Comprehensive Examination of Induction of Drug Metabolizing Enzymes in Human upcyte® Hepatocytes

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Introduction

Drugs that induce drug metabolizing enzymes (DMEs) responsible for their own metabolism, or that of a co-administered drug, are a major source of concern in drug discovery. Human upcyte® hepatocytes are proliferating hepatocytes that retain many characteristics of primary human hepatocytes and are an important model for studying drug-drug interactions (DDI).

We conducted a comprehensive evaluation of altered gene expression in upcyte® cells treated with a selection of reference DME inducers. Cells were treated with prototypical agonists of Pregnane X Receptor (PXR), Constitutive Androstane Receptor (CAR), Aryl Hydrocarbon Receptor (AhR), Farnesoid X Receptor (FXR), Liver X Receptors (LXR), Peroxisome Proliferator-Activated Receptor Alpha (PPARA), Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) or Liver Receptor-Homology 1 (LRH-1).

Next-Generation Sequencing (NGS) was used to quantify the altered gene expression induced by these drugs with a focus on DMEs that can affect DDI.

Aims and Objectives

- A comprehensive evaluation of altered gene expression in upcyte® hepatocytes treated with a selection of reference DME inducers.
- Using upcyte® hepatocytes as a model to predict DDI and potential drug mechanism of hepatotoxicity

Materials and Methods

Cell culture and treatments. upcyte® human hepatocytes (donor 10-03) were treated with prototypical agonists of PXR (Rifampicin; 20 μM), CAR (CITCO; 2 μM), AhR (MeBIO; 1 μM), FXR (GW4064; 1 μM), LXR (T0901317; 3 μM), PPARA (GW590735; 1 μM), Nrf2 (Sulforaphane; 10 μM); and LRH-1 (ML179; 20 μM) diluted in INDIGO Assay Medium. Treatments were replenished after 24hr, and continued for a total of 48h.

RNA Purification and Ampliseq® Transcriptome Analysis. Total RNA was purified using the SV96 total RNA isolation system (Promega). RNA libraries used for sequencing were prepared using Ion Ampliseq Transcriptome Human Gene Expression kit (Life Technologies). cDNA quality was confirmed using the Agilent® dsDNA high sensitivity kit. 100 pM of pooled barcoded libraries were used for templating and sequencing using Ion PI™ Hi-Q™ IC kit (Life Technologies), Ion Chef and Ion Proton. Fastq raw sequence files were aligned to the human Hg19 reference sequences by the Ion torrent browser plug-in using the default parameters. Aligned BAM files were uploaded to the StrandNGS for further analysis. After filtering, the aligned reads were normalized and quantified using the DeSeq algorithm by the StrandNGS program. Statistical analyses were performed using the Moderated T-test comparing each treatment to the control group (DMSO; 0.1%). Fold change analysis was performed on those data found to be statistically significant.

Ingenuity Pathways Analysis (IPA) and IBIPlots. Genes that were significantly modulated in response to treatments were analyzed using Ingenuity Pathway Analysis (IPA, Qiagen). P-value of overlap of genes associated with hepatotoxicity and pathogenesis pathways, as described by Sutherland *et. al*, 2017, were determined by IPA and visualized using IBIPlots™.

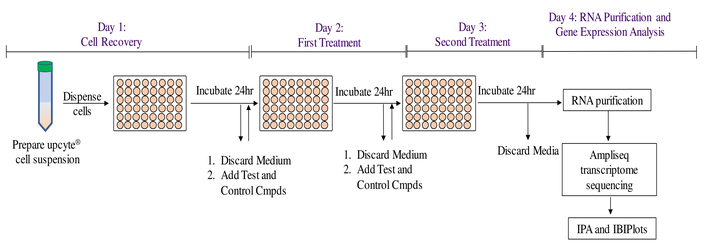


Figure 1: upcyte® hepatocyte culture and assay work flow

Transcriptome analysis, upcyte® human hepatocytes

Table 1. Summary of NGS Results: Top regulated genes in response to prototypical agonists of various nuclear receptors listed in the table below. Red= gene increased relative to control; Green= gene decreased relative to control.

