Expression patterns and action analysis of genes associated with physiological responses during rat liver regeneration: Cellular immune response

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Abstract

AIM: To study the cellular immune response during rat liver regeneration (LR) at a transcriptional level.

METHODS: Genes associated with the cellular immune response were obtained by collecting the data from databases and retrieving articles. Gene expression changes during LR were detected by rat genome 230 2.0 array.

RESULTS: A total of 127 genes were found to be associated with LR. The number of initially and totally expressing genes in the initial phase of LR [0.5-4 h after partial hepatectomy (PH)], transition from G_0-G_1 (4-6 h after PH), cell proliferation (6-66 h after PH), cell differentiation and structure-function reconstruction (66-168 h after PH) was 54, 11, 34, 3 and 54, 49, 70, 49 respectively, illustrating that the associated genes were mainly triggered at the initiation of LR, and worked at different phases. According to their expression similarity, these genes were classified into 41 up-regulated, 21 predominantly up-regulated, 41 down-regulated, 14 predominantly down-regulated, 10 similarly up-regulated and down-regulated genes, respectively. The total up- and down-regulated expression times were 419 and 274, respectively, demonstrating that the expression of most genes was increased while the expression of a small number of genes was decreased. Their time relevance was classified into 14 groups, showing that the cellular physiological and biochemical activities were staggered during LR. According to the gene expression patterns, they were classified into 21 types, showing the activities were diverse and complicated during LR.

CONCLUSION: Antigen processing and presentation are enhanced mainly in the forepart, prophase and anaphase of LR. T-cell activation and antigen elimination are enhanced mainly in the forepart and prophase of LR. A total of 127 genes associated with LR play an important role in cellular immunity.

INTRODUCTION

After antigenic stimulus, antigens are processed and presented to lymphocytes by antigen-presenting cells, and lymphocytes are activated[1] to proliferate and differentiate into effector cells that eliminate antigens[2]. This process is called immune response which is the self-protective mechanism of organisms developed during a long evolutionary history, closely associated with high animal survival[3]. Usually, the immunoreactions are classified into cellular and humoral immune responses according to different effectors[4]. The cellular immune response performs in brief as following: firstly the antigens were specifically recognized and presented by T-cells[5], then the effector cells, including T-cells, macrophages *etc.*, work by clearing the antigens. Injured cells and cell remnants caused by partial hepatectomy (PH)[6] are harmful to the organism[7], and wound areas are also susceptible to infection with antigen and xenobiotics[8]. Whether the cellular immune system plays a part in this process is worthy of an in-depth study.

In addition, PH[9] also activates the remnant hepatocytes to enter into the cell cycle to compensate for the lost liver mass, which is called liver regeneration (LR)[10]. Usually, based on the cellular physiological activities, the process

is classified into 4 phases: initiation (0.5-4 h after PH), transition from G_0 to G_1 (4-6 h after PH), cell proliferation (6-66 h after PH), cell differentiation and structure-function reorganization (66-168 h after PH)[11]. According to a time course, it is divided into 4 phases: 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)[12], in which many physiological and biochemical events, such as cell activation, de-differentiation, proliferation and its regulation, re-differentiation, reorganization of structure-function[13], are involved and regulated by many factors such as cellular immune response[14]. The relevance between cellular immune and liver regeneration has been studied at the transcriptional level[12,15,16]. The expression changes of genes in regenerating liver after PH can be detected by rat genome 230 2.0 array[17,18] containing 213 genes participating in the cellular immune response. A total of 127 genes have been identified to be associated with LR[19]. Their expression changes, patterns and action were primarily analyzed in the present study.

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 randomly divided into groups, 6 rats in each group (male: female = 1:1). PH was performed as previously described[9], the left and middle lobes of liver were removed. The 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 3 times in PBS at 4℃, 100-200 mg was taken from the middle parts of the right lobe. Six samples were collected from each group, mixed into 1-2 g (0.1-0.2 g × 6) liver tissue, and stored at -80℃. The sham-operation (SO) groups underwent the same PH without removal of liver lobes. The animal protection laws of China were strictly followed.

