PO Box 2345, Beijing 100023, China
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BASIC RESEARCH

Expression patterns and action analysis of genes associated with blood coagulation responses during rat liver regeneration

Li-Feng Zhao, Wei-Min Zhang, Cun-Shuan Xu

Li-Feng Zhao, Faculty of Life Science and Technology, Ocean University of China, Qingdao 260003, Shandong Province, China
Cun-Shuan Xu, College of Life Science, Henan Normal University, Xinxiang 453007, Henan Province, China
Li-Feng Zhao, Wei-Min Zhang, Key Laboratory for Cell Differentiation Regulation, Xinxiang 453007, Henan Province, China
Supported by the National Natural Science Foundation of China, No. 30270673
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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Abstract

AIM: To study the blood coagulation response after partial hepatectomy (PH) at transcriptional level.

METHODS: After PH of rats, the associated genes with blood coagulation were obtained through reference to the databases, and the gene expression changes in rat regenerating liver were analyzed by the Rat Genome 230 2.0 array.

RESULTS: It was found that 107 genes were associated with liver regeneration. The initially and totally expressing gene numbers occurring in initiation phase of liver regeneration (0.5-4 h after PH), G_0/G_1 transition (4-6 h after PH), cell proliferation (6-66 h after PH), cell differentiation and structure-function reconstruction (66-168 h after PH) were 44, 11, 58, 7 and 44, 33, 100, 71 respectively, showing that the associated genes were mainly triggered in the forepart and prophase, and worked at different phases. According to their expression similarity, these genes were classified into 5 groups: only up-, predominantly up-, only down-, predominantly down-, up- and down-regulation, involving 44, 8, 36, 13 and 6 genes, respectively, and the total times of their up- and down-regulation expression were 342 and 253, respectively, demonstrating that the number of the up-regulated genes was more than that of the down-regulated genes. Their time relevance was classified into 15 groups, showing that the cellular physiological and biochemical activities were staggered during liver regeneration. According to gene expression patterns, they were classified into 29 types, suggesting that their protein activities were diverse and complex during liver regeneration.

CONCLUSION: The blood coagulation response is enhanced mainly in the forepart, prophase and anaphase of liver regeneration, in which the response in the forepart, prophase of liver regeneration can prevent the bleeding caused by partial hepatectomy, whereas that in the anaphase contributes to the structure-function reorganization of regenerating liver. In the process, 107 genes associated with liver regeneration play an important role.

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

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INTRODUCTION

The liver is the main site where coagulation factors are synthesized[1]. Tissue damage is often companied with angiorrhexis, bleeding and blood coagulation. Blood coagulation is a complex hemostatic process in which zymogens convert into coagulation factors, and promote blood coagulation at the site of wound[2]. This process is classified into extrinsic pathway and intrinsic pathway[3]. A proportion of coagulation factors of the former originate from blood, while that of the latter all from blood. Blood coagulation abnormity can lead to hereditary prothrombin deficiency, vitamin K deficiency, haemophilia and hepatic coagulation defects[4,5].

Partial hepatectomy (PH)[6] causes blood vessel injury and bleeding. Meanwhile, the remnant hepatocytes activated by it enter into cell cycle to compensate for the lost liver tissue, which process is called liver regeneration (LR)[7,8]. Generally, based on the physiological activities of the cells, the regeneration process is classified into 4 phases: the initiation (0.5-4 h after PH), the transition from G_0 to G_1 (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)[9]. According to time course, it is also divided 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)[10], which are regulated by many factors including blood coagulation response[11]. To study the relationship between the blood coagulation response after PH and liver regeneration at transcriptional level[10,12], we examined the expression changes of genes in regenerating liver after partial hepatectomy by Rat Genome 230 2.0 array[13] containing 174 blood coagulation-associated genes. One hundred and seven genes were identified which were associated with liver regeneration[14], and we primarily studied the expression changes, patterns and functions of these genes.

