Toxicology and Applied Pharmacology 231 (2008) 216–224

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Expression profiling of interindividual variability following xenobiotic exposures in primary human hepatocyte cultures

Katy M.O. Goyak [a], Mary C. Johnson [a], Stephen C. Strom [b], Curtis J. Omiecinski [a,*]

[a] Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA 16802, USA
[b] Department of Pathology, University of Pittsburgh, Pittsburgh, PA 15261, USA

ARTICLE INFO

Article history:
Received 11 January 2008
Revised 14 April 2008
Accepted 23 April 2008
Available online 10 May 2008

Keywords:
DNA microarray
Primary human hepatocytes
Interindividual variability

ABSTRACT

To examine the magnitude of human variability across the entire transcriptome after chemical challenge, we profiled gene expression responses to three different prototypic chemical inducers in primary human hepatocyte cultures from ten independent donors. Correlation between basal expression in any two hepatocyte donors ranged from r^2 values of 0.967 to 0.857, and chemical treatment tended to negatively impact correlation between donors. Including anticipated target genes, 10,812, 8373, and 7847 genes were changed in at least one donor by Aroclor 1254 (A1254), di(2-ethylhexyl) phthalate (DEHP), and phenobarbital (PB), respectively. A subset of these gene targets ($n=41$) were altered with a high level of reproducibility in at least 9 donors, gene responses that correlated well with literature-reported mechanism of action. Filtering responses to the level of gene subsets clarified the biological impact associated with the respective chemical effectors, in lieu of substantial interindividual variation among donor responses. In these respects, the use of hierarchical clustering methods successfully grouped seven of the ten donors into chemical-specific rather than donor-specific clusters. However, at the whole-genome level, the magnitude of conserved gene expression changes among donors was surprisingly small, with fewer than 50% of the gene responses altered by a single chemical conserved in more than one donor. The use of higher level descriptors, such as those defined by the PANTHER classification system, may enable more consistent categorization of gene expression changes across individuals, as increased reproducibility was identified using this method.

Introduction

Primary human hepatocyte cultures are recognized as the most appropriate *in vitro* system with which the *in vivo* liver can be modeled for studies of xenobiotic metabolism and biotransformation (Gomez-Lechon et al., 2003; LeCluyse, 2001). Although advantages of the human primary hepatocyte model, such as elimination of metabolism extrapolation across species and increased predictability potential for idiosyncratic toxicity, are attractive, interindividual variability is problematic, as widespread differences in metabolism among donors and hepatocyte preparations have been cited as a limitation for data reproducibility (Liguori et al., 2005; Slatter et al., 2006). Issues of genetics as well as technical factors such as the surgical, storage and transfer conditions and pathological state of the donor liver tissue may all additionally contribute to variability in response (Baccarani et al., 2003; Fisher et al., 2001; Lloyd et al., 2004).

In studies that examine genome-wide transcriptional responses to chemical treatment, the potentially complicating nature of interindividual variability has led to difficulty in how to construe differences manifested at the transcriptome scale. In an effort to minimize variability across samples, some studies have pooled RNA from multiple donors (Huang et al., 2007; Piton et al., 2005), while others have only used hepatocytes from a single donor (Keum et al., 2006; Ryu et al., 2006). Variability across donors complicates the determination of which genes are regulated, in that some studies report findings in each individual donor (Thum and Borlak, 2007), some follow a stringent criteria such that genes of interest must be changed in all donors (Harris et al., 2004; Liguori et al., 2005; Radaeva et al., 2002), some apply a filtering system so that genes of interest are those changed in a particular number of donors (Richert et al., 2003), while others report average expression across donors (Li et al., 2007; Rae et al., 2001). Overall, a limited number of donors are generally included ($n=2$–6), and surprisingly few genes survive the filtering conditions: Richert et al. parsed a 38 gene dataset based on the >12,000 genes on their array; Thum and Borlak, 44 of 302 possible genes; Liguori et al., 142 of >22,000; Harris et al., 867 of 31,000; and Radaeva et al., 53 of 12,000. The apparent trend emerging from these studies is that, regardless of the nature of the chemical of interest, only small sets of responsive genes are regulated reproducibly by xenobiotic exposures among hepatocytes across individual donors, whereas large subsets of genes are uniquely regulated within

* Corresponding author. Center for Molecular Toxicology and Carcinogenesis, 101 Life Sciences Building, The Pennsylvania State University, University Park, PA 16802, USA. Fax: +1 814 863 1696.
E-mail address: cjo10@psu.edu (C.J. Omiecinski).

0041-008X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2008.04.024

Table 1
Competitive PCR primer sequences

Gene	Primer name	Sequence
CYP1A2	External FP	TGCGGCAGGGCGACGATTTC
	External RP	GCAGGGCAGGGTTAGGCAGGTA
	Internal FP	TGATGGCCAGAGCTTGACGAGCATGTGAGCAAGGAG
	Internal RP	CTCCTTGCTCACATGCTCGTCAAGCTCTGGCCATCA
CYP4A11	External FP	AGGCAAAGTTCGTGTCCAGCTCTA
	External RP	TGGATCACTTGGTCTGTGTGCTGA
	Internal FP	TCCTGGCTCCATGGATTGCTGGACACCATCATGAAG
	Internal RP	CTTCATGATGGTGTCCAGCAATCCATGGAGCCAGGA

individual donors. Despite this trend, there is often little consideration given to the apparent vast magnitude of genes changed in a donor-specific manner.

To broaden these analyses regarding the magnitude of human variability existing in whole-transcriptome responses to chemical challenge, we performed whole-genome expression profiling analyses following treatments with three different prototypic chemical inducers using hepatocytes from ten independent donors, maintained under primary culture techniques that largely preserve highly differentiated character endpoints associated with mature hepatocytes (Olsavsky et al., 2007; Page et al., 2007). Comparisons among donors were performed both at basal conditions and after treatments with Aroclor 1254 (A1254), an environmental contaminant that consists of a mixture of polychlorinated biphenyls (PCBs), di(2-ethylhexyl) phthalate (DEHP), a plasticizer agent belonging to the peroxisome proliferator class of chemicals that activate the rodent PPARα receptor, and phenobarbital (PB), a barbiturate drug used as an anti-seizure agent. These chemicals were selected both for their diverse mechanisms of action and for the wealth of information available on the effects that these chemicals have in a variety of other model systems. Using ten human hepatocyte donors, we report findings in agreement with those from previous studies, that large subsets of genes were regulated according to individual donors, whereas a relatively

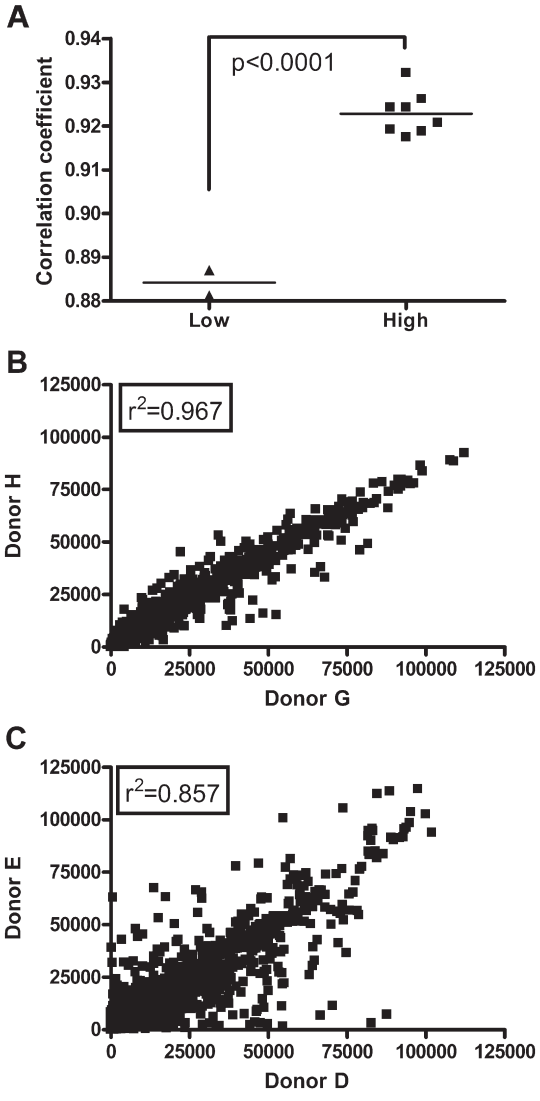

Fig. 1. Overall correlation in basal gene expression between donors is significantly higher in eight of ten donors compared to the remaining two donors. For each donor, expression of all genes on the array was compared to each of the nine other donors in order to obtain a mean correlation coefficient for each donor pair. Significance between high and low correlation donors was determined by a two-tailed *t*-test (A; $p<0.0001$). Examples of the scatter plots of the donor pairs with the highest and lowest correlation are shown in B and C, respectively.

