Expression patterns and action analysis of the toxic liver injury-associated genes during rat liver regeneration after partial hepatectomy☆

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Abstract

Objective. The aim of this paper was to study the actions of the genes associated with toxic liver injury during liver regeneration (LR) at transcriptional level. *Methods*. The toxic liver injury-associated genes were obtained by collecting the data of databases and referring to thesis, and the gene expression changes in rat regenerating liver after partial hepatectomy (PH) were checked by the Rat Genome 230 2.0 Array. *Results*. A total of 87 genes were found to be liver regeneration-associated. The initial and total expressing number of 87 gene occurring in initial phase (0.5 – 4 h after PH), G0/G1 transition (4 – 6 h after PH), cell proliferation (6 – 66 h after PH), cell differentiation and structure-functional reconstruction (72 – 168 h after PH) were 31, 4, 13, 1 and 31, 23, 41, 34, respectively, illustrating that the associated genes were triggered mainly at early stage of LR, and worked at different stages. According to expression similarity, these genes were classified into 5 types including only up-regulated, predominantly up-, only down-, predominantly down-, and equally up- and down-, involving 21, 8, 9, 5 and 2 genes respectively; and the total frequencies of up-regulation and down-regulation were 236 and 106 respectively, demonstrating that expression level of most genes was increased during LR, while that of the minor was decreased. The classification of their time relevance and expression patterns into 15 and 21 groups respectively, show that the cellular physiological and biochemical activities during LR were staggered, diverse and complicated. *Conclusion*. The drug or hepatotoxin metabolism-involved genes were mainly up-regulated between 18 – 120 h; oxidative stress-induced apoptotic-related genes and proliferative-related genes acted together to increase liver cell number at a fixed rate. [Life Science Journal. 2007; 4(3): 42 – 48] (ISSN: 1097 – 8135).

Keywords: partial hepatectomy; Rat Genome 230 2.0 Array; genes; toxic liver injury; liver regeneration

1 Introduction

Partial hepatectomy (PH)[1], liver injury or hepatotoxin administration[2], acting as stimulus, readily induce the rapid replication of remnant hepatocytes to restore liver mass, which is called liver regeneration (LR). Generally, based on hepatic physiological activities, the regenerative process is divided into initial phase (0.5 – 4 h after PH), G0/G1 transition (4 – 6 h after PH), cell proliferation (6 – 66 h after PH), cell differentiation and structure-functional reorganization (72 – 168 h after PH)[3], and involves many physiological and biochemical events including cell activation, de-differentiation, proliferation and re-differentiation[4], which undergo the regulation by many factors, such as cytokines, hormones, drugs and hepatotoxin[5].

The liver is the main detoxification organ of the body[6]. And the impairment of liver detoxification function would lead to severe liver failure[7]. The toxic liver injury is an acute or chronic liver disease caused by hepatotoxin[8] and mainly occurs to hepatocyte. The pathogenesis is that the oxidative poison caused lipocyte and hepatocyte to undergo denaturalization, necrosis, peroxisome hyperplasia, H_2O_2 metabolism perturbation, the depression of antidotic function of liver, which leads to cells stress injury. Furthermore, some other hepatotoxin may

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inhibit or interfere with protein synthesis, bile secretion, haemachrome synthesis or induce cells mutation and subsequently bring liver diseases by disturbing metabolism obstacle respectively.

Both PH and toxic liver injury can cause compensatory proliferation of the liver cells more or less[11,12]. Studying the correlation between the physiological and biochemical activities in LR and that in liver toxicity will contribute to clarifying the molecular mechanism of LR and to establishing the treatment, prevention methods of the toxic liver injury[18]. Therefore, we employed the Rat Genome 230 2.0 Array containing 87 toxic liver injury-involved genes to check genes expression changes in rat regenerating liver post PHx, finding 45 of them to be LR-associated. And the expression dynamics, patterns and actions of these genes in LR were primarily analyzed.

