Expression patterns and action analysis of genes associated with the responses to drugs, toxins, oxidation and unfolded proteins during rat liver regeneration☆

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Abstract

Objective. The aim is to investigate the responses to drugs, toxins, oxidation and unfolded proteins after partial hepatectomy (PH) at transcriptional level. *Methods*. The genes associated with the responses to drugs, toxins, oxidation and unfolded proteins were obtained by collecting the data and thesis. Their expression changes in regenerating liver were checked by Rat Genome 230 2.0 Array. *Results*. It was found that 35, 14, 91 and 25 genes in sequence involved in the responses to drugs, toxins, oxidation, unfolded proteins were associated with liver regeneration (LR). The initial and total expressing gene numbers at four phases of LR, i.e., the initiation (0.5 – 4 hours after PH), the transition from G0 to G1 (4 – 6 hours after PH), the cell proliferation (6 – 66 hours after PH), the cell differentiation and structure-function reorganization (66 – 168 hours after PH) were 78, 21, 65, 2 and 78, 61, 137, 89, respectively, demonstrating the genes associated with LR were mainly triggered at the early phase, and worked at different phases. Based on their expression similarity, the genes were classified into 5 groups including only up, predominantly up, only down, predominantly down, and up/down regulation, involving in 55, 27, 40, 16 and 7 genes, respectively. The total times of their up and down-regulated expression were respectively 667 and 283, and the expression of the most genes was increased, whereas the minority decreased. According to the time relevance, they were classified into 13 groups, displaying that the cellular physiological and biochemical activities were staggered during LR. Their expression patterns were classified into 24 types, showing that the activities mentioned above were diverse and complicated during LR. *Conclusion*. The responses to drugs and oxidation were increased mainly in the early phase, prophase and late phases during LR, and the responses to toxins and unfolded proteins predominantly in the middle and late phases. 145 genes associated with LR played an important role. [Life Science Journal. 2007;4(1):43 – 51] (ISSN: 1097 – 8135).

Keywords: partial hepatectomy; Rat Genome 230 2.0 Array; responses to drugs, toxins, oxidation and unfolded proteins; genes; liver regeneration

1 Introduction

When organisms undergo distinct stimuli including physics[1-5], chemistry[5-9], biology[10, 11], physiology[12-19] and so on, the relevant stress protein (SP) genes are activated to protect organisms against these harmful stimuli. The stress response to one stimulus can usually increase cell tolerance to another stimulus. It implies stress proteins induced by different stimuli have functional cross[20]. After partial hepatectomy (PH)[21], the remnant hepatocytes were activated to proliferate to compensate the lost liver mass, which is called liver regeneration (LR)[22, 23]. According to the cellular physiological activities, the regeneration process is usually categorized into four stages including initiation phase (0.5 – 4 hours after PH), transition from G0 to G1 (4 – 6 hours after PH), cell proliferation (6 – 66 hours after PH), cell differentiation and reorganization of the structure-function (66 – 168 hours after PH)[23]. According to time course, it was classified into four phases including forepart (0.5 – 4 hours after PH), prophase (6 – 12 hours after PH), metaphase (16 – 66 hours after PH), and anaphase (72 – 168 hours after PH)[24]. In addition, PH, as an injurious stimulus, can induce many stress responses including the responses to drugs, toxins, oxidation and unfolded proteins. The above responses involved numerous genes and proteins, hence, it is almost impossible to clarify the action of genes associated with the above responses during LR at transcriptional level unless high-throughput gene expression arrays[25, 26]. So, we used the Rat Genome 230 2.0 Array

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containing 65 genes associated with drugs response, 23 genes to toxins, 179 genes to oxidation and 50 genes to unfolded proteins to detect gene expressions changes after PH[27,28]. And 145 genes among them were found to be associated with LR[29]. Moreover, their expression character, patterns and actions in regenerating liver were further analyzed.

2 Materials and Methods

2.1 Regenerating liver

Healthy Sprague-Dawley rats weighing 200 – 250 g were from the Animal Center of Henan Normal University. The rats were separated into two groups randomly, hepatectomized group and sham-operation (SO) group. Each group included 6 rats (male: female = 1:1). PH was performed according to Higgins and Anderson[21], by which the left and middle lobes of liver were removed. Rats were killed by cervical vertebra dislocation at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 54, 66, 72, 120, 144 and 168 hours after PH and the regenerating livers were observed at corresponding time point. The livers were rinsed three times in PBS at 4 ℃, and then 100 – 200 mg livers from middle parts of right lobe of each group (total 1 – 2 g livers, 0.1 – 0.2 g × 6 samples, per group) were gathered and mixed together, then stored at – 80 ℃. SO group was the same as hepatectomized group except the liver lobes unremoved. The laws of animal protection of China were enforced strictly.

