Comparing *In Vitro* and *In Vivo* Genomic Profiles Specific to Liver Toxicity Induced by Thioacetamide

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Abstract – As it is needed to assay possible feasibility of extrapolation between *in vivo* and *in vitro* systems and to develop a new *in vitro* method for toxicity testing, we investigated global gene expression from both animal and cell line treated with thioacetamide (TAA) and compared between *in vivo* and *in vitro* genomic profiles. For *in vivo* study, mice were orally treated with TAA and sacrificed at 6 and 24 h. For *in vitro* study, TAA was administered to a mouse hepatic cell line, BNL CL.2 and sampling was carried out at 6 and 24 h. Hepatotoxicity was assessed by analyzing hepatic enzymes and histopathological examination (*in vivo*) or lactate dehydrogenase (LDH) assay and morphological examination (*in vitro*). Global gene expression was assessed using microarray. In high dose TAA-treated group, there was centrilobular necrosis (*in vivo*) and cellular toxicity with an elevation of LDH (*in vitro*) at 24 h. Statistical analysis of global gene expression identified that there were similar numbers of altered genes found between *in vivo* and *in vitro* at each time points. Pathway analysis identified several common pathways existed between *in vivo* and *in vitro* system such as glutathione metabolism, bile acid biosynthesis, nitrogen metabolism, butanoate metabolism for hepatotoxicity caused by TAA. Our results suggest it may be feasible to develop toxicogenomics biomarkers by comparing *in vivo* and *in vitro* genomic profiles specific to TAA for application to prediction of liver toxicity.

Keywords □ thioacetamide, *in vivo*, *in vitro*, toxicogenomics, hepatotoxicity, microarray

INTRODUCTION

It is needed to develop new technologies to evaluate them more quickly and accurately not only for safety concern but also for cost-effectiveness, owing to occurrence of many new foods, drugs and chemicals. Among them, toxicogenomic approaches employing microarray technology allows to investigate expressions of thousands of genes affected simultaneously in biological experiment such as chemical-induced toxicity test, and may serve as a valuable tool to evaluate new food and drugs. Toxicogenomics combines transcript, protein and metabolite profiling with conventional toxicology (Waters and Fostel 2004), and its approach should help not only to discover highly sensitive and predictive biomarkers for toxicity but also to understand molecular cellular mechanism of toxicity, including the fields of hepatotoxicity,

nephrotoxicity and genotoxicity (Pennie *et al.* 2004).

It is expected that it will compensate evaluation of toxicity measured by conventional ways, and it seems that toxicogenomics will provide powerful tool that may show gene and protein changes earlier, even at treatment levels below the limits of detection of traditional measures of toxicity, and it may be possible to apply toxicogenomics data into regulatory decision making (Boverhof and Zacharewski 2006) after biologic validation of toxicogenomics-based test methods (Corvi *et al.* 2006) and reviewing and analyzing toxicogenomics data (Leighton *et al.* 2006).

Thioacetamide (TAA) induced liver cell damage, fibrosis and/or cirrhosis, associated with increase of oxidative stress and activation of hepatic stellate cells (Kang *et al.* 2005; Li *et al.* 2002; Muller *et al.* 1988; Porter *et al.* 1979). It was metabolized via CYP450, and induced hepatocellular toxicities including hepatic cell necrosis (James *et al.* 2003; Porter *et al.* 1979).

Even though it seems that compounds with similar toxic mechanisms produce similar changes in gene expression *in vivo* and *in vitro* system (Waring *et al.* 2001a), there was also

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discrepancy for gene expressions between animal and cell line system (Perkins *et al.* 2006). Therefore it may be worthwhile to compare transcriptional responses in livers of animals and hepatocyte cells line after exposure to chemicals to determine how faithfully *in vitro* model system reflects *in vivo* responses using microarray.

In this study, we investigated global gene expression from both mouse liver and mouse hepatic cell line treated with TAA to gain a better understanding of molecular mechanisms. And we further compared between *in vivo* and *in vitro* profiles, and assessed the feasibility of the extrapolation between two systems to compare of *in vitro* gene expression profiles to *in vivo* system.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin-streptomycin were purchased from Invitrogen (Carlsbad, CA). CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay kit was obtained from Promega Co. (Madison, WI) while cytotoxicity detection kit was from Roche Molecular Biochemicals (Indianapolis, IN). Mouse Genome Survey Microarray gene chips were supplied by Applied Biosystems (Foster City, CA). This microarray platform has 33,012 probes, which are 60-mers, lie mostly within 1,500 base pairs of the 3' end of the source transcript. TAA was obtained from Sigma (St. Louis, MO).