RNA isolation and purification
Total RNA was isolated from frozen livers according to the manual of the Trizol kit (Invittrogen)[22] and purified the guide for the RNeasy mini kit (Qiagen)[21]. Agarose electrophoresis (180V, 0.5 h) showed that the total RNA samples had a 2:1 ratio of 28S to 18S rRNA intensities. Total RNA concentration and purity were estimated by optical density measurements at 260/280 nm[22].

cDNA, cRNA synthesis and purification
Total RNA (1-8 μg) was used as a template for cDNA synthesis. cDNA and cRNA synthesis was proceeded as previously described[17]. cRNA labeled with biotin was synthesized using 12 μL synthesized cDNA as a template, and cDNA and cRNA were purified[17]. Measurement of concentration, purity and quality of cDNA and cRNA was conducted as previously reported[22].

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 microarry produced by Affymetrix, and 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 with GeneChip Scan 3000 (Affymetrix Inc., USA), and the signal values of gene expression were observed[18].

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[18].

Normalisation of microarray data
To minimize errors in microarray analysis, each analysis was performed 3 times by rat genome 230 2.0 array. Results with a maximal total ratio (Rm) and an average of three housekeeping genes β-actin, hexokinase and glyceraldehyde-3-phosphate dehydrogenase approaching to 1.0 (Rh) were taken as a reference. Modified data were generated by applying a correction factor (Rm/Rh) multiplying the ratio of each gene in Rh at each time point. To remove spurious gene expression changes resulting from errors in microarray analysis, the gene expression profiles at 0-4 h, 6-12 h and 12-24 h after PH were reorganized by a normalization analysis program (NAP) software according to the cell cycle progression of regenerating hepatocytes. Data statistics and cluster analysis were done using GeneMath, GeneSpring, Microsoft Excel software[18,23,24].

Identification of genes associated with liver regeneration
First, nomenclature of the cellular immune response was adopted from the GENEONTOLOGY database (www.geneontology.org) and inputted into the cellular immune response at NCBI (www.ncbi.nlm.nih.gov) and RGD (rgd.mcw.edu) to identify the rat, mouse and human genes associated with the activities mentioned above. According to the 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), genes associated with blood coagulation were collated. The results of this analysis were codified and compared with those obtained in humans and mice in order to identify human and mouse genes which are different from those of rats. Comparing these genes with the analysis output of the rat genome 230 2.0 array, genes showing more than twofold change in expression level as meaningful expression changes[19], were referred to as rat homologous genes or rat specific genes associated with cellular immune response. Genes displaying reproducible results in three independent analyses with the chip and more than twofold change in expression level at least at one time point during LR with a significant difference ($P \leq 0.01 < 0.05$) or an extremely significant difference ($P \leq 0.01$) between PH and sham operation (SO), were referred to as genes associated with LR.