2 Materials and Methods

2.1 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 two groups at random and each group included 6 rats (male:female = 1:1). PH was performed according to Higgins and Anderson[19], 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, 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 point. The livers were rinsed three times in PBS at 4 °C, and then total 1 – 2 g livers (100 – 200 mg livers from middle parts of right lobe of samples) were gathered and stored at – 80 °C. The sham-operation (SO) groups were the same procedure as PH groups except the liver lobes unresolved. 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 reagent (Invitrogen Corporation, Carlsbad, California, USA)[20] and then purified base on the guide of RNeasy mini kit (Qiagen, Inc, Valencia, CA, USA)[21]. 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 h). Total RNA concentration and purity were estimated by optical density measurements at 260/280 nm[22].

2.3 cDNA, cRNA synthesis and purification

1 – 8 µg total RNA as template was used for cDNA synthesis. cDNA purification was based on the way established by Affymetrix[23]. cRNA labeled with biotin was synthesized using cDNA as the template, and cDNA and cRNA were purified according to the purification procedure of GeneChip Analysis[23]. 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 at 94 °C for 35 min was digested into 35 – 200 bp fragments. The hybridization buffer prepared according to the way Affymetrix provided was added to the prehybridized Rat Genome 230 2.0 microarray produced by Affymetrix, then hybridization was carried out at 45°C for 16 h on a rotary mixer at 60 rpm. 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[24].

2.5 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 GCOS (GeneChip operating software) 1.2[24].

2.6 Normalisation of the microarray data

To minimize error from the microarray analysis, each analysis was performed three times. Results that a total ratio were maximal (R^m) and that the average of three housekeeping genes β-actin, hexokinase and glyseraldehyde-3-phosphate dehydrogenase approached 1.0 (R^h) were 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 (normalization analysis program) software according to the cell cycle progression of the regenerating hepatocytes. Data statistics and cluster analysis were done using GeneMath, GeneSpring (Silicon Genetics, San Carlos, CA), Microsoft Excel (Microsoft, Redmond, WA) software[24-26].

2.7 Identification of genes associated with LR

Firstly, the nomenclature of a biological process (e.g. toxic liver injury) was adopted from the GENEONTOLOGY database (www. geneontology. org), and inputted 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 specific biological process. 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 this analysis were codified, and compared with the results obtained for mouse and human searches in order to identify human genes which are different from those of rat. Comparing these genes with the analysis output of the Rat Genome 230 2.0 Array, those genes which showed more than twofold changes in expression level were referred to as rat homologous or rat specific genes associated with the biological process under evaluation. Genes, which displayed reproducible results with three independent analysis with the chip and which showed more than twofold change in expression level in at least one time point during LR with significant difference ($0.01 \leq P < 0.05$) or extremely significant difference ($P \leq 0.01$) between PH and SO, were referred to as associated with LR.

3 Results

3.1 General statement of expression of the toxic liver injury-associated genes during LR

According to the data of databases at NCBI, GENEMAP, KEGG and BIOCARTA, 87 genes were associated with liver diseases. In which, 75 genes were contained in Rat Genome 230 2.0 Array. Among them, 45 genes revealed meaningful expression changes at least at a time point after PH, showed significant difference or extremely significant difference in expression when comparing PH with SO and displayed reproducible results with three detection with Rat Genome 230 2.0 Array, suggesting that the genes were associated with LR. The analysis indicated that 21 genes were up-, 9 genes down-, and 15 genes up/down-regulated during LR. The range of up-regulation was from 2 to 128 times higher than control, and that of down-regulation was 2 – 12 folds (Table 1).