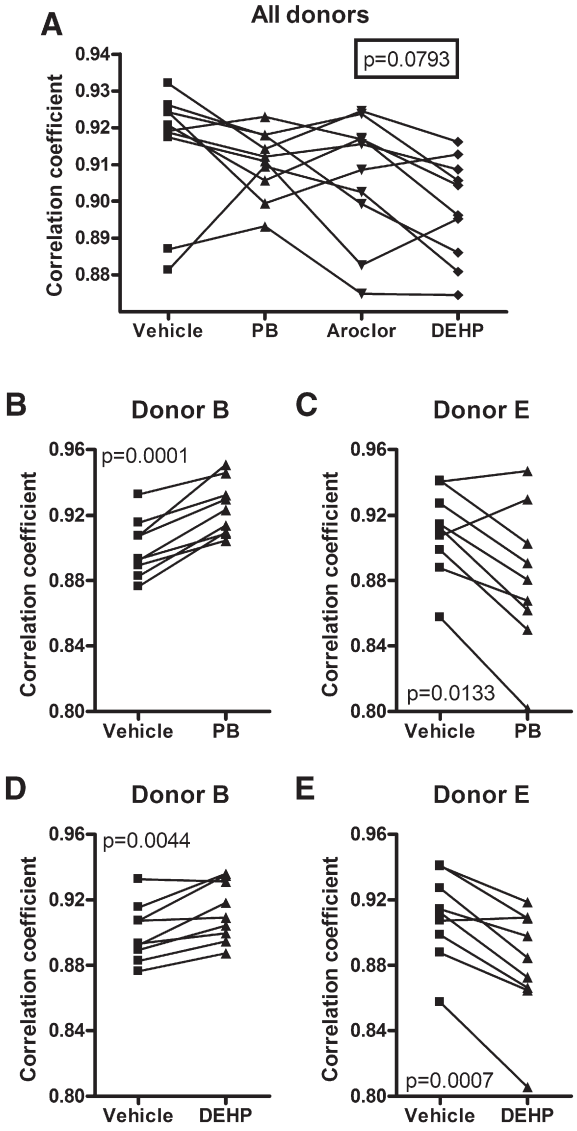

Fig. 2. Effect of chemical treatment on correlation of gene expression between donors is highly donor-specific. For each donor, expression of all genes on the vehicle-, PB-, A1254-, and DEHP-treated arrays was compared to the expression of all genes on the appropriate array corresponding to the nine other donors to obtain mean correlation coefficients between each donor pair at each treatment condition. Significance between treatment conditions was determined using one-way ANOVA in combination with Tukey's multiple-comparison post-test (A; $p<0.01$). Significance between correlation of donor B and all other donors at basal and PB (B) or DEHP (D) conditions and between correlation of donor E and all other donors at basal and PB (C) or DEHP (E) conditions was determined using a two-tailed *t*-test ($p<0.05$).

small set of target genes was regulated consistently in response to individual chemical treatment across donors. However, we show that by using a PANTHER-based functional category analysis rather than a gene-level approach, more consistent identification of transcriptional patterns is detected across donors treated with common agents, patterns that are concordant with known mechanisms of action for the respective agents and indicative of biological themes identified in other model systems.

Methods

Cell culture. Primary human hepatocytes were obtained from the University of Pittsburgh, through the Liver Tissue Cell Distribution System, NIH Contract #N01-DK-7-0004/HHSN267200700004C. Available donor information has been described in detail previously (Olsavsky et al., 2007). Hepatocytes were plated on collagen-coated T25 flasks, and within 48 h a dilute overlay of Matrigel (225 μg/ml; BD Biosciences, San Jose, CA) was added dropwise to the cultures in William's Media E supplemented with 1% penicillin/streptomycin, 10 mM HEPES, 20 μM glutamine, 25 nM dexamethasone, 10 mM insulin, 30 mM linoleic acid, 1 mg/ml BSA, 5 ng/ml selenious acid, and 5 μg/ml transferrin. Selected cultures were treated with either DMSO, 10 μg/ml A1254, 100 μM DEHP, or 500 μM PB for 24 h after either 66 (Donors G and I) or 90 h (Donors A through F, H and J) in culture, depending on the condition of the cells. If not specified otherwise, all culturing materials were purchased from Invitrogen (Carlsbad, CA).

RNA isolation. After a 24 h treatment period, RNA was extracted from the hepatocytes with TRIzol® Reagent (Invitrogen) according to the manufacturer's instructions. Contaminating DNA was removed using DNA-free™ DNase Treatment and Removal Reagents (Ambion, Inc., Austin, TX) following the manufacturer's protocol for rigorous DNase treatment. Concentration of extracted RNA was assessed by UV absorbance at 260 nm using a SmartSpec 3000 Spectrophotometer (BioRad, Hercules, CA).

Quantitative real-time PCR. A High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) was used to reverse transcribe RNA into cDNA, according to recommendations from the manufacturer. TaqMan® Assays-on-Demand™ Gene Expression assays (Applied Biosystems) were set up according to the manufacturer's instructions for a 50-μl reaction volume, subsequently divided into duplicate 25-μl reactions in a 96-well plate, and read in an Applied Biosystems 7300 Real-Time PCR System. Expression of CYP2B6 (Assay ID Hs00167937_m1) was calculated using the ΔΔCt method (Livak and Schmittgen, 2001), using 18S (Assay ID Hs99999901_s1) expression in the vehicle-treated sample from the appropriate donor as the reference standard. ΔΔCt was transformed into fold change using the formula, fold change $=2^{-\Delta\Delta Ct}$.

Competitive PCR. Internal standards were created for CYP1A2 and CYP4A11 by a three step method (Anderson et al., 1997). Initially, an approximately 500 bp fragment was amplified from human hepatocyte cDNA using the external primers listed in Table 1 in a 50-μl reaction containing 0.25 mM dNTPs, 0.5 μM forward and reverse primers, 2.5 U of Taq polymerase, 1× reaction buffer, and 0.5 μg cDNA. Thermal cycling consisted of 30 cycles of a 1 min denaturation step at 95 °C, a 45 s annealing step at 58 °C, and a 2 min extension step at 72 °C. Using these amplicons as a template, two small fragments were amplified following the same reaction and thermocycler conditions; a 5' fragment was created with an overhang specific for the internal FP by using the external FP and internal RP, and a 3' fragment was created with an overhang specific for the internal RP by using the internal FP and external RP. In the final step, the 5' and 3' fragments were annealed due to the presence of these overhangs, using five annealing cycles consisting of a 2 min denaturation step at 94 °C, a 3 min annealing step at 58 °C, and a 4 min extension step at 72 °C and 30 cycles of amplification as described above. After every step, the resulting amplicon was purified using a QIAquick Gel Extraction kit (Qiagen Inc., Valencia, CA), resulting in approximately 300 bp internal standards. CYP1A2 and CYP4A11 expression were determined in each sample using a standard curve consisting of PCR reactions. PCR reaction and thermal cycling conditions were identical to those used to create the internal standard described above, and target gene concentration equaled the concentration of the internal standard when endogenous gene and the internal standard were present in a 1:1 ratio.

Microarray protocol. Forty RNA samples (ten donors at four treatment conditions) were hybridized to Human Genome U133 Plus 2.0 Arrays (Affymetrix, Santa Clara, CA) by Paradigm Array Labs, Inc. (Icoria, Research Triangle Park, NC) as described previously (Olsavsky et al., 2007). Resulting data was analyzed with GeneChip® Operating Software (GCOS; Affymetrix) to determine the signal intensity of each probe set on each array via the MAS5 algorithm, as well as detection (P, present; A, absent; or M, marginal) and change calls (I, increase; MI, marginal increase; NC, no change; MD, marginal decrease; or D, decrease). The signal log ratio of each probe set on the treated arrays was subsequently calculated using the vehicle-treated arrays from the appropriate donor as the baseline. When the signal log ratio was a positive number, signal log ratio was transformed to fold change by the formula, fold increase $=2^{\text{signal log ratio}}$, or, when the signal log ratio was a negative number, fold decrease $=-(2^{(-\text{signal log ratio})})$. Only genes with a change call of I or D ($p<0.002$) were considered significantly changed.