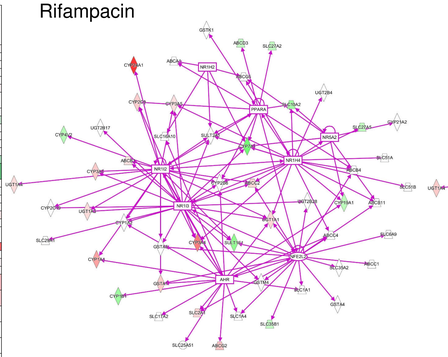
Compound (target receptor)	P<0.05, FC all	P<0.05, 2-fold	P<0.05 , 3-fold	Top regulated genes
Rifampicin (PXR)	2208	645	226	CYP24A1, SEC14L4, C12orf36, HSPB3, OR4F7P, THBD
CITCO (CAR)	2906	822	266	CYP24A1, CYP1A1, CYP26A1, HSPB3, HAO2, GPR37
GW4064 (FXR)	3011	809	288	FGF19, CD3G, STK32C, CTD-2619J13.8, TDO2, ADRA2A
MeBIO (AhR)	4318	1735	735	CYP1A1, CYP1B1, CYP1B1-AS1, P2RY4, GPR37, RP11-98D18.9
T0901317 (LXR)	3006	1417	602	CYP24A1, PLEKHA8, RAP1GAP2, FFO1, TAS2R10, ADRA2A
GW590735 (PPARA)	2576	640	226	PLEKHA8, ZNF527, PDK4, NDUFS1, GCSHP3, FAM127A
L-Sulforaphane (Nrf2)	4417	1560	583	AKR1B10, TRIM16, ZNF527, FAM127A, ADRA2A, HIST1H3D
ML-179 (LRH-1)	4108	1773	638	ZNF527, PLEKHA8, ANK1, GRIN3B, GCSHP3, FAM127A

Drug Metabolism Enzymes (DMEs)