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[6]: the left and middle lobes of the liver were removed. Rats were sacrificed by cervical vertebra dislocation at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 54, 66, 72, 120, 144 and 168 h after PH and the regenerating livers were examined at corresponding time points. The livers were rinsed three times in PBS at 4℃. Then 100-200 mg liver tissues from middle parts of the right lobe, six samples of each group were gathered and mixed together to 1-2 g (0.1-0.2 g × 6) of total liver tissue, then stored at -80℃. The sham-operation (SO) groups were treated the same with partial hepatectomy ones except that the liver lobes were not removed. The guidelines of animal protection of China were followed strictly.

RNA isolation and purification

Total RNA was isolated from the frozen livers according to the manual of Trizol kit (Invitrogen)[15] and then purified based on the guide of RNeasy mini kit (Qiagen)[16]. In brief, total liver tissues frozen at -80℃ were homogenized liquid nitrogen, and homogenates were split in TRIzol reagent, followed by chloroform extraction and isopentyl alcohol precipitation. The total RNA integrity was confirmed by agarose electrophoresis and checked by the ratio of 28S to 18S. Total RNA concentration and purity were estimated by optical density measurements at 260/280 nm[17].

cDNA and cRNA synthesis and purification

As template, 0.4 ng total RNA was used for cDNA synthesis. cDNA purification was proceeded based on the methods established by Affymetrix[18]. cRNA labeled with biotin was synthesized using cDNA as the template and then purified[18]. Measurement of cDNA, cRNA concentration and purity were the same as above.

cRNA fragmentation and microarray detection

Fifteen microliter (1 μg/μL) cRNA incubated with 5 × fragmentation buffer at 94℃ for 35 min was digested into 35-200 bp fragments. The hybridization buffer was added to the prehybridized Rat Genome 230 2.0 microarray produced by Affymetrix, then hybridization was carried out for 16 h at 45℃ on a rotary mixer at 60 rotation/min. 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[13].

Microarray data analysis

Signal values were quantified and normalized with GCOS1.2 software. Quantified signal intensities were obtained by deducting foreground signal values. Signal intensities were replaced by 200 when they were < 200. When experiment/control (Ri) was between 0.1 and 10, Ri was taken as natural logarithms to generate lnRi, and the normalize coefficient factor (ND) was taken by averaged Ri. The modified signal values were generated by ND multiplying control, and were replaced by 200 when it was < 200[13].

Normalization of microarray data

To minimize error from the microarray analysis, each analysis was performed three times. Results whose total ratio was maximal (R^m) and that whose average value 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 using 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 with 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[13,19,20].

Identification of genes associated with liver regeneration

Firstly, the nomenclature of blood coagulation was adopted from the GENEONTOLOGY database (www.geneontology.org), and input into blood coagulation at NCBI (www.ncbi.nlm.nih.gov) and RGD (rgd.mcw.edu) to identify the rat, mouse and human genes associated with the blood coagulation. 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 blood coagulation were collated. The results of the analysis were codified, 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 which showed a greater than twofold change in expression level, regarded as meaningful[14] rat homologous genes, were referred to as rat specific genes associated with blood coagulation response under evaluation. Genes, which displayed reproducible results on three independent analyses with the chip and which showed a greater than

Table 1 Expression abundance of 107 blood coagulation response-associated genes during rat liver regeneration