Table 1 Expression of 127 cellular immune 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
1 Antigen precessing, presentation			Igf1r		0.4	Tlr4	3	0.5	Gadd45g		8.0, 0.4
Cd3d	3	4.0, 0.4	[1]Igh-1a		0.3	Tnf	3	3.2	Il1rl2		3.2, 0.1
Cd3e	3	2.7	Il15		0.4	Tnfsf12		0.3	Il2	2	3.5, 0.3
Cd3g	3	0.2	[1]Il1b		0.4	[1]Tp53		2.9	Il4	2	2.6, 0.1
Csf2		0.3	Il2	3	3.5, 0.3	Traf1		0.4	Inha		0.2
Cx3cr1	3	6.8, 0.4	Il4	3	2.6, 0.1	Usf1		2.0, 0.5	Itgb1	1	2.6
Itgb1	3	2.6	Il6		6.1, 0.3	Usf2		3.3	Klrc2	2	2.3, 0.4
Lgals3bp	3	10.6	Irf1		0.3	Vipr1		2.3	Klrg1		0.4
Prkra		4.2, 0.1	Itgam		0.3	Xbp1		4.3, 0.3	Lgals3bp	1	10.6
Tap1	3	2.2	Itgb2		0.5	Zap70		0.4	Lsp1		0.3
Tlr2	3	10.6	Kdr		2.4, 0.4	**3 Antigen elimination**			Mif		3.2
Tnfrsf4	3	2.3, 0.3	Kit		0.4	Adm		8.0	Mnda		5.3, 0.5
2 T-cell activation, proliferation			Klrc2	3	2.3, 0.4	Adora2a		0.5	Muc1		6.8, 0.2
Ager	3	0.4	Map3k7		0.5	Ager	2	0.4	Ncf1		3.7, 0.2
Akt1		3.9	Mapk8		19.7, 0.5	App		6.4	Ocil		9.1, 0.1
Apoe		0.1	Mmp9		9.5, 0.5	Art2b		0.3	Pfn1		4.2
Arhgdib		13.0	Myd88		2.1	[1]Bcl10		2.3	Pla2g4a		2.0
B7h3		3.5	Nfatc1		0.3	Bdnf		2.6, 0.4	Plaur		13.9
[1]Bcl2		0.3	Nfkb1		2.3, 0.4	[1]C3ar1		2.3, 0.3	Pnliprp2		36.8, 0.5
Bcl2l1		2.1, 0.4	P2rx7		2.5, 0.4	[1]C5r1		2.6, 0.4	Prf1		0.2
[1]Bmi1		2.0	Pawr		0.3	Ccl17		0.1	Pten		0.5
Btk		2.0	Plau		3.0, 0.4	Ccr1		27.9, 0.4	Ptprc	2	3.0, 0.1
Card11		2.3, 0.2	Ppbp		2.1, 0.1	[1]Ccr6		4.3, 0.3	Rela	2	0.5
Cd244	3	0.3	Prkca		4.6	Cd244	2	0.3	Slc16a1		3.2
Cd80		3.0, 0.3	Prkcq		3.0, 0.4	Cd3d	1	4.0, 0.4	Sod2		5.6
[1]Cd86		2.6	Psme2		4.0	Cd3e	1	2.7	Spn	2	4.0, 0.2
Cebpg		0.4	Ptgs2		2.1, 0.1	Cd3g	1	0.2	Spp1		2.7, 0.5
Chuk		0.3	Ptk2b		3.6	Cfh		2.5	Tap1	1	2.2
Cxcl12		0.2	Ptprc	3	3.0, 0.1	Crhr1		3.8, 0.5	Tcrb	2	0.2
Ddx58		11.8	Rela	3	0.5	Csf2	1	0.3	Tcrg	2	0.3
Erbb2		0.1	Rhog		0.5	Ctgf		13.9	Tff1		0.1
[1]F3		2.0, 0.2	Socs1		2.4, 0.5	Ctse		0.4	Tgfb2		2.9, 0.5
Fcgr1		2.6	Socs3		2.5, 0.1	[1]Ctsk		10.3	Tlr4	2	0.5
Fyn		0.4	Spn	3	4.0, 0.2	Cx3cr1	1	6.8, 0.4	Tnf	2	3.2
Glmn		6.0, 0.4	Tcrb	3	0.2	E2f1		21.2	Tnfrsf4	1	2.3, 0.3
Gzmb		7.5	Tcrg	3	0.3	[1]Ebi3		0.2	Umod		3.0, 0.4
Icam1		3.0	Tert		5.3, 0.3	[1]F2		0.3	Zfp148		2.5
Ifng		6.5	Tgfb1		4.0	Fosl1		2.3			