Animals and chemicals treatment

In vivo experiment, male 5-week old ICR mice were supplied by the Department of Laboratory Animal Resources, the National Institute of Toxicological Research, Food and Drug Administration, Seoul, Korea. The animals were housed in polycarbonated cages with hardwood chips in a room with 12/12 h light/dark cycles and controlled humidity and temperature. They were allowed free access to pellet chow during the experiment. All procedures were approved by the Institutional Animal Care and Use Committee of National Institute of Toxicological Research.

Six-week-old mice (n=32) were randomly allocated to four groups as follow: groups 1 as control, group 2 as vehicle (saline), groups 3 and 4 as TAA low and high dose treatment (20, 200 mg/kg, respectively, dissolved in saline, i.p.). Mice were sacrificed at 6 and 24 h after the treatment.

Initial and final body weights were measured. At the end of

the experiment, all animals were fasted overnight and euthanized by exsanguination under ether anesthesia. Blood was taken from the abdominal aorta, and serum biochemistry was performed for the following parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) using Prestige 24i (Tokyo Boeki Medical System, Japan).

At necropsy, half of livers were fixed in 10% phosphate-buffered formalin, and routinely processed for embedding in paraffin, and staining of 4 μ m sections with hematoxylin and eosin for histopathological examination. And the remaining samples from all the animals were snap-frozen in liquid nitrogen for RNA extraction and subsequent analysis.

Cell line, cell culture and chemicals treatment

In vitro experiment, murine embryonic normal hepatic cell line, BNL CL.2 cells (ATCC TIB-73) was purchased from American Type Culture Collection (Manassas, VA). The cells were cultured in DMEM medium supplemented with 100 units of penicillin-streptomycin, 2 mM L-glutamine, and 10% FBS at 37°C in a 5% CO₂ atmosphere.

TAA was dissolved in dimethyl sulfoxide (DMSO) and were freshly diluted in culture media for each experiment. Vehicle concentrations were less than 0.5% in all experiments. At 6 or 24 h after treatment, cells were harvested for RNA extraction.

Cytotoxicity assay

Cytotoxicity was assessed using Cytotoxicity Detection Kit according to manufacture's instruction (Roche, Germany). In brief, BNL CL.2 (2X10⁵ cells/ml) was treated with TAA (0-10000 μ M) or 0.5% DMSO (as vehicle control) and was incubated for 6 or 24 h, and supernatant was mixed with dye solution and catalyst for 30 min. The absorbance at 490 nm of the solution was measured using a spectrophotometer (Benchmark PlusTM, Bio-Rad Laboratories, Hercules, CA).

RNA isolation and Microarray gene expression profiling

Total RNAs were extracted for gene expression analysis using the RNeasy Mini kit (Qiagene, Valencia, CA). The yield of RNA was determined spectrophotometrically by measuring the optical density at 260 nm. Total mRNA was converted into double-stranded cDNA using a Chemiluminescent RT-IVT labeling Kit (Applied Biosystems) and an oligo(dT)₂₄ primer. Digoxigenin-labeled cRNA was generated from the double-stranded cDNA using a Chemiluminescent RT-IVT labeling kit. Labeled cRNA was purified using cRNA purification kit

(Applied Biosystems). The quality and quantity of RNA and cRNA was evaluated using spectrometry and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

Each cRNA sample was fragmented by incubation for 30 min at 60°C in fragmentation buffer. The Mouse Genome Survey Microarray gene chip (Applied Biosystems) was hybridized with the fragmented digoxigenin-labeled cRNAs at 55°C for 16 h and then washed. After washing procedure, the chemiluminescent detection, image acquisition and analysis were performed using the Chemiluminescent Detection Kit and Applied Biosystems 1700 Chemiluminescent Microarray Analyzer following the manufacturer's protocols. The chemiluminescent signals from the scanned images were quantified, corrected for background, and spot- and spatially-normalized using the 1700 Chemiluminescent Microarray Analyzer (Applied Biosystems). Microarray analyses were performed for each RNA sample (3 samples/ group).