2.2 RNA isolation and purification

Total RNA was isolated from frozen livers according to the manual of Trizol kit (Invitrogen Corporation, Carlsbad, California, USA)[30] and then purified base on the guide of RNeasy mini kit (Qiagen, Inc, Valencia, CA, USA)[31]. Total RNA samples were checked to exhibit a 2:1 ratio of 28S rRNA to 18S rRNA intensities by agarose electrophoresis (180 V, 0.5 hour). Total RNA concentration and purity were estimated by optical density measurements at 260/280 nm[32].

2.3 cDNA, cRNA synthesis and purification

As template, 1 – 8 μg total RNA was used for cDNA synthesis. cDNA purification was based on the way established by Affymetrix[27]. cRNA labeled with biotin was synthesized using cDNA as the template, and cDNA and cRNA were purified according to the GeneChip Analysis[27]. Measurement of cDNA, cRNA concentration and purity were the same as above.

2.4 cRNA fragmentation and microarray detection

15 μl (1 μg/μl) cRNA incubated with 5 × fragmentation buffer was digested into 35 – 200 bp fragments at 94 ℃ for 35 minutes. Rat Genome 230 2.0 microarray produced by Affymetrix was prehybridized, then the hybridization buffer was added at 45 ℃, 60 rpm for 16 hours. The microarray was washed and stained by GeneChip fluidics station 450 (Affymetrix Inc, Santa Clara, CA, USA). The chips were scanned by GeneChip Scan 3000 (Affymetrix Inc, Santa Clara, CA, USA), and the signal values of gene expression were observed[28].

2.5 Microarray data analysis

The normalized signal values, signal detections (P, A, M) and experiment/control (Ri) were obtained by quantifing and normalizing the signal values using GCOS (GeneChip operating software) 1.2[28].

2.6 Normalization of the microarray data

To minimize error from the microarray analysis, each sample at each time point during LR was measured three times with Rat Genome 230 2.0 microarray. A total ratio was maximal (R^m) and the average of three housekeeping genes β-actin, hexokinase and glyseraldehyde-3-phosphate dehydrogenase approached 1.0 (R^h) was taken as a reference. The modified data were generated by applying a correction factor (R^m/R^h) multiplying the ratio of every gene in R^h at each time point. To remove spurious gene expression changes resulting from errors in the microarray analysis, the gene expression profiles at 0 – 4 hours, 6 – 12 hours and 12 – 24 hours after PH were reorganized by normalization analysis program (NAP) software according to the cell cycle progression of the regenerating hepatocytes. Data statistics and cluster analysis were done using GeneMath, GeneSpring, Microsoft Excel software[28, 33, 34].

2.7 Identification of genes associated with LR

Firstly, the nomenclature of four physiological responses mentioned above was adopted from the GENEONTOLOGY database (www.geneontology.org) and input into the databases of NCBI (www.ncbi.nlm.nih.gov) and RGD (rgd.mcw.edu) to identify the rat, mouse and human genes associated with the responses to drugs, toxins, oxidation and unfolded proteins. In addition, according to maps of biological pathways embodied by GENMAPP (www.genmapp.org), KEGG (www.genome.jp/kegg/pathway.html # amino) and BIOCARTA (www.biocarta.com/genes/index.asp), the genes associated with the biological process were collated. The results of these analysis were codified, and compared with the results obtained from mouse and human searches to identify human and mouse genes which are different from those of rat. These genes (human and mouse genes differed from those of rat) were compared with the analysis output of the Rat Genome 230 2.0 Array. Those genes which showed more than twofold changes at expression level, observed as meaningful expression changes[29], were referred to as rat homologous or rat specific genes associated with the responses to drugs, toxins, oxidation and unfold proteins under evaluation. Genes, which displayed reproducible results with three independent analysis with the chip and which showed more than twofold changes at expression level in at least one time point in LR with significant difference ($0.01 \leqslant P < 0.05$) or extremely significant difference ($P \leqslant 0.01$) between PH and SO, were referred to as associated with LR.