[1]Reported genes associated with liver regeneration; Associated with others: involved in other process of cellular immune response.
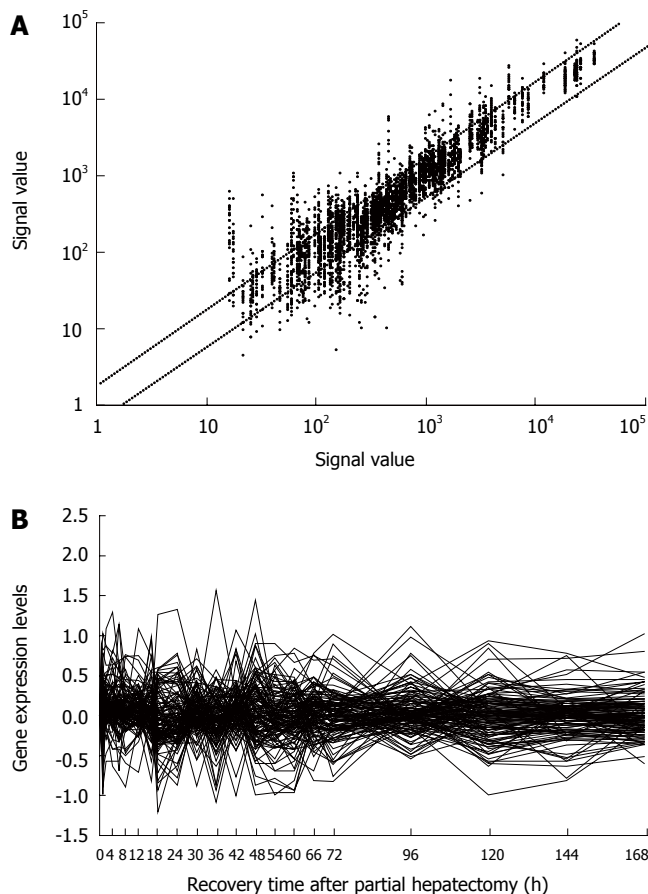
RESULTS

Expression changes of genes associated with cellular immune response during liver regeneration

According to the data from databases at NCBI, GENMAPP, KEGG, BIOCARTA and RGD, 468 genes were involved in the cellular immune response, of which 213 were contained in the rat genome 230 2.0 array. The expression of 127 genes displayed meaningful changes at least at one time point after PH, showing significant or extremely significant differences in expression compared with those after PH and SO, and reproducible results detected by three analyses of rat genome 230 2.0 array, suggesting that the genes were associated with LR. Ranges of the expression of up-regulated and down-regulated genes were respectively 2-37 fold and 2-10 fold of the control (Table 1). Up-regulated, down-regulated and up-/down-regulated expressions were observed in 41, 41 and 45 genes, respectively during LR. The up- and down-regulated expression times were 419 and 274, respectively (Figure 1A). At the initial phase (0.5-4 h after PH), 32 genes displayed up-regulated expression, 19 down-regulated expression, and 2 up-/down-regulated expression. At the transition phase from G_0 to G_1 (4-6 h after PH), 29 genes showed up-regulated expression, and 11 down-regulated expression. At the cell proliferation phase (6-66 h after PH), 43 genes exhibited up-regulated expression, 44 down-regulated expression, and 29 up-/down-regulated expression. At the cell differentiation and structure-functional reorganization phase (66-168 h after PH), expression was up-regulated in 49 genes, down-regulated in 46 genes, and up-/down-regulated in 15 (Figure 1B).

Initial expression time of genes associated with cellular immune response during liver regeneration

At each time point of LR, the number of initially up-regulated and down-regulated as well as the total number of up-regulated and down-regulated genes were 15 and 6 at 0.5 h; 7, 10 and 17, 13 at 1 h; 7, 1 and 21, 3 at 2 h; 5, 3 and 27, 6 at 4 h; 1, 3 and 19, 10 at 6 h; 0, 0 and 17, 6 at
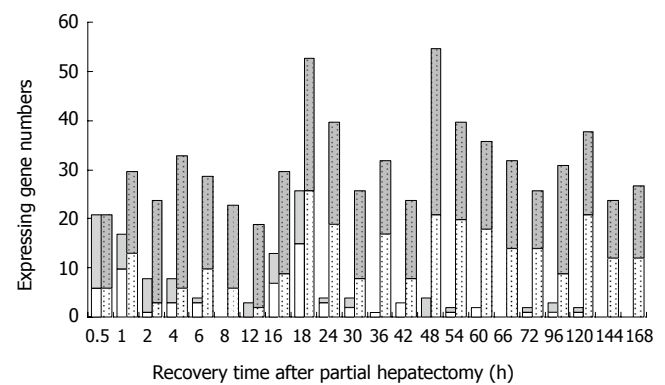
A

B

Figure 1 Expression frequency (**A**) and changes (**B**) of 127 cellular immune response-associated genes during rat liver regeneration. Detection data of rat genome 230 2.0 array were analyzed and graphed by Microsoft Excel. The dots above bias represent the expression of genes up-regulated more than two fold, those under bias represent down-regulated more than two fold; the total times of up-regulated, down-regulated expression under bias are 419, 274, respectively. The ones between biases have meaningless alteration. The expression of 88 genes is up-regulated 2-37 fold and that of 86 genes is down-regulated 2-10 fold.