Data analysis

For comparison of gene expression profiles, control, vehicle control, 20 and 200 mg/kg TAA (*in vivo*), and control, 0 (vehicle control), and 1000 and 10000 μ M TAA (*in vitro*) were tested. Two time points for cell harvest of 6 and 24 h after the treatment were chosen to investigate the time-relating pattern in animals or cells exposed to TAA. Data were analyzed from three independent experiments with samples at 6 and 24 h after TAA treatment in mice and cell line.

Gene expression data from microarray were input to GenPlex (Istech Co. Ltd., Korea). The signal log ratio values, which represent ratios of hybridization signals between control and treated cells, were calculated after quantile normalization. Significantly altered genes induced by TAA were extracted by One-Way ANOVA ($p < 0.05$).

K-means clustering was performed to identify genes that have a similar differential expression profile across conditions using AVADIS (Strand Life Sciences, Redwood city, CA). Pathway analyses were conducted using DAVID (The Database for Annotation, Visualization and Integrated Discovery) (<http://david.abcc.ncifcrf.gov/>) and the PANTHER (Protein ANalysis THrough Evolutionary Relationships) (<http://www.pantherdb.org/>). The extracted genes were categorized based on location, cellular components, and reported or suggested biochemical, biologic, and molecular functions.

Statistical analysis

Statistical analyses for body weights, liver weights and

serum biochemical parameters were performed with the Tukey-Kramer method using the JMP program (SAS Institute, Cary, NC). For all comparisons, probability values less than 5% ($p < 0.05$) were considered to be statistically significant.

RESULTS

Body and liver weight in mice

During experiment, there was no death, and there were no differences of body weights and absolute and relative liver weights between control and TAA-treated groups (data not shown).

Histopathological examination of liver in mice

In high dose TAA treatment group, there were severe centrilobular necrosis and hemorrhage at 24 h after treatment (Fig. 1). However, there was no histopathological change at 6 h in low and high dose and at 24 h in low dose treatment of TAA (data not shown).

Fig. 1. Histopathological findings of livers of mice. Mice were sacrificed at 24 h after the treatment as following treatment. A: control; B: TAA 200 mg/kg

Fig. 2. Changes of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) in serum of TAA-treated mice. Blood samples are collected at hours 6 and 24 after administration. Data are expressed as mean $\pm$ SD from three or four mice. **,*** Significantly different from control group ($p<0.05$, $p<0.01$, respectively)

Fig. 3. LDH assay in TAA-treated mouse cell line. Samples are collected at 24 h after TAA treatment. Data are expressed as mean $\pm$ SD.

Serum biochemistry in mice

Data for serum biochemistry are shown in Fig. 2. There were significant increases of AST, ALT and LDH in high dose treatment of TAA at 24 h ($p<0.01$). There were no alterations of these parameters at 6 h after TAA treatment (data not shown).

Cytotoxicity and morphological examination on hepatic cells, BNL CL.2

In vitro toxicity test showed that TAA treatment induced cellular toxicity over 5000 μ M (TAA) at 24 h after TAA treatment (Fig. 3). We set a high dose as 10000 μ M as apparent cellular toxicity-inducing dose, and one-tenth dose as low dose as not showing apparent cellular toxicity. Morphological

Fig. 4. Morphological aspect of mouse hepatic cell line, BNL CL.2. Cells were treated with following materials and examined at 24 h after the treatment. A: control; B: vehicle; C: TAA 10000 μ M.

examination of chemical-treated cell lines, cellular toxicity was evident at 24 h after treatment (Fig. 4).

Microarray analysis of gene expression patterns in BNL CL.2

Global gene expression showing a significance over 0.05 by One-Way ANOVA showed that there were 677 differentially expressed genes (*in vivo*) and 1973 genes (*in vitro*) at 6 h and 1583 (*in vivo*) and 2217 (*in vitro*) at 24 h by TAA treatment, and there were commonly altered genes of 213 and 516 at 6 and 24 h, respectively, between *in vivo* and *in vitro*. By time-dependent analysis, numbers of commonly up- or down-regu-

Table I. Commonly altered genes between *in vivo* and *in vitro* caused by TAA treatment