Correlation analysis. All probe sets on the arrays (54,675) were included for the correlation analysis. To obtain an estimate of similarity in global expression at the basal level, a Pearson correlation coefficient was calculated for each donor in comparison to the nine other donors, where the mean value for these nine measurements was called the mean correlation coefficient ± standard deviation (SD). A two-tailed *t*-test was used to determine significance between donors with high and low correlation to the other donors.

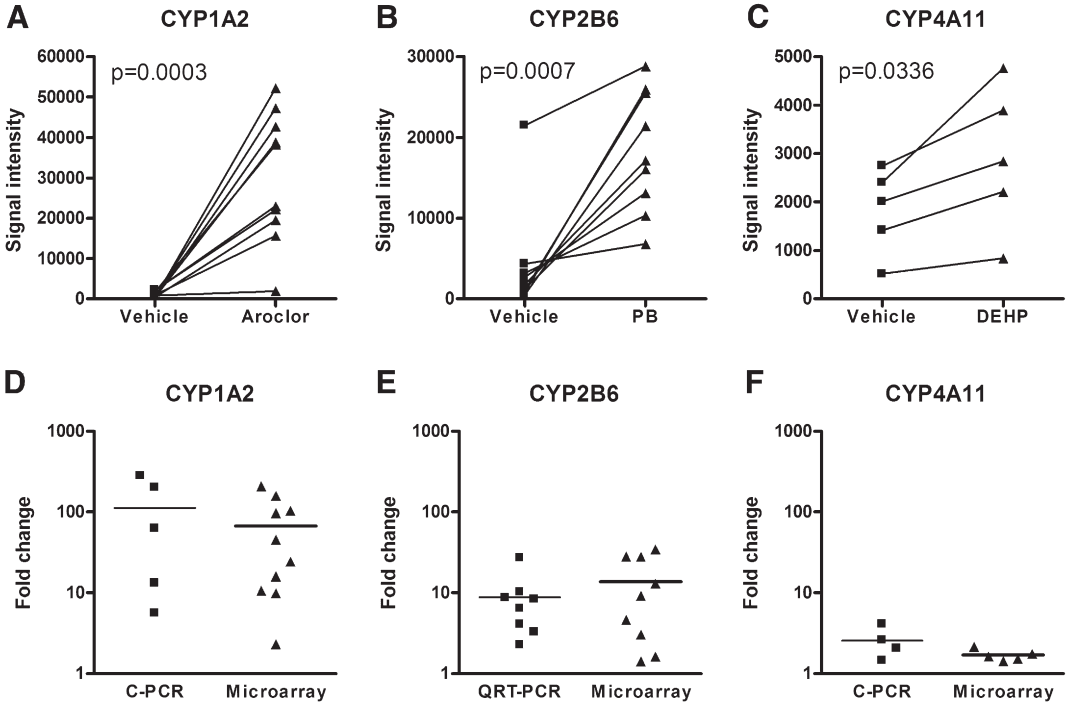

Fig. 3. Hallmark target genes are increased in human hepatocytes treated *in vitro* with A1254 (A, D), PB (B, E), or DEHP (C, F). Panels A–C show signal intensity values for individual arrays representing the ten donors in which CYP1A2 was increased, the nine donors in which CYP2B6 was increased, and the five donors in which CYP4A11 was increased. Significance in expression difference between vehicle and treatment conditions was determined by a paired *t*-test ($p<0.05$). Panels D–F show the fold change of CYP1A2, CYP2B6, and CYP4A11 in the donors mentioned above, as measured by microarray analysis and by either quantitative RT-PCR (QRT-PCR) or competitive PCR (C-PCR).

Mean correlation coefficients were also calculated for each donor treated with chemical in comparison to the other donors receiving a similar treatment. One-way analysis of variance (ANOVA) in combination with Tukey's multiple-comparison post-test was used to determine significance of the difference in correlation between hepatocytes treated with DMSO, A1254, DEHP, and PB. All statistical analyses were performed using GraphPad Prism v4.00 for Windows (GraphPad Software, San Diego, CA).

Hierarchical clustering. To estimate similarity between gene expression in hepatocytes from multiple donors under different treatment conditions, the 40 samples were subjected to hierarchical clustering analysis using the small reproducible response gene set ($n=41$; changed in at least 9 of 10 similarly-treated donors). Clustering was done with the publically-available program PermutMatrix version 1.9.3 (Caraux and Pinloche, 2005) using a Euclidean distance measurement and an average linkage.

Transcriptome-scale reproducibility. To compare reproducibility in expression changes across human hepatocytes from different donors, differentially expressed genes in each sample were subjected to gene ontology over-representation analysis using the PANTHER classification system version 6.0. For each of 241 biological process and 254 molecular function ontology categories, PANTHER calculates the number of genes identified in that category in both the list of differentially regulated genes from a particular sample and the list of genes on the array and compares the two numbers using the binomial test to determine if there are more genes than expected in the differentially regulated list (Thomas et al., 2006). Over-representation is defined as $p<0.05$. To avoid inclusion of both larger ontology categories and the small sub-categories within the same node, larger categories determined as over-represented were excluded when a smaller sub-category within the same node was also identified. One-way ANOVA was used to determine if there was a significant difference in the

Table 2
Probe sets identified as either increased or decreased in at least 9 human hepatocyte donors treated with either A1254, DEHP, or PB

Chemical[a]	Probe set name	Gene title	Gene symbol	Change[b]
A, D, P	206755_at; 217133_x_at	Cytochrome P450, family 2, subfamily B, polypeptide 6	*CYP2B6*	Inc
A, D, P	206754_s_at	Cytochrome P450, family 2, subfamily B, polypeptide 7 pseudogene 1	*CYP2B7P1*	Inc
A, D, P	218245_at	Tsukushin	*TSKU*	Inc
A, D	229477_at; *1553583_a_at*	Thyroid hormone responsive (SPOT14 homolog, rat)	*THRSP*	Dec
A, P	1494_f_at; 207244_x_at 211295_x_at 214320_x_at 207718_x_at	Cytochrome P450, family 2, subfamily A, polypeptide 6	*CYP2A6*	Inc
A, P	208147_s_at	Cytochrome P450, family 2, subfamily C, polypeptide 8	*CYP2C8*	Inc
A, P	205939_at	Cytochrome P450, family 3, subfamily A, polypeptide 7	*CYP3A7*	Inc
A, P	205342_s_at; 211470_s_at	Sulfotransferase family, cytosolic, 1C, member 1	*SULT1C1*	Inc
A	222592_s_at	Acyl-CoA synthetase long-chain family member 5	*ACSL5*	Inc
A	201491_at	AHA1, activator of heat shock 90kDa protein ATPase homolog 1 (yeast)	*AHSA1*	Inc
A	206561_s_at	Aldo-keto reductase family 1, member B10 (aldose reductase)	*AKR1B10*	Inc
A	205633_s_at	Aminolevulinate, delta-, synthase 1	*ALAS1*	Inc
A	224461_s_at	Apoptosis-inducing factor, mitochondrion-associated, 2	*AIFM2*	Inc
A	205749_at	Cytochrome P450, family 1, subfamily A, polypeptide 1	*CYP1A1*	Inc
A	207608_x_at; 207609_s_at	Cytochrome P450, family 1, subfamily A, polypeptide 2	*CYP1A2*	Inc
A	216025_x_at	Cytochrome P450, family 2, subfamily C, polypeptide 19 /// cytochrome P450, family 2, subfamily C, polypeptide 9	*CYP2C19 /// CYP2C9*	Inc
A	214421_x_at	Cytochrome P450, family 2, subfamily C, polypeptide 9	*CYP2C9*	Inc
A	202581_at	Heat shock 70kDa protein 1B	*HSPA1B*	Inc
A	210338_s_at	Heat shock 70kDa protein 8	*HSPA8*	Inc
A	204679_at	Potassium channel, subfamily K, member 1	*KCNK1*	Inc
A	227055_at	Methyltransferase like 7B	*METTL7B*	Inc
A	201468_s_at	NAD(P)H dehydrogenase, quinone 1	*NQO1*	Inc
A	203814_s_at	NAD(P)H dehydrogenase, quinone 2	*NQO2*	Inc
A	225252_at	Sulfiredoxin 1 homolog (*S. cerevisiae*)	*SRXN1*	Inc
A	212665_at	TCDD-inducible poly(ADP-ribose) polymerase	*TIPARP*	Inc
A	230830_at	Transmembrane protein 177	*TMEM177*	Inc
A	208596_s_at; 215125_s_at	UDP glucuronosyltransferase 1 family, polypeptide A10 /// UDP glucuronosyltransferase 1 family, polypeptide A8 /// UDP glucuronosyltransferase 1 family, polypeptide A7 /// UDP glucuronosyltransferase 1 family, polypeptide A6	*UGT1A10 /// UGT1A8 /// UGT1A7 /// UGT1A6 /// UGT1A5 /// UGT1A9 /// UGT1A4 /// UGT1A1 /// UGT1A3*	Inc
A	204719_at	ATP-binding cassette, subfamily A (ABC1), member 8	*ABCA8*	Dec
A	219803_at	Angiopoietin-like 3	*ANGPTL3*	Dec
A	210143_at	Annexin A10	*ANXA10*	Dec
A	1552389_at; 1552390_a_at	Chromosome 8 open reading frame 47	*C8orf47*	Dec
A	205776_at	Flavin containing monooxygenase 5	*FMO5*	Dec
A	205498_at	Growth hormone receptor	*GHR*	Dec
A	225424_at	Glycerol-3-phosphate acyltransferase, mitochondrial	*GPAM*	Dec
A	219313_at	GRAM domain containing 1C	*GRAMD1C*	Dec
A	203914_x_at; 211548_s_at	Hydroxyprostaglandin dehydrogenase 15-(NAD)	*HPGD*	Dec
A	1558603_at	Plasminogen-like B2 /// plasminogen-like B1	*PLGLB2 /// PLGLB1*	Dec
A	220786_s_at	Solute carrier family 38, member 4	*SLC38A4*	Dec
A	202687_s_at; 202688_at	Tumor necrosis factor (ligand) superfamily, member 10	*TNFSF10*	Dec
A	201009_s_at; 201010_s_at	Thioredoxin interacting protein	*TXNIP*	Dec
P	239492_at	SEC14-like 4 (*S. cerevisiae*)	*SEC14L4*	Inc