3.2 Expression changes of the toxic liver injury-associated genes during LR

At each time point of LR, the numbers of initial up-, down-regulated and total up-, down-regulated gene were in sequence: both 13 and 6 at 0.5 h; 1, 3 and 13, 8 at 1 h; 4, 0 and 14, 2 at 2 h; 3, 1 and 17, 4 at 4 h; 1, 0 and 14, 3 at 6 h; 0, 0 and 12, 4 at 8 h; 0, 0 and 9, 6 at 12 h; 3, 1 and 10, 5 at 16 h; 2, 3 and 14, 10 at 18 h; 0, 1 and 12, 9 at 24 h; 1, 0 and 5, 3 at 30 h; 0, 0 and 5, 5 at 36 h; 1, 0 and 8, 4 at 42 h; 0, 0 and 13, 5 at 48 h; 0, 0 and 8, 6 at 54 h; 0, 0 and 11, 4 at 60 h; 0, 0 and 8, 2 at 66 h; 0, 0 and 9, 6 at 72 h; 0, 0 and 8, 1 at 96 h; 1, 0 and 13, 2 at 120 h; 0, 0 and 10, 7 at 144 h; 0, 0 and 10, 4 at 168 h. In the aspect of the initial expression of above 45 genes, 30 initially up-regulated genes and 15 initially down-regulated were confirmed during LR, respectively; at initial stage of LR (0.5 – 4 h after PH), G0/G1 transition (4 – 6 h after PH), cell proliferation (6 – 66 h after PH), cell differentiation and the structure-function reorganization (72 – 168 h after PH), the number of initially up and initially down-regulated genes were 21 and 10, 4 and 1, 8 and 5, 1 and 0. The whole situation of genes expression was that the total frequencies of up and down-regulated expression were 236 and 106 respectively; at the above-mentioned four phases of LR, that of up-regulation and down-regulation was in sequence 57 and 20, 31 and 7, 129 and 66, 50 and 20 (Figure 1).

3.3 Expression similarity and time relevance of the toxic liver injury-associated genes during LR

The 45 genes mentioned above during LR could be characterized based on their similarity in expression as follow: only up-, predominantly up-, only down-, predominantly down-, and up-/down-regulated, involved in 21, 8, 9, 5 and 2 genes, respectively (Figure 2). They could also be classified based on 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 and 42 h, 36 h, 48 h, 54 and 60 h, 66 and 72 h, 96 h, 120 h, 144 and 168 h, in which the times of up-regulation and down-regulation of these genes were 26 and 14, 14 and 2, 31 and 7, 12 and 4, 9 and 6, 10 and 5, 26 and 19, 13 and 7, 5 and 5, 13 and 5, 19 and 10, 17 and 8, 8 and 1, 13 and 2, 20 and 11, respectively (Figure 2).

3.4 Expression patterns of the toxic liver injury-associated genes during LR

The 45 genes mentioned above during LR might be categorized according to the changes in expression into 21 types of patterns: (1) up-regulation at one time point, i.e. 0.5, 30, 120 h after the rat partial hepatectomy (Figure 3A), 3 genes involved; (2) up-regulation at two time points, i.e. 16 and 96 h (Figure 3B), 1 gene involved; (3) up-regulation at three time points (Figure 3B), 1 gene involved; (4) up-regulation at two phases, i.e. 16-24 and 42 – 48 h(Figure 3C), 1 gene involved; (5) up-regulation at three phases (Figure 3C), 1 gene involved; (6) up-regulation at one time point/phase, i.e. 42 – 48 and 96 h; 48 and 2 – 24 h (Figure 3D), 2 genes involved; (7) up-regulation at one time point/two phases (Figure 3E), 3 genes involved; (8) up-regulation at one time point/three phases (Figure 3F), 1 gene involved; (9) up-regulation at two time points/one phase (Figure 3F), 1 gene involved; (10) up-regulation at two time points/three phases (Figure 3G), 1 gene involved; (11) up-regulation at three time points/two phases (Figure 3H), 2 genes involved; (12) down-regulation at two time points, i.e. 1 and 168, 24and 144, 18

Table 1. Expression abundances of the 45 toxic liver injury-associated genes during rat LR

