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BASIC RESEARCH

Expression patterns and action analysis of genes associated with drug-induced liver diseases during rat liver regeneration

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Abstract

AIM: To study the action of the genes associated with drug-induced liver diseases at the gene transcriptional level during liver regeneration (LR) in rats.

METHODS: The genes associated with drug-induced liver diseases were obtained by collecting the data from databases and literature, and the gene expression changes in the regenerating liver were checked by the Rat Genome 230 2.0 array.

RESULTS: The initial and total expression numbers of genes occurring in phases of 0.5-4 h after partial hepatectomy (PH), 4-6 h after PH (G0/G1 transition), 6-66 h after PH (cell proliferation), 66-168 h after PH (cell differentiation and structure-function reconstruction) were 21, 3, 9, 2 and 21, 9, 19, 18, respectively. It is illustrated that the associated genes were mainly triggered at the initial stage of LR and worked at different phases. According to their expression similarity, these genes were classified into 5 types: only up-regulated (12 genes), predominantly up-regulated (4 genes), only down-regulated (11 genes), predominantly down-regulated (3 genes), and approximately up-/down-regulated (2 genes). The total times of their up- and down-expression were 130 and 79, respectively, demonstrating that expression of most of the genes was increased during LR, while a few decreased. The cell physiological and biochemical activities during LR were staggered according to the time relevance and were diverse and complicated in gene expression patterns.

CONCLUSION: Drug metabolic capacity in regenerating liver was enhanced. Thirty-two genes play important roles during liver regeneration in rats.

Key words: Partial hepatectomy; Rat Genome 230 2.0 array; Drug-induced liver diseases; Genes associated with liver regeneration

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INTRODUCTION

The liver has a very strong capacity to regenerate[1]. Liver cells proliferate rapidly to compensate for lost liver tissues after liver injury or drug stimulus, which is called liver regeneration (LR)[2]. The LR process is usually categorized based on hepatic physiological activities divided into four stages: initiation phase [0.5-4 h after partial hepatectomy (PH)], transition from G0 to G1 (4-6 h after PH), cell proliferation (6-66 h after PH), cell differentiation and reorganization of the structure-function (66-168 h after PH)[3]. The process involves hepatic cell activation, dedifferentiation, proliferation and its regulation, redifferentiation, structure-functional reorganization[4].

Liver is a vital organ of drug metabolism[5]. Disorder of drug metabolism in liver could cause drug-induced liver diseases[6]. It is indicated that 182 genes are associated with drug-induced liver diseases. In addition, there are gene-gene, protein-protein, gene-regulator, and protein-regulator interactions. It is hardly possible to highlight the role of the genes in LR unless gene expression profiles is analyzed with high-throughput[7,8]. Therefore, we used the Rat Genome 230 2.0 array containing 84 genes associated with drug-induced liver diseases to detect gene expression changes after PH, finding that 32 of them were associated with LR, and analyzed these genes expression changes, patterns and actions during LR primarily.

MATERIALS AND METHODS

Regenerating liver preparation

Healthy SD rats weighing 200-250 g were obtained from the Animal Center of Henan Normal University. The rats were separated into groups at random and each group included 6 rats (male:female = 1:1). PH was performed according to Higgins and Anderson[9], the left and middle lobes of the liver were removed. Rats were killed by cervical vertebra dislocation at 0.5, 1, 2, 4, 6, 8, 12, 16, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 96, 120, 144 and 168 h after PH and the regenerating livers were observed at corresponding time points. The livers were rinsed three times in PBS at 4℃, then 100-200 mg liver from the middle parts of the right lobe, six samples of each group were collected, wrer mixed with 1-2 g (0.1-0.2 g × 6) total liver tissues, and stored at -80℃. The sham-operation (SO) groups were treated the same with the PH group except that the liver lobes were unremoved. The laws of animal protection of China were enforced strictly.