Figure 2 Initial and total expression profiles of 127 cellular immune response-associated genes at each time point of liver regeneration. Grey bars: up-regulated genes; white bars: down-regulated genes; blank bars: initial expressing genes in which up-regulated genes are predominant in the forepart and prophase and down-regulated genes in the metaphase, whereas very few genes in the anaphase; dotted bars: total expressing genes in which some genes are up-regulated and others are down-regulated during the whole LR.

Figure 3 Expression similarity and time relevance clusters of 127 cellular immune 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 antigen processing and presentation; green represents down-regulated genes mainly associated with antigen elimination; black represents the genes whose expressions are meaningless. The upper and right trees showing the expression similarity cluster and time series cluster respectively, by which these genes can be classified into 5 and 14 groups, respectively.
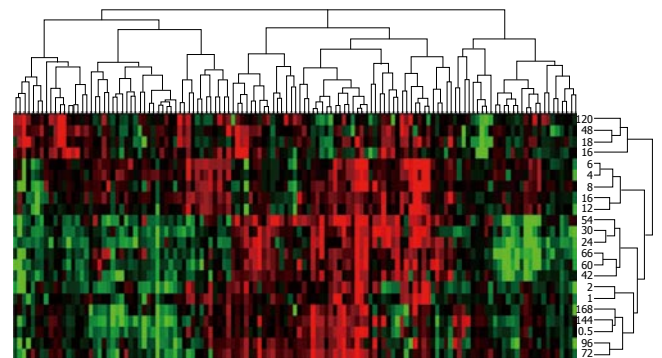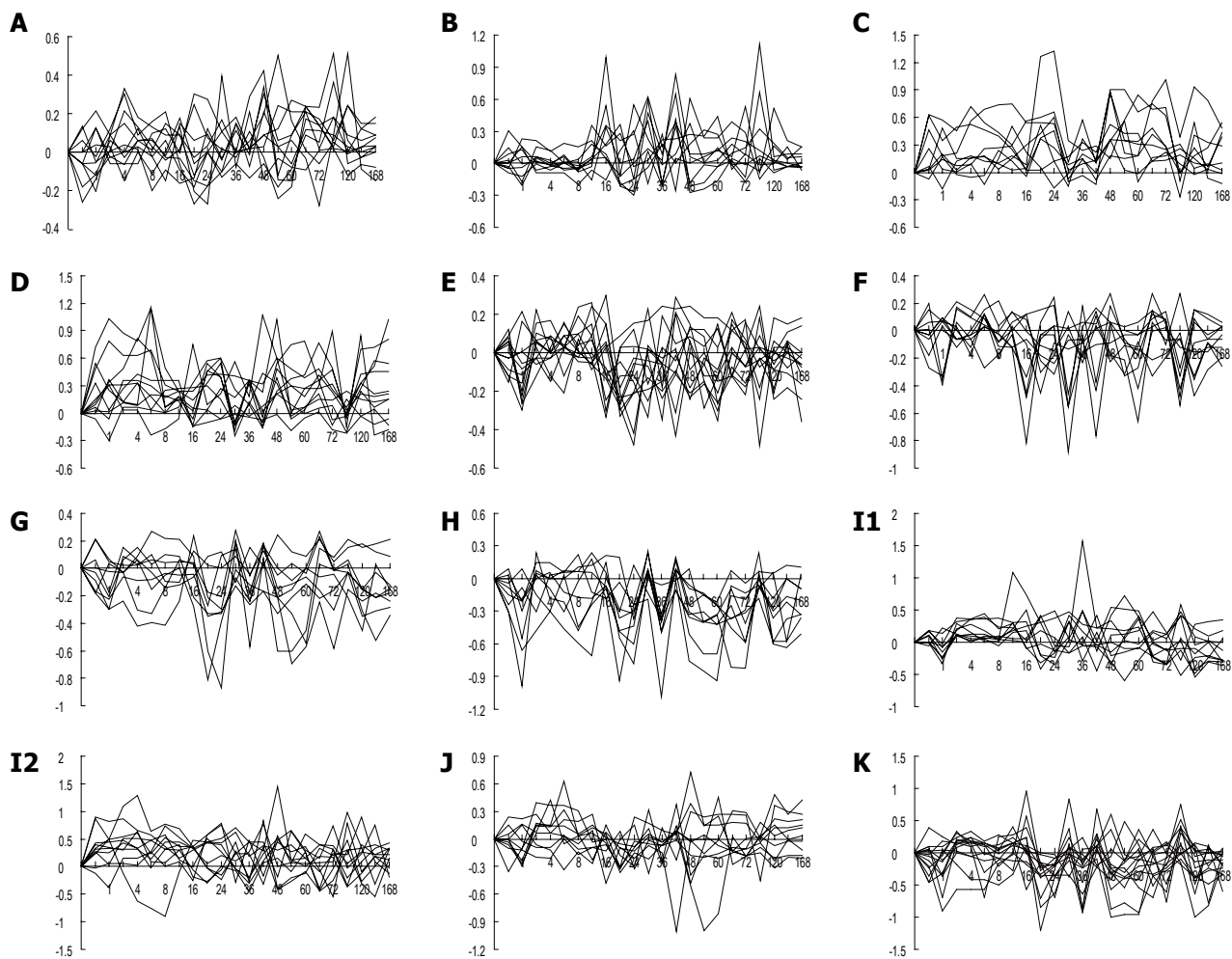
8 h; 3, 0 and 17, 2 at 12 h; 6, 7 and 21, 9 at 16 h; 11, 15 and 27, 26 at 18 h; 1, 3 and 21, 19 at 24 h; 2, 2 and 18, 8 at 30 h; 0, 1 and 15, 17 at 36 h; 0, 3 and 16, 8 at 42 h; 4, 0 and 34, 20 at 48 h; 1, 1 and 20, 20 at 54 h; 0, 2 and 18, 18 at 60 h; 0, 0 and 18, 14 at 66 h; 1, 1 and 12, 14 at 72 h; 2, 1 and 22, 9 at 96 h; 1, 1 and 17, 21 at 120 h; 0, 0 and 12, 12 at 144 h; 0, 0 and 15, 12 at 168 h (Figure 2). Generally, gene expression changes occurred during the whole LR. The up- and down-regulated expression times were 419 and 274, respectively. The initially up-regulated genes were predominant in the forepart and prophase and the down-regulated genes were predominant in the metaphase, whereas only a few of down-regulated genes were found in the anaphase.