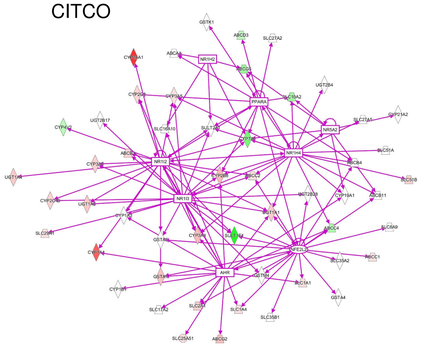
Table 2. Significantly regulated DMEs

Symbol	Entrez Gene Name	Rifampicin	CITCO	GW4064	MeBio	T0901317	GW590735	Sulforaphane	ML 179
ABCA1	ATP binding cassette subfamily A member 1					5.1	1.6		2.3
ABCA3	ATP binding cassette subfamily A member 3	1.4		1.5			1.8		
ABCA5	ATP binding cassette subfamily A member 5	1.5	1.2	2.2		2.1			
ABCA6	ATP binding cassette subfamily A member 6		4.3						
ABCA7	ATP binding cassette subfamily A member 7				-2.4			-2.3	
ABCB1	ATP binding cassette subfamily B member 1	1.7	2.4		2.3	2.7	3.0	6.0	2.6
ABCB10P1	ATP binding cassette subfamily B member 10 pseudogene 3	-1.3		-1.5		-1.7			
ABCB11	ATP binding cassette subfamily B member 11			21.8				1.8	1.9
ABCB4	ATP binding cassette subfamily B member 4			6.7					-3.4
ABCC1	ATP binding cassette subfamily C member 1		1.8	1.7			1.5		2.0
ABCC2	ATP binding cassette subfamily C member 2	2.4	1.8	1.6		3.0		1.9	1.8
ABCC3	ATP binding cassette subfamily C member 4		-3.8	-3.0	-1.6	-2.0	-1.5		
ABCC5	ATP binding cassette subfamily C member 5								2.1
ABCC6	ATP binding cassette subfamily C member 6					-3.7			-3.1
ABCC6P1	ATP binding cassette subfamily C member 6 pseudogene 1		-3.6	-3.7	-5.8	-4.2			-7.1
ABCD1	ATP binding cassette subfamily D member 1					2.5			
ABCD3	ATP binding cassette subfamily D member 3	-1.3	-1.5	-1.8	-1.5	-2.1		-1.2	1.3
ABCD4	ATP binding cassette subfamily D member 4			1.6					
ABCE1	ATP binding cassette subfamily E member 1		2.4					2.9	
ABCG2	ATP binding cassette subfamily G member 2	3.5	3.7	2.3	24.4	2.9			3.5
ABCG5	ATP binding cassette subfamily G member 5		-2.3					-8.1	
CYP19A1	cytochrome P450 family 19 subfamily A member 1	-1.5		-1.5	-1.5		-1.5	-1.5	
CYP1A1	cytochrome P450 family 1 subfamily A member 1	5.2	9.1		4.5	9.6			14.2
CYP1A2	cytochrome P450 family 1 subfamily A member 2				4.7				
CYP1B1	cytochrome P450 family 1 subfamily B member 1	-2.2		-2.0	45.2				2.9
CYP1B1-AS1	CYP1B1 antisense RNA 1	-2.6		-2.2	38.3	-2.9			2.4
CYP21A1P	cytochrome P450 family 21 subfamily A member 1, pseudogene						4.2	5.2	6.8
CYP21A2	cytochrome P450 family 21 subfamily A member 2								5.5
CYP24A1	cytochrome P450 family 24 subfamily A member 1	12.4	11.6			20.6			2.3
CYP26A1	cytochrome P450 family 26 subfamily A member 1	7.0	9.0			8.8			
CYP26C1	cytochrome P450 family 26 subfamily C member 1		1.7	2.1					
CYP27B1	cytochrome P450 family 27 subfamily B member 1				2.1				
CYP2B6	cytochrome P450 family 2 subfamily B member 6		3.8	-4.9		-10		6.3	
CYP2C18	cytochrome P450 family 2 subfamily C member 18								4.9
CYP2C19	cytochrome P450 family 2 subfamily C member 19		2.4					-2.2	3.2
CYP2C9	cytochrome P450 family 2 subfamily C member 9	2.8	2.2	-3.9	-1.8		1.4	3.9	4.0
CYP2E1	cytochrome P450 family 2 subfamily E member 1		-4.4	-4.3		-3.4			-3.0
CYP2R1	cytochrome P450 family 2 subfamily R member 1							-3.2	-3.4
CYP2S1	cytochrome P450 family 2 subfamily S member 1		6.5	7.6		6.9		4.0	3.9
CYP3A4	cytochrome P450 family 3 subfamily A member 4	7.3	3.7						
CYP3A5	cytochrome P450 family 3 subfamily A member 5	1.8	1.4	-1.9	-2.1			-1.8	-3.0
CYP3A7	cytochrome P450 family 3 subfamily A member 7	3.0	2.7	-5.5				-2.6	
CYP4F11	cytochrome P450 family 4 subfamily F member 11							12.0	
CYP4F12	cytochrome P450 family 4 subfamily F member 12	2.0				3.5		2.1	2.0
CYP4F22	cytochrome P450 family 4 subfamily F member 22		-3.9						
CYP4F3	cytochrome P450 family 4 subfamily F member 3	1.7		-1.9				2.3	
CYP4V2	cytochrome P450 family 4 subfamily V member 2	-1.8	-1.6		-1.9	-5.3	-1.6	-3.7	
CYP4X1	cytochrome P450 family 4 subfamily X member 1						-4.4		2.8
CYP7A1	cytochrome P450 family 7 subfamily A member 1	-3.1	-3.1	-3.1		-3.1		-3.0	
GSTA1	glutathione S-transferase alpha 1	2.9	3.5	3.6	-2.4	2.3		3.6	
GSTA4	glutathione S-transferase alpha 4			-4.7					
GSTA5	glutathione S-transferase alpha 5					2.1			
GSTK1	glutathione S-transferase kappa 1								-2.0
GSTM4	glutathione S-transferase mu 4			-3.7					
GSTO2	glutathione S-transferase omega 2	-2.1	-1.5	-2.0		-2.1			
SULT1A2	sulfotransferase family 1A member 2					1.3			
SULT1B1	sulfotransferase family 1B member 1			-5.5		-4.8			
SULT1C2	sulfotransferase family 1C member 2		2.5	-2.1				-1.6	1.5
SULT1E1	sulfotransferase family 1E member 1	-2.8	-5.0	-12	-2.6				
SULT2A1	sulfotransferase family 2A member 1			-3.2			2.0	2.1	-1.5
UGT1A1	UDP glucuronosyltransferase family 1 member A1	2.2	2.8			3.1	1.7	4.5	1.6
UGT1A3	UDP glucuronosyltransferase family 1 member A3	2.0	3.0			3.6	2.4	3.8	1.4
UGT1A4	UDP glucuronosyltransferase family 1 member A4	2.0	2.9			3.6	2.4	3.7	1.3
UGT1A5	UDP glucuronosyltransferase family 1 member A5	2.1	3.1			3.7	2.5	3.9	1.4
UGT2A3	UDP glucuronosyltransferase family 2 member A3			1.5				1.7	
UGT2B11	UDP glucuronosyltransferase family 2 member B11							1.9	
UGT2B15	UDP glucuronosyltransferase family 2 member B15	-1.6	-2.1	1.3	-3.0	-2.1	-1.5		
UGT2B17	UDP glucuronosyltransferase family 2 member B17				-3.4				
UGT2B28	UDP glucuronosyltransferase family 2 member B28							3.3	
UGT2B4	UDP glucuronosyltransferase family 2 member B4			1.5	-1.1		-1.3	2.1	1.3
UGT2B7	UDP glucuronosyltransferase family 2 member B7	-1.3	-1.4	-1.2	-1.3	-2.4	-2.3	-1.8	1.9