Gene Abbr.	Associated with	Fold difference	Gene Abbr.	Associated with	Fold difference	Gene Abbr.	Associated with	Fold difference	Gene Abbr.	Associated with	Fold difference
C2	1	2.1	Anxa8	1, 2	0.4	Fbn1	1, 2	2.8	Rab27a	1, 2	3.4
Cd59	1	0.5	Anxa9	1, 2	4.6	Fgb	1, 2	4.3	Scube1	1, 2	3.2
Coch	1	0.2, 2.3	App	1, 2	6.4	Fgl2	1, 2	3.1	Tbxa2r	1, 2	0.2, 2.0
Daf1	1	0.2	B4galt1	1, 2	3.1	Fli1	1, 2	4.9	Tm4sf3	1, 2	3.6
Hrg	1	0.5, 2.8	Bdkrb2	1, 2	0.4	G7c	1, 2	3.9	[3]Tnf	1, 2	3.2
Il1b	1	0.4	C1qA	1, 2	0.3	Ggcx	1, 2	0.3, 2.6	Trove2	1, 2	0.4
[3]Il6	1	0.3, 6.1	C1qr1	1, 2	5.5	Gna12	1, 2	2.5	[3]A2m	2	0.4, 46.2
Lman1	1	2.0	C3	1, 2	0.2	Gnaq	1, 2	2.5	Adora2a	2	0.5, 2.0
Masp1	1	3.0	C3ar1	1, 2	2.3	[3]Hgf	1, 2	0.4	C8g	2	0.4, 2.0
Mbl2	1	0.2	C4a	1, 2	0.5	[3]Hnf4a	1, 2	0.1, 4.5	Cfh	2	2.5
Nfe2	1	0.1	C4bpa	1, 2	2.0	Hs6st2	1, 2	0.1, 2.6	Crp	2	0.5, 2.0
Ptgdr	1	0.3, 2.0	C5r1	1, 2	0.4, 2.6	Itgb3	1, 2	0.2	Cspg2	2	0.4, 6.8
Ptger2	1	0.5	Cd36	1, 2	0.1	Klkb1	1, 2	0.4	Ctrl	2	0.2, 4.6
Ptgs1	1	3.4	Cfi	1, 2	6.4	Kng1	1, 2	2.1	Dcbld2	2	0.5, 4.3
Ptgs2	1	0.1, 2.1	Clca2	1, 2	0.5	Kptn	1, 2	0.2	Gc	2	3.4
Prss1	1	0.1	Clca3	1, 2	0.2	Mmrn1	1, 2	0.1	Lrp1	2	0.1, 2.0
Prss2	1	0.4, 39.4	Clca4	1, 2	0.3, 5.7	P2rx1	1, 2	9.7	Phyh	2	2.3
Serpind1	1	0.1	Cr2	1, 2	6.0	P2ry12	1, 2	0.1	Proc	2	0.3, 2.0
Tf	1	2.7	Crry	1, 2	2.4	P2y12	1, 2	0.2	Procr	2	6.5
Tfpi	1	4.4	Edn1	1, 2	0.4, 2.6	Pabpc4	1, 2	0.3, 2.1	Pros1	2	2.1
Tfpi2	1	0.4	Efemp2	1, 2	0.5, 2.4	Plat	1, 2	0.4, 4.9	Serpina5	2	0.1, 7.8
Tp53	1	2.9	Entpd2	1, 2	0.4	Plau	1, 2	0.4, 3.0	Serpinb9	2	5.3
Anxa2	1, 2	4.5	F10	1, 2	0.5	Plaur	1, 2	13.9	Serpine1	2	16.7
Anxa3	1, 2	3.9	F2	1, 2	0.3	Plg	1, 2	2.1	Serpinf2	2	0.2, 2.0
Anxa4	1, 2	2.0	F2rl2	1, 2	0.2	Plscr1	1, 2	7.5	Serpini2	2	0.4, 7.0
Anxa5	1, 2	2.3	F3	1, 2	0.1, 2.0	Ppbp	1, 2	0.1, 2.1	[3]Thbd	2	9.6
Anxa7	1, 2	6.8	F5	1, 2	0.5	Rab11fip1	1, 2	0.3, 8.6			

1: Extrinsic blood coagulation pathway; 2: Intrinsic blood coagulation pathway; [3]Reported genes associated with liver regeneration.

twofold change in expression level at least at one time point during liver regeneration with significant difference ($0.01 \leqslant P < 0.05$) or extremely significant difference ($P \leqslant 0.01$) between PH and SO, were referred to as associated with liver regeneration.

RESULTS

Expression changes associated with blood coagulation response during liver regeneration

According to the databases at NCBI, GENMAPP, KEGG, BIOCARTA and RGD, 357 genes were involved in blood coagulation, in which, 174 genes were contained in the Rat Genome 230 2.0 array. Among them, the expression of 107 genes displayed meaningful changes at least at one time point after PH, showed significant or extremely significant differences in expression between PH and SO, and displayed reproducible results at three detections with Rat Genome 230 2.0 array, suggesting that the genes were associated with LR. Fold changes in up-regulation range were more than 2 to 46 fold compared with control, and down-regulation more than 2 to 10 fold (Table 1). The analysis indicated that 44 genes were up-regulated, 36 genes down-, and 27 genes up/down- during liver regeneration. The total up- and down-regulation times were 342 and 253, respectively (Figure 1A). At the initiation phase (0.5-4 h after PH), 24 genes displayed up-regulation, 14 genes down, and 2 genes up/down; at the transition phase from G_0 to G_1 (4-6 h after PH), 24 genes up, and 8 genes down; at the cell proliferation phase (6-66 h after PH), 45 genes up, 42 genes down, and 11 genes up/down; at cell differentiation and structure-function reorganization phase (66-168 h after PH), 38 genes up, and 27 genes down, and 5 genes up/down (Figure 1B).