Gene Abbr.	In-volved in others	Recovery time (h) after partial hepatectomy (PH)																						
		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 Lipid metabolism and peroxidation																								
Abcb1		1	0.54	0.58	0.87	0.81	0.87	0.72	1.32	0.61	***4.59***	***3.75***	0.52	***3.03***	0.64	1.41	1.62	1.32	***3.98***	***2.77***	0.54	1.23	0.71	0.87
Cat		1	***2.83***	1.48	1.07	1.28	1.12	1.73	1.08	1.12	0.73	0.62	1.31	0.71	1.09	0.62	1.00	1.07	0.77	1.34	1.00	1.05	1.58	1.05
Fmo3		1	1.15	1.76	1.74	1.74	***2.00***	***2.19***	***2.26***	***3.01***	0.76	1.24	***4.48***	0.81	***4.16***	0.93	0.66	0.66	0.93	1.20	1.15	1.41	1.23	1.32
Lpin1		1	***9.55***	***15.01***	***14.47***	***12.29***	***10.70***	***10.50***	1.43	0.77	0.96	1.43	0.70	0.77	1.04	1.35	1.62	1.01	0.99	***2.19***	0.87	***3.45***	***2.68***	***3.45***
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***
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*
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
CD36		1	*0.10*	*0.22*	*0.20*	*0.20*	*0.41*	*0.24*	*0.23*	*0.25*	*0.27*	*0.11*	1.95	*0.14*	1.47	*0.13*	*0.27*	*0.23*	0.53	*0.37*	0.93	0.62	*0.50*	0.62
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*
Hmgcr		1	*0.35*	0.88	0.90	*0.24*	*0.25*	*0.37*	*0.48*	1.09	0.81	*0.28*	0.72	0.65	1.08	*0.28*	0.73	0.67	1.03	0.67	0.85	0.88	0.90	0.75
Fmo1		1	***2.46***	***2.86***	***2.00***	1.87	0.71	0.67	*0.44*	*0.42*	*0.38*	0.54	0.97	1.52	1.37	0.66	1.07	1.23	1.86	1.83	1.32	***2.64***	1.62	1.87
Hao1		1	1.41	***2.32***	0.93	1.15	0.81	0.72	*0.47*	0.61	1.23	1.42	0.74	1.07	0.79	1.07	1.41	1.32	1.86	1.96	1.23	1.74	***2.00***	1.74
2 Other metabolism																								
Aldoa		1	1.07	1.08	1.07	1.00	1.74	1.66	1.41	1.98	1.23	1.75	0.91	1.41	***2.08***	***2.14***	1.15	1.23	1.62	1.20	***3.48***	1.15	1.07	1.00
Aldob		1	1.00	1.24	0.93	0.76	0.81	0.63	0.71	1.22	0.87	1.08	***2.40***	0.87	1.94	0.93	0.93	0.81	1.14	1.20	1.52	1.15	1.07	0.93
Ccl2	3	1	***7.46***	***2.67***	1.62	1.62	1.23	1.09	***5.66***	***8.12***	***10.56***	***18.47***	1.20	***14.93***	1.20	***1.28***	***4.92***	***8.57***	***4.58***	***2.77***	1.15	***2.46***	0.57	0.66
Fbp1		1	0.93	1.24	0.87	1.07	1.07	1.09	0.81	***3.01***	1.00	1.15	1.38	0.87	1.81	0.81	1.15	1.07	1.31	1.20	***2.00***	1.15	1.07	1.00
Got1		1	***3.73***	***5.72***	***6.06***	***6.96***	***12.13***	***12.37***	***3.48***	***2.84***	***2.30***	***3.50***	1.20	***2.30***	1.04	***2.00***	1.74	***2.83***	***3.24***	***2.96***	1.00	***4.29***	***3.73***	***3.73***
Pck1		1	***2.14***	***3.07***	1.87	1.74	1.74	***2.04***	1.15	1.40	1.62	***2.01***	1.12	1.23	1.12	1.23	1.87	1.41	***2.13***	***2.41***	1.32	***3.25***	***2.30***	***2.14***
Pklr		1	***3.51***	***3.16***	***3.46***	***6.59***	***4.66***	0.79	1.13	1.01	***2.20***	***2.49***	1.08	0.51	1.09	1.37	1.22	0.77	0.84	1.22	1.00	0.81	***2.80***	***3.17***
Ttr		1	0.93	1.24	0.87	0.93	1.07	0.95	0.71	***6.91***	0.87	0.94	***2.76***	0.93	1.94	1.00	0.93	1.00	1.14	1.38	***3.48***	1.52	1.23	1.07
Irs1		1	1.41	1.16	1.74	1.74	1.74	1.35	1.23	0.93	0.87	*0.44*	1.70	1.07	0.97	0.87	0.71	1.23	1.07	0.85	1.07	0.87	*0.50*	0.81
Ca5b		1	***3.01***	***2.05***	***2.15***	***2.38***	1.51	1.64	1.75	0.57	***2.30***	1.39	*0.37*	1.42	0.64	***2.32***	***2.47***	***3.65***	1.93	1.19	0.63	***2.83***	1.93	1.68
Mapk8	3	1	***7.46***	***3.07***	***12.20***	***19.70***	***4.29***	***5.77***	***4.92***	***2.28***	***3.73***	***5.68***	1.70	1.74	*0.49*	***3.03***	***4.59***	***2.00***	1.41	***5.53***	0.93	1.74	***5.66***	0.66