3 Results

3.1 Expression changes of the genes associated with the responses to drugs, toxins, oxidation and unfolded proteins during LR

According to the data of NCBI, GENEMAP, KEGG, BIOCARTA and RGD, the responses to drugs, toxins, oxidation and unfolded proteins involved 74, 25, 195 and 58 genes respectively, in which 65, 23, 179 and 50 genes were contained in Rat Genome 230 2.0 Array separately. Among them, 35, 14, 91 and 25 genes displayed meaningful changes in expression at least at one time point after PH, showed significant or extremely significant differences in expression when comparing PH with SO, and were repeatable in three times of detection by Rat Genome 230 2.0 Array. The results suggested that the genes were associated with LR. Up-regulation was 2 – 59.7 folds of control, and down-regulation 2 – 33.3 (Table 1). 55 genes were up-regulated, 40 genes down, 50 genes up/down during LR. The total up and down-regulated times were 667 and 283, respectively (Figure 1A). At the initiation stage (0.5 – 4 hours after PH), 52 genes were up-regulated, 26 genes down; at the transition phase from G0 to G1 (4 – 6 hours after PH), 42 genes revealed up-regulated, 19 genes down-regulated; at cell proliferation phase (6 – 66 hours after PH), 62 genes showed up-regulated, 41 genes down-regulated, 34 genes up/down-regulated; at cell differentiation and reorganization of the structure-function stage (66 – 168 hours after PH), 56 genes were up-regulated, 21 genes down-regulated, 12 genes up/down-regulated (Figure 1B).

Table 1. Expression abundance of 145 genes associated with responses to drugs, toxins, oxidation and unfolded proteins during rat LR

Gene Abbr.	Accosiated to others	Fold difference
1 Drug		
Abca3	3	9.8
Abcb1		4.6
Abcb4		0.4
Abcc1	3	0.2
Abcc6		0.4
Abp1		2.6
Ahr		2.2
Ahrr		0.1
Akr1c12		0.2, 2.22
Akr1c6		0.03, 4.0
Arnt	2	0.2, 3.0
Arnt2	2	0.4, 6.8
Blmh	2	3.7
Bphl	2	0.4
Cat	3	2.8
Ces2	2	3.2
Cyp2c		52.6
Cyp2d6		0.3
Drd1a		14.0
Drd2		8.6
Ebp		0.4
Ephx1	2, 3	0.4, 2.8
Fpgs		0.5, 3.75
Gabrg3		0.4
Gprk5		0.3, 9.1
Gsta5		5.6
Gstm2	3	4.5
Msra	3	0.5
Nat8		5.2
Oprs1		0.3
Pbp		3.7
Slc18a1		0.4, 7.8
Snca	3	0.2
Sult1a1		0.2, 2.2
2 Toxin		
Ace		0.5
Arnt	1	0.2, 3.0
Arnt2	1	0.4, 6.8
As3mt		0.5
Blmh	1	3.7
Bphl	1	0.4
Ces2	1	3.2
Ephx1	1, 3	0.4, 2.8
Gsta2	3	0.2, 7.0
Gucy2c		0.4, 9.8
Mpst		0.1, 2.6
Pon1		0.4
3 Oxidation		
Aass		3.8
Abca3	1	9.8
Abcc1	1	0.2
Als2		0.5
Apoe		0.1
Atm		0.3
Atox1		2.8
Bcl2		0.3
Bcl2a1		0.3, 5.3
Btg1		2.7
Btg3		5.3
Ca3		14.0
Casp8		10.6
Cat	1	2.8
Cav		10.6
Chek1		0.3, 5.3
Creb1		0.5
Crisp2		0.2
*Crp		0.5
Ctsb		3.6
Cycs		2.2
Cyp1a1		59.7
Ddit3		0.5, 2.7
Dhcr24		0.1
Drd2		8.6
Dusp1		0.4, 6.0
Edn1		0.4, 2.6
*Egr1		18.6
Ephx1	1, 2	0.4, 2.8
Falz		0.1
Fbp1		3.0
Foxm1		13.9
Ggtla1		4.0
Gstm2	1	4.5
H6pd		2.9
Hao1		0.5, 2.3
Hmox2		0.4
Hnf4a		0.1, 4.5
Hspb1	4	11.0
Htatip		0.4
*Idh1		0.2, 2.4
*Ifng		6.5
Igf1r		0.4
*Jak2		6.5
Jun		6.9
Kdr		0.4, 2.4
Lpl		2.0
Mafk		0.3, 2.1
Mapk1		2.7
Mapk8		0.5, 19.7
Matk		0.3, 4.9
*Mbl2		0.2
*Mgst1		0.5
Mmp9		0.5, 9.5
Msra	1	0.5
Muc1		0.2, 6.8
Nfkb1		0.4, 2.3
Nos3		0.3, 2.1
Nox1		0.3, 8.6
Parg		4.8
Pdlim1		0.5, 3.2
Plaur		13.9
Plcd1		0.2, 5.5
Ppp1r15b		0.1, 3.2
Prdx3		0.5, 2.6
Prdx5		0.5
Prdx6		2.3
Prkaa2		0.2, 5.3
Prkca		4.6
*Prkcb1		8.2
Pten		0.5
Ptgs1		3.4
Rps6ka5		5.6
Snca	1	0.2
Sod2		5.6
Tap1		2.2
Tert		0.3, 5.3
*Tgfb1		4.0
Tgfb1i1		0.5, 2.0
*Tnf		3.2
*Tp53		2.9
Trpm2		0.4, 4.0
Txnip		2.9
Txnrd2		0.3
Ucp3		0.5, 2.2
Ugt1a7		2.1
Vegfa		0.1, 4.5
Xpa		0.2
Xrcc5		4.7
*Ptgs2		0.1, 2.1
Gsta2	2	0.2, 7.0
4 Unfolded protein		
Cebpb		3.1
Dnajb5		0.4
Dnajb9		0.3
Dnajc3		0.4
*Hspa1a		0.2
Hspa1b		9.2
Hspa2		11.0
Hspa5		0.1, 5.7
Hspa8		0.4, 9.8
Hspb1	3	3.4
Hspb2		4.7
Hspb3		0.5, 2.6
Hspb7		0.2
Hspb8		0.5, 6.8
Hspca		2.0
Hspe1		0.5, 2.8
Hyou1		0.4, 3.5
*Lman1		0.3, 4.3
Npm1		0.4, 3.6
Osp94		0.5, 4.6
Park2		2.9
Per1		2.4
Serpinh1		2.1
*Tp53		2.9
Xbp1		0.4