RNA isolation and purification

Total RNA was isolated from frozen livers according to the manual of the Trizol kit (Invittrogen)[10] and purified based on the RNeasy mini-kit (Qiagen)[11]. Total RNA samples exhibited a 2:1 ratio of 28S to 18S rRNA intensities by agarose electrophoresis (180V, 0.5h). Total RNA concentration and purity were estimated by optical density measurement at 260/280 nm[12].

cDNA, cRNA synthesis and purification

As a template, 1-8 μg total RNA was used for cDNA synthesis. cDNA and cRNA synthesis was proceeded by the established method of Affymetrix[13]. cRNA labeled with biotin was synthesized using 12 μL of the above synthesized cDNA as the template, and cDNA and cRNA were purified[13]. Concentration, purity and quality of cDNA and cRNA were measured by the same method mentioned above[12].

cRNA fragmentation and microarray detection

Fifteen μL (1 μg/μL) cRNA incubated with 5 × fragmentation buffer at 94℃ for 35 min was digested into 35-200 bp fragments. The hybridization buffer was added to the prehybridized Rat Genome 230 2.0 microarray produced by Affymetrix, then hybridization was carried out for 16 h at 45℃ on a rotary mixer at 60 rpm. The microarray was washed and stained by GeneChip fluidics station 450 (Affymetrix Inc., USA). The chips were scanned by GeneChip Scan 3000 (Affymetrix Inc., USA), and the signal values of gene expression were observed[14].

Microarray data analysis

The normalized signal values, signal detections (P, A, M) and experiment/control (Ri) were obtained by quantifying and normalizing the signal values using GCOS1.2[14].

Normalization of microarray data

To minimize error in the microarray analysis, each analysis was performed three times by Rat Genome 230 2.0 microarray. Results with a total ratio was maximal (R^m) and when the average of three housekeeping genes (β-actin, hexokinase and glyceraldehyde-3-phosphate dehydrogenase) approached 1.0 (R^h), it 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 h, 6-12 h and 12-24 h after PH were reorganized by NAP software (normalization analysis program) according to the cell cycle progression of the regenerating hepatocytes. Data statistics and cluster analysis were done using GeneMath, GeneSpring, Microsoft Excel software[14-16].

Identification of genes associated with liver regeneration

The nomenclature of a liver disease (e.g. drug-induced liver diseases) was adopted from the GENEONTOLOGY database (www.geneontology.org), and input into the databases at NCBI (www. ncbi.nlm.nih.gov) and RGD (rgd. mcw.edu) to identify the rat, mouse and human genes associated with the above liver diseases. Then the genes associated with the drug-induced liver diseases were collated. The results of this analysis were codified, and compared with the results obtained for human and mouse searches in order to identify the difference of human and mouse genes from rats. In comparison to these genes with the analysis output of the Rat Genome 230 2.0 array, the genes, showing a greater than twofold change in expression level as meaningful expression changes[17] , were referred to as rat homologous genes or rat specific genes associated with drug-induced liver diseases. Genes, which displayed reproducible results with three independent analyses with the chip and showed a greater than twofold change in expression level in at least one time point during liver regeneration 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 liver regeneration.

RESULTS

Expression changes of genes associated with drug-induced liver diseases during LR

According to the data from databases at NCBI, GENEMAP, KEGG and BIOCARTA, 182 genes were associated with drug-induced liver diseases. Among them, 84 genes were contained in the Rat Genome 230 2.0 array. Thirty-two of them revealed meaningful changes in expression at least at one time point after PH. There was significant difference or extremely significant difference in expression between PH and SO, and reproducible results were obtained with three analyses with Rat Genome 230 2.0 array, suggesting that the genes were associated with LR (Table 1). The analysis indicated that 12 genes were up-, 11 genes down-, and 9 were up/down-regulated during liver regeneration. Total expression times of up- and down-expressed genes were 130 and 79, respectively (Figure 1A). At the initial stage of liver regeneration (0.5-4 h after PH), 13 genes displayed up-, 7 genes down-，1 gene up/down-regulation; at the transition phase from G_0 and G_1 (4-6 h after PH), 10 genes revealed up-, 2 genes down-regulation; at cell proliferation phase (6-66 h after PH), 12 genes showed up-, 8 genes down-, 5 genes up/down-regulation; and at cell differentiation and structure-function reorganization phase (66-168 h after PH), 9 genes displayed up-, 10 genes down-, 5 genes up/down-regulation (Figure 1B).

Table 1 Expression abundance of 32 genes associated with drug-induced liver diseases during liver regeneration