Time & state	Gene Symbol	Gene Title	mRNA Accession No	<i>In vivo</i> (mg/kg)			<i>In vitro</i> (μM)		
				Vehicle	20	200	DMSO	100	1000
6 hr									
Up-regulated	Egr1	early growth response 1	NM_007913	0.4711065	-0.533091	6.0834074	1.075264	2.1965866	3.3196907
	Cyr61	cysteine rich protein 61	NM_010516	-0.363033	1.6927719	4.7499895	0.608985	0.3927651	1.6033726
	Brd2	bromodomain containing 2	NM_001025387; NM_010238	0.0157681	0.6906452	2.5885897	0.3498917	1.0804014	1.7204275
	Bhlhb2	basic helix-loop-helix domain containing, class B2	NM_011498	0.217659	0.1298513	2.455925	0.2699194	0.9475002	1.4352331
	Eaf1	ELL associated factor 1	NM_028932	0.3048029	1.0060234	2.0910797	0.2132931	0.9494963	1.0152721
	Vldlr	very low density lipoprotein receptor	NM_013703	-0.385152	-0.233289	1.4303284	0.6748552	1.2519007	1.0504208
	Sirt1	sirtuin 1 ((silent mating type information regulation 2, homolog) 1 (S. cerevisiae)	XM_975350; NM_019812	0.0789547	0.6221294	1.4199762	0.3536739	1.4564581	1.257844
	Gna13	guanine nucleotide binding protein, alpha 13	NM_010303	-0.066282	0.3500481	1.3125257	0.4579668	0.7073698	1.1964998
	Sgk	serum/glucocorticoid regulated kinase	NM_011361	-0.091127	0.0876694	1.24471	0.6271582	1.4130945	1.5765734
	Obfc2a	oligonucleotide/oligosaccharide-binding fold containing 2A	NM_028696	0.7143288	0.7684889	1.1428185	0.4273176	2.5966148	2.8336105
Down-regualted	Tpd52l1	tumor protein D52-like 1	NM_009413	-0.504838	-0.836243	-1.222363	-0.401925	-0.82837	-1.27316
24 h									
Up-regulated	Egr1	early growth response 1	NM_007913	3.990447	5.692029	1.529541	1.1671934	2.3108444	2.7765312
	Dnajc5	DnaJ (Hsp40) homolog, subfamily C, member 5	XM_990872; NM_016775	-0.002933	0.0024271	1.6610451	0.6511717	2.6408472	2.3038568
	Gsr;LOC630729	glutathione reductase 1; similar to Glutathione reductase, mitochondrial precursor (GR) (GRase)	NM_010344; XM_904332	-0.52001	-0.299269	2.245201	-0.379162	1.6973562	1.9085684
	Ccl2	chemokine (C-C motif) ligand 2	NM_011333	0.5813847	1.6273861	2.719284	-0.042987	1.8100033	1.8199978
	Tpd52	tumor protein D52	NM_001025261; NM_001025262; NM_001025263; NM_001025264; NM_009412	-0.828274	-0.261851	3.9038076	-0.02171	1.3979578	1.467206
	LOC632337;Odc1	similar to Ornithine decarboxylase (ODC);ornithine decarboxylase, structural 1	NM_013614	0.1163139	0.5939121	1.4938564	-0.640778	0.8450623	1.3277531
	Gpiap1	GPI-anchored membrane protein 1	NM_016739	-0.694784	-0.414566	1.1523285	-0.474688	1.5330992	1.2523527
	Rdh10	retinol dehydrogenase 10 (all-trans)	NM_133832	0.543747	0.6505814	3.9891615	-0.488431	0.8506956	1.2116423
	Crip1	cysteine-rich protein 1 (intestinal)	NM_007763	0.5737352	0.4232197	5.8757067	0.3290539	0.9495201	1.0674477
	AW146242	expressed sequence AW146242	NM_146168	-0.064575	0.4170895	3.7042484	0.3326998	1.0475569	1.0639467
	Axl	AXL receptor tyrosine kinase	NM_009465	0.2459059	0.3151503	5.077778	0.2044811	1.0880184	1.0308094
	Down-regualted	Suc1g1	succinate-CoA ligase, GDP-forming, alpha subunit	NM_019879	0.1262913	0.1956635	-1.545529	0.3596268	-0.486233
	Gpx1	glutathione peroxidase 1	NM_008160	-0.111317	0.1314945	-2.807049	0.5258656	-0.134722	-1.137102
	Elov16	ELOVL family member 6, elongation of long chain fatty acids (yeast)	NM_130450	-1.044017	-1.017226	-1.29104	-1.785263	-1.468534	-1.200234
	Aqp8	aquaporin 8	NM_007474	-0.718494	-0.933998	-4.214431	-1.863714	-2.123119	-1.854801

lated genes between *in vivo* and *in vitro* caused by TAA treatment were ten and one at 6 h and eleven and four at 24 h, respectively (Table I).