* Reported genes associated with LR; Associated to others: involved in other responses

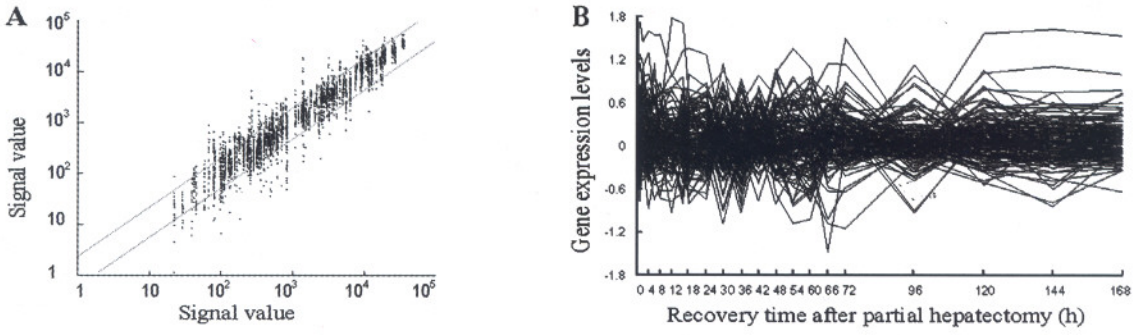

Figure 1. Expression frequency, abundance and changes of 145 genes associated with responses to drugs, toxins, oxidation and unfolded proteins during rat LR. Detection data of Rat Genome 230 2.0 Array were analyzed and graphed by Microsoft Excel. A. Gene expression frequency. The dots above bias represented the genes up-regulated more than twofold, and total times of up-regulation were 667; those under bias represented the genes down-regulated more than twofold, and down-regulation were 283 times; and the ones between biases represented the genes insignificantly changed. B. Gene expression abundance and changes. 105 genes were 2 – 59.7 folds up-regulated, and 90 genes 2 – 33.3 folds down-regulated.

3.2 Initiation expression time of the genes associated with the responses to drugs, toxins, oxidation and unfolded proteins during LR

At each time point of LR, the numbers of initial up, down and total up, down-regulated genes were in sequence: both 25 and 9 at 0.5 hour; 16, 6 and 38, 11 at 1 hour; 8, 1 and 36, 5 at 2 hours; 3, 10 and 36, 14 at 4 hours; 4, 4 and 33, 11 at 6 hours; 2, 2 and 32, 11 at 8 hours; 0, 4 and 24, 15 at 12 hours; 11, 7 and 36, 15 at 16 hours; 5, 7 and 36, 18 at 18 hours; 2, 4 and 34, 12 at 24 hours; 3, 2 and 22, 16 at 30 hours; 0, 3 and 30, 19 at 36 hours; 0, 1 and 29, 8 at 42 hours; 1, 1 and 37, 17 at 48 hours; 0, 2 and 29, 20 at 54 hours; 0, 0 and 30, 22 at 60 hours; 0, 0 and 30, 10 at 66 hours; 0, 0 and 31, 7 at 72 hours; 0, 0 and 21, 12 at 96 hours; 2, 0 and 32, 10 at 120 hours; 0, 0 and 24, 11 at 144 hours; 0, 0 and 22, 6 at 168 hours (Figure 2). Generally, gene expression changes occurred during the whole LR, and the up and down-regulation were respectively 667 and 283 times. The initially up-regulated genes were predominantly expressed in the forepart, and the down- in the prophase, pre-metaphase and mid-metaphase, whereas only few genes were initially up-expressed in the late metaphase and anaphase.