Gene	Recovery time (h) after partial hepatectomy (PH)																					
Abbr.	0	0.5	1	2	4	6	8	12	16	18	24	30	36	42	48	54	60	66	72	96	120	144	168
1 Cholestasis																							
Ace	1	0.80	**0.48**	1.29	0.88	1.39	0.82	1.14	1.16	1.00	0.88	0.93	1.18	1.03	1.18	1.03	1.39	0.56	0.71	0.78	0.75	0.56	**0.47**
Akt1	1	0.81	0.76	0.98	0.86	1.01	0.87	1.11	1.12	0.62	0.71	*3.91*	0.65	*2.60*	0.53	0.57	0.68	1.10	0.97	1.17	1.02	0.94	1.01
Apoe	1	0.93	1.24	0.87	0.93	1.07	0.95	0.76	**0.15**	0.93	1.08	**0.13**	0.93	**0.17**	1.00	1.00	0.93	1.07	1.20	**0.33**	1.41	1.23	1.07
Bdnf	1	0.97	**0.46**	1.98	1.43	1.04	1.04	*2.00*	*2.08*	0.63	0.58	*2.12*	0.52	1.43	**0.45**	1.25	*2.50*	1.06	1.32	*2.56*	**0.39**	0.52	**0.50**
Cyp2d6	1	0.76	**0.33**	1.62	1.00	1.15	1.35	1.62	1.14	1.15	1.24	0.60	0.57	0.74	0.81	0.81	0.66	1.73	0.65	1.07	0.66	**0.44**	**0.50**
Ephx1	1	1.52	1.53	1.32	0.93	1.00	0.63	**0.41**	0.86	0.76	0.62	0.97	*2.46*	1.12	*2.14*	1.74	*2.00*	*2.82*	*2.77*	0.54	1.74	1.62	1.62
Il6	1	1.87	1.08	*3.25*	*3.25*	*3.03*	*3.81*	1.32	0.99	*3.25*	1.87	0.91	**0.50**	1.28	*2.83*	1.87	*2.30*	0.87	**0.28**	*6.06*	0.81	1.52	0.93
Mmp9	1	*2.05*	*2.16*	*2.10*	*2.07*	1.37	*2.81*	*2.75*	*2.13*	1.12	1.58	1.27	1.83	*5.89*	1.00	1.20	1.15	1.16	1.21	*9.54*	*2.22*	**0.48**	*2.03*
Slc22a1	1	0.62	0.88	0.76	0.66	0.66	0.59	0.71	0.86	0.81	0.82	1.20	0.71	1.47	**0.41**	0.71	0.62	1.31	1.05	1.23	0.81	0.76	0.71
Slc22a2	1	1.23	1.01	**0.50**	0.93	0.87	**0.39**	**0.38**	**0.33**	**0.38**	**0.33**	**0.08**	0.87	0.69	1.07	1.07	1.62	**0.18**	0.98	**0.38**	0.66	1.15	1.32
2 Fatty liver																							
Esr1	1	1.07	0.62	*3.48*	*4.92*	*4.92*	1.17	1.41	1.31	*2.14*	1.08	1.29	1.23	1.37	1.74	2.64	4.29	1.51	1.48	1.23	*4.59*	*6.06*	*2.30*
Fabp1	1	0.87	1.16	0.81	0.81	1.00	0.77	0.66	1.40	0.87	0.94	1.29	0.87	1.12	0.87	0.93	0.93	1.07	1.29	**0.50**	1.23	1.00	0.93
Hiat1	1	0.93	1.43	1.07	1.07	1.32	1.90	1.15	1.14	1.15	1.33	**0.10**	1.00	**0.14**	1.41	1.07	0.93	1.41	1.20	**0.15**	1.32	1.32	1.32
Hsd11b1	1	*4.92*	*6.13*	*2.64*	*4.92*	*2.83*	*3.81*	1.07	0.99	*2.14*	*3.50*	1.04	0.87	0.97	*2.83*	1.87	*2.46*	0.93	*2.77*	0.93	*3.73*	*5.28*	*4.92*
Il5	1	1.15	0.67	1.00	0.76	0.76	1.26	1.52	0.86	*2.83*	1.52	0.85	1.32	1.28	*3.03*	*3.25*	*3.48*	1.51	1.70	1.07	0.93	1.32	1.32
Mthfr	1	**0.47**	**0.44**	1.87	*3.25*	*2.64*	*3.19*	*3.73*	1.50	**0.50**	**0.38**	1.48	0.66	1.69	0.66	0.71	0.87	**0.46**	**0.40**	*2.00*	0.57	0.66	**0.50**
Tnf	1	0.87	0.62	1.23	1.41	1.15	1.17	0.93	0.93	0.71	0.66	0.79	1.41	0.85	1.62	1.07	1.15	0.93	0.52	1.23	*3.25*	0.81	0.71
Trp63	1	*3.00*	*2.44*	*2.40*	1.06	0.97	1.70	*2.03*	1.58	1.81	1.35	0.84	1.19	1.33	1.55	2.12	1.62	1.23	1.12	1.11	0.74	1.19	0.90
Ugt1a1	1	0.93	1.43	1.28	1.23	1.15	0.99	0.71	*2.06*	1.04	0.88	1.36	1.04	1.25	0.95	0.95	1.08	1.51	1.34	0.69	1.42	1.12	1.08
3 Liver ischemic injury																							
