

## Expression patterns and action analysis of genes associated with inflammatory responses during rat liver regeneration

Heng-Yi Shao, Li-Feng Zhao, Cun-Shuan Xu

Heng-Yi Shao, Cun-Shuan Xu, College of Life Science, Henan Normal University, Xinxiang 453007, Henan Province, China  
Li-Feng Zhao, Key Laboratory for Cell Differentiation Regulation, Xinxiang 453007, Henan Province, China  
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Correspondence to: Professor Cun-Shuan Xu, College of Life Science, Henan Normal University, Xinxiang 453007, Henan Province, China. xucs@x263.net

Telephone: +86-373-3326001 Fax: +86-373-3326524

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associated with liver regeneration, in which 239 LR-associated genes play an important role.

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**Key words:** Partial hepatectomy; Rat Genome 230 2.0 array; Inflammatory response; Genes associated with liver regeneration

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### Abstract

**AIM:** To study the relationship between inflammatory response and liver regeneration (LR) at transcriptional level.

**METHODS:** After partial hepatectomy (PH) of rats, the genes associated with inflammatory response were obtained according to the databases, and the gene expression changes during LR were checked by the Rat Genome 230 2.0 array.

**RESULTS:** Two hundred and thirty-nine genes were associated with liver regeneration. The initial and total expressing gene numbers found in initiation phase (0.5-4 h after PH), G<sub>0</sub>/G<sub>1</sub> transition (4-6 h after PH), cell proliferation (6-66 h after PH), cell differentiation and structure-function reconstruction (66-168 h after PH) of liver regeneration were 107, 34, 126, 6 and 107, 92, 233, 145 respectively, showing that the associated genes were mainly triggered at the beginning of liver regeneration, and worked at different phases. According to their expression similarity, these genes were classified into 5 groups: only up-regulated, predominantly up-, only down-, predominantly down-, up- and down-, involving 92, 25, 77, 14 and 31 genes, respectively. The total times of their up- and down-regulated expression were 975 and 494, respectively, demonstrating that the expressions of the majority of genes were increased, and that of a few genes were decreased. Their time relevance was classified into 13 groups, showing that the cellular physiological and biochemical activities were staggered during liver regeneration. According to gene expression patterns, they were classified into 33 types, suggesting that the activities were diverse and complex during liver regeneration.

**CONCLUSION:** Inflammatory response is closely

### INTRODUCTION

Partial hepatectomy (PH)<sup>[1]</sup>, being severely harmful to the body, can cause inflammatory response<sup>[2]</sup>. Furthermore, it also activates the remaining liver cells to enter into cell cycle to replenish the lost livers, which is called liver regeneration (LR)<sup>[3,4]</sup>. Generally, an inflammatory response goes through the following processes: the change of vascular permeability<sup>[5]</sup> as a result of the tissue injury<sup>[6]</sup>, leukocytes' escape from blood vessel, activation and proliferation of target cells induced by released cytokines, elimination of foreign materials, wound repair and so on<sup>[6]</sup>. It is a defense mechanism developed during the long-term evolutionary process, being closely linked to survival of living organisms<sup>[7]</sup>.

Usually, the process of LR is classified based on the cellular physiological activities into 4 phases consisting of the initiation (0.5-4 h after PH), the G<sub>0</sub>/G<sub>1</sub> transition (4-6 h after PH), the cell proliferation (6-66 h after PH), the cell differentiation and structure-function reorganization (66-168 h after PH)<sup>[8]</sup>, or divided according to time course into 4 phases including forepart (0.5-4 h after PH), prophase (6-12 h after PH), metaphase (16-66 h after PH), and anaphase (72-168 h after PH)<sup>[9]</sup>, in which a variety of physiological and biochemical events including cell activation, de-differentiation, proliferation and its regulation, re-differentiation, reorganization of structure-function<sup>[10]</sup> are involved. The process is regulated by many factors including inflammation<sup>[11]</sup>. Studying the relationship between inflammatory response and liver regeneration at transcriptional level is helpful to clarify the molecular mechanism of liver regeneration<sup>[9,12]</sup>. In the current study,

the expression changes of genes in regenerating liver were detected by Rat Genome 230 2.0 array<sup>[13,14]</sup> containing 409 genes associated with inflammatory response. Two hundred and thirty-nine genes were found associated with liver regeneration<sup>[15]</sup>, and their expression changes, patterns and roles in LR were preliminarily analyzed.

## 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<sup>[3]</sup>: 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, 24, 36, 54, 66, 72, 120, 144 and 168 h after partial hepatectomy and the regenerating livers were examined at corresponding time points. The livers were rinsed three times in PBS at 4°C. Then 100-200 mg liver tissues from middle parts of the right lobe, six samples of each group were gathered and mixed together to a total of 1-2 g (0.1-0.2 g × 6) of liver tissue, and stored at -80°C. The sham-operation (SO) groups were treated the same way with partial hepatectomy ones except that the liver lobes were unremoved. The laws of animal protection of China were followed strictly.

### *RNA isolation and purification*

Total RNA was isolated from frozen livers according to the manual of Trizol kit (Invitrogen)<sup>[16]</sup> and then purified based on the guide of RNeasy mini kit (Qiagen)<sup>[17]</sup>. Total RNA samples were examined and exhibited a 2:1 ratio of 28S 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<sup>[18]</sup>.

### *cDNA, cRNA synthesis and purification*

As template, 1-8 µg total RNA was used for cDNA synthesis. cDNA and cRNA synthesis were proceeded according to the methods established by Affymetrix<sup>[13]</sup>. cRNA labeled with biotin was synthesized using 12 µL of the above synthesized cDNA as the template, and cDNA and cRNA were purified<sup>[13]</sup>. Measurement of concentration, purity and quality of cDNA and cRNA was the same as above.