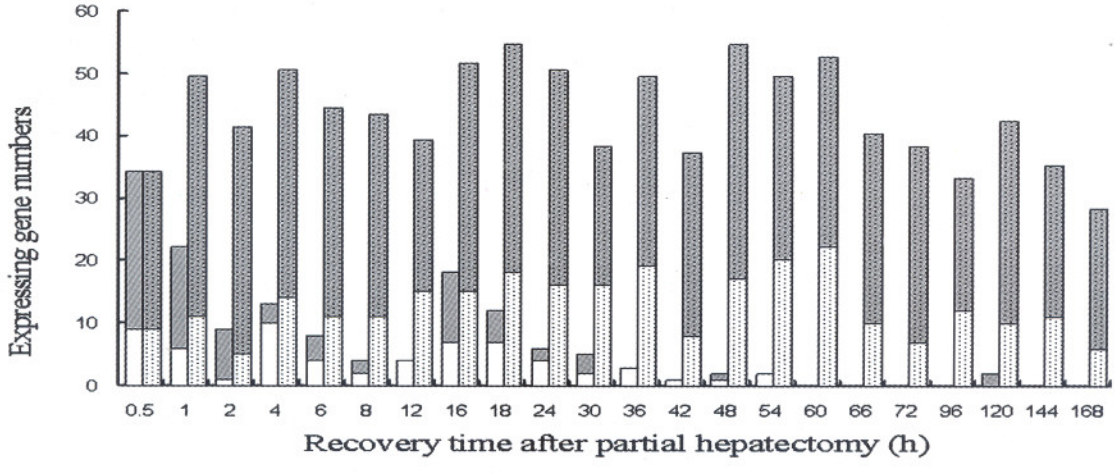

Figure 2. The initial and total expression profiles of 145 genes associated with responses to drugs, toxins, oxidation and unfolded proteins at each time point of LR. Grey bars: Up-regulated genes; White bars: Down-regulated genes. Blank bars represent initial expressing genes, in which up-regulation genes are predominant in the forepart, and the down- prophase, pre-metaqhase and mid-metaphase, whereas almost none in the late metaphase and anaphase. Dotted bars represent the total expressing genes, in which some genes are up-regulation and others down-regulation during LR.

3.3 Expression similarity and time relevance of the genes associated with the responses to drugs, toxins, oxidation and unfolded proteins during LR

145 genes mentioned above during LR could be characterized based on their similarity in expression as following: only up, predominantly up, only down, predominantly down, and up/down-regulated, involving 55, 27, 40, 16 and 7 genes, respectively (Figure 3). 145 genes could also be classified based on time relevance into 13 groups including 0.5 and 144 hours, 1 and 2 hours, 4 and 6 hours, 8 and 12 hours, 16 hours, 18 hours, 24 and 30 hours, 36 and 48 hours, 42 and 54 hours, 60 and 66 hours, 72 and 96 hours, 120 hours, 168 hours, in which the up and down-regulated gene numbers were 49 and 20; 74 and 16; 69 and 25; 56 and 26; 36 and 15; 36 and 18; 56 and 32; 67 and 36; 58 and 38; 60 and 32; 52 and 19; 32 and 10; 22 and 6, respectively (Figure 3). The up-regulation genes were mainly associated with oxidative stress, drug metabolism and transport, folding and transport of peptide. The down-regulation genes were mostly those associated with anti-apoptosis and oxidative injury.