Ces2	1	0.81	1.08	1.32	1.32	1.23	1.44	1.00	*2.81*	1.23	1.42	0.85	1.62	*2.23*	1.74	1.62	1.52	*3.24*	*2.77*	1.23	1.87	1.41	1.52
Ces3	1	1.74	*3.29*	*2.46*	*3.03*	*3.03*	*2.88*	1.32	0.70	0.87	1.00	0.64	**0.44**	0.60	**0.27**	**0.44**	0.57	**0.33**	1.48	0.71	1.23	1.62	1.62
Creb1	1	0.93	1.01	0.93	**0.50**	1.00	0.55	0.66	1.06	1.23	0.94	0.91	0.87	0.91	0.87	1.41	1.15	1.14	0.85	0.93	1.23	1.07	1.00
Fos	1	*28.43*	*16.11*	*8.54*	*3.07*	*4.82*	*2.07*	*3.72*	*3.50*	*2.83*	*2.63*	*3.21*	1.77	*2.03*	*2.76*	1.65	1.69	1.90	1.71	1.12	*2.44*	0.88	1.00
Per1	1	*3.03*	*3.49*	*4.29*	*3.91*	*3.73*	*3.09*	0.93	1.61	**0.38**	0.71	1.38	**0.44**	1.81	**0.44**	**0.33**	**0.47**	0.75	0.79	1.15	*3.48*	*4.00*	*3.48*
4 Drug hepatitis																							
Bcl2	1	0.82	0.64	1.12	1.00	1.19	1.17	1.15	0.85	**0.33**	**0.37**	1.80	**0.32**	0.79	**0.44**	**0.39**	**0.46**	0.81	**0.38**	0.84	0.50	0.68	0.64
Cyp2d6	1	0.76	**0.33**	1.62	1.00	1.15	1.35	1.62	1.14	1.15	1.24	0.60	0.57	0.74	0.81	0.81	0.66	1.73	0.65	1.07	0.66	**0.44**	**0.50**
Egr1	1	*17.15*	*18.59*	*13.93*	*2.30*	*2.00*	*2.68*	*3.48*	0.86	1.62	*2.84*	0.64	*3.03*	0.56	*2.83*	*3.25*	*3.73*	*4.58*	*2.25*	0.66	*3.03*	1.00	0.93
Esr1	1	1.07	0.62	*3.48*	*4.92*	*4.92*	1.17	1.41	1.31	*2.14*	1.08	1.29	1.23	1.37	1.74	*2.64*	*4.29*	1.51	1.48	1.23	*4.59*	*6.06*	*2.30*
Gstm1	1	1.32	*2.17*	1.32	1.52	1.52	1.26	0.66	1.61	1.23	1.42	1.12	1.23	1.69	1.41	1.52	1.52	1.73	*2.10*	1.07	1.62	1.52	1.32
Il5	1	1.15	0.67	1.00	0.76	0.76	1.26	1.52	0.86	*2.83*	1.52	0.85	1.32	1.28	*3.03*	*3.25*	*3.48*	1.51	1.70	1.07	0.93	1.32	1.32
Mthfr	1	**0.47**	**0.44**	1.87	*3.25*	*2.64*	*3.19*	*3.73*	1.50	**0.50**	**0.38**	1.48	0.66	1.69	0.66	0.71	0.87	**0.46**	**0.40**	*2.00*	0.57	0.66	**0.50**
Nat2	1	1.00	1.89	1.07	*2.30*	1.32	1.09	1.41	0.99	*3.25*	*4.02*	1.29	1.07	1.04	*3.48*	*2.83*	*3.25*	*2.63*	1.70	1.41	*3.48*	1.74	*2.46*
Pten	1	0.66	1.08	0.93	**0.50**	**0.47**	0.72	0.76	0.86	1.23	1.33	0.79	1.41	1.04	1.74	1.32	1.23	1.62	1.12	1.41	1.52	1.32	1.23
Ptgs2	1	0.57	0.90	*2.14*	1.23	1.41	1.17	1.41	1.31	0.62	0.66	0.85	**0.12**	1.12	**0.47**	0.71	1.15	**0.31**	0.69	1.00	**0.44**	**0.33**	**0.47**
Trp63	1	*3.00*	*2.44*	*2.40*	1.06	0.97	1.70	2.03	1.58	1.81	1.35	0.84	1.19	1.33	1.55	*2.12*	1.62	1.23	1.12	1.11	0.74	1.19	0.90
5 Granulomatous disease of the liver																							
Cyp2d6	1	0.76	**0.33**	1.62	1.00	1.15	1.35	1.62	1.14	1.15	1.24	0.60	0.57	0.74	0.81	0.81	0.66	1.73	0.65	1.07	0.66	**0.44**	**0.50**
6 Peliosis hepatis																							
Erbb2	1	**0.50**	**0.10**	1.74	0.66	0.62	**0.34**	0.62	1.40	**0.29**	**0.17**	1.12	**0.41**	1.47	**0.50**	**0.41**	**0.47**	**0.15**	**0.15**	1.15	**0.25**	**0.23**	**0.31**
Sult1a1	1	1.62	*2.17*	1.41	1.52	1.41	1.26	0.76	**0.22**	1.00	1.24	1.12	1.15	**0.49**	1.87	1.32	1.41	1.62	1.70	0.71	1.74	1.62	1.62