### *cRNA fragmentation and microarray detection*

Fifteen microliters (1 µg/µL) cRNA incubated with 5 × fragmentation buffer at 94°C for 35 min was digested into 35-200 bp fragments. Rat Genome 230 2.0 microarray produced by Affymetrix was prehybridized, then the hybridization buffer was added at 45°C, and spun at 60 rotations per min for 16 h. 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<sup>[14]</sup>.

### *Microarray data analysis*

The normalized signal values, signal detections (P, A, M)

and experiment/control (R<sub>i</sub>) were obtained by quantifying and normalizing the signal values using GCOS1.2<sup>[14]</sup>.

### *Normalization of microarray data*

To minimize error in the microarray analysis, each analysis was performed three times by Rat Genome 230 2.0 microarray. Result with a total ratio was maximal (R<sup>m</sup>) and that of which the average of three housekeeping genes (β-actin, hexokinase and glyseraldehyde-3-phosphate dehydrogenase) approached 1.0 (R<sup>h</sup>) were taken as a reference. The modified data were generated using a correction factor (R<sup>m</sup>/R<sup>h</sup>) multiplying the ratio of every gene in R<sup>h</sup> 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, and Microsoft Excel software<sup>[14,19,20]</sup>.

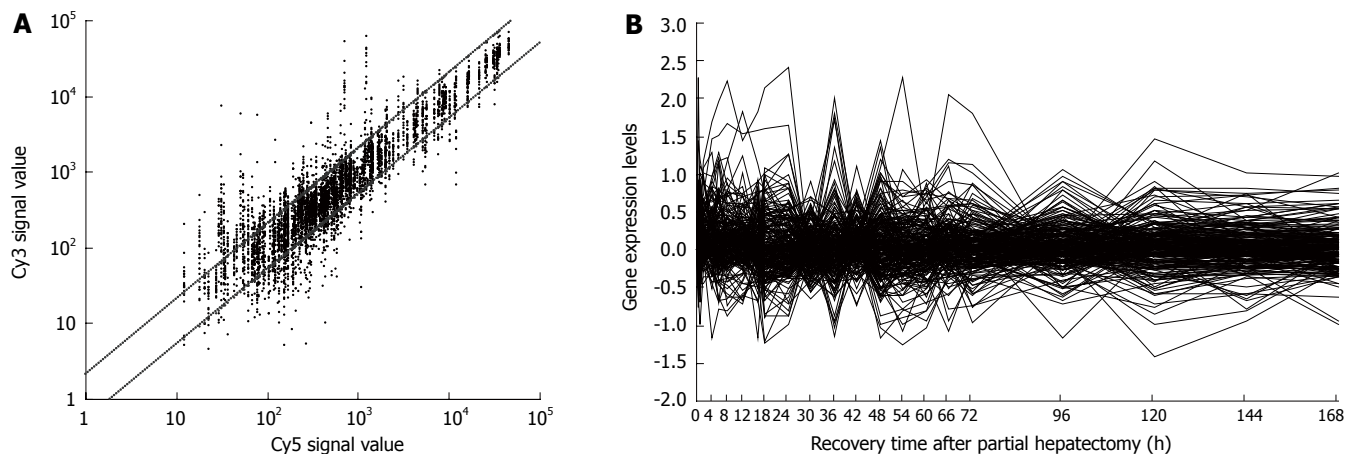
### *Identification of genes associated with liver regeneration*

Firstly, the nomenclature of inflammatory response was adopted from the GENEONTOLOGY database (www.geneontology.org), and then input into NCBI (www.ncbi.nlm.nih.gov) and RGD (rgd.mcw.edu) to identify the rat, mouse and human genes associated with the inflammatory response. 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 inflammatory response were collated. The results of this analysis were coded, and compared with the results from human and mouse studies in order to identify human and mouse genes which are different from those of rat. In comparison of these genes with the analysis output of the Rat Genome 230 2.0 array, those genes that showed a greater than twofold change in expression level, regarded as meaningful expression changes<sup>[15]</sup>, were referred to as rat homologous genes or rat specific genes associated with inflammatory response. Genes that displayed reproducible results with three independent analyses with the chip and showed a greater than twofold change in expression level at least at one time point during liver regeneration with significant difference (0.01 ≤ P < 0.05) or extremely significant difference (P ≤ 0.01) between PH and sham operation (SO), were referred to as associated with liver regeneration.

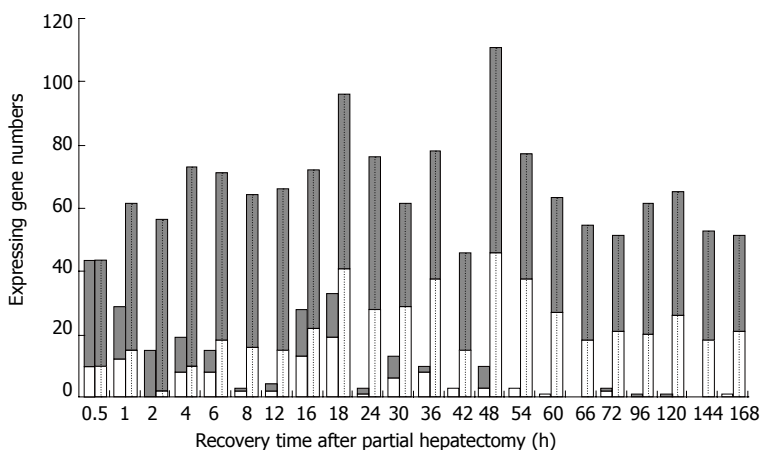
## RESULTS

### *Expression changes of genes associated with inflammatory response during liver regeneration*

According to the databases at NCBI, GENMAPP, KEGG, BIOCARTA and RGD, 661 genes were involved in inflammatory response, in which, 405 genes were contained in the Rat Genome 230 2.0 array. Among them, the expression of 239 genes displayed meaningful changes at least at one time point after PH, showed significant or extremely significant differences in expression when compared between PH and SO, and displayed reproducible



**Figure 1** Expression frequency, abundance and changes of 239 inflammatory response-associated genes during rat liver regeneration. Detection data of Rat Genome 230 2.0 array were analyzed and graphed by Microsoft Excel. **A:** Gene expression frequency. The dots above bias represent the genes up-regulated more than two fold, and total times of up-regulation were 975; those below bias down-regulated more than two fold, and times of down-regulation were 494; and the ones between biases no-sense alteration; **B:** Gene expression abundance and changes. One hundred and sixty-two genes were 2-257 fold up-regulated, and 147 genes 2-25 fold down-regulated.