Hierarchical analysis showed that there were similar patterns between *in vivo* and *in vitro* at 6 h after treatment. However, at 24 h, there was a cluster showing alteration at low dose as well as high dose treatment in *in vitro* system (Fig. 5). *k*-means clustering analysis showed there were some clusters showing alteration at 6 and 24 h after high dose as well as low dose treatment of TAA (Fig. 6). These clusters contained several genes such as mitogen activated protein kinase 14, tumor necrosis factor, alpha-induced protein 3, dead box polypeptide 1, thioredoxin reductase 1, cyclin G2. PCA analysis showed groups of TAA treatment were located at a different position compared to control group (Fig. 7). High dose treatment group represented a clear distinction at 6 h both *in vivo* and *in vitro* system. On the while, in *in vitro* system at 24 h, low dose and high dose groups showed closed location. Pathway analysis for differentially expressed genes by DAVID Bioinformatic Resources (NIAID/NIH) identified that there were 11 (*in vivo*) and 22 (*in vitro*) pathways by TAA treatment, and identified several common pathways between *in vivo* and *in vitro* system such as glutathione metabolism, bile acid biosynthesis, nitrogen metabolism, butanoate metabolism for TAA-induced hepatotoxicity (Table II).

DISCUSSION

Hierarchical clustering analysis showed that there were

Fig. 5. Hierarchical clustering analysis of genes altered by TAA treatment. A: *in vivo* at 6 h; B: *in vitro* at 6 h; C: *in vivo* at 24 h; D: *in vitro* at 24 h; Lane 1: control; Lane 2: Vehicle; Lane 3: Low dose treatment; Lane 4: High dose treatment.

generally similar patterns between *in vivo* and *in vitro*. However, *in vitro*, there were several clusters showing simultaneous alteration at both 6 and 24 h in low and high dose group of TAA treatment by hierarchical clustering and *k*-means analysis. PCA analysis also showed that low dose and high dose groups showed closed location at 24 h in *in vitro* system,

Fig. 6. *k*-means clustering analysis. It represents there are some clusters showing alteration both low and high dose treatment of TAA in *in vitro*.

Fig. 7. Principal Component Analysis (PCA) for altered genes by TAA treatment. *In vivo* at 6 and 24 h: Control as grey; Vehicle as lemon color; Low dose treatment as deep yellow; High dose treatment as orange color; *In vitro* at 6 and 24h: Control as grey; Vehicle as lemon color; Low dose treatment as deep yellow; High dose treatment as orange color

representing higher sensitivity to low dose treatment of TAA-induced toxicity than *in vivo*. Even though there were no toxicological responses at 6 h after TAA treatment, our microarray data represents *in vitro* may detect toxic response more sensitive way, even at low dose. And our PCA data provided a clear distinction between control or vehicle treatment group and toxicant-treated groups. It suggests that gene expression data can be used to discern different hepatotoxic agents and toxicity endpoints as shown in a previous report (Huang *et al.* 2004).

Pathway analysis for differentially expressed genes identified that there were 11 (*in vivo*) and 22 (*in vitro*) pathways by TAA treatment, representing *in vitro* system has more biological pathways than *in vivo* system. It seems there may be more defense mechanism existed in *in vivo* more than *in vitro*. And our experiments also showed there were many gene alterations relating to oxidative stress both *in vivo* and *in vitro*. As pathway analysis represented that there were several common pathways between *in vivo* and *in vitro* system, it suggests that it may be feasible to extract common biomarkers by comparing the pathways from *in vivo* and *in vitro* genomic profiles. Statistical analysis of global gene expression identified that there were 677 genes (*in vivo*) and 1913 genes (*in vitro*) at 6 h and 1583 and 2217 at 24 h caused by TAA. It represented more numbers of genes found in *in vitro* than *in vivo*. Our

Table II. Pathway analysis altered by TAA treatment (KEGG)