Italic numbers: Genes are up-regulated more than twofold; Bold numbers: Genes are down-regulated more than twofold.

Initial expression time of genes associated with drug-induced liver diseases during LR

At each time point of LR, the numbers of initial up-, down-regulation and total up-, down-regulation genes were in the sequence: both 6 and 2 at 0.5 h; 3, 3 and 9, 5 at 1 h; 3, 1 and 10, 1 at 2 h; 1, 2 and 10, 2 at 4 h; 0, 0 and 8, 1 at 6 h; 0, 0 and 8, 2 at 8 h; 0, 1 and 6, 2 at 12 h; 2, 1 and 5, 3 at 16 h; 1, 1 and 6, 5 at 18 h; 0, 0 and 4, 4 at 24 h; 1, 1 and 3, 3 at 30 h; 0, 0 and 2, 6 at 36 h; 0, 0 and 4, 3 at 42 h; 0, 1 and 7, 7 at 48 h; 0, 0 and 5, 4 at 54 h; 0, 0 and 8, 3 at 60 h; 0, 0 and 4, 5 at 66 h; 0, 0 and 5, 4 at 72 h; 0, 1 and 4, 4 at 96 h; 1, 0 and 8, 3 at 120 h; 0, 0 and 3, 4 at 144 h , 0, 0 and 5, 6 at 168 h (Figure 2).

Expression similarity and time relevance of genes associated with drug-induced liver diseases during LR

Thirty-two genes mentioned above during LR could be characterized based on their similarity in expression as follows: only up-, predominantly up-, only down-, predominantly down-, and up-/down-regulated, involving 13, 4, 11, 3 and 2 genes, respectively (Figure 3). They could also be classified according to the time relevance into 15 groups, including 0.5 and 1 h, 2 h, 4 and 6 h, 8 h, 12 h, 16 h, 18 and 24 h, 30 h, 36 h, 42 and 96 h, 48 h, 54 h and 60 h, 66 and 72 h, 120 h, 144 and 168 h. Their times of up- and down-regulation genes were respectively 15 and 7, 10 and 1, 18 and 3， 8 and 2, 6 and 2， 6 and 3, 10 and 9, 3 and 3, 6 and 2, 8 and 7, 7 and 7, 13 and 7, 9 and 9, 8 and 3, 8 and 10 (Figure 3).

Expression patterns of genes associated with drug-induced liver diseases during LR

