Induction of ADME-Associated Gene Expression and Activity in the Human Hepatocyte Cell Line - HepaRG

Jodi Morrison[1], Tina Catalano[1], Sophie Pan[1], Julia Izhakova[1], Claire Guyomard[2], Christophe Chesne[2] and Rob Shipman[1], [1]NoAb BioDiscoveries Inc., [2]BIOPREDIC International

Purpose

To investigate the changes in ADME-associated gene expression levels and activity levels in drug-treated HepaRG cells.

Methods

A human hepatocyte cell line [HepaRG] was obtained from BioPredic and cultured according to BioPredic guidelines. The HepaRG cell line was treated with drugs for 24 and 48 hours. Cryo-preserved human hepatocytes were obtained from Invitrogen-CellzDirect and cultured according to Invitrogen-CellzDirect instructions. The human hepatocytes were treated with drugs for 24 and 48 hours. CYP induction was assessed using prototypical inducers followed by either bioanalysis or P450-Glo assays [Fisher-Promega]. Gene expression analysis was performed using the human DTEx™ microarray. Total RNA was isolated from each sample using the RNeasy kit [Qiagen]. Total RNA was amplified using the MessageAmpII aRNA amplification kit [ABI-Ambion]. Labelled cDNA was generated from the amplified RNA using SuprScript II reverse transcriptase [Invitrogen] and CY5-random pentadecamers [Eurofins MWG Operon]. DTEx™ microarrays were hybridised for 18 hours at 55°C, washed and air-dried prior to scanning. Microarray images were acquired with ScanArray [Perkin Elmer] and image analysis was performed with QuantArray [Perkin Elmer]. Data analysis, cluster dendrograms and matrix plots were generated in GeneLinker Gold [Improved Outcomes Software].

Results

1. Cytochrome P450 gene expression and activity levels tended to increase over time for each drug used in both the HepaRG cell line and human primary hepatocytes.

2. Nuclear xenobiotic receptor gene expression levels tended to remain constant or increase slightly over time for each drug used in both the HepaRG cell line and human primary hepatocytes.

3. Drug transporter gene expression levels tended to remain constant or increase slightly over time for each drug used in both the HepaRG cell line and human primary hepatocytes.

4. Transferase gene expression levels tended to remain constant or increase slightly over time for each drug used in both the HepaRG cell line and human primary hepatocytes.

Conclusions

1. Gene expression levels for most of the ADME-associated genes on the human DTEx microarray are inducible in both the HepaRG cell line and human primary hepatocytes.

2. Induction of CYP gene expression correlates with the induction of CYP activity in both the HepaRG cell line and human hepatocytes.

3. The HepaRG cell line is equivalent to human primary hepatocytes in the induction of CYP gene expression and activity.

4. The HepaRG cell line represents an alternative to human primary hepatocytes for ADME-associated gene expression or ADME-associated gene activity induction studies.

5. DTEx™ microarrays can be used to assess both temporal and drug-associated changes in ADME-associated gene expression in the HepaRG cell line and human primary hepatocytes.

Figure 1. Induction of ADME-associated gene expression levels in the HepaRG cell line.
Total RNA isolated from drug-treated HepaRG cells was labelled and hybridised to individual DTEx™ microarrays as described in the *Methods*. Gene expression values represent the normalised, mean value [relative to a stable, constitutively expressed control gene] from at least 3 individual microarray hybridisations [n=12 for each gene].

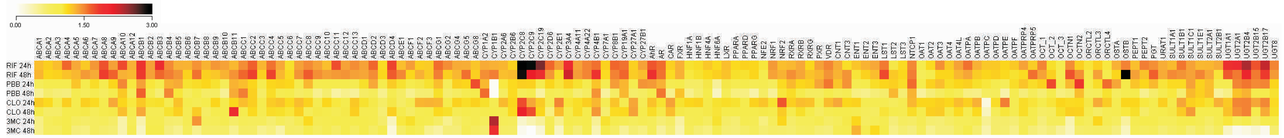


Figure 2. Comparison of gene expression induction levels between drug-treated HepaRG cells and drug-treated hepatocytes.
Total RNA isolated from drug-treated HepaRG cells and six drug-treated hepatocyte samples was labelled and hybridised to individual DTEx™ microarrays as described in the *Methods*. Gene expression values represent the normalised, mean value [relative to a stable, constitutively expressed control gene] from at least 3 individual microarray hybridisations [n=12 for each gene]. Gene expression induction values for ABCB and ABCC drug transporters, Cytochrome P450s and transferases represnet the ratio between drug-treated and vehicle-treated samples and were used to generate histograms using Microsoft Excel.