<i>In vivo</i> pathway	Number of related genes
GLUTATHIONE METABOLISM	5
BILE ACID BIOSYNTHESIS	4
NITROGEN METABOLISM	5
BUTANOATE METABOLISM	7
ASCORBATE AND ALDARATE METABOLISM	6
FRUCTOSE AND MANNOSE METABOLISM	7
GALACTOSE METABOLISM	7
TETRACHLOROETHENE DEGRADATION	4
BISPHENOL A DEGRADATION	4
NUCLEOTIDE SUGARS METABOLISM	4
LINOLEIC ACID METABOLISM	7
<i>In vitro</i> pathway	
GLUTATHIONE METABOLISM	11
BILE ACID BIOSYNTHESIS	6
NITROGEN METABOLISM	4
BUTANOATE METABOLISM	10
ASCORBATE AND ALDARATE METABOLISM	4
FRUCTOSE AND MANNOSE METABOLISM	11
GALACTOSE METABOLISM	9
TETRACHLOROETHENE DEGRADATION	3
BISPHENOL A DEGRADATION	5
OXIDATIVE PHOSPHORYLATION	30
CELL CYCLE	16
TIGHT JUNCTION	25
RNA POLYMERASE	6
GLUTAMATE METABOLISM	8
D-GLUTAMINE AND D-GLUTAMATE METABOLISM	3
PYRIMIDINE METABOLISM	13
LYSINE DEGRADATION	10
GLYCINE, SERINE AND THREONINE METABOLISM	9
N-GLYCAN DEGRADATION	6
LIMONENE AND PINENE DEGRADATION	5
PORPHYRIN AND CHLOROPHYLL METABOLISM	5
B CELL RECEPTOR SIGNALING PATHWAY	10

attempt to identify commonly altered genes between *in vivo* and *in vitro* showed oxidative stress and lipid metabolism related genes were involved in TAA-induced toxicity. TAA is generally thought to be bioactivated by CYP and/or flavin-containing monooxygenase (FMO) systems to sulfinic (sulfoxide) and sulfenic (sulfone) metabolites (Hunter *et al.* 1977; Porter *et al.* 1979). Phenobarbital pretreatment potentiated TAA toxicity, parallel to the induction of CYP and FMO (Hunter *et al.* 1977; Zaragoza *et al.* 2000). Among metabolizing enzymes, CYP2E1

is associated with elevated hepatotoxicity (Nieto *et al.* 1999; Wang *et al.* 2005) and increases TAA-induced liver injury in diabetic (Wang *et al.* 2000) or diet-restricted rats (Ramaiah *et al.* 2001). And CYP2E1-null mice show no toxicity when treated with TAA (Kang, J.S. *et al.*, 2007), suggesting that CYP2E1 is the principal enzyme responsible for the metabolic conversion of this chemical to their active hepatotoxic metabolite. However, our experiment showed that the expression of CYP2E1 was not picked up as shown in previous report (Minami *et al.* 2005).

Generally, it seems that there were similar pattern between *in vivo* and *in vitro* in our experiments. However, there were somewhat discrepancies of expression pattern by time. In TAA-treated mice, there were evident changes of serum biochemical parameters at 24 h, associated with manifest histopathological lesions at the same time. It was reported that there were good correlation between the histopathology, clinical chemistry, and gene expression profiles induced by hepatotoxicants (Waring *et al.* 2001b). However, our data suggest there may be time-dependent alterations of genes. Therefore, it is very important to set a relevant exposure time for toxicants, with an awareness of relations between early time and later time profiles. As there has been a trend recently in focusing on toxicogenomic profiles after short-term treatment of some chemicals *in vivo* (Bulera *et al.* 2001; Ellinger-Ziegelbauer *et al.* 2004; Michel *et al.* 2003), further studies are thus warranted to analyze detailed alterations from early to late stages of hepatotoxic process, so that any hepatopreventive strategy can be based on a firm foundation.

As expected, there was not an exact correlation between two systems. It should be reminded that liver tissue and primary cells express different suites of genes and it suggests they have fundamental differences in their cell physiology, and also indicating that care must be taken in extrapolating from primary cells to whole animal organ toxicity effects (Perkins *et al.* 2006). Further multidimensional data set for liver toxicity provide an informatics challenge requiring appropriate computational methods for integrating various toxicological data into profiles and models predicting toxicity (Dix *et al.* 2007).

Taken together, our results suggest it may be feasible to develop toxicogenomics biomarkers by comparing *in vivo* and *in vitro* genomic profiles specific to hepatotoxic chemical(s) for application to prediction of liver toxicity.

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