Thirty-two genes mentioned above during LR might be

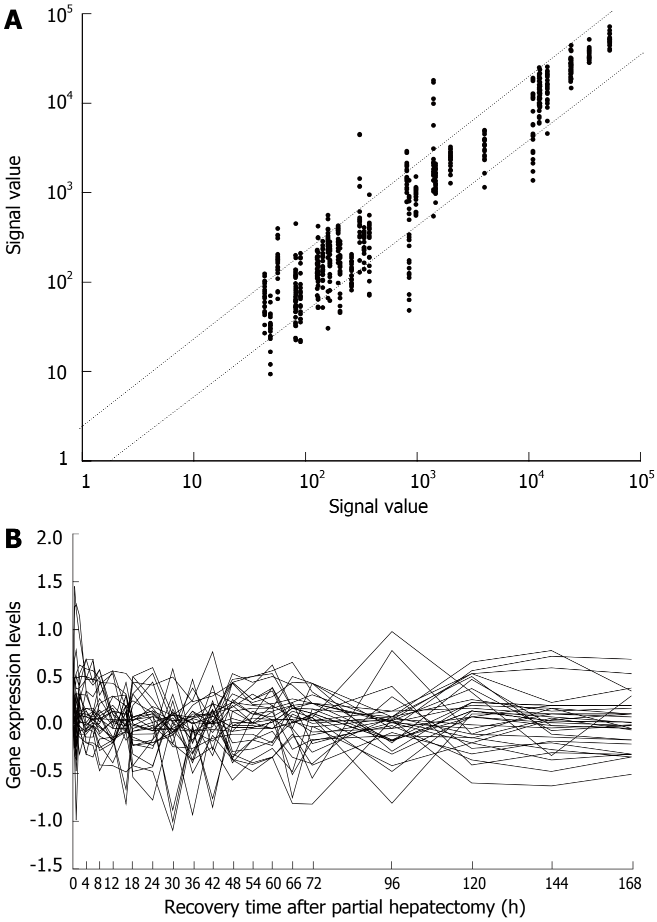

Figure 1 Expression profiles of 32 genes associated with drug-induced liver diseases during liver regeneration. **A**: The abundance and frequency of gene expression, each point represents the signal value of one gene at corresponding time point. The dots above bias represent the genes up-regulated by more than twofold, those under bias represent the genes down-regulated by more than twofold, and the ones between biases represent the genes meaningless alteration in expression. The farer the genes from the bias, the greater the folds of gene change; **B**: The expression changes of genes associated with LR.

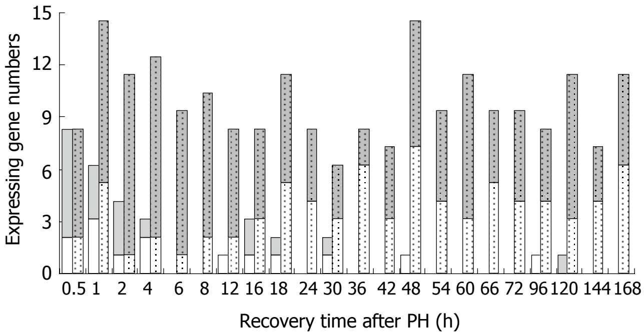

Figure 2 The initial and total expression profiles of 32 genes associated with drug-induced liver diseases at each time point of liver regeneration. Blank bars: Initial expressing gene number; Dotted bars: Total expressing; Grey bars: Up-regulated; White bars: Down-regulated.

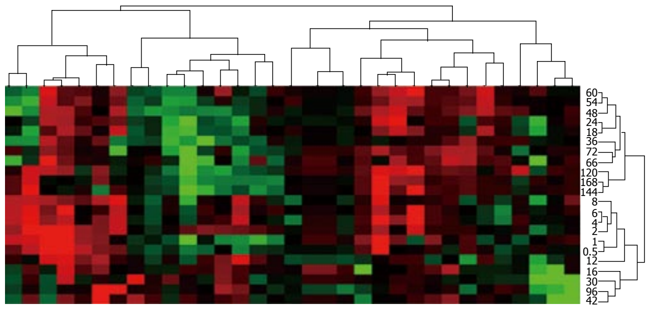

Figure 3 Expression similarity and time relevance clusters of 32 genes associated with drug-induced liver diseases during liver regeneration. Detection data of Rat Genome 230 2.0 array were analyzed by H-clustering. Red: Up-regulation genes; Green: Down-regulation; Black: No-sense in expression change; The upper and right trees respectively show expression cluster and time series cluster.

categorized according to the changes in expression into 20 types of patterns: (1) up-regulation at one time point, i.e. at 16, 120 h after PH, (Figure 4A), 2 genes; (2) up- at two time point, i.e. at 1 and 72 h, 30 and 42 h, (Figure 4B), 2 genes; (3) up- at one time point/phase, i.e. at 18 and 48-72 h (Figure 4C), 1 gene; (4) up- at one time point/two phases (Figure 4C), 1 gene; (5) up- at one time point/three phases (Figure 4C), 1 gene; (6) up- at two time points/one phase (Figure 4D), 2 genes; (7) up- at three time points/two phases (Figure 4E), 2 genes; (8) up- at three time points/phases (Figure 4D), 1 gene; (9) down- at one time point, at 0.5, 48 or 96 h (Figure 4F), 3 genes; (10) down- at two time points, i.e. at 1 and 168 h (Figure 4G), 1 gene; (11) down- at three time points(Figure 4G), 1 gene; (12) down- at more time points (Figure 4G), 1 gene; (13) down- at one phase, i.e. at 1-6 h (Figure 4H), 1 gene; (14) down- at one time point/phase, i.e. at 1 and 144-168 h (Figure 4H), 1 gene; (15) down- at two time points/phases (Figure 4H), 1 gene; (16) down- at two time points/four phases (Figure 4H), 1 gene; (17) down- at three time points/one phase (Figure 4H), 1 gene; (18) predominantly up- (Figure 4I), 4 genes; (19) predominantly down- (Figure 4J), 3 genes; (20) up/down- approximately (Figure 4K), 2 genes.