Figure 2. Comparison of Cytochrome P450 gene expression and activity levels in drug-treated HepaRG cells.
Total RNA isolated from drug-treated HepaRG cells was labelled and hybridised to individual DTEx™ microarrays as described in the *Methods*. Gene expression values represent the normalised, mean value [relative to a stable, constitutively expressed control gene] from at least 3 individual microarray hybridisations [n=12 for each gene]. P450-Glo assays for CYP1A, CYP2C, CYP3A and CYP4A were performed on parallel cultures of drug-treated HepaRG cells. Fold-change or induction values for CYP gene expression and activity were used to generate histograms using Microsoft Excel.

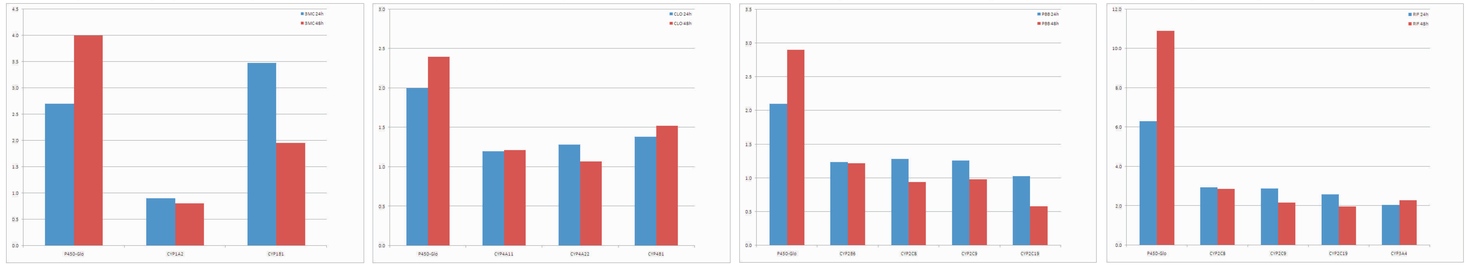


Figure 3. Comparison of CYP activity levels in drug-treated HepaRG cells assessed by P450-Glo assays or bioanalysis.

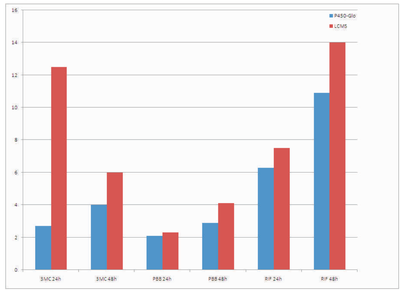


Figure 4. Comparison of CYP3A4 gene expression and CYP3A4 activity levels in drug-treated HepaRG cells assessed by DTEx™ microarray, Q-PCR, P450-Glo assays and bioanalysis.

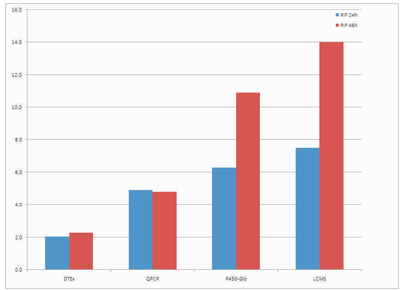
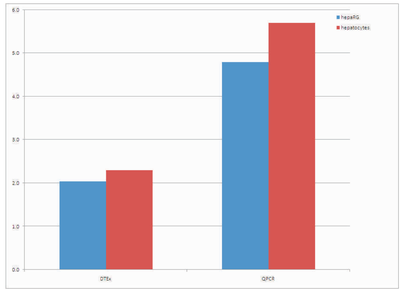


Figure 5. Comparison of CYP3A4 gene expression levels in drug-treated HepaRG cells and drug-treated hepatocytes assessed by DTEx™ microarray and Q-PCR



NoAb BioDiscoveries

BIOPREDIC INTERNATIONAL