**Figure 2** The initial and total expression profiles of 239 inflammatory response-associated genes at each time point of liver regeneration. Grey bars: Up-regulated gene; White bars: Down-regulated. Blank bars represent initial expressing genes, in which up-regulated genes were predominant in the forepart, and the down-regulated in the prophase and metaphase, whereas very few in the anaphase. Dotted bars represent the total expressing genes, in which some genes were up-regulated, and the others down-regulated during the whole LR.

results by three detections with Rat Genome 230 2.0 array, suggesting that the genes were associated with LR. Change in up-regulation ranged from 2 to 257 times higher than control, and down-regulation ranged from 2 to 25 times lower than control (Table 1). The data indicated that 92 genes were up-regulated, 77 genes down-, and 70 genes up/down-during liver regeneration. The total up- and down-regulated times were 975 and 494, respectively (Figure 1A). At the initiation phase (0.5-4 h after PH), 77 genes displayed up-regulation, 27 genes down, 3 genes up/down; at the G0/G1 transition (4-6 h after PH), 70 genes up-regulated, 20 genes down-, and 2 genes up/down-; at the cell proliferation phase (6-66 h after PH), 102 genes up-regulated, 84 genes down-, and 47 genes up/down-; at cell differentiation and structure-function reorganization phase (66-168 h after PH), 82 genes up-regulated, 44 genes down-, and 19 genes up/down- (Figure 1B).

#### Initial expression time of genes associated with inflammatory response during liver regeneration

At each time point of liver regeneration, the numbers of initial up-, down-regulated and total up-, down-regulated genes are as followings: both 34 and 10 at 0.5 h; 17, 12 and 47, 15 at 1 h; 15, 0 and 55, 2 at 2 h; 11, 8 and 64, 10 at 4 h;

7, 8 and 54, 18 at 6 h; 1, 2 and 49, 16 at 8 h; 2, 2 and 52, 15 at 12 h; 15, 13 and 51, 22 at 16 h; 14, 19 and 56, 41 at 18 h; 2, 1 and 49, 28 at 24 h; 7, 6 and 33, 29 at 30 h; 2, 8 and 41, 38 at 36 h; 0, 3 and 31, 15 at 42 h; 7, 3 and 66, 46 at 48 h; 0, 3 and 40, 38 at 54 h; 0, 1 and 37, 27 at 60 h; 0, 0 and 37, 18 at 66 h; 1, 2 and 31, 21 at 72 h; 1, 0 and 42, 20 at 96 h; 1, 0 and 40, 26 at 120 h; 0, 0 and 35, 18 at 144 h; 0, 1 and 31, 21 at 168 h (Figure 2). Generally, gene expression changes occurred during the whole liver regeneration, and the up- and down-regulations were 975 and 494 times, respectively. There were predominantly initial up-regulated genes in the forepart, and the down-regulated genes in the prophase and metaphase, whereas only a few in the anaphase.

#### Expression similarity and time relevance of genes associated with inflammatory response during liver regeneration

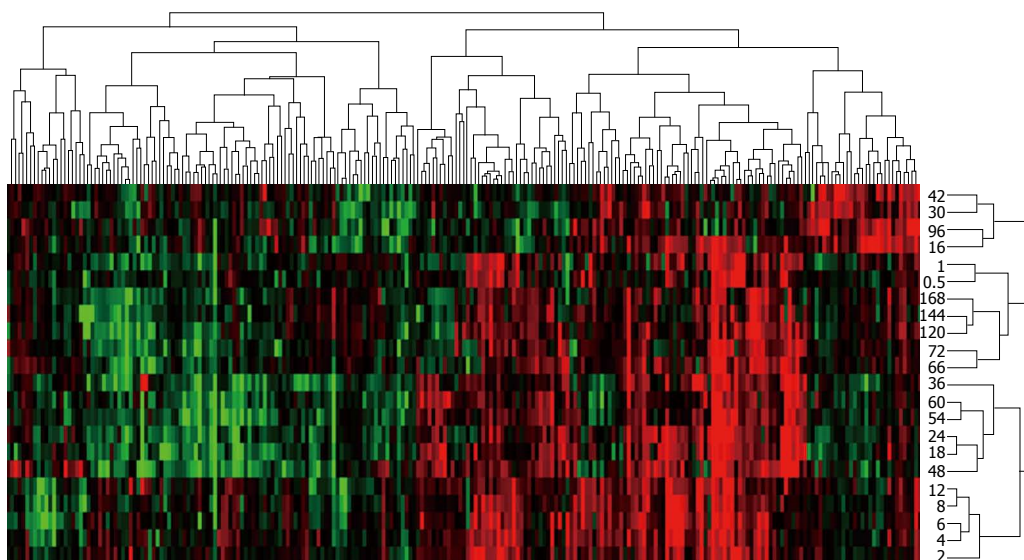
Two hundred and thirty-nine genes could be characterized based on their similarity in expression as following: only up-, predominantly up-, only down-, predominantly down-, up/down-regulated, involving 92, 25, 77, 14 and 31 genes, respectively (Figure 3). According to time relevance, they were classified into 13 groups, including 0.5 and 1 h, 2 h, 4 and 6 h, 8 and 12, 16 and 96 h, 18 and 24 h, 30 and 42 h, 36 h, 48 h, 54 and 60 h, 66 and 72 h, 120 and 144 h,