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***
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*
Plau	3	1	1.50	1.36	***2.10***	***2.19***	***2.47***	1.72	1.68	1.07	1.51	1.18	0.68	1.19	*0.36*	1.31	1.34	1.54	***3.00***	1.40	0.53	1.89	***2.08***	***2.19***
Ptpn1		1	0.77	0.88	0.93	***2.15***	1.46	1.60	*0.26*	1.45	0.73	1.16	***2.88***	0.81	***2.79***	1.01	1.06	1.07	1.23	1.13	1.48	1.07	1.23	1.04
Scnn1g		1	1.10	*0.39*	1.43	1.51	***2.03***	1.47	1.44	1.17	*0.38*	0.52	1.60	1.16	***2.14***	0.56	1.19	1.17	0.64	0.79	1.28	1.34	1.25	0.741
3 Regulation of cell proliferation, apoptosis and cell necrosis																								
Ca3		1	***11.46***	***13.96***	***5.96***	***8.63***	***6.89***	***4.40***	1.37	0.74	***3.24***	1.75	0.68	1.52	0.62	1.06	***4.41***	***3.19***	1.37	***6.50***	0.97	***10.39***	***12.85***	***9.99***
Cav		1	***2.46***	***2.55***	***2.64***	***3.73***	***5.28***	***2.04***	***3.48***	***2.62***	***4.00***	***4.02***	1.48	***6.50***	1.20	***10.56***	1.41	***9.19***	***4.90***	***4.19***	0.81	***4.29***	***2.14***	***2.46***
Ccl2	2	1	***7.46***	***2.67***	1.62	1.62	1.23	1.09	***5.66***	***8.12***	***10.56***	***18.47***	1.20	***14.93***	1.20	***1.28***	***4.92***	***8.57***	***4.58***	***2.77***	1.15	***2.46***	0.57	0.66
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***
IL1rn		1	1.87	1.43	***2.83***	***8.00***	***14.93***	***16.32***	***2.46***	***2.63***	***3.03***	***3.75***	1.48	0.81	1.58	***2.83***	0.93	1.00	0.81	0.79	1.41	0.71	0.76	0.81
Tgfb1		1	0.87	0.51	1.15	1.15	1.07	1.02	1.15	***2.13***	***3.71***	***4.02***	1.82	1.23	***2.75***	***2.14***	0.93	1.15	1.62	0.74	1.41	0.93	0.76	0.87
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
Tp53		1	0.85	1.50	0.96	0.89	0.93	1.74	1.32	0.91	***2.40***	***2.87***	0.85	1.38	1.00	***2.26***	***2.22***	***2.05***	1.97	1.76	***2.01***	1.11	1.23	1.07
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
IL1b		1	1.00	1.01	0.87	0.93	1.32	0.63	1.07	0.81	*0.35*	0.82	0.74	0.81	1.04	1.15	*0.47*	0.54	1.41	0.79	1.87	0.66	0.76	0.76
Prf1		1	*0.50*	*0.22*	*0.34*	*0.50*	1.07	*0.36*	0.57	0.81	*0.33*	*0.25*	0.97	0.87	0.97	1.23	*0.41*	0.38	0.57	*0.26*	0.81	1.15	*0.27*	1.07
Edn1		1	1.23	0.62	1.62	1.07	1.32	1.09	1.23	0.71	*0.41*	*0.38*	***2.57***	1.00	1.47	1.00	0.71	1.32	0.75	0.69	1.32	0.81	0.71	0.71
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
Mapk8	2	1	***7.46***	***3.07***	***12.20***	***19.70***	***4.29***	***5.77***	***4.92***	***2.28***	***3.73***	***5.68***	1.70	1.74	*0.49*	***3.03***	***4.59***	***2.00***	1.41	***5.53***	0.93	1.74	***5.66***	0.66
Osmr		1	*0.25*	*0.10*	0.71	0.62	1.62	1.09	1.23	*0.28*	1.23	0.94	*0.17*	0.81	*0.18*	***2.46***	0.66	0.81	1.31	0.60	0.87	*0.50*	*0.25*	*0.29*
Plau	2	1	1.50	1.36	***2.10***	***2.19***	***2.47***	1.72	1.68	1.07	1.51	1.18	0.68	1.19	*0.36*	1.31	1.34	1.54	***3.00***	1.40	0.53	1.89	***2.08***	***2.19***
Retn		1	*0.44*	*0.36*	0.87	1.15	1.07	1.44	0.54	1.40	0.71	*0.29*	0.74	*0.33*	***2.23***	*0.44*	*0.29*	0.57	*0.20*	*0.28*	1.23	*0.18*	*0.29*	0.76
Spp1		1	1.14	1.45	1.19	***2.68***	0.89	1.09	1.16	0.91	*0.47*	0.94	0.88	0.85	0.97	0.66	0.64	0.69	0.82	0.77	0.91	0.57	0.99	0.76
Vegfa		1	0.54	0.88	0.66	*0.13*	*0.38*	*0.41*	*0.44*	*0.38*	*0.20*	*0.47*	1.12	*0.22*	***4.46***	*0.22*	*0.08*	*0.09*	0.81	0.69	***4.29***	1.00	1.00	1.23