Initial expression time points associated with blood coagulation 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 follows in sequence: both 9 and 6 at 0.5 h; 6, 10 and 12, 14 at 1 h; 8, 0 and 16, 3 at 2 h; 4, 1 and 17, 4 at 4 h; 4, 2 and 21, 7 at 6 h; 1, 0 and 13, 5 at 8 h; 0, 5 and 12, 10 at 12 h; 7, 7 and 15, 13 at 16 h; 5, 8 and 17, 24 at 18 h; 3, 0 and 18, 16 at 24 h; 2, 3 and 11, 11 at 30 h; 0, 4 and 23, 18 at 36 h; 1, 1 and 9, 9 at 42 h; 1, 2 and 22, 24 at 48 h; 0, 0 and 12, 13 at 54 h; 0, 0 and 22, 11 at 60 h; 2, 0 and 21, 9 at 66 h; 0, 1 and 15, 11 at 72 h; 0, 0 and 11, 10 at 96 h; 4, 0 and 13, 9 at 120 h; 0, 0 and 15, 9 at 144 h; 0, 0 and 12, 13 at 168 h (Figure 2). Generally, gene expression changes occurred during the whole liver regeneration, with the up- and down-regulation times of 342 and 253, respectively. The initially up-regulated genes were predominantly expressed in the forepart, and the down-regulated genes in the prophase and metaphase, whereas there was little initial expression in the anaphase.

Expression similarity and time relevance associated with blood coagulation response during liver regeneration

Totally 107 genes could be characterized based on their similarity in expression as following: only up-,

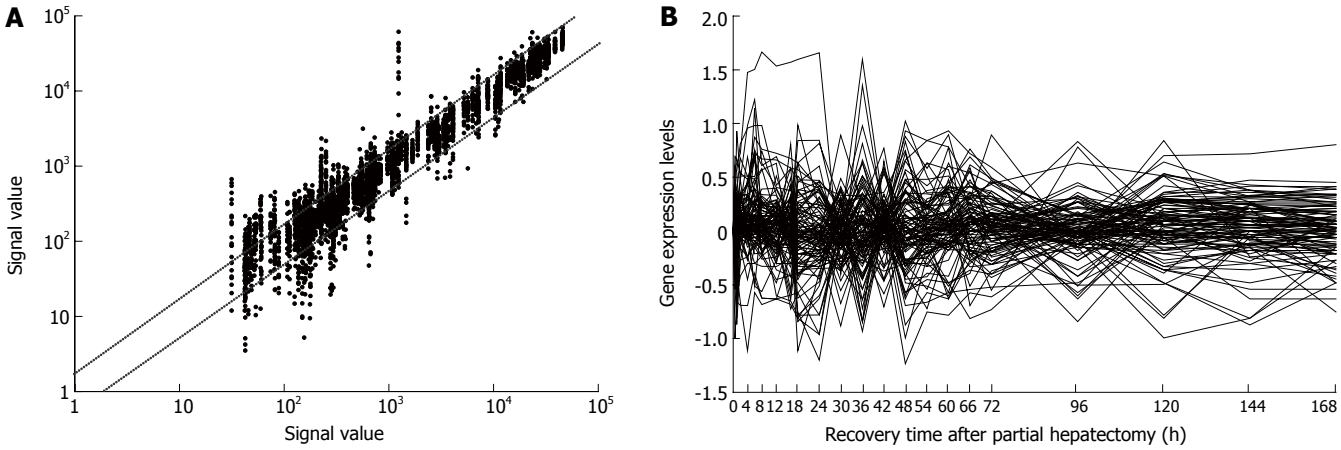

Figure 1 Expression frequency, abundance and changes of 107 blood coagulation response-associated genes during rat liver regeneration. Detection data of Rat Genome 230 2.0 array were analyzed and graphed with Microsoft Excel. **A**: Gene expression frequency. The dots above bias represent the genes up-regulated more than two fold, and total times of up-regulation were 342; those below bias down-regulated more than two fold, and times of down-regulation were 253; and the ones between biases no-sense alteration; **B**: Gene expression abundance and changes. Seventy-six genes were 2-46 fold up-regulated, and 62 genes 2-10 fold down- regulated.