When more than one probe set is listed as changed by more than one chemical, all probe sets were changed by each chemical listed unless underlined or italicized. Underlining signifies that that probe set was changed only by A1254, and italicizing signifies that that probe set was changed only by DEHP.

[a] A, Aroclor 1254; D, DEHP; P, PB.

[b] Inc, Increased; Dec, Decreased; Change call was determined by GCOS; $p<0.002$.

numbers of probe sets, genes and over-represented gene ontology categories found in n donors, where $n=2$ through 9 ($p<0.05$). For the purposes of ontology-based hierarchical clustering, p-values were transformed first by the formula $y=1/y$ in order to label the most significantly over-represented categories with the largest values. To reduce the range of the resulting values, a subsequent transformation was done following either the formula $y=\log(y)$ when a category was associated with increased genes or $y=-1*\log(y)$ when associated with decreased genes.

Results

Correlation between hepatocyte donors

To assess the magnitude of variation between donors across the entire transcriptome, our initial analysis consisted of obtaining correlation coefficients as a global measure of similarity between donors. A comparison of mean correlation coefficients revealed a high level of correlation between eight of the ten donors, with mean correlation values ranging from $r^2=0.917\pm0.022$ to $r^2=0.932\pm0.028$ (standard deviation; Fig. 1A). The remaining two donors, D and E, had significantly lower correlation with hepatocytes from the other donors, with mean correlation coefficients of 0.887 ± 0.044 and 0.881 ± 0.043, respectively ($p<0.0001$). The highest correlation between any two donors was $r^2=0.967$ between donors G and H (Fig. 1B) and the lowest was $r^2=0.857$ between donors D and E (Fig. 1C).

In comparison to results at basal conditions, chemical treatment tended to impart a negative impact on correlation between donors, although not significantly in all cases. When all ten donors were taken into account, there was no significant difference in mean correlation between hepatocytes treated with vehicle, PB, A1254 or DEHP (Fig. 2A; $p=0.0793$). However, if hepatocytes from the two donors with poor basal correlation relative to the other donors (donors D and E) were excluded from the analysis, a significant difference in mean correlations based on treatment was detected ($p=0.0042$), with the most significant difference noted between vehicle- and DEHP-treated hepatocytes ($p<0.01$). The decrease noted in correlation between basal and induced conditions in hepatocytes suggests that responses to chemical challenge are more variable across individuals than are the basal profiles of global gene expression. However, the extent of interindividual variability to chemical treatment in this system did fluctuate considerably from donor to donor. For example, treatment with PB and DEHP actually increased the correlation coefficient of global gene expression in donor B when compared to basal expression of all other donors (Figs. 2B and D; basal range: $r^2=0.876$ to $r^2=0.933$; PB range: $r^2=0.904$ to $r^2=0.951$; DEHP range: $r^2=0.887$ to $r^2=0.936$), whereas, exposures to these same chemicals decreased the correlation coefficient of global gene expression in donor E when compared to basal expression in all other donors (Figs. 2C and E; basal range: $r^2=0.857$ to $r^2=0.941$; PB range: $r^2=0.802$ to $r^2=0.947$; DEHP range: $r^2=0.805$ to $r^2=0.919$).

Target gene validation

Transcript levels of classically responsive target genes for each chemical agent were increased in hepatocytes from most donors, providing evidence that our culturing methodology is appropriate for measures of expression changes after chemical challenge (Figs. 3A–C; $p<0.05$). For example, A1254 induced one of its target genes, CYP1A2, most consistently, as induction was apparent in all ten donors, ranging from 2.3-fold in donor F to 208-fold in donor J (Fig. 3A), and PB increased a target gene, CYP2B6, in nine of ten donors, ranging from 1.4- (donor F) to 34-fold (donor H) (Fig. 3B). The induction of CYP2B6 in these hepatocyte donors is consistent with results generated via a different algorithm, PLIER, that were reported in a previous study comparing gene expression in human liver and in primary hepatocytes (Olsavsky et al., 2007). Additionally, these induction responses are in agreement with previous results from a comprehensive study utilizing hepatocytes from multiple donors ($n=62$) maintained under conditions comparable to those described here (Madan et al., 2003). The final chemical used in this study, DEHP, did not produce consistent induction patterns of its anticipated target, CYP4A11, as CYP4A11 levels were increased in hepatocytes from only five of the ten donors, ranging from 1.4-fold in donor A to 2.1-fold in donor D (Fig. 3C).

Both competitive PCR and quantitative RT-PCR techniques were used to confirm the array-detected increases in target gene expression. Using the former method, CYP1A2 was increased in A1254-treated hepatocytes from five donors, ranging from a 6-fold induction in donor A to a 283-fold induction in donor H (Fig. 3D), and CYP2B6 was increased in eight donors, ranging from 2.3-fold in donor E to 27-fold in donor J (Fig. 3E). Competitive PCR confirmed that CYP4A11 was inconsistently

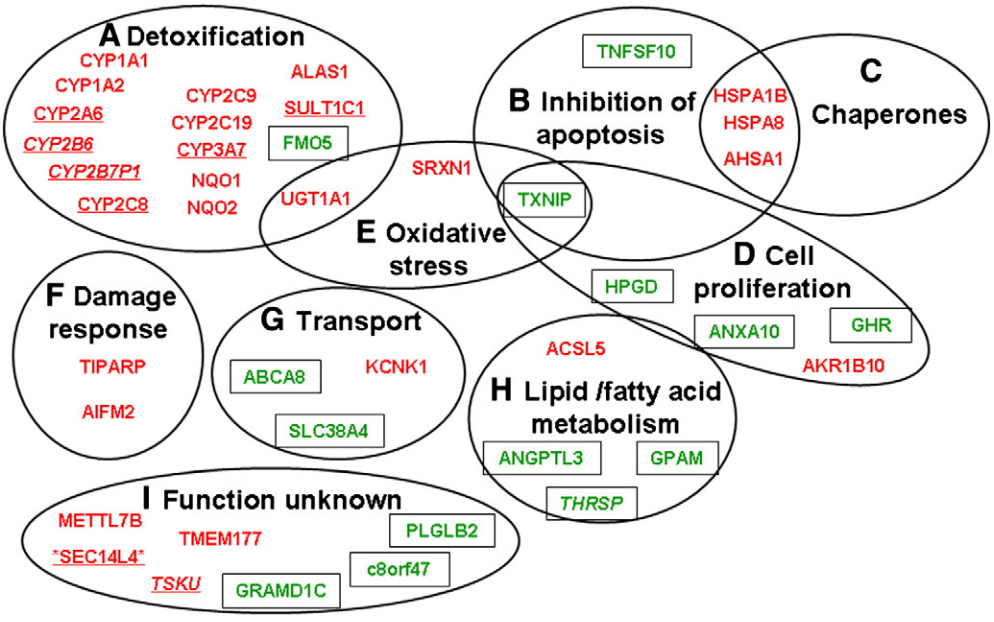

Fig. 4. Target genes that were increased or decreased in at least nine hepatocyte donors by a single chemical reflect known functional effects of chemical treatment *in vivo*. The 40 genes regulated by A1254, 4 by DEHP (italicized), and 8 by PB (underlined) were categorized according to function. A single gene, SEC14L4, was regulated by PB but not Aroclor (indicated by *). Genes decreased by chemical treatment are enclosed in a box; otherwise genes are increased.

increased in the hepatocytes, as this gene was induced in four of five donors, ranging from 1.5-fold in donor H to 4.1-fold in donor B (Fig. 3F). Overall, no significant difference was found in fold change measured by the two methods for any of the target genes, supporting the validity of our microarray analysis method for the quantification of mRNA levels.