1: Lipid metabolism and peroxidation; 2: Other metabolisms; 3: Regulation of cell proliferation, apoptosis and cell necrosis; Bold italic: the genes up-regulated 2-fold or more at some time points in LR; Italic: the genes down-regulated 2-fold or more at some time points in LR.

and 54 h (Figure 3I), 3 genes involved; (13) down-regulation at three time points (Figure 3J), 1 gene involved; (14) down-regulation at one time point/one phase, i.e. 1 and 144-168 h (Figure 3J), 1 gene involved; (15) down-regulation at two time points/two phases (Figure 3J), 1 gene involved; (16) down-regulation at three time points /one phase (Figure 3K), 1 gene involved; (17) down-regulation at three time points /two phases (Figure 3K), 1 gene involved; (18) down-regulation at three time points /three phases (Figure 3K), 1 gene involved; (19) first up- and then down-regulated (Figure 3L), 1 gene involved; (20) first down- and then up-regulated (Figure 3L), 1 gene involved; (21) up/down-regulated mixed (Figure 3M1 – M2), 13 genes involved.

4 Discussion

This paper mainly addresses the roles of toxic liver injury-associated genes in rat LR. It has been known that lipid metabolism disorder and oxidative stress induced by hepatotoxin metabolites are the primary intrinsic causes of toxic liver damage. Interestingly, as demonstrated in Table 1, some pathogenesis-associated genes, such as the drug or hepatotoxin metabolism-involved genes *abcb*1, *nat*2, *fmo*1, *fmo*3 and *cyp*2*d6*[28-31], the lipid metabolism-related genes *lpin*1[21], *apoe*[22], *cd*36[23] and *hmgcr*[24], as well as the lipid peroxidation-participating genes *ace*[25], *hao*1[26] and *cat*[27], exhibited the meaningful expression changes after PH. And the chip detection displayed that above 5 drug or hepatotoxin metabolism-involved genes (that's *abcb*1, *nat*2, *fmo*1, *fmo*3 and *cyp*2*d*6) were dominantly up-regulated between 18 and 120 h, suggesting that the metabolic process is most likely to be increased during this period. In addition, there had been preceding an extremely significant and sustained up-regulation (> 10 folds) of lipid oxidation and degradation-associated *lpin*1 from 0.5 – 8 h, implying that it is key to lipid degratation in early phase of rat LR.