Table 1 Expression abundance of 239 inflammatory response-associated genes during rat liver regeneration

Gene abbr.	Associated with others	Fold difference	Gene abbr.	Associated with others	Fold difference	Gene abbr.	Associated with others	Fold difference	Gene abbr.	Associated with others	Fold difference
<b>1 Vascular reaction</b>			S100a9		4.9	<sup>1</sup> Serpine1		16.7	Ndst1		0.5, 2.1
Adcyap1		3.5	Scube1	2 <sup>b</sup>	3.2	Socs1		0.5, 2.4	Nfatc4		0.5
Alox5		0.2, 2.5	<sup>1</sup> Socs3		0.1, 2.5	Tgfb2		0.5, 2.9	Nfkb1		0.4, 2.3
Alox5ap		4.9	Stab1		0.4	Tnfrsf11b		0.3, 8.7	Nrf1		0.2, 2.4
Aoc3		6.1	Tac1		0.2	Tp53		2.9	Pap		68.6
Bdkrb2		0.4	Tlr2		10.6	Traf1		0.4	Pecam1		3.5
Cr2		6.0	Tlr4		0.5	<b>3 Cell activation, proliferation and differentiation</b>			Prdx5		0.5
Crp		0.5	Vps45		12.6	<b>a Cell activation</b>			Prg2		0.3
Ctsb		3.6	Zap70		0.4	Acadm		6.9	Prg3		0.2
Ctsc		0.4	Zfp36		0.1, 4.6	Adprt1		0.5	Prkca		0.5, 4.6
Cysltrl		0.1, 2.1	<b>b Cytokine</b>			Ager	4	0.4	Proc		0.3
Edg3		4.0	<sup>1</sup> A2m		0.4, 46.2	Ambp		5.1	Procr		6.5
F2	2 <sup>b</sup>	0.3	Abcb1		4.6	Aox1		0.3	Prok2		8.6
F3	2 <sup>b</sup>	0.1, 2.0	Agtr1a		0.4	Baat		0.2	Pten		0.5
Ggtla1		4.0	Atrn		4.4	C3		0.2	Rage		0.4
Itga1		2.9	Bcl2a1		0.3, 5.3	C3ar1		0.3, 2.3	Rela	2 <sup>a</sup>	0.5
Klkb1		0.4	Bdnf		0.4, 2.6	C4a		0.5	Ripk2		0.4
Kng1		2.1	Casp1		3.0	C5r1		0.4, 2.6	Sele	3 <sup>b</sup>	12.9
Ltb4r	3 <sup>a</sup>	0.5, 8.7	Ccl17		0.1	Casp8		10.6	Slpi		2.1
Nos3	2 <sup>b</sup>	0.3, 2.1	Ccl19		3.9	Cd80		0.3, 3.0	Sod2		5.6
Oldlr1		0.3, 6.5	Ccl2		18.5	Cd86		2.6	Spn		0.2, 4.0
Ptafr		7.1	Ccl20		8.0	Chuk		0.3	Tgfb1		4.0
Ptger3	2 <sup>a</sup>	0.2	Ccl21b		0.1, 2.0	Cnr2		0.5, 2.6	Tmem23		0.2, 4.3
Ptges		4.5	Ccl24		4.0	Crp		0.3	<sup>1</sup> Tnf		3.2
Ptgs2		0.1, 2.1	Ccl27		0.3	Crebbp		0.1	Tnfaip6		0.2, 2.1
Reg3g		0.3, 7.5	Ccl4		0.2, 3.0	Csf2		0.3	Tnfrsf4		0.3, 2.3
Spin2a		0.2, 4.3	Ccl7		22.6	<sup>1</sup> Ctgf		13.9	Tnfsf11		2.3
Spink5		0.1	Ccr1		0.4, 27.9	Cybb	4	2.5	Tnfsf4		0.2, 2.3
<b>2 Release cytokine</b>			Cd274		0.3	Dusp1		0.4, 6.0	<b>b Cell proliferation</b>		
<b>a Process of release</b>			Cklf1		8.3	Dysf		0.5, 4.9	Akt1		3.9
Abcf1		2.1	Clec7a		0.2	Fabp4		29.9	B7h3		3.5