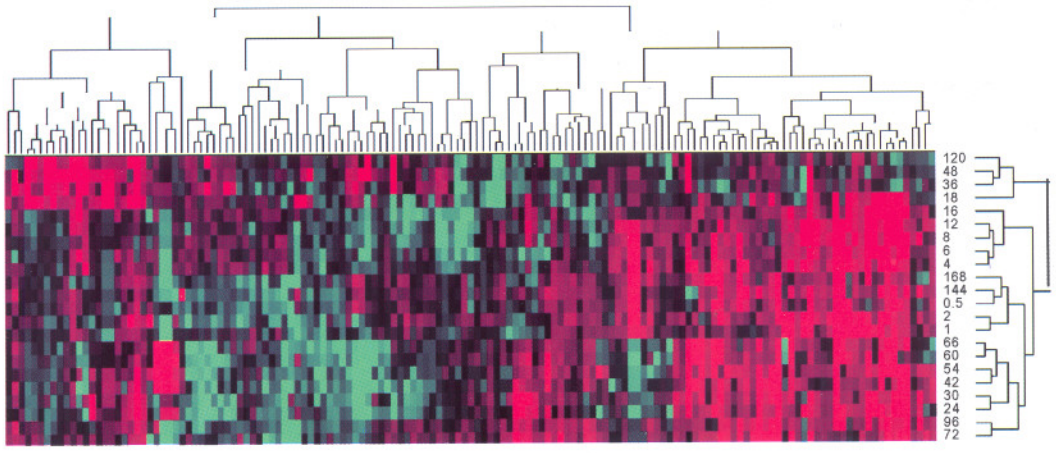

Figure 3. Expression similarity and time relevance cluster of 145 genes associated with responses to drugs, toxins, oxidation and unfolded proteins during LR. Detection data of Rat Genome 230 2.0 Array were analyzed by H-clustering. Red represents up-regulation genes mainly associated with oxidative stress, drug metabolism and transport, folding and transport of peptide; Green represents the down- ones mostly with anti-apoptosis and oxidative injury; Black indicates the genes with nonsense change in expression. The upper and right trees respectively show expression similarity and time series clusters, by which the above genes were classified into 5 and 13 groups separately.

3.4 Expression patterns of the genes associated with the responses to drugs, toxins, oxidation and unfolded proteins during LR

145 genes mentioned above during LR might be categorized into 24 clusters according to the changes in expression changes: (1) up-regulation at one time point, at 0.5, 6, 16, 30, 36, 48, 120 hours after PH (Figure 4A), involved in 9 genes; (2) up at two time points, at 16 and 42 hours, 16 and 96 hours, 30 and 42 hours (Figure 4B), in 4 genes; (3) up at three time points (Figure 4C), in 2 genes; (4) up at four time points (Figure 4C), in 3 genes; (5) up at one phase, 0.5－6 hours, 0.5－8 hours (Figure 4D), in 2 genes; (6) up at three phases (Figure 4D), in 2 genes; (7) up at one time point/one phase, at 8 and 1－4 hours, 120 and 1－24 hours, 120 and 6－8 hours (Figure 4D), in 3 genes; (8) up at two time points/one phase (Figure 4E), in 5 genes; (9) up at one time point/two phases (Figure 4F), in 5 genes; (10) up at two time points/two phases (Figure 4G), in 4 genes; (11) up at three time points/two phases (Figure 4H), in 4 genes; (12) up at two time points/three phases (Figure 4I), in 6 genes; (13) at more time points or phases (Figure 4J), in 6 genes; (14) down at one time point, at 0.5, 4, 6, 12, 16, 24, 30, 36, 42, 54 hours (Figure 4K), in 11 genes; (15) down at two time points, at 1 and 66 hours, 1 and 168 hours, 2 and 12 hours, 12 and 36 hours, 16 and 30 hours, 24 and 54, 48 and 60 (Figure 4L), in 7 genes; (16) down at three time points (Figure 4M), in 2 genes; (17) down at four time points (Figure 4M), in 4 genes; (18) down at one phase, at 0.5－2 hours, 4－6 hours, 54－60 hours (Figure 4N), in 3 genes; (19) down at one time point/one phase, at 1 and 144－168 hours, 8 and 120－144 hours, 36 and 54－60 hours, 48 and 12－24 hours (Figure 4N), in 5 genes; (20) down at one time point/two phases (Figure 4N), in 2 genes; (21) down at more time points or phases (Figure 4O), in 6 genes; (22) first up and then down (Figure 4P), in 12 genes; (23) first down and then up (Figure 4Q), in 10 genes; (24) up/down mixed (Figure 4R), in 28 genes.