What's more, metabolites of drug or toxin also directly affect the cell physiological and biochemical processes. For example, the intracellular oxidative stress can lead to apoptotic, necrotic cell death, or even cell prolifera-

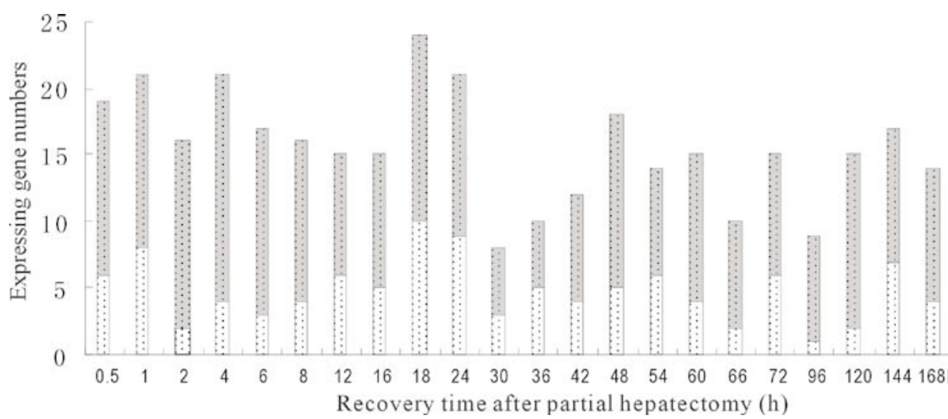

Figure 1. The initial and total expression profiles of the 45 toxic liver injury-associated genes at each time point of LR. Blank bars: Initial expressing gene number; Dotted bars: Total expressing gene number; Grey bars: Up-regulated genes; White bars: Down-regulated genes. Expression change of the genes spans the whole live regeneration. Initially up-regulated genes are predominate at 0.5 – 6, 16 – 18, 30, 42 and 120 h after PH; initially down-regulated genes are overwhelm at 0.5 – 1, 4, 16 – 24 h; there are no initially expressed genes at 8 – 12, 36, 48 – 96, 144 – 168 h.

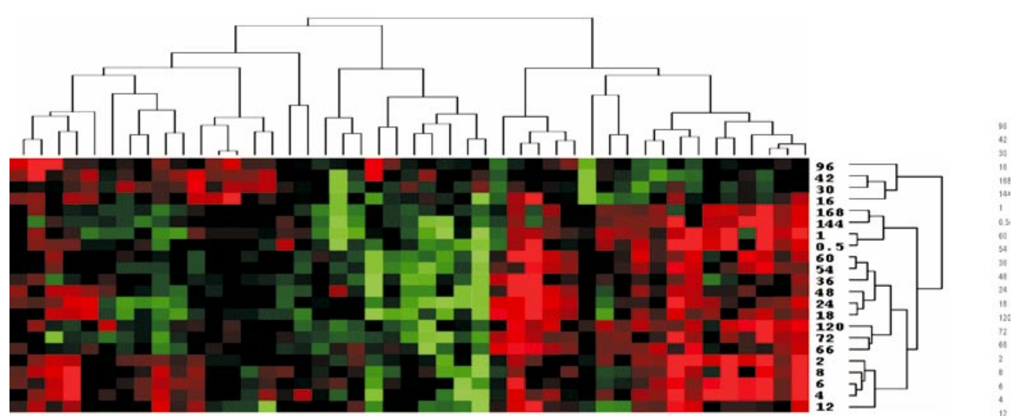

Figure 2. Expression similarity and time relevance clusters of the 45 toxic liver injury-associated genes during LR. Red: Up-regulation genes; Green: Down-regulation; Black: No-sense in expression change; The upper and right trees respectively show expression similarity cluster and time relevance cluster.