Abcf2		0.4	Ctla4		0.3	Fos		28.4	Bcl6		8.6
Abcf3		0.5, 2.3	Cxcl10		0.3, 9.2	<sup>1</sup> Foxm1		13.9	Cd22		0.4
Adm		8.0	Cxcl12		0.2	Gal		11.3	Hdac7a		0.5, 4.3
Adora2a		0.5	Cxcl16		0.3	<sup>1</sup> Hgf		0.4	Igh-1a		0.3
<sup>1</sup> Anxa1		4.3	Ddt		4.4	Hmox2		0.4	Il15	3 <sup>a</sup>	0.4
Apoa2		2.9	Dfy		0.4, 8.5	<sup>1</sup> Hpse		0.3, 6.3	Il2		0.3, 3.5
Apol3		2.6	Dmd		0.3	Icam1		3.0	Il4	3 <sup>a,c</sup>	0.1, 2.6
App		6.4	Ebi3		0.2	Ighe		0.2, 2.5	Il5	3 <sup>c</sup>	3.5
Blvra		3.5	Ela2		0.5, 52.0	Ikbkb		0.3	Il7	3 <sup>c</sup>	2.8
C1qr1	4	5.5	F2	1	0.3	Ikbkg		0.4	Indo		0.4, 2.3
Cd163		0.2	F3	1	0.1, 2.0	Il15	3 <sup>b</sup>	0.4	Odc1		3.3
Cd74		0.4	Gif		0.1	Il16		0.4, 3.1	Plp		0.1
Chst1		0.0	Gsk3b		0.4	<sup>1</sup> Il1B		0.4	Ppara		0.3
Chst2		4.4	Hrh1		0.5, 9.9	Il1f5		0.4, 2.8	Sele	3 <sup>a</sup>	12.9
Col1a2		3.0	Hrh4		7.5	Il1f8		0.5	Spp1	3 <sup>c</sup>	0.5, 2.7
Col3a1		0.3, 6.5	<sup>1</sup> Ifng		6.5	Il1r1		0.5	<b>c Cell differentiation</b>		
Crbpb		3.1	Igfbp4		0.5	Il1rn		16.3	Il12rb2		2.8
Esrra		0.2	Il17f		0.1	Il22ra2		0.1	Il4	3 <sup>a,b</sup>	0.1, 2.6
Fcgr1		2.6	Il6		0.3, 6.1	IL2ra		0.3, 4.3	Il5	3 <sup>b</sup>	3.5
Itgb2		0.5	Il6r		3.0	Il2rg		0.2, 5.1	Il7	3 <sup>b</sup>	2.8
Klrg1		0.4	Ins1		2.1	Il4	3 <sup>b,c</sup>	0.1, 2.6	Spp1	3 <sup>b</sup>	0.5, 2.7
Lcn2		0.5, 257.2	Jak2		6.5	Il5ra		7.0	Stat5a		0.2
Map2k3		0.4	Jun		1.0, 6.9	Itgam	4	3.4	Stat5b		0.3
Mapk1		2.7	Lif		0.4, 3.0	Lama5		3.8	Tslp		0.5, 6.1
Mbp		0.4	Loc297568		0.4	Lamc1		0.4, 2.5	<b>4 Phagocytosis</b>		
Ms4a1	3 <sup>a</sup>	0.1	Mapk8		0.5, 19.7	Ltb4r	1	0.5, 8.7	Ager	3 <sup>a</sup>	0.4
Ncf1		0.2, 3.7	<sup>1</sup> Mmp9		0.5, 9.5	Mbl2		0.2	C1qr1	2 <sup>a</sup>	5.5
Nfkbiz		6.6	Nos3	1	0.3, 2.1	Mcpt6		0.2	Cybb	3 <sup>a</sup>	2.5
Nostrin		8.0	P2rx7		0.4, 2.5	Mif		3.2	Itgam	3 <sup>a</sup>	3.4
Orm1		2.8	Pbef1		8.1	Mob		7.0	Itih4		0.3
Parg		4.8	Pik3cd		0.3	Ms4a1	2 <sup>a</sup>	0.1	Lyz		0.4, 3.7
Pla2g4b		2.0	Plaur		13.9	Ms4a2		0.4, 5.3	Nox1		0.3, 8.6
Psen2		0.2	Pbp		0.1, 2.1	Mug1		0.1, 3.5	Nox4		0.1, 3.0
Ptger3	1	0.2	Ptgs1		0.7, 3.4	Myd88		2.1			
Reg3a		0.1, 64.0	Rgs16		0.1, 8.6	Ncr3		0.3			
Rela	3 <sup>a</sup>	0.5	Serpib5		0.5, 12.1						