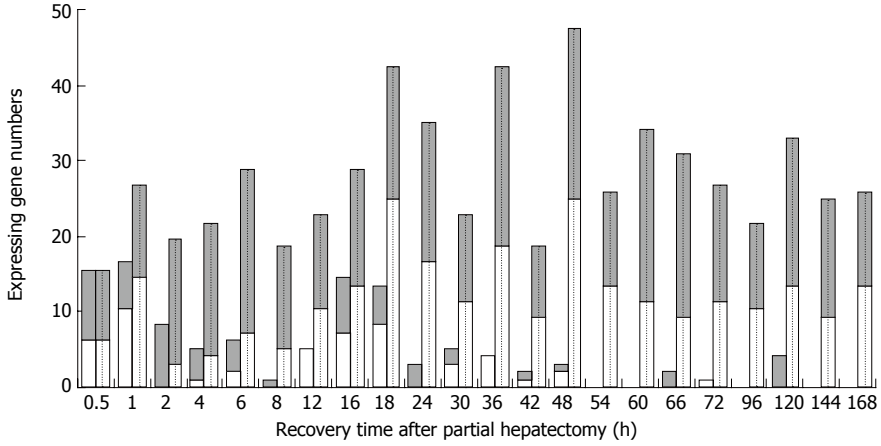

Figure 2 The initial and total expression profiles of 107 blood coagulation response-associated genes at each time point of liver regeneration. Grey bars: Up-regulated gene; White bars: Down-regulated. Blank bars represent initial expressing genes, in which up-regulated genes were predominant in the forepart, and the down-regulated genes in the prophase and metaphase, whereas there was little initial expression 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.

predominantly up-, only down-, predominantly down-, up-/down-regulated, involving 44, 8, 36, 13 and 6 genes, respectively (Figure 3). According to time relevance, they were classified into 15 groups, including 0.5 h, 1 h, 2 h, 4 and 6 h, 8 and 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, and the up- and down-regulation times were 9 and 6, 12 and 14, 16 and 3, 38 and 11, 25 and 15, 15 and 13, 35 and 40, 20 and 20, 23 and 18, 22 and 24, 34 and 24, 35 and 20, 11 and 10, 19 and 13, 27 and 22, respectively (Figure 3). The up-regulated expression genes were chiefly associated with promoting blood coagulation, and the down-regulated expression genes mostly associated with inhibiting blood coagulation.

Expression patterns of the genes associated with blood coagulation response during liver regeneration

The 107 genes were categorized into 29 patterns, according to the changes in their expression. (1) up-regulation at one time point, i.e. 6, 16, 30, 66, 120 h after partial hepatectomy (Figure 4A), 8 genes involved; (2) up at two time points, i.e. 6 and 42 h, 30 and 42 h (Figure 4B), 2 genes involved; (3) up at three time points (Figure 4B), 2 genes involved; (4) up at multiple time points (Figure 4C), 5 genes involved; (5) up at one phase, i.e. 1-48, 4-6, 120-168 h (Figure 4D), 3 genes involved; (6) up at one time point/phase, i.e. 0.5 and 4-6 h, 24 and 66-72 h, 42 and 120-168 h, 66 and 120-168 h (Figure 4E), 4 genes involved; (7) up at one time point/two phases (Figure 4F), 2 genes involved; (8) up at one time point/three phases (Figure 4F), 1 gene involved; (9) up at two time points/one phase (Figure 4G), 5 genes involved; (10) up at two time points/phases (Figure 4H), 2 genes involved; (11) up at two time points/three phases (Figure 4H), 3 genes involved; (12) up at three time points/one phase (Figure 4I), 3 genes involved; (13) up at three time points/two phases (Figure 4I), 1 gene involved; (14) up at multiple time points/phases (Figure 4J), 3 genes involved; (15) down at one time point, i.e. 16, 30, 36, 42, 48, 72 h (Figure 4K), 8 genes involved; (16) down at two time points, i.e. 0.5 and 48 h, 1 and 72 h, 16 and 30 h, 16 and 96 h, 18 and 48 h, 18 and 54 h, 30 and 48 h, 30 and 96 h, 36 and 48 h (Figure 4L), 10 genes involved; (17) down at multiple time points (Figure 4M), 4 genes involved; (18) down at one phase, i.e. 6-12 h (Figure 4N), 1 gene involved; (19) down at two phases, i.e. 18-24 and 48-54 h (Figure 4N), 1 gene involved; (20) down at one time point/phase, i.e. 1 and 96-120 h, 18 and 120-144 h, 36 and 12-24 h, 96 and 16-24 h (Figure 4O), 4 genes