DISCUSSION

In this paper, the roles of 84 genes associated with drug-induced liver diseases during liver regeneration were analyzed. Of the 36 genes associated with drug-induced abnormality of cell proliferation and apoptosis, cocaine addiction-associated cAMP responsive element binding protein 1 (CREB1)[18] and estradiol-induced interleukin 6 (IL6)[19] were related to liver regeneration initiation[20]. Cocaine-induced V-fos FBJ murine osteosarcoma viral oncogene homolog (FOS)[21], troglitazone-induced early growth response 1 (EGR-1)[22], prostaglandin-endoperoxide synthase 2 (PTGS2) repressed by cyclophosphamide[23], estradiol-activated Akt (v–akt) murine thymoma viral oncogene homolog 1 (AKT1)[24] and estradiol-induced brain derived neurotrophic factor (BDNF)[25] all promote cell growth or cell division[26,27]. Valproic acid-restrained estrogen receptor 1 (ESR1)[28] inhibits cell division[29]. Period homolog 1 (Drosophila) (PER1) promotes apoptosis[30]. Cyclophosphamide-induced B-cell leukemia/lymphoma 2 (BCL2) restrains apoptosis[31]. Diethylstilbestrol-restrained transformation related protein 63 (TRP63)[32] is associated with differentiation[33]. Estradiol-induced matrix metallopeptidase 9 (MMP9)[34] is involved in the breakdown of extracellular matrix. Indomethacin-induced phosphatase and tensin homolog (PTEN)[35] blocks tumor cell proliferation and migration[36].