Expression similarity and time relevance of genes associated with cellular immune response during liver regeneration

A total of 127 genes could be characterized based on their similarity in expression: 41 up-regulated, 21 predominantly up-regulated, 41 down-regulated, 14 predominantly down-regulated, 10 up-/down-regulated genes, respectively

(Figure 3). According to the time relevance, they were classified into 14 groups (0.5 and 144 h, 1 and 2 h, 4 and 6 h, 8 h, 12 and 16 h, 18 and 48 h, 24 and 30 h, 36 h, 42 h, 54 h, 60 and 66 h, 72 and 96 h, 120 h, 168 h). The up- and down-regulated expression times were 21 and 7, 64 and 22, 42 and 8, 72 and 16, 32 and 9, 37 and 34, 62 and 49, 69 and 55, 54 and 28, 57 and 32, 41 and 35, 27 and 25, 20 and 16, 16 and 20, respectively (Figure 3). The up-regulated expression genes were chiefly associated with antigen processing and presentation, and the down-regulated genes were significantly associated with antigen elimination.

Expression patterns of genes associated with cellular immune response during liver regeneration

A total of 127 genes were categorized into 21 patterns according to the changes in their expression: 11 up-regulated genes at one time point (i.e. 4, 18, 30, 48, 54, 96, 120 h) after PH (Figure 4A); 5 up-regulated genes at two

Figure 4 Twenty-one gene expression patterns of 127 cellular immune response-associated genes during liver regeneration. Expression patterns were obtained by analysis of detection data with rat genome 230 2.0 array using Microsoft Excel. **A-D**: 41 up-regulated genes; **E-H**: 41 down-regulated genes; **I-K**: 45 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.

time points (i.e. 12 and 60 h, 18 and 54 h, 24 and 48 h, 30 and 42 h, 72 and 120 h) (Figure 4B); 2 up-regulated genes at three time points (Figure 4B); 3 up-regulated genes at four time points (Figure 4B); 2 up-regulated genes at one time point/two phases (Figure 4C); 4 up-regulated genes at two time points/one phase (Figure 4C); 3 up-regulated genes at two time points/phases (Figure 4C); 4 up-regulated genes at multiple time points/phases (Figure 4D); 13 down-regulated genes at one time point (i.e. 16, 18, 24, 30, 36, 42, 60, 96, 168 h) (Figure 4E); 4 down-regulated genes at two time points (i.e. 16 and 30 h, 18 and 54 h, 30 and 96 h, 42 and 60 h) (Figure 4F); 3 down-regulated genes at three time points (Figure 4F); 3 down-regulated genes at four time points (Figure 4F); 4 down-regulated genes at one phase (i.e. 4-6 h, 4-8 h, 54-60 h and 120-168 h) (Figure 4G); 3 down-regulated genes at one time point/phase (i.e. 48 and 18-24 h, 72 and 120-144 h, 120 and 18-24 h) (Figure 4G); 2 down-regulated genes at one time point/two phases (Figure 4G); 2 down-regulated genes at two time points/one phase (Figure 4H); 2 down-regulated genes at two time points/phases (Figure 4H); 5 down-regulated genes at multiple time points/phases (Figure 4H); 21 predominantly up regulated genes (Figure

4I); 14 predominantly down regulated genes (Figure 4J); 10 similarly up-/down-regulated genes (Figure 4K).

DISCUSSION