<sup>1</sup>Indicates the reported genes associated with liver regeneration; the letters "a" and "b" at the top right corner of the number "2" respectively indicate "Process of release" and "Cytokine" during the process of release cytokine, the letters "a", "b" and "c" at the top right corner of the number "3" respectively refer to "Cell activation", "Cell proliferation" and "Cell differentiation" in the process of cell activation, proliferation and differentiation.





**Figure 3** Expression similarity and time relevance clusters of 239 inflammatory response-associated genes during liver regeneration. Detection data of Rat Genome 230 2.0 array were analyzed by H-clustering. Red represents up-regulated genes chiefly associated with promotion of inflammatory response; Green represents down-regulation ones mainly associated with suppression of inflammatory response; Black: No-sense in expression change. 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.

168 h and the up- and down-regulated times were 81 and 25, 55 and 2, 118 and 28, 101 and 31, 93 and 42, 105 and 69, 64 and 44, 41 and 38, 66 and 46, 77 and 65, 68 and 39, 75 and 44, 31 and 21 respectively (Figure 3). The up-regulated genes were chiefly ones promoting inflammatory response, and the down-regulated genes were mostly ones suppressing inflammatory response.

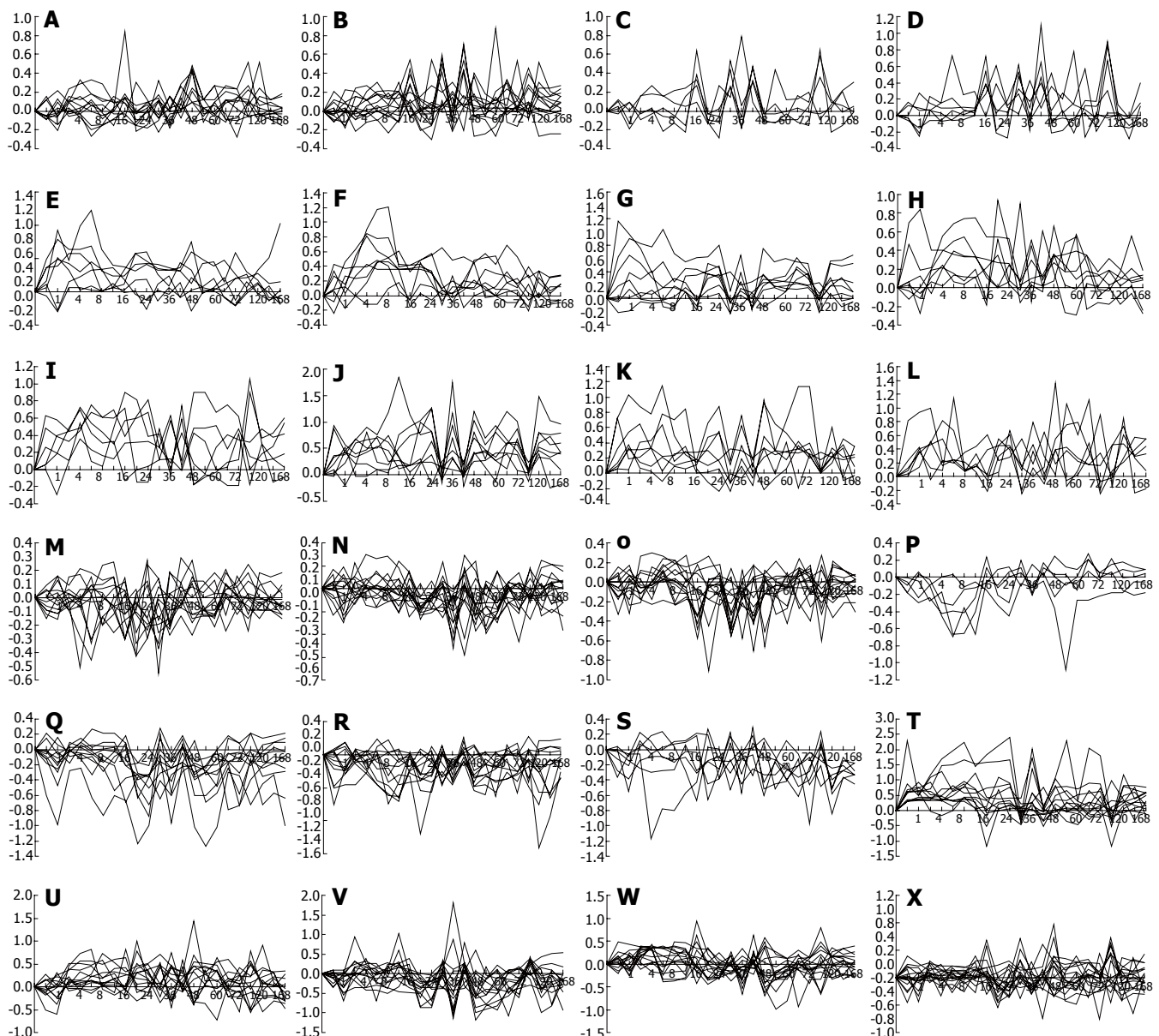
#### Expression patterns of genes associated with inflammatory response during liver regeneration

Two hundred and thirty-nine genes were categorized into 33 patterns, according to the changes in their expression. (1) up-regulation at one time point, i.e. 4, 6, 16, 18, 30, 48, 96, 120 h after partial hepatectomy (Figure 4A), 11 genes involved; (2) up at two time points, i.e. 6 and 66 h, 12 and 60 h, 16 and 42 h, 16 and 96 h, 24 and 48 h, 30 and 42 h, 36 and 66 h, 48 and 120 h, 72 and 120 h (Figure 4B), 13 genes involved; (3) up at three time points (Figure 4C), 5 genes involved; (4) up at multiple time points (Figure 4D), 6 genes involved; (5) up at one time phase, i.e. 0.5-6 h, 0.5-8 h, 1-48 h, 6-12 h (Figure 4E), 4 genes involved; (6) up at two time phases, i.e. 16-24 h and 42-48 h (Figure 4E), 1 gene involved; (7) up at three time phases (Figure 4E), 1 gene involved; (8) up at multiple time phases (Figure 4E), 1 gene involved; (9) up at one time point/phase, i.e. 0.5 and 4-12 h, 18 and 48-60 h, 48 and 2-24 h, 54 and 6-24 h, 66 and 6-24 h, 96 and 1-24 h, 120 and 2-72 h, 120 and 0.5-8 h (Figure 4F), 8 genes involved; (10) up at one time point/two phases (Figure 4G), 5 genes involved; (11) up at one time point/three phases (Figure 4G), 3 genes involved; (12) up at two time points/one phase (Figure 4H), 7 genes involved; (13) up at two time points/phases (Figure 4I), 6 genes involved; (14) up at two time points/three phases (Figure 4J), 8 genes involved; (15) up at three time points/one phase (Figure 4K), 1 gene involved; (16) up at three time points/two phases (Figure 4K), 6 genes involved; (17) up at multiple time points/phases (Figure 4L), 6 genes involved; (18) down at one time point, i.e. 4, 6, 16, 18, 30, 36, 42, 48, 54, 60, 72, 168 h (Figure 4M, 4N), 28 genes involved; (19) down at two time points, i.e. 0.5 and 48 h, 1 and 72 h, 16 and 42 h, 16 and 30 h, 18 and 54 h, 18 and

168 h, 30 and 42 h, 30 and 96 h, 42 and 66 h (Figure 4O), 11 genes involved; (20) down at three time points (Figure 4O), 2 genes involved; (21) down at multiple time points (Figure 4O), 1 gene involved; (22) down at one phase i.e. 4-8 h, 4-6 h, 4-12 h, 6-12 h (Figure 4P), 4 genes involved; (23) down at two phases, i.e. 18-24 and 48-54 h (Figure 4P), 1 gene involved; (24) down at one time point/phase, i.e. 36 and 48-60 h, 48 and 12-30 h, 48 and 18-24 h, 54 and 18-24 h, 72 and 120-144 h (Figure 4Q), 5 genes involved; (25) down at one time point/two phases (Figure 4Q), 4 genes involved; (26) down at one time point/three phases (Figure 4Q), 2 genes involved; (27) down at two time points/one phase (Figure 4R), 6 genes involved; (28) down at two time points/phases (Figure 4R), 6 genes involved; (29) down at three time points/one phase (Figure 4S), 2 genes involved; (30) down at multiple time points/phases (Figure 4S), 5 genes involved; (31) predominantly up (Figure 4T and U), 25 genes involved; (32) predominantly down (Figure 4V), 14 genes involved; (33) similarly up/down (Figure 4W and X), 31 genes involved.