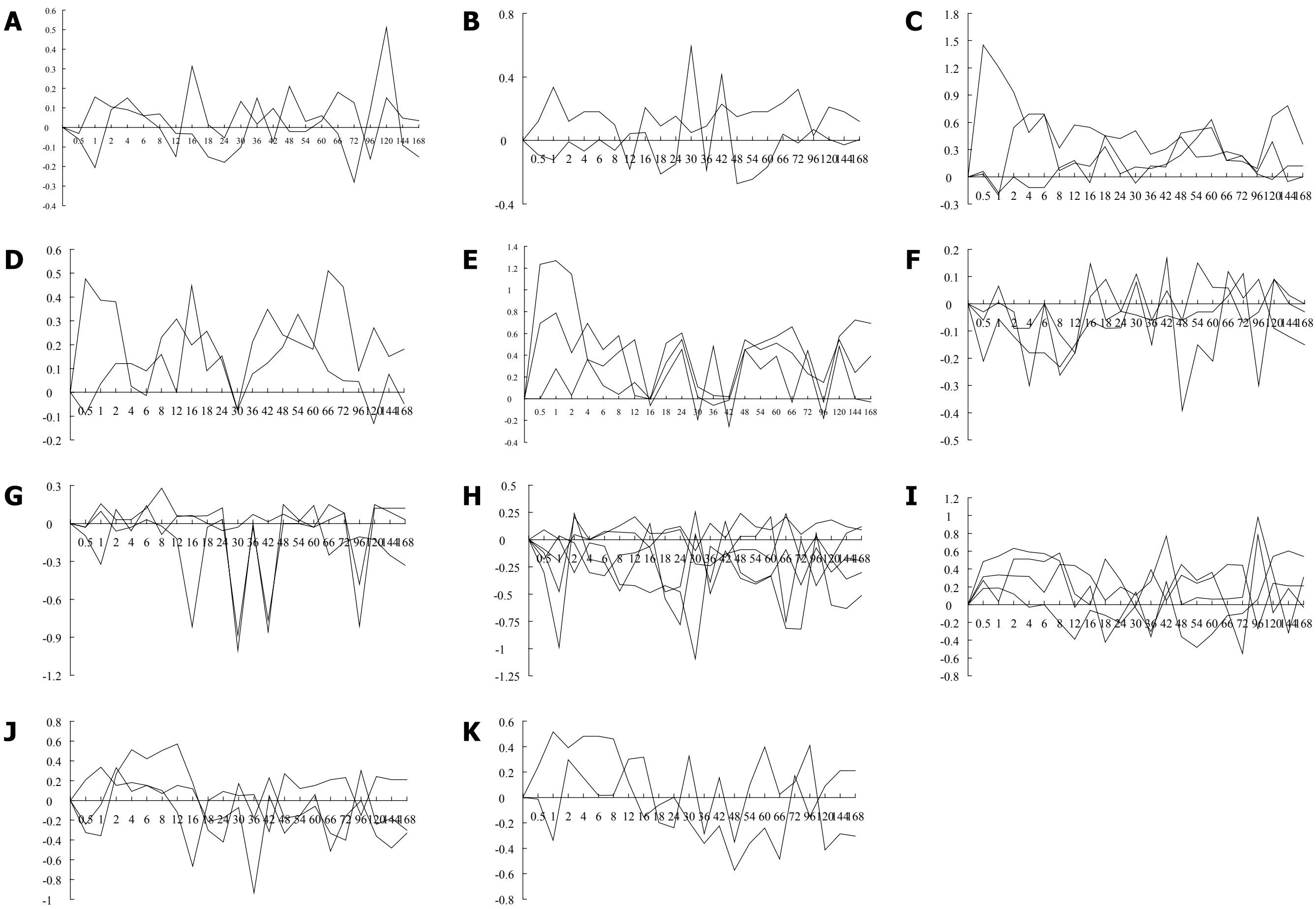

Figure 4 Expression patterns of 32 genes associated with drug-induced liver diseases during liver regeneration. These genes exhibit 20 types of expression patterns. **A-E**: Up-regulation in expression; **F-H**: Down-regulation; **I-J**: Up/down-regulation mixed. X-axis represents recovery time after PH (h), Y-axis shows logarithm ratio of the signal values of genes at each time point to the control.

Angiotensin I converting enzyme (ACE), whose activity is inhibited by captopril[37], participates in the control of blood pressure. The sameness or similarity in some time points, and the difference in other points of meaningful expression changes of these genes during LR may indicate that they regulate the mass of regenerating liver together.

Of the 21 genes associated with drug-induced disorder of lipid metabolism or amino-acid metabolism, estradiol-induced apolipoprotein E (APOE)[38] and fatty acid binding protein 1, liver (FABP1) play a part in the metabolism and transport of lipid[39,40]. One of the tamoxifens' target proteins: epoxide hydrolase 1, microsomal (EPHX1)[41], and tetracyclin-induced tumor necrosis factor (TNF)[42] participate in lipid metabolism, and 5, 10-methylenetetrahy drofolate reductase (MTHFR)[43] plays a role in methionine biosynthesis. That meaningful expression changes of these genes are the same or similar in some time points, then different in other points during LR perhaps regulate the metabolism of lipid and/or amino-acid together.

Of the 27 genes associated with drug metabolism disorder, six genes including solute carrier family 22 member 1, 2, (SLC22A1, SLC22A2), UDP glucuronosyltransferase 1 family A1 (UGT1A1), glutathione S-transferase M1 (GSTM1), amitriptyline-restrained cytochrome P450 family 2 subfamily D 6 (CYP2D6)[44] and sulfotransferase family cytosolic 1A phenol-preferring member 1 (SULT1A1) are involved in drug metabolism[45-47]. Hippocampus abundant gene transcript 1 (HIAT1) is responsible for transmembrane of tetracyclin[48]. N-acetyltransferase 2 (NAT2) catalyzes decomposition of isoniazid. Hydroxysteroid (11-β) dehydrogenase 1 (HSD11B1) can inactivate cortisol[49]. Carboxylesterase 2, 3 (CES2，CES3) catalyze the hydrolysis of fatty acids and cocaine[50]. Interleukin 5 (IL5) is associated with corticosteroid resistance[51] and inflammation[52]. V-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (ERBB2) can impede the function of tamoxifen[53]. The expression changes of the genes mentioned above were the same or similar at some time points and different at other time points during LR, speculating that they promote drug metabolism together.

In conclusion, some genes associated with drug-induced liver diseases are up-regulated and the others are down-regulated during liver regeneration. In liver regeneration, some drug-induced liver diseases-related genes regulate the liver cell number by adjusting cell proliferation and apoptosis, some control lipid metabolism or amino acid metabolism, and others participate and modulate drug metabolism, demonstrating that they are closely in line with liver regeneration. We will use northern blotting, protein array, RNA interference etc. to further confirm the above results at the cell level in the future.

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