Reproducible response genes

To address conserved transcriptional responses, we identified a set of genes that were consistently regulated by each chemical among hepatocytes from nearly all donors. Although 10,812, 8373, and 7847 genes were changed in at least one donor by A1254, DEHP, and PB, only 41 genes met the stringent criteria of being increased or decreased in at least 9 of 10 donors treated by a single chemical (Table 2). Of the three chemicals, A1254 clearly had the most distinctive, reproducible effect on transcription, as 40 genes (54 probe sets) were similarly changed in nearly all donors. Seven of the eight genes consistently regulated by PB were also regulated by A1254, which was anticipated, since A1254 acts as a broad mixed-function oxidase inducer, activating both PB-response genes and dioxin-response genes (Safe, 1993). The final chemical in our study, DEHP, consistently regulated only four genes, all of which were also regulated by A1254.

Functional analysis of reproducible response genes

All of the identified response genes sorted to at least one of eight functional categories. Although PB and DEHP consistently changed relatively few genes in common across donors, the genes that were identified as responders to these agents agreed with anticipated functional categories. For example, nearly all of the genes consistently regulated by PB in different donors were genes encoding enzymes active in biotransformation/xenobiotic metabolism, including CYP2A6, CYP2B6, CYP2C8, CYP3A7, and SULT1C1 (Fig. 4A). DEHP also regulated biotransformation genes, CYP2B6 and a likely pseudogene, CYP2B7P1. Activation of THRSP, another DEHP-response gene, is more consistent with known mechanisms of peroxisome proliferators, due to its role in lipid metabolism, a biological process regulated by the activation of PPARα. The functions of the remaining PB- and DEHP-response genes, SEC14L4 and TSKU, have not yet been fully described.

A1254 exhibited the most reproducible transcriptional effects across donors. Consistent with its role as a mixed-function oxidase effector, A1254 regulated, with the exception of SEC14L4, all of the PB-response genes, as well as known dioxin-response genes also involved in biotransformation, including CYP1A1, CYP1A2, NQO1, NQO2, and UGT1A1 (Fig. 4A). In our analyses, A1254 was found to inhibit pro-apoptotic genes (Fig. 4B) and suggested tumor suppressors (Fig. 4D) and to increase expression of chaperones (Fig. 4C), antioxidants (Fig. 4E), and damage response (Fig. 4F) genes. Genes within the transport (Fig. 4G) and lipid and fatty acid metabolism (Fig. 4H) categories were also regulated consistently by A1254.

Hierarchical clustering of reproducible genes

When all 40 samples were subjected to hierarchical clustering, the reproducible gene set was sufficient to overcome the influence of interindividual variation for seven of the ten donors. When considering only these seven donors, chemical-specific clusters were apparent, as the majority of samples within each of the three treatment groups clustered according to treatment, despite the evident influence of interindividual variability (Fig. 5). The most distinctive results were apparent for the A1254-treated samples, as all 7 samples in this cluster were treated with this agent. DEHP and PB treatments resulted in less distinctive profiles within this sample set, as only 6 of 9 and 4 of 5 samples within the "DEHP" and "PB" clusters, respectively, were treated with the chemical of interest. Interestingly, genes within this reproducible gene set were often grouped nearest to genes that share similar functions. Specifically, genes were grouped together based on their roles in biotransformation/xenobiotic metabolism (Figs. 4A; 5, purple line), stress response (Figs. 4C, E, and F; 5, blue line), lipid and fatty acid metabolism (Figs. 4H; 5, orange line) and tumor suppressor properties (Figs. 4D; 5, green line).

Transcriptome-scale reproducibility

Although the identification of reproducible target genes is supportive of the use of human hepatocytes as a model system that reflects known mechanism of action despite the source of donor, our goal was ultimately to determine the magnitude of conserved gene changes in response to chemical treatment across donors. Overall, at a whole-transcriptome scale, a surprising lack of conserved regulation of genes was found (Fig. 6A), even for A1254 treatment, which elicits a more distinctive gene expression profile than either of the other two chemicals. In A1254-treated hepatocytes, only 11 out of 14,102 total changed genes were increased or decreased in all ten donors. In other

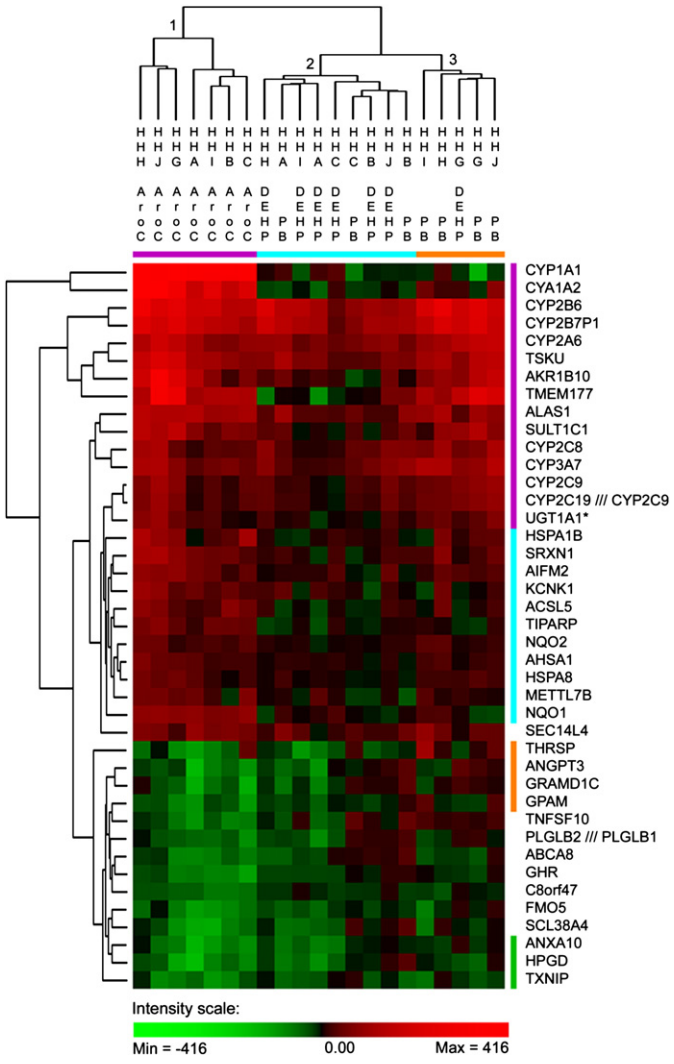

Fig. 5. Hierarchical clustering groups hepatocyte donors by chemical treatment when the dataset consists of genes regulated similarly by one chemical in at least 9 donors. Numbered clusters and horizontal lines indicate chemical-specific clusters: 1, purple line, A1254; 2, blue line, DEHP; and 3, orange line, PB. Vertical lines represent groups of genes with similar function: purple line, biotransformation; blue line, stress response; orange line, lipid and fatty acid metabolism; and green line, tumor suppressor activity. Dataset consisted of 41 genes. Intensity scale represents fold change. * indicates that this probe set cannot distinguish between various UGT1A isoforms. Clustering was done using a Euclidean distance measure and average linkage.

words, less than a tenth of a percent of all changed genes were always changed. Using less stringent criteria, 1470 genes were changed consistently in at least five donors; still, approximately only 10 percent of all changed genes. Further, only about half of the genes were changed in at least two donors, leaving half of all genes identified as changed found to be done so in only a single donor. This pattern is similar for the other two chemicals used in this study, although the percentage of genes regulated in multiple donors was lower in DEHP- and PB-treated hepatocytes than for A1254-treated hepatocytes at each number of donors (data not shown).

Because this lack of reproducibility may have important implications for studies that rely on a small number of donors to verify responses in a limited number of genes, we examined the use of higher level descriptors to attempt to increase the level of reproducibility in changes across donors. A total of 292, 341, and 324 distinct ontology categories were over-represented among transcriptional changes induced by A1254, DEHP, and PB, respectively, in at least one donor. When considering the transcriptional changes induced in at least five, six, seven or eight donors, a significantly higher level of reproducibility among individual hepatocyte donors was obtained when over-represented gene ontology categories were compared rather than probe sets or genes ($p<0.05$; Fig. 6B).

