drug treatment → qPCR

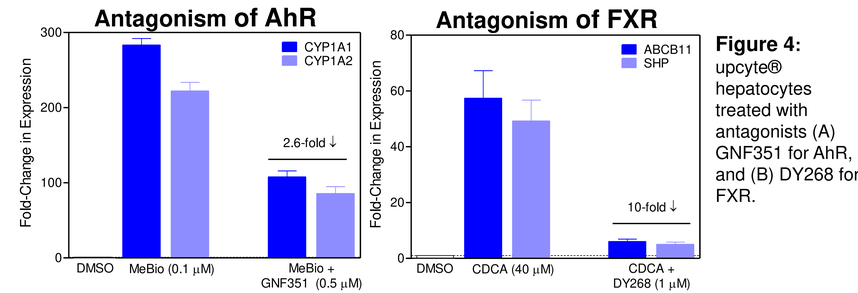


Figure 4: upcyte® hepatocytes treated with antagonists (A) GNF351 for AhR, and (B) DY268 for FXR.

Results and Conclusions

- upcyte® hepatocytes provide ligand-specific agonism/antagonism of the xenobiotic-sensing Nuclear Receptors, resulting in the altered expression of the clinically-relevant drug metabolizing enzymes and transporters that they regulate.
- These results confirm the utility of upcyte® hepatocytes in assessing drug-induced modulation of clinically relevant Nuclear Receptor target genes, which are predictive of a drug's potential liability to promote drug-drug interactions.