Cellular immune response is a self-protection mechanism formed during the long-term evolutionary processes, closely associated with higher animal. Of the proteins associated with antigen processing and presentation, five proteins including toll-like receptor 2 (TLR2) can activate the immune response by recognizing many kinds of pathogens[25]. Five proteins including lectin galactoside-binding soluble 3 binding protein (LGALS3BP) activate antigen presenting cells[26]. Transporter 1 ATP-binding cassette subfamily B (TAP1) speeds up antigen translocation[27]. The meaningful expression profiles of the genes encoding the above proteins are identical or similar at some time points while different at other time points, indicating that they may co-regulate antigen processing and presentation. In the present study, *tlr2* and *lgals3bp* were all up-regulated at multiple time points, reaching their peaks that were both 10.6 fold of the control respectively at 168 h and 48 h after PH. It is suggested that these genes play a

key role in antigen processing and presentation during LR.

Of the proteins associated with T-cell activation and proliferation, 6 proteins including granzyme B (GZMB) can activate T-cells[28], 4 proteins including transforming growth factor beta 1 (TGFB1) can activate CD4+T cells[29], 10 proteins including Rho GDP dissociation inhibitor beta (ARHGDIB) increase connection between antigen presenting cells and T-cells by promoting T-cell proliferation[30], 16 proteins including protein kinase C alpha (PRKCA) increase cytokine synthesis and expression of IFNγ and IgG[31], apolipoprotein E (APOE) facilitates endocytosis[32], 6 proteins including T-cell receptor beta chain (TCRB) activate the T-cell-dependent signaling pathway[33], 5 proteins including DEAD (Asp-Glu-Ala-Asp) box 58 (DDX58) promote the immunologic response[34]; intercellular adhesion molecule 1 (ICAM1) facilitates leucocyte transport[35]; interferon-gamma (IFN-γ) interacts with IL-12 and TNFα to augment immunological competence [36]; insulin-like growth factor I receptor (IGF1R) conducts signals of autoimmune inflammation[37]; integrin beta 2 (ITGB2) promotes leucocyte adherence and phagocytosis[38]; 4 proteins including Fyn proto-oncogene (FYN) suppress the Th2-mediated immune response[39], 7 proteins including kit oncogene (KIT) inhibit T lymphocyte proliferation[40]; vasoactive intestinal peptide receptor 1 (VIPR1) combines T-cell to enhance HIV infection[41]. In the present study, the meaningful expression profiles of the genes encoding the above proteins were identical or similar at some time points while different at other time points, indicating that they may co-regulate T-cell activation and proliferation. *gzmb* expression was up-regulated at 0.5-8, 36, 48-66 and 168 h and reached 7.5 fold at 48 h after PH. *arhgdib* expression was up-regulated at 16, 42 and 96 h and reached 13 fold at 96 h after PH. *prkca* expression was up-regulated at 16, 30, 42 and 96 h and reached 4.6 fold at 96 h after PH. *ddx58* expression was up-regulated at 1, 16, 30 and 42-48 h, reached 11.8 fold at 42 h after PH. *ifng* expression was up-regulated at 1-6, 18-24, 36, 48-66 and 144-168 h and reached 6.5 fold at 4 h after PH, indicating that they are crucial in T-cell activation and proliferation during LR.