Providing further support for ontology-based analyses, chemical-specific clusters similar to those presented for the reproducible gene set were apparent when ontology categories consistently regulated across donors were used as the basis for clustering (Fig. 7). Similar to the gene-level clustering results, A1254-treated samples from the six included donors grouped together, while there was less distinction between DEHP- and PB-treated samples. Not surprisingly, many of the ontology categories found to be over-represented consistently among A1254-treated donors were closely related to the functional themes identified by the reproducible gene set, such as 'lipid and fatty acid transport', 'fatty acid metabolism', and 'chaperone'.

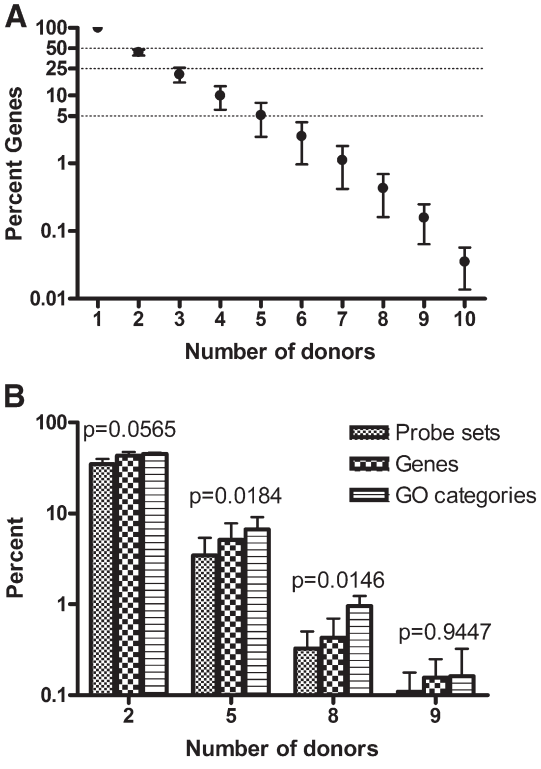

Fig. 6. Globally, transcriptional changes in response to chemical treatment are not highly conserved between individual human hepatocyte donors. The percentage of total changed genes found in at least n number of donors ($n=1$ through 10) is shown in Panel A. Error bars reflect results from each of the three different chemicals used to treat human hepatocytes. The mean number of probe sets, genes, and over-represented GO categories identified in n number of donors is shown in Panel B. Results from $n=2$, 5, 8, and 9 donors are shown. Significant difference in the means is considered as $p<0.05$ (one-way ANOVA).

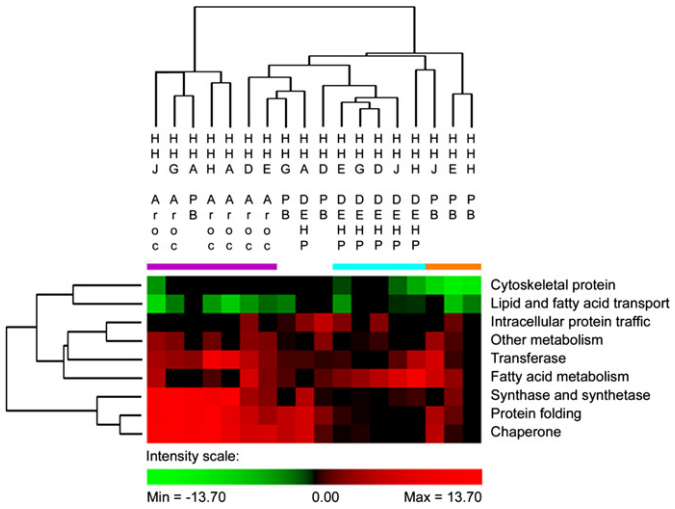

Fig. 7. Hierarchical clustering groups hepatocyte donors by chemical treatment when the dataset is based on over-represented gene ontology categories. Significance of over-representation was considered as $p<0.05$. p-values were transformed by $y=1/y$ followed by $y=\log(y)$ if a category was over-represented among increased genes or $y=-1*\log(y)$ if over-represented among decreased genes. Intensity scale represents the transformed values, where max and min values are the most significantly over-represented categories among the increased and decreased genes, respectively. Horizontal lines indicate chemical-specific groups: purple line, A1254; blue line, DEHP; and orange line, PB.

Discussion

In this study, global transcriptional responses were examined in primary hepatocyte cultures from ten independent donors. Basal gene expression profiles as well as responses to three prototypic chemical inducers were assessed, with the intent of evaluating both trends in reproducibility across donors and mechanisms that may be conserved among donors, unique for each chemical class. Overall, the data obtained revealed that only small sets of genes were regulated consistently across donors, whereas relatively large gene sets were regulated uniquely according to individual. Further, the degree of interindividual variability across donors was substantial, such that, of the three chemicals studied, only A1254 affected a large enough number of consistently regulated gene responses to permit analysis of conserved biological mechanisms. PB exposures resulted in surprisingly few conserved gene responses across individuals, although PB did evoke consistent induction of the known target gene, CYP2B6, as well as other anticipated response genes. These hallmark PB responses engendered confidence in the validity of the overall donor response profile associated with this chemical (Figs. 3B and E; 4). On the other hand DEHP less consistently induced its anticipated target gene, CYP4A11 across individuals (Figs. 3C and F), and the reproducible response genes that DEHP did perturb in hepatocytes were not all obviously consistent with reported DEHP mechanisms of action. These latter data suggest that human hepatocytes are not particularly impacted by DEHP exposures under the conditions evaluated. A similar chemical-specific phenomenon was reported by Liguori et al. (2005), in which only one of six quinolone agents regulated a set of genes as defined by significant change in hepatocytes from all four donors treated with that specific quinolone but not with the remaining quinolone agents.

In the case of A1254, where the number of conserved gene sets of responsive genes was large enough to permit functional analysis, results were consistent with literature-reported functional changes

ascribed to A1254, or PCB exposures in general. In agreement with the hypothesis that dioxin-like tumor promoters, such as A1254, promote carcinogenesis through inhibition of apoptosis (Park and Matsumura, 2006), two proapoptotic genes were identified as decreased by A1254, TNFSF10 (Yamanaka et al., 2000) and TXNIP (Wang et al., 2006). Similarly, chaperones, particularly Hsp70 family members, also contribute to inhibition of apoptosis (Bivik et al., 2007). Our study revealed that two Hsp70 family members, HSPA1B and HSPA8, as well as a cofactor for Hsp90, AHSA1, were increased in nine of ten A1254-treated donors. Also relating to a role as a modulator of carcinogenesis, A1254 exposures in the hepatocyte model resulted in reduced expression of three suggested tumor suppressor genes, TXNIP (Yoshida et al., 2005), HPGD (Yan et al., 2004), and ANXA10 (Liu et al., 2002), and in increased expression of a potential oncogene, AKR1B10 (Fukumoto et al., 2005).

Possibly related to tumor promotion, PCBs have been implicated in increasing reactive oxygen species (ROS) (Saito, 1990; Srinivasan et al., 2001). In concert with this scheme, multiple chaperones, often increased under stressful conditions, are consistently up-regulated in the human hepatocyte donors, as were two antioxidants, SRXN1 (Findlay et al., 2005) and UGT1A1 (Yueh and Tukey, 2007). Although enhanced cellular ROS damages DNA, proteins, and lipids, DNA appears particularly sensitive to damage following PCB exposures, as PCBs have been reported to increase DNA strand breaks and adduct formation (Srinivasan et al., 2001). Similarly, our study identified two up-regulated genes involved in the DNA damage response. TIPARP is a DNA-binding protein activated by ROS-generated DNA strand breaks, including those produced by PCB exposure, that appears to play a role in the decision of the cell to undergo apoptosis or necrosis (Cole and Perez-Polo, 2004). One mode of TIPARP signaling may involve the initiation of apoptosis by mediating the translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus, where it initiates DNA fragmentation (Yu et al., 2002). Interestingly, our results identified AIFM2, a closely related homolog to AIF that triggers a functionally similar caspase-independent apoptotic pathway (Wu et al., 2002), as consistently up-regulated by A1254. The goal of our functional analysis was not to force every identified response gene into a single role, but to create a view of possible conserved mechanisms based on the genes changed upon chemical treatment and direction of those changes. To this end, we found that conserved sets of genes exhibiting change across individuals sorted well into known functional categories defined by PANTHER, therefore supporting the use of the primary human hepatocyte culture system as a biologically relevant model for capturing responses occurring *in vivo*.