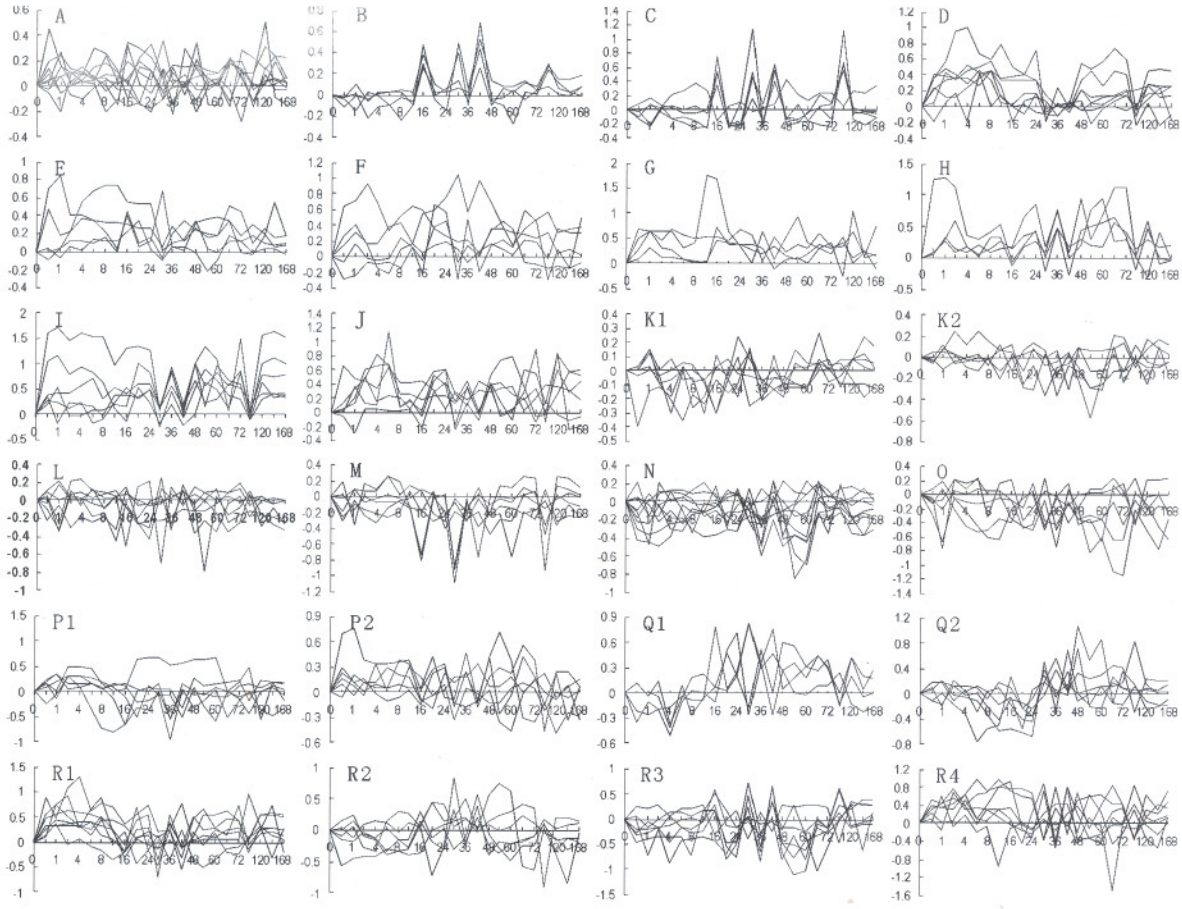

Figure 4. Twenty-four expression patterns of 145 genes associated with responses to drugs, toxins, oxidation and unfolded proteins during LR. Expression patterns were obtained by the analysis of detection data of Rat Genome 230 2.0 Array with Microsoft Excel. A－I: 55 up-regulated genes; J－O: 40 down-regulated genes; P－R: 50 up/down-regulated genes. X-axis represents recovery time after PH (h); Y-axis shows logarithm ratio of the signal values of genes at each time point to control.

4 Discussion

Liver is an important organ metabolizing drugs and toxins[35]. It can also respond to stress including oxidation and unfolded protein. In which, 15 kinds of proteins associated with drug metabolism, such as cytochrome P450 subfamily Ⅱ C (CYP2C) etc., participate in transportation and catabolism of drugs[36]. Dopamine receptor 1A, 2 (DRD1A, DRD2) are involved in drug addiction. Aryl hydrocarbon receptor repressor (AHRR) suppresses activation of aryl hydrocarbon receptor[37]. Aryl hydrocarbon receptor nuclear translocator (ARNT) promotes secretion of trypsin[38]. Aldo-keto reductase family 1 member C6 (AKR1C6) accelerates bile acid synthesis[39]. Catalase (CAT) can protect cells against oxidation damage by decomposing H_2O_2[40]. Folylpolyglutamate synthase (FPGS) plays an important role in synthesis of nucleic acid[41]. G protein-coupled receptor kinase 5 (GPRK5) can raise blood pressure[42]. Phosphatidylethanolamine binding protein (PBP) is concerned with biomembrane formation[43]. Amiloride-binding protein 1 (ABP1) can open Na^+ channel of epithelia[44]. Gamma-aminobutyric acid A receptor subunit gamma 3 (GABRG3) is associated with liver diseases depended on ethanol[45]. N-acetyltransferase 8 (NAT8) debases cell conglutination[46]. The meaningful expression changes of these genes showing the sameness or the similarity in some time points, then difference in other during LR perhaps co-regulate the response to drug. Especially, *cyp2c* displayed significant up-regulation during almost the LR, and had the highest abundance at 16 hours that was 9.1-fold of control. *drd1 a* was up at 16, 30, 42 and 96 hours post PH, and had peak expression showing 14-fold of control at 30 hours. It is speculated that the two play crucial roles in the drugs response.