Rifampacin



CITCO



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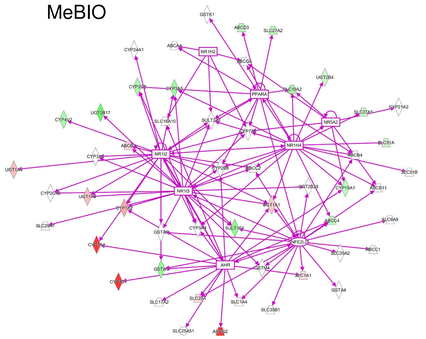
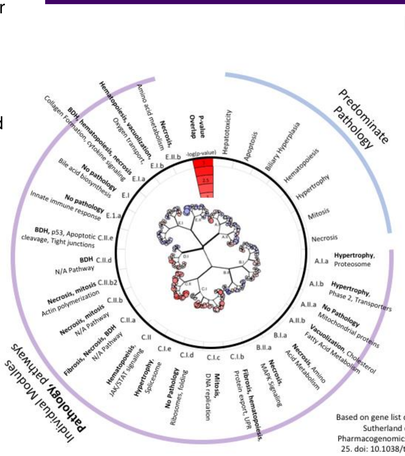


Figure 2: Drug Metabolism Enzyme (DME) Transcription Network. The DMEs shown in Table 1, plus the nuclear receptor targets (PXR, CAR, AhR, FXR, LXR, PPARA, Nrf2, LRH-1) were connected using IPA. Shown are the transcription networks for three prototypical inducers of DME. Gene symbols colored red are increased relative to control, green symbols are decreased relative to control.

Hepatotoxicity



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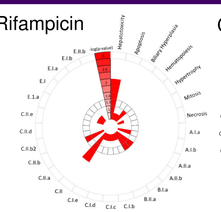

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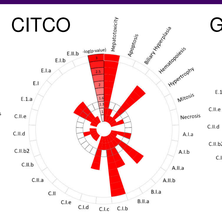

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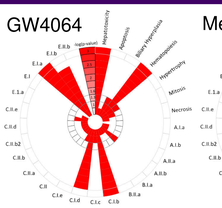

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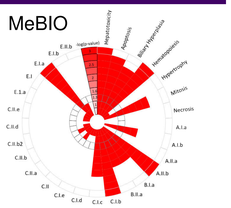

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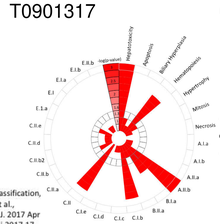

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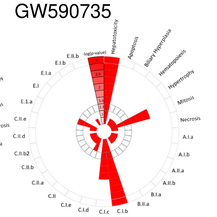
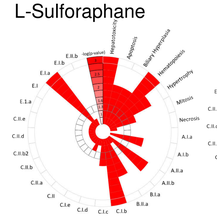


L-Sulforaphane



ML-179

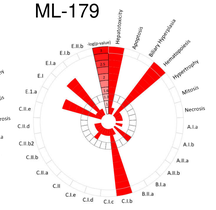


Figure 3: Hepatotoxic module association. Genes associated with hepatotoxicity as described by Sutherland *et al.,* were compared to the list of significantly regulated genes (see Table 1). IBIPlots™ were used to visualize the p–value of overlap for each gene list. -log(p-value)>1.3 is considered a statistically significant overlap.

Results and Conclusions

- upcyte® human hepatocytes are responsive to a variety of prototypical DME inducers including PXR, CAR and AhR agonists.
- In addition to predicted induction of Phase 1 DMEs (CYP3A4, CYP2B6, CYP1A2), changes in Phase 2 (UGT1A1, GSTA1) and Phase 3 transporters (ABCA1) transcripts are evident
- Genes affected by these prototypical inducers is consistent with different aspects of hepatotoxicity. Research is on-going to determine if alterations in specific subsets of genes is predictive of pathology.
- These results demonstrate the utility of upcyte® hepatocytes in assessing drug-induced modulation of DME, and predict potential DDI or drug-induced hepatotoxicity.

Reference

Toxicogenomic module associations with pathogenesis: a network-based approach to understanding drug toxicity. *Sutherland JJ et. al, The Pharmacogenomics Journal 00, 1-14*; 2017.