Despite these considerations, interindividual variability remains problematic for toxicological assessments. Throughout the course of our study, hepatocytes from two of the ten human donors were repeatedly marked as outliers, as correlation analysis identified donors D and E as exhibiting significantly lower correlation to all other samples than did the remaining donors (Fig. 1A). Additionally, Donor E appeared to regulate a particularly unique subset of genes in response to PB and DEHP, as the correlation of gene expression in this donor to expression in all other donors decreased when treated with PB and DEHP when compared to basal level expression patterns (Figs. 2C and E). Donor D was the only donor to not significantly increase the PB target gene, CYP2B6 (Fig. 3B). Consequently, donors D and E, as well as F, were omitted from the hierarchical clustering analysis, since the PB- and DEHP-treated samples from these donors clustered uniquely by donor, rather than by treatment (Fig. 5). Although the factors contributing to this outlier status cannot be defined, the finding that some hepatocytes are of sufficiently sub-optimal quality as to mark these donors as significantly different from other donors has implications for studies in which genetic material is pooled from multiple donors.

Further, studies that compare gene expression even in a limited number of donors is problematic, as it has been reported that mean and variance data are misleading due to the fact that gene expression, particularly of those genes associated with metabolic processes, does not follow a normal distribution across donors (Slatter et al., 2006). Our study provides further evidence of this contention, in that the standard deviations in fold induction of both CYP1A2 and CYP2B6 are nearly as large as the "average" fold induction itself (data not shown). Our results are consistent with other reports suggesting that the use of biological theme as a comparator, rather than reliance on probe set or individual gene responses, increases reproducibility in results across laboratories and microarray platforms (Bammler et al., 2005; Currie et al., 2005; Guo et al., 2006). The analyses presented here further support the concept that the use of higher level descriptors, such as those engendered in the PANTHER classification used here is a valuable approach, as these methods enable focus on biological processes and functions, and provide a viable method for managing the otherwise large degree of interindividual variability data that are encountered when focusing on individual gene descriptors as the primary analysis tool. Expanding on previous ontology structures, the PANTHER system queries extended database content and was built using a combination of scientific curation and computational algorithms that sort genes into groups based on the function of the gene product (Thomas et al., 2003). These attributes provide the system with an enhanced ability to extract biologically meaningful information.

Despite the many factors contributing to extensive variability in basal and inducer-related gene expression profiles across hepatocytes from individual donors, the data reported here, and in our earlier studies (Olsavsky et al., 2007; Page et al., 2007), validate the use of primary human hepatocytes, cultured under highly defined conditions, as a valuable and biologically relevant *in vitro* model system that largely reproduces conserved profiles of gene expression changes that are comparable to results obtained with *in vivo* model systems.

Acknowledgments

This research was funded by a Toxicogenomics Research Consortium grant from the National Institutes of Environmental Health Sciences, U19 ES11387 and a grant from the National Institute of General Medical Sciences, GM66411.

References

Anderson, K.M., Cheung, P.H., Kelly, M.D., 1997. Rapid generation of homologous internal standards and evaluation of data for quantitation of messenger RNA by competitive polymerase chain reaction. J. Pharmacol. Toxicol. Methods 38, 133–140.

Baccarani, U., Sanna, A., Cariani, A., Sainz-Barriga, M., Adani, G.L., Zambito, A.M., Piccolo, G., Risaliti, A., Nanni-Costa, A., Ridolfi, L., Scalamogna, M., Bresadola, F., Donini, A., 2003. Isolation of human hepatocytes from livers rejected for liver transplantation on a national basis: results of a 2-year experience. Liver Transplant. 9, 506–512.

Bammler, T., Beyer, R.P., Bhattacharya, S., Boorman, G.A., Boyles, A., Bradford, B.U., Bumgarner, R.E., Bushel, P.R., Chaturvedi, K., Choi, D., Cunningham, M.L., Deng, S., Dressman, H.K., Fannin, R.D., Farin, F.M., Freedman, J.H., Fry, R.C., Harper, A., Humble, M.C., Hurban, P., Kavanagh, T.J., Kaufmann, W.K., Kerr, K.F., Jing, L., Lapidus, J.A., Lasarev, M.R., Li, J., Li, Y.J., Lobenhofer, E.K., Lu, X., Malek, R.L., Milton, S., Nagalla, S.R., O'malley, J.P., Palmer, V.S., Pattee, P., Paules, R.S., Perou, C.M., Phillips, K., Qin, L.X., Qiu, Y., Quigley, S.D., Rodland, M., Rusyn, I., Samson, L.D., Schwartz, D.A., Shi, Y., Shin, J.L., Sieber, S.O., Slifer, S., Speer, M.C., Spencer, P.S., Sproles, D.I., Swenberg, J.A., Suk, W.A., Sullivan, R.C., Tian, R., Tennant, R.W., Todd, S.A., Tucker, C.J., Van Houten, B., Weis, B.K., Xuan, S., Zarbl, H., 2005. Standardizing global gene expression analysis between laboratories and across platforms. Nat. Methods 2, 351–356.

Bivik, C., Rosdahl, I., Ollinger, K., 2007. Hsp70 protects against UVB induced apoptosis by preventing release of cathepsins and cytochrome *c* in human melanocytes. Carcinogenesis 28, 537–544.

Caraux, G., Pinloche, S., 2005. PermutMatrix: a graphical environment to arrange gene expression profiles in optimal linear order. Bioinformatics 21, 1280–1281.

Cole, K., Perez-Polo, J.R., 2004. Neuronal trauma model: in search of Thanatos. Int. J. Dev. Neurosci. 22, 485–496.

Currie, R.A., Orphanides, G., Moggs, J.G., 2005. Mapping molecular responses to xenoestrogens through Gene Ontology and pathway analysis of toxicogenomic data. Reprod. Toxicol. 20, 433–440.

Findlay, V.J., Tapiero, H., Townsend, D.M., 2005. Sulfiredoxin: a potential therapeutic agent? Biomed. Pharmacother. 59, 374–379.

Fisher, R.L., Gandolfi, A.J., Brendel, K., 2001. Human liver quality is a dominant factor in the outcome of *in vitro* studies. Cell Biol. Toxicol. 17, 179–189.

Fukumoto, S., Yamauchi, N., Moriguchi, H., Hippo, Y., Watanabe, A., Shibahara, J., Taniguchi, H., Ishikawa, S., Ito, H., Yamamoto, S., Iwanari, H., Hironaka, M., Ishikawa, Y., Niki, T., Sohara, Y., Kodama, T., Nishimura, M., Fukayama, M., Dosaka-Akita, H., Aburatani, H., 2005. Overexpression of the aldo-keto reductase family protein AKR1B10 is highly correlated with smokers' non-small cell lung carcinomas. Clin. Cancer Res. 11, 1776–1785.

Gomez-Lechon, M.J., Donato, M.T., Castell, J.V., Jover, R., 2003. Human hepatocytes as a tool for studying toxicity and drug metabolism. Curr. Drug Metab. 4, 292–312.

Guo, L., Lobenhofer, E.K., Wang, C., Shippy, R., Harris, S.C., Zhang, L., Mei, N., Chen, T., Herman, D., Goodsaid, F.M., Hurban, P., Phillips, K.L., Xu, J., Deng, X., Sun, Y.A., Tong, W., Dragan, Y.P., Shi, L., 2006. Rat toxicogenomic study reveals analytical consistency across microarray platforms. Nat. Biotechnol. 24, 1162–1169.

Harris, A.J., Dial, S.L., Casciano, D.A., 2004. Comparison of basal gene expression profiles and effects of hepatocarcinogens on gene expression in cultured primary human hepatocytes and HepG2 cells. Mutat. Res. 549, 79–99.

Huang, J., Hao, P., Zhang, Y.L., Deng, F.X., Deng, Q., Hong, Y., Wang, X.W., Wang, Y., Li, T.T., Zhang, X.G., Li, Y.X., Yang, P.Y., Wang, H.Y., Han, Z.G., 2007. Discovering multiple transcripts of human hepatocytes using massively parallel signature sequencing (MPSS). BMC Genomics 8, 207.

Keum, Y.S., Han, Y.H., Liew, C., Kim, J.H., Xu, C., Yuan, X., Shakarjian, M.P., Chong, S., Kong, A.N., 2006. Induction of heme oxygenase-1 (HO-1) and NAD[P]H: quinone oxidoreductase 1 (NQO1) by a phenolic antioxidant, butylated hydroxyanisole (BHA) and its metabolite, tert-butylhydroquinone (tBHQ) in primary-cultured human and rat hepatocytes. Pharm. Res. 23, 2586–2594.