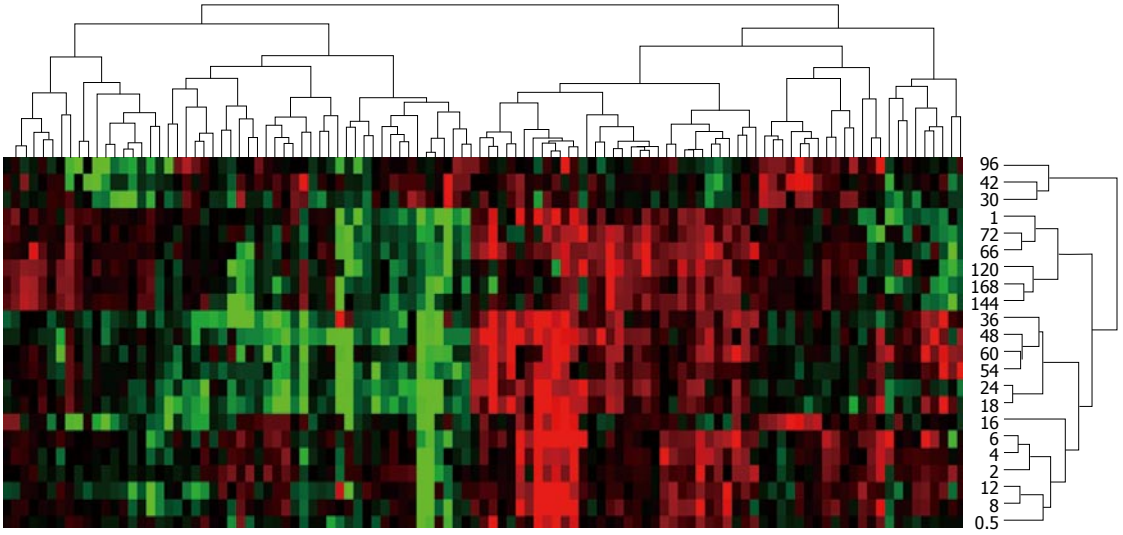

Figure 3 Expression similarity and time relevance clusters of 107 blood coagulation response-associated genes during liver regeneration. Detection data of Rat Genome 230 2.0 array were analyzed by H-clustering. Red represents up-regulated genes chiefly associated with promoting blood coagulation; Green represents down-regulated ones mainly associated with inhibiting blood coagulation; 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 15 groups separately.

involved; (21) down at one time point/two phases (Figure 4O), 1 gene involved; (22) down at one time point/three phases (Figure 4O), 1 gene involved; (23) down at two time points/one phase (Figure 4P), 3 genes involved; (24) down at two time points/phases (Figure 4P), 1 gene involved; (25) down at three time points/two phases (Figure 4Q), 1 gene involved; (26) down at multiple time points/phases (Figure 4Q), 1 gene involved; (27) predominantly up (Figure 4R), 8 genes involved; (28) predominantly down (Figure 4S), 13 genes involved; (29) similarly up/down (Figure 4T), 6 genes involved.