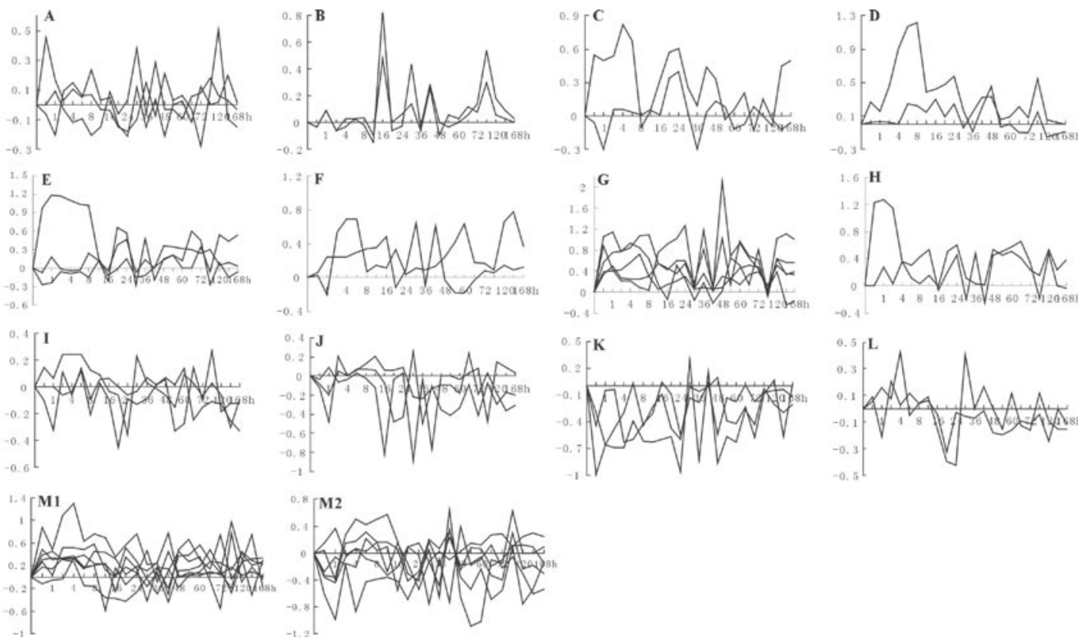

Figure 3. Expression patterns of the 45 toxic liver injury-associated genes during LR. These genes exhibit 21 types of expression patterns. A – H: Up-regulation in expression; L – K: Down-regulation; L – M: Up-/down-regulation mixed. X-axis represents recovery time after PH (h), Y-axis shows logarithm ratio of the signal values of genes at each time point to control.

tion, depending on the balance of factors that activate and inhibit apoptotic/proliferation-related proteins, such as the bcl2 family of proteins and the caspases, which determines the final total liver cell numbers. Table 1 showed that among the genes encoding these proteins, seven genes *il*1*b*, *il*6, *osmr*, *cav*, *tp*53, *tgfb*1 and *tnf* all have the capacity of preventing hepatocyte cell proliferation[32-36] and inducing apoptosis[37]. The expression level of them were elevated mainly at 16 – 24, 48 and 60 h post PH [38,39], meanwhile, *edn*1, supporting cell proliferation by mediating G-protein coupled receptor protein signaling pathway[40], was decreased in mRNA level at 18 – 24 h, which is likely that they can hold down overproliferation of the cell at corresponding stages during LR. Whereas the genes *ca*3, *bcl*2, *vegfa* and *spp*1 are capable of inhibiting cell apoptosis[41-44]. According to the microarray data, the expression of above four genes were strikingly down-regulated at the same period as previously mentioned, perhaps helpful for cell reproduction. Taken together, the above genes regulate the balance between cell proliferation and apoptosis together. Among the necrosis-involved genes, *prf*1 positively regulates necrotic cell death *via* cytolysis[45] and was down regulated almost during the whole LR; *spp*1and *retn*[44] also may protect against necrosis *via* respectively inhibiting fibrosis and massive adipocyte differentiation[46], and expression of the two genes were decreased in LR, which demonstrated they may co-modulate hepatocyte necrotic death caused by metabolism turbulence. Among them, *cav* expression was increased almost during the whole LR, reaching its peak abundance with 10.6 times higher than control at 48 h; *ca*3 exhibited the persistent high expression level at 0.5 – 8 h and arrived at a peak of 14-fold of control at 1 h post PHx. It was presumable that the two genes are important in regulating the balance between cell proliferation and apoptosis during rat LR.

To sum up, expression changes of the toxic liver injury-related genes after rat PH were detected with high-throughput gene expression analysis at transcriptional level, and found there were 27 up-regulated genes, 9 down-regulated and 15 up/down-regulated in LR. These genes together play the role in cell metabolism (e.g. lipid degradation), cell proliferation and apoptosis so that they contribute to liver regenerative progress. And the above results need to be further analyzed with the techniques, such as Northern blotting, protein chip, RNA interference, protein-interaction etc.

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