## DISCUSSION

Inflammatory response is a self-protection mechanism formed during the long evolution, closely bound up with higher animal survival. In the process, six proteins including leukotriene B4 receptor (LTB4R), are associated with vascular reactions, through enhancing vascular permeability<sup>[21]</sup>. In addition, seven other proteins including platelet-activating factor receptor (PTAFR) have a role in increasing vasodilatation and permeability<sup>[22]</sup>. Four proteins including oxidized low density lipoprotein receptor 1 (LDLR1) promote leukocytes and proteins to exude from vessels<sup>[23]</sup>. Four proteins including regenerating islet derived 3 gamma (REG3G) accelerate vasodilatation<sup>[24]</sup>. Four proteins including amine oxidase copper containing 3 (AOC3) restrain leucocytes to migrate to sites of inflammation<sup>[25]</sup>. The meaningful expression profiles of the genes encoding the above proteins were the same or similar at some points while different at others, indicating that they may co-regulate vascular reaction. Among them,



**Figure 4** Thirty-three gene expression patterns of 239 inflammatory response-associated genes during liver regeneration. Expression patterns were obtained by the analysis of detection results of Rat Genome 230 2.0 array with Microsoft Excel. **A-L**: 92 up-regulated genes; **M-S**: 77 down-regulated genes; **T-X**: 70 up/down-regulated genes. X-axis represents recovery time after partial hepatectomy (h); Y-axis shows logarithm ratio of the signal values of genes at each time point to control.

*aoc3* was up-regulated at 36, 54 and 168 h after PH, and reached a peak at 36 h, which was 6.1 fold of control. *lib4r* was up- at 6, 18, 36, 48 and 60 h after PH, and reached a peak at 60 h, which was 6 fold of control. *oldlr1* was up- at 2-6, 18-24 and 48-66 h after PH, and reached a peak at 6 h, which was 6.5 fold of control. *ptafr* was up- at 16, 30, 42 and 96 h after PH, and reached a peak at 96 h, which was 7.1 fold of control. *reg3g* was up- at 12 and 36 h after PH, and reached a peak at 36 h, which was 7.5 fold of control. It is assumed that the above genes play a key role in vascular reaction during liver regeneration.

Ten proteins including lipocalin 2 (LCN2), associated with cytokine release, are activated by interleukins to promote inflammation<sup>[26]</sup>. Integrin beta 2 (ITGB2) stimulates leucocytes to secrete IL-12<sup>[27]</sup>. Nine proteins including regenerating islet-derived 3 alpha (REG3A) accelerate cytokines synthesis<sup>[28]</sup>. Fourteen proteins

including toll-like receptor 2 (TLR2) increase activation of macrophages<sup>[29]</sup>. Nine proteins including vacuolar protein sorting 45 (VPS45) stimulate the release of inflammatory factors, such as histamine, bradykinin<sup>[30]</sup>. The meaningful expression profiles of the genes encoding these proteins, being the same or similar at some points while different at others, indicate that they may co-regulate cytokine release. Among them, *len2* was up-regulated at 0.5-24, 36, 48-72 and 120-168 h after partial hepatectomy, and reached a peak at 24 h, which was 257 fold of control. *reg3a* was up- at 12, 36 and 96 h after PH, and reached a peak at 36 h, which was 64 fold of control. *tlr2* was up- at 0.5-8, 18-66 and 120-168 h after PH, and reached a peak at 168 h, which was 10.6 fold of control. *nps45* was up- at 16, 30, 42 and 96 h after PH, and reached a peak at 42 h, which was 12.6 fold of control. It is suggested that the above genes play a key role in cytokine release during liver regeneration.

Twelve proteins including chemokine C-C motif ligand 7 (CCL7), associated with inflammatory response, accelerate T cell migration to sites of inflammation<sup>[31]</sup>. Nine proteins including plasminogen activator urokinase receptor (PLAUR) promote leukocyte adhesion<sup>[32]</sup>. Eight proteins including serine or cysteine peptidase inhibitor clade E member 1 (SERPINE1) promote inflammatory cell migration<sup>[33]</sup>. Ten proteins including mitogen-activated protein kinase 8 (MAPK8) promote neutrophils, macrophages and T cells to ooze from blood vessel<sup>[34]</sup>. ATP-binding cassette sub-family B member 1 (ABCB1) enhances the transduction of inflammatory signaling<sup>[35]</sup>. Three proteins including alpha-2-macroglobulin (A2M) restrain the activities of various proteases including trypsin, thrombin and collagenase<sup>[36]</sup>. Twelve proteins including chemokine C-C motif receptor 1 (CCR1) inhibit the activities of leukocyte and inflammatory protein (MIP)-1 by enhancing the chemotaxis of neutrophils, monocytes, lymphocytes and eosinophils<sup>[37]</sup>. The meaningful expression profiles of these genes showed the sameness or similarity at some points while exhibited difference at others, suggesting that they may regulate the cytokine-mediated inflammation together. Among them, *a2m* was up- at 0.5-24, 36 and 48-54 h, and reached a peak at 8 h, which was 46.2 fold of control. This is generally consistent with the result reported by Scotte *et al.*<sup>[38]</sup>. *serpine1* was up- at 1-48 h, and reached a peak at 6 h, which was 16.7 fold of control. This is in conformity with the result reported by Mueller *et al.*<sup>[39]</sup>. *cl7* was up- at 1-4, 12, 24, 48, 66 and 144-168 h, and reached a peak at 48 h, which was 22.6 fold of control. *crr1* was up- at 8-36, 48 and 120 h, and reached a peak at 48 h, which was 27.9 fold of control. *mapk8* was up- at 0.5-24, 48-60, 72 and 144 h, having a peak at 4 h, which was 12 fold of control. *plaur* was up- at 1, 6, 18-24, 48, 72 and 120 h, and had the highest abundance of 13.9 fold at 6 h. It indicates that these genes play a key role in inflammation during liver regeneration.