DISCUSSION

Blood coagulation plays an important physiological role. PH induces blood coagulation, which results from direct or indirect interaction between various proteins. Tissue factor (TF), associated with extrinsic blood coagulation, initiates extrinsic blood coagulation by acting on coagulation factor 7 (F7)[21]. Interleukin 6 (IL6) accelerates the transformation from fibrinogen to fibrin[22]. Twelve proteins including protease serine 2 (PRSS2) inhibit blood coagulation by suppressing the activities of TF and coagulation factors[23-25]. Seven proteins including annexin A 7 (ANXA7) accelerate the transformation from plasminogen to plasmin by inhibiting the activities of factor 10 (F10) and thrombin, repress blood coagulation, and induce migration and tube repair of blood vessel endothelial cells as well[26-28]. The meaningful expression profiles of the proteins encoding the above genes were the same or similar at some points while different at others, indicating that they may co-regulate extrinsic blood coagulation. Among them, *il6* was up-regulated at 2-8, 18, 48, 60 and 96 h, and reached a peak at 96 h, which was 6.1 times higher than the control. This is generally in line with the result reported by Takatori *et al*[29]. *tf* was up-regulated at 16, 30 and 96 h during liver regeneration, and reached a peak at 16 h, which was 2.7 times higher than the control. *prss2* was up-regulated mainly at 18, 36 and 54-60 h, and reached a peak at 36 h, which was 39.4 times higher than the control. *anxa7* was up-regulated at 16, 30, 42 and 96 h, and reached a peak at 96 h, which was 6.8 times higher than the control. It suggests that these genes play a key role in blood coagulation during liver regeneration.

Moreover, six proteins including phospholipid scramblase 1 (PLSCR1), associated with intrinsic blood coagulation, promote blood coagulation by activating coagulation factors[30-33]. Amyloid beta precursor protein (APP) and beta-1,4-galactosyltransferase 1 (B4GALT1) accelerate blood coagulation[34,35]. Nine proteins including alpha-2-macroglobulin (A2M) inhibit blood coagulation by reducing thrombin and collagenase[36-39]. Five proteins including serine peptidase inhibitor clade E member 1 (SERPINE1) depress fibrinolysis by inhibiting the activities of plasma serine protease protein C and plasminogen activator[40,41]. Twelve proteins including plasminogen activator tissue (PLAT) inhibit blood coagulation by converting plasminogen to plasmin through cleaving the Arg-Val bond[42-44]. Thrombomodulin (THBD) restrains blood coagulation[45]. The meaningful expression profiles of the genes encoding the proteins mentioned above were the same -ness or similarity at some points while difference at others, suggesting that they may co-regulate intrinsic blood coagulation. Among them, *thbd* showed up-regulation during almost the whole LR, and had the highest abundance of 9.6 times higher than control at 6 h, which is consistent with the result reported by Takatori *et al*[46]. *a2m* was up-regulated at 0.5-24, 36 and 48-54 h, and reached a peak at 8 h, which was 46.2 times higher than the control. This is generally in conformity with the result reported by Scotte *et al*[47]. *plscr1* was up-regulated at 2-24, 36 and 48-120 h during liver regeneration, and reached a peak at 6 h, which was 2.7 times higher than the control. *app* was up-regulated at multiple phases after PH, and reached a peak at 168 h, which was 3.1 times higher than the control. *serpine1* was up-regulated at 1-48 h, and reached a peak at 6 h, which was 16.7 times