LeCluyse, E.L., 2001. Human hepatocyte culture systems for the *in vitro* evaluation of cytochrome P450 expression and regulation. Eur. J. Pharm. Sci. 13, 343–368.

Li, Y., Mezei, O., Shay, N.F., 2007. Human and murine hepatic sterol-12-alpha-hydroxylase and other xenobiotic metabolism mRNA are upregulated by soy isoflavones. J. Nutr. 137, 1705–1712.

Liguori, M.J., Anderson, M.G., Bukofzer, S., McKim, J., Pregenzer, J.F., Retief, J., Spear, B.B., Waring, J.F., 2005. Microarray analysis in human hepatocytes suggests a mechanism for hepatotoxicity induced by trovafloxacin. Hepatology 41, 177–186.

Liu, S.H., Lin, C.Y., Peng, S.Y., Jeng, Y.M., Pan, H.W., Lai, P.L., Liu, C.L., Hsu, H.C., 2002. Down-regulation of annexin A10 in hepatocellular carcinoma is associated with vascular invasion, early recurrence, and poor prognosis in synergy with p53 mutation. Am. J. Pathol. 160, 1831–1837.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods 25, 402–408.

Lloyd, T.D., Orr, S., Patel, R., Crees, G., Chavda, S., Vadyar, H., Berry, D.P., Sherlock, D., Dennison, A.R., 2004. Effect of patient, operative and isolation factors on subsequent yield and viability of human hepatocytes for research use. Cell Tissue Bank 5, 81–87.

Madan, A., Graham, R.A., Carroll, K.M., Mudra, D.R., Burton, L.A., Krueger, L.A., Downey, A.D., Czerwinski, M., Forster, J., Ribadeneira, M.D., Gan, L.S., LeCluyse, E.L., Zech, K., Robertson Jr., P., Koch, P., Antonian, L., Wagner, G., Yu, L., Parkinson, A., 2003. Effects of prototypical microsomal enzyme inducers on cytochrome P450 expression in cultured human hepatocytes. Drug Metab. Dispos. 31, 421–431.

Olsavsky, K.M., Page, J.L., Johnson, M.C., Zarbl, H., Strom, S.C., Omiecinski, C.J., 2007. Gene expression profiling and differentiation assessment in primary human hepatocyte cultures, established hepatoma cell lines, and human liver tissues. Toxicol. Appl. Pharmacol. 222, 42–56.

Page, J.L., Johnson, M.C., Olsavsky, K.M., Strom, S.C., Zarbl, H., Omiecinski, C.J., 2007. Gene expression profiling of extracellular matrix as an effector of human hepatocyte phenotype in primary cell culture. Toxicol. Sci. 97, 384–397.

Park, S., Matsumura, F., 2006. Characterization of anti-apoptotic action of TCDD as a defensive cellular stress response reaction against the cell damaging action of ultra-violet irradiation in an immortalized normal human mammary epithelial cell line, MCF10A. Toxicology 217, 139–146.

Piton, A., Le Ferrec, E., Langouet, S., Rauch, C., Petit, E., Le Goff, F., Guillouzo, A., Morel, F., 2005. Oltipraz regulates different categories of genes relevant to chemoprevention in human hepatocytes. Carcinogenesis 26, 343–351.

Radaeva, S., Jaruga, B., Hong, F., Kim, W.H., Fan, S., Cai, H., Strom, S., Liu, Y., El Assal, O., Gao, B., 2002. Interferon-alpha activates multiple STAT signals and down-regulates c-Met in primary human hepatocytes. Gastroenterology 122, 1020–1034.

Rae, J.M., Johnson, M.D., Lippman, M.E., Flockhart, D.A., 2001. Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays. J. Pharmacol. Exp. Ther. 299, 849–857.

Richert, L., Lamboley, C., Viollon-Abadie, C., Grass, P., Hartmann, N., Laurent, S., Heyd, B., Mantion, G., Chibout, S.D., Staedtler, F., 2003. Effects of clofibric acid on mRNA expression profiles in primary cultures of rat, mouse and human hepatocytes. Toxicol. Appl. Pharmacol. 191, 130–146.

Ryu, H.M., Park, S.G., Yea, S.S., Jang, W.H., Yang, Y.I., Jung, G., 2006. Gene expression analysis of primary normal human hepatocytes infected with human hepatitis B virus. World J. Gastroenterol. 12, 4986–4995.

Safe, S., 1993. Toxicology, structure-function relationship, and human and environmental health impacts of polychlorinated biphenyls: progress and problems. Environ. Health Perspect. 100, 259–268.

Saito, M., 1990. Polychlorinated biphenyls-induced lipid peroxidation as measured by thiobarbituric acid-reactive substances in liver subcellular fractions of rats. Biochim. Biophys. Acta 1046, 301–308.

Slatter, J.G., Templeton, I.E., Castle, J.C., Kulkarni, A., Rushmore, T.H., Richards, K., He, Y., Dai, X., Cheng, O.J., Caguyong, M., Ulrich, R.G., 2006. Compendium of gene expression profiles comprising a baseline model of the human liver drug metabolism transcriptome. Xenobiotica 36, 938–962.

Srinivasan, A., Lehmler, H.J., Robertson, L.W., Ludewig, G., 2001. Production of DNA strand breaks *in vitro* and reactive oxygen species *in vitro* and in HL-60 cells by PCB metabolites. Toxicol. Sci. 60, 92–102.

Thomas, P.D., Kejariwal, A., Campbell, M.J., Mi, H., Diemer, K., Guo, N., Ladunga, I., Ulitsky-Lazareva, B., Muruganujan, A., Rabkin, S., Vandergriff, J.A., Doremieux, O., 2003. PANTHER: a browsable database of gene products organized by biological function, using curated protein family and subfamily classification. Nucleic Acids Res. 31, 334–341.

Thomas, P.D., Kejariwal, A., Guo, N., Mi, H., Campbell, M.J., Muruganujan, A., Lazareva-Ulitsky, B., 2006. Applications for protein sequence-function evolution data: mRNA/protein expression analysis and coding SNP scoring tools. Nucleic Acids Res. 34, W645–W650.

Thum, T., Borlak, J., 2007. Detection of early signals of hepatotoxicity by gene expression profiling studies with cultures of metabolically competent human hepatocytes. Arch. Toxicol.

Wang, Z., Rong, Y.P., Malone, M.H., Davis, M.C., Zhong, F., Distelhorst, C.W., 2006. Thioredoxin-interacting protein (txnip) is a glucocorticoid-regulated primary response gene involved in mediating glucocorticoid-induced apoptosis. Oncogene 25, 1903–1913.

Wu, M., Xu, L.G., Li, X., Zhai, Z., Shu, H.B., 2002. AMID, an apoptosis-inducing factor-homologous mitochondrion-associated protein, induces caspase-independent apoptosis. J. Biol. Chem. 277, 25617–25623.

Yamanaka, T., Shiraki, K., Sugimoto, K., Ito, T., Fujikawa, K., Ito, M., Takase, K., Moriyama, M., Nakano, T., Suzuki, A., 2000. Chemotherapeutic agents augment TRAIL-induced apoptosis in human hepatocellular carcinoma cell lines. Hepatology 32, 482–490.

Yan, M., Rerko, R.M., Platzer, P., Dawson, D., Willis, J., Tong, M., Lawrence, E., Lutterbaugh, J., Lu, S., Willson, J.K., Luo, G., Hensold, J., Tai, H.H., Wilson, K., Markowitz, S.D., 2004. 15-Hydroxyprostaglandin dehydrogenase, a COX-2 oncogene antagonist, is a TGF-beta-induced suppressor of human gastrointestinal cancers. Proc. Natl. Acad. Sci. U. S. A. 101, 17468–17473.

Yoshida, T., Nakamura, H., Masutani, H., Yodoi, J., 2005. The involvement of thioredoxin and thioredoxin binding protein-2 on cellular proliferation and aging process. Ann. N.Y. Acad. Sci. 1055, 1–12.

Yu, S.W., Wang, H., Poitras, M.F., Coombs, C., Bowers, W.J., Federoff, H.J., Poirier, G.G., Dawson, T.M., Dawson, V.L., 2002. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science 297, 259–263.

Yueh, M.F., Tukey, R.H., 2007. Nrf2-Keap1 signaling pathway regulates human UGT1A1 expression *in vitro* and in transgenic UGT1 mice. J. Biol. Chem. 282, 8749–8758.