Among the proteins associated with the inflammatory cell activation, connective tissue growth factor (CTGF) enhances macrophage activity via M-CSF (macrophage colony-stimulating factor)<sup>[40]</sup>. Eight proteins including cysteine aspartate-specific protease 8 (CASP8) lead inflammatory response by accelerating neutrophil apoptosis<sup>[41]</sup>. Ten proteins including v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS) accelerate inflammatory response by activating MAPK<sup>[42]</sup>. Six proteins including forkhead box M1 (FOXM1) induce inflammatory response when they are over expressed<sup>[43]</sup>. Seven proteins including pancreatitis-associated protein (PAP) inhibit inflammatory response by suppressing the activity of NFκB<sup>[44]</sup>. Six proteins including fatty acid binding protein 4 (FABP4) inhibit inflammatory response by reducing the activities of NFκB and IκB of macrophages<sup>[45]</sup>. Eight proteins including interleukin 1 receptor antagonist (IL1RN) decrease inflammatory response by inhibiting the activities of IL1A and IL1B<sup>[46]</sup>. Four proteins including galanin (GAL) suppress inflammatory response by inhibiting proliferation of thymus cells and macrophages<sup>[47]</sup>. The meaningful expression profiles of the genes encoding the proteins mentioned above showed the sameness or similarity at some point while exhibited

difference at others, implying that they may co-regulate the activity of inflammatory cell. Among them, *ctgf* was up- at 0.5-8, 18-24, 36, 54 and 72 h after PH, and reached a peak at 6 h, which was 13.9 fold of control. This is consistent with the results reported by Pi *et al.*<sup>[48]</sup>. *foxm1* was up- at 1, 18-24, 36, 48-72 and 120 h after PH, and reached a peak at 66 h, which was 13.9 fold of control. This is in line with the results reported by Wang *et al.*<sup>[43]</sup>. *casp8* was up- at 1, 18-24, 36, 48-72 and 120-168 h, and reached a peak at 48 h, which was 10.6 fold of control. *fabp4* was up- at 4, 12-24, 36, 48-72 and 120-168 h, and reached a peak at 120 h, which was 29.9 fold of control. *fos* was up- at 0.5-30, 42-48 and 120 h after PH, and reached a peak at 0.5 h, which was 28.4 fold of control. *gal* was up- at 1-16, 30, 42 and 96-120 h after PH, and reached a peak at 96 h, which was 11.3 fold of control. *il1rn* was up- at 2-24 and 48 h after PH, and reached a peak at 8 h, which was 16.3 fold of control. *pap* was up- at 1-24, 36, 48-54, 66 and 120-144 h after PH, and reached a peak at 12 h, which was 68.6 fold of control. It is assumed that these genes play vital roles in inflammatory cell activation during LR.

Nine proteins including B-cell CLL/lymphoma 6 (BCL6), associated with inflammatory cell proliferation, promote the proliferation of T cells and B cells<sup>[49]</sup>. Five proteins including selectin E (SELE) promote recruitment of white blood cells to inflammation sites by activating neutrophils<sup>[50]</sup>. Three proteins including cluster of differentiation 276 (CD276) induced by LPS inhibit T cell proliferation<sup>[51]</sup>. The meaningful expression profiles of the genes encoding these proteins showed the sameness or similarity at some points while difference at others, indicating that they may modulate inflammatory cell proliferation together. Remarkably, *sele* showed up-regulation at 0.5-2, 16-24, 36, 54-66 and 96-120 h with the highest abundance of 12.9 fold of control at 66 h after PH. *bcl6* was up- at 0.5-12, 24, 48-54, 72 and 120-168 h, and reached a peak at 0.5 h, which was 8.6 times higher than the control. It is supposed that they are important in inflammatory cell proliferation during liver regeneration.

Interleukin 12 receptor beta 2 (IL12RB2), associated with inflammatory cells differentiation, promotes Th1 cells differentiation<sup>[52]</sup>. Three proteins including thymic stromal lymphopoietin (TSLP) facilitate the differentiation of CD4(+)T cells to Th2 cells<sup>[53]</sup>. The meaningful expression profiles of the genes encoding these proteins exhibited the sameness or similarity at some points while difference at others, indicating that they may co-regulate inflammatory cell differentiation. Remarkably, *tslp* displayed up-regulation at 18-24, 36, 48-60, 72 and 120-144 h with the highest abundance of 6.1 fold at 18 h after PH, indicating that it is essential to inflammatory cell differentiation during liver regeneration.

Five proteins including complement component 1 q subcomponent receptor 1 (C1QR1), associated with inflammation elimination, can clear dead cells by promoting monocytes, neutrophils and epithelial cells to secrete inflammatory factors<sup>[54]</sup>. Lysozyme (LYZ) promotes bacterialepicyte dissolution<sup>[55]</sup>. NADPH oxidase 1, 4 (NOX1, NOX4) accelerate cell aging by catalyzing production of active oxygen<sup>[56]</sup>. The meaningful expression changes of these genes displaying sameness or similarity at some



points while difference at others indicate that they may co-regulate subsidence of inflammation. Especially, *h3* was up-regulated at 1-4, 48 and 96 h, and reached a peak at 48 h, which was 3.7 times higher than the control. *nax1* was up- at 0.5-8, 18 and 120 h, and had a peak expression at 2 h, which was 8.6 times higher than the control. *clqr1* was up- at 0.5-12, 24, 36, 60-72 and 120-168 h, reaching a peak at 8 h, which was 5.5-fold of control. It is supposed that the three genes are of importance in eliminating inflammation during liver regeneration.

In conclusion, the expression changes of the inflammatory response-associated genes after partial hepatectomy in rats have been investigated with high-throughput gene expression analysis. It is preliminarily confirmed that inflammatory response is enhanced during liver regeneration; that Rat Genome 230 2.0 array is a useful tool for analysis of the inflammatory response at the transcriptional level. However, the process of DNA→mRNA→protein→function is influenced by many factors including protein interaction. Therefore, further analyses of our results using techniques, such as Northern blotting, protein chip, RNA interference, protein-interaction are needed.

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