Among proteins associated with response to toxin, uanylate cyclase 2c (GUCY2c) mediates acute secretory diarrhea induced by heat-stable enterotoxins[47]. Arsenic

+3 oxidation state methyltransferase (AS3MT) prevents regenerating liver from toxicity of arsenic +3 by catalyzing transfer of a methyl group to trivalent arsenicals[48]. Mercaptopyruvate sulfurtransferase (MPST) plays a role in catabolism of cysteine and cyanide[49]. Glutathione-S-transferase alpha type 2 (Gsta2) is concerned with detoxification by binding to hepatocyte nuclear factor 1 (HNF1)[50]. Paraoxonase 1 (PON1) is involved in decomposition of lipid peroxides and organophosphorus compounds[51]. UDP glycosyltransferase 1 family A7 (UGT1A7) is concerned with elimination of multifold toxin[52]. Angiotensin I converting enzyme (ACE) protects tissues from chronic hypoxia to maintain the stabilization of cardiopulmonary function by converting angiotensin I into angiotensin II[53]. The sameness or the similarity in some time points, then difference in others of meaningful expression changes of these genes during LR perhaps regulate the response to toxin in regenerating liver together. Remarkably, *gucy2c* was up during almost the whole LR, having a peak that was 9.8 folds of the control. *gsta2* was up at 30,42 and 96 hours after PH, and reached a peak at 42 hours showing 7 times higher than control. It was presumed that the two play key roles in the response to toxin in regenerating liver.

Among proteins associated with response to oxidation, 18 kinds of proteins including cytochrome P450 subfamily 1 member A1 (CYP1A1) regulate oxidation reaction during LR and protect cells from oxidative damage[54]. 3 kinds of proteins including lipoprotein lipase (LPL) participate in decomposing lipid through oxidation[55]. Poly ADP-ribose glycohydrolase (PARG) resists inflammation[56]. Adenosine monophosphate-activated protein kinase α2 catalytic subunit (PRKAα2) regulates the balance between energy supply and demand[57]. Dual specificity phosphatase 1 (DUSP1) is relative to the release of endotoxin[58]. The meaningful expression changes of these genes showing the sameness or the similarity in some time points, and difference in others during LR perhaps co-regulate the response to oxidation. Among them, *cyp1a1* was up during almost the whole LR, showing peak expression at 12 hours that was 59.7 folds of control, presuming that it plays a critical role in the oxidative stress.

Among the proteins associated with the response to unfolded protein, eleven kinds of proteins including 10 kDa heat shock protein 1 (HSPE1) play the role in the folding of proteins and the degradation of wrong folded proteins[59]. 27 kDa heat shock protein 2 (HSPB2) restrains apoptosis by maintaining functions of mitochondrion[60]. 27 kDa heat shock protein 3 (HSPB3) is involved in cells survival and differentiation[61]. Hsp40 homolog subfamily C member 3 (DNAJC3) accelerates the expression of p58 (a protein kinase) and depresses eukaryote translation initiation factor 2 (eIF2)[62]. Hsp40 homolog subfamily B member 9 (DNAJB9) resists cell apoptosis[63]. 70 kDa heat shock protein 1B (HSPA1B) can protect cell depending on CO and regulate immunoreaction[64]. 70 kDa heat shock protein 2 (HSPA2) promotes the transition from G1 to S[65]. 70 kDa heat shock protein 4-like (HSPA4L /OSP94) accelerates the secretion of CD1d and sends antigen to T cells[66]. Nucleophosmin (NPM1) hastens the assembly of ribosome and maintains the manifold enzymatic activity in liver[67]. That the meaningful expression changes of these genes are same or similar in some points, then different in others during LR perhaps presumably regulate the response to unfolded protein together. Among them, *hspa8* was up during almost the whole LR, and had a peak at 12 hours that was 9.8 folds of control. *hspa1b* showed up-regulation at multiple time points after PH, and had the highest abundance having 8.2-fold increase at 60 hours. *hspa2* was up-regulated at metaphase, anaphase, and reached a peak having a 10-fold increase at 30 hours. It was supposed that the three have key roles in the response to unfolded protein in regenerating liver.

In conclusion, the responses to drugs, toxins, oxidation and unfolded proteins were investigated using high-throughput gene expression profiles commencing from long time (0.5 hour－7 days after PH) and multiple time points (total 23). It was primarily proved that the regenerating liver had an increase in the responses to drugs, toxins, oxidation and unfolded proteins, that Rat Genome 230 2.0 Array was a useful tool analyzing the above responses at transcriptional level. However, the processes, namely, DNA→ mRNA→ protein, were influenced by many factors including protein interaction. Therefore, later the above results will be further analyzed by the techniques, such as Northern blotting, protein chip, RNA interference, protein-interaction etc.

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