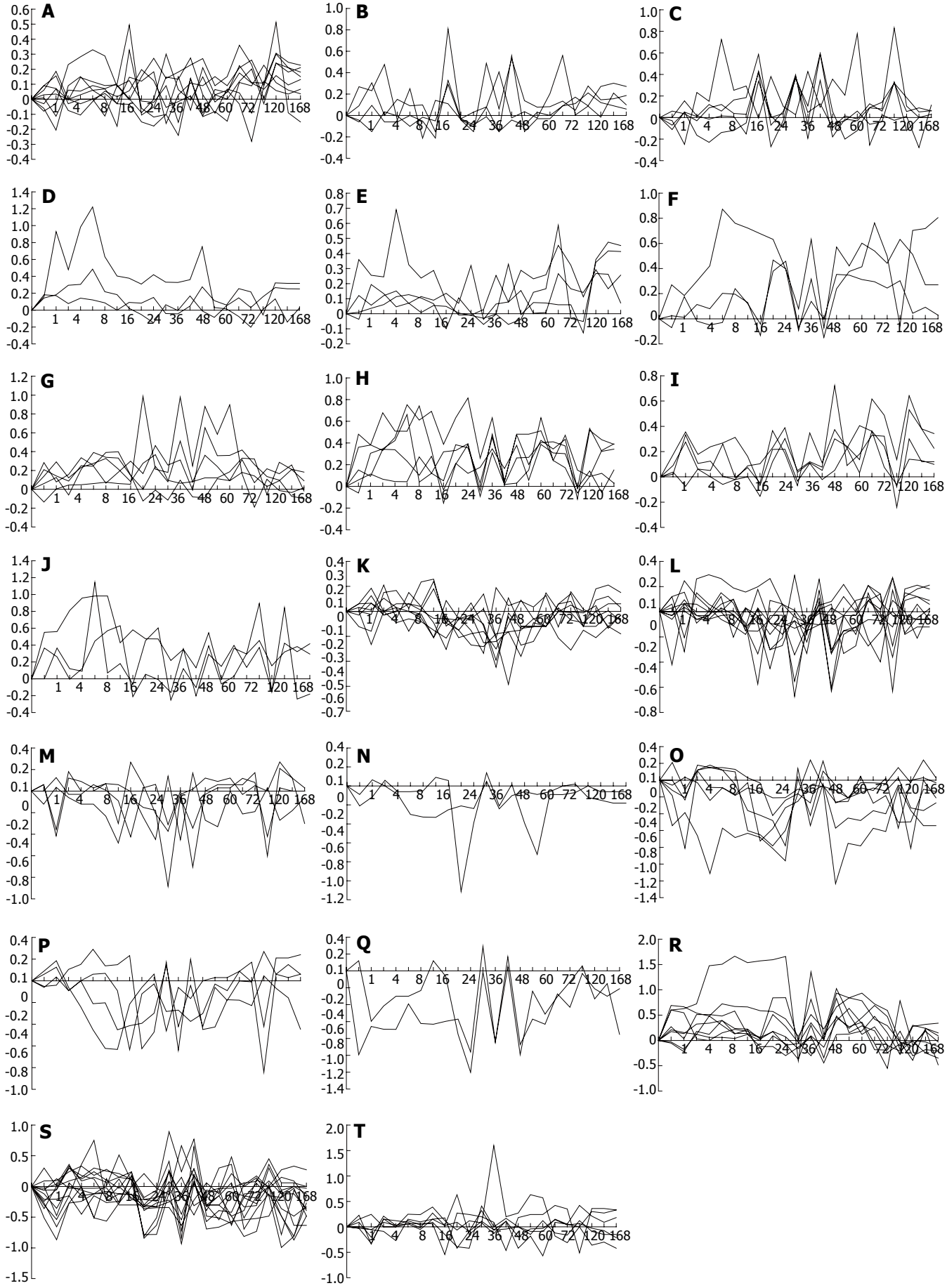

Figure 4 Twenty-nine gene expression patterns of 107 blood coagulation response-associated genes during liver regeneration. Expression patterns were obtained by the analysis of detection data of Rat Genome 230 2.0 array with Microsoft Excel. **A**-**J**: 44 up-regulated genes; **K**-**Q**: 36 down-regulated genes; **R**-**T**: 27 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.

higher than the control. *plat* was up-regulated at multiple phases after PH, and reached a peak at 66 h, which was 4.9 times higher than the control. It implies that these genes play a crucial role in blood coagulation during liver regeneration.

In summary, the expression changes of the genes associated with blood coagulation during liver regeneration have been investigated by high-throughput gene expression analysis and in long time range (0.5 h-7 d after PH) and at multiple time points (totally 23). It is preliminarily proved that PH can cause various physiological responses including blood coagulation, and that Rat Genome 230 2.0 array is a useful tool for analysis of the blood coagulation responses at gene transcriptional level. However, the processes of DNA→mRNA→protein are influenced by many factors including protein interactions. Therefore, our results need to be further analyzed using techniques, such as Northern blotting, protein chip, RNA interference, protein-interaction.

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