Of the proteins associated with antigen elimination, profilin 1 (PFN1) accelerates antibody processing and modification [42]; 9 proteins including E2F transcription factor 1 (E2F1) and pancreatic lipase-related protein 2 (PNLIPRP2) increase immune response by speeding up the activities of lymphocytes and cytokines[43,44]; zinc finger protein 148 (ZFP148) accelerates the differentiation of monocytes into macrophages by inhibiting the activity of integrin CD11b[45]; complement factor H (CFH) promotes complement activation[46]; cathepsin κ (CTSK) promotes immunologic response *via* bactericidal action[47]; 6 proteins including superoxide dismutase 2 (SOD2) inhibit tumor cell proliferation and migration[48]; lymphocyte specific 1 (LSP1) and uromodulin (UMOD) suppress chemotaxis of macrophages and neutrophils[49,50]; chemokine receptor 1 (CCR1) conducts signals of inflammatory response[51]; 3 proteins including connective tissue growth factor (CTGF) accelerate wound repair by increasing expression of chemotactic factors[52]; transforming growth factor beta 2 (TGFB2) combines IL-10 to suppress immunologic response induced by bacterial infection[53]; myeloid cell nuclear differentiation antigen (MNDA) blocks combination of ligands and receptors[54]; 8 proteins including adrenomedullin (ADM) control the activity of effector lymphocytes and cytokines[55]; plasminogen activator urokinase receptor (PLAUR) promotes cancer cell spread[56]. The meaningful expression profiles of the genes encoding these proteins are identical or similar at some time points while different at other time points, indicating that they may co-regulate antigen elimination. In the present study, *ctsk* expression was up-regulated at 1, 18-24, 48 and 66-168 h and reached its peak at 72 h, which was 10.3 times that of the control and is basically in line with the result reported by Dransfeld[15]. *e2f1* expression was up-regulated at 18-30, 54-72 and 120 h, and reached its peak at 24 h, which was 21.2 times that of the control. *pnliprp2* expression was up-regulated at 12-18 and 36 h, and reached its peak at 36 h, which was 36.8 fold that of the control. *sod2* expression was up-regulated at 0.5, 4-24 and 48 h, and reached its peak at 12 h, which was 5.6 times that of the control. *ctgf* expression was up-regulated at 0.5-8, 18-24, 36, 54 and 72 h, and reached its peak at 1 h, which was 10.7 fold that of the control. *adm* expression was up-regulated at 0.5-24, 36, 48-72 and 168 h, and reached its peak at 54 h, which was 8-fold that of the control. *plaur* expression was up-regulated at 1, 6, 18-24, 48, 72 and 120 h, and reached its peak at 6 h, which was 13.9 times that of the control. These findings suggest that the seven genes are of importance in antigen elimination during liver regeneration.

In conclusion, the expression changes of cellular immune response-associated genes after rat partial hepatectomy can be investigated by high-throughput gene expression analysis. Cellular immune response is enhanced during liver regeneration. Rat genome 230 2.0 array is a useful tool for analyzing the response at the transcriptional level. However, DNA→ mRNA→ protein→ function is influenced by many factors including protein interaction. So we will further analyze the results using such techniques as Northern blotting, protein chip, RNA interference, protein-interaction *etc*.

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