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

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 liver regeneration 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), G0/G1 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

Zhao LF, Zhang WM, Xu CS. Expression patterns and action analysis of genes associated with blood coagulation responses during rat liver regeneration. World J Gastroenterol 2006; 12(42): 6842-6849

INTRODUCTION

The liver is the main site where coagulation factors are synthesized[1]. Tissue damage is often accompanied with angiorrhexitis, 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 abnormality 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 G0 to G1 (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\(^{[8]}\); 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°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 1-2 g (0.1-0.2 g × 6) of total liver tissue, then stored at -80°C. The sham-operation (SO) groups were treated the same (0.1-0.2 g × 6) of total liver tissue, then stored at -80°C.

**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 RNaseasy mini kit (Qiagen)\(^{[16]}\). In brief, total liver tissues frozen at -80°C were homogenized in 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°C 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°C 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\(^{[18]}\).

**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\(^{[18]}\).

**Normalization of microarray data**

To minimize error from the microarray analysis, each analysis was performed three times. Results whose total ratio was maximal (R\(^{+}\)) and that whose average value of three housekeeping genes (β-actin, hexokinase and glyceraldehyde-3-phosphate dehydrogenase) approached 1.0 (R\(^{-}\)) were taken as a reference. The modified data were generated using a correction factor (R\(^{+}\)/R\(^{-}\)) multiplying the ratio of every gene in R\(^{+}\) 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\(^{[15,20]}\).

**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
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 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.

| Gene Abb. | Associated with fold difference | Gene Abb. | Associated with fold difference | Gene Abb. | Associated with fold difference | Gene Abb. | Associated with fold difference |
|-----------|---------------------------------|-----------|---------------------------------|-----------|---------------------------------|-----------|---------------------------------|
| C2        | 1.2                             | Anxa8     | 1.2                             | 0.4       | Fbrl1                            | 1.2       | 0.2                             | 3.8       |
| Cd59      | 1.0                             | Anxa9     | 1.2                             | 4.6       | Fgb                              | 1.2       | 4.3                             | 3.2       |
| Coch      | 0.2                             | App       | 1.2                             | 6.4       | Fg2                              | 1.2       | 3.1                             | 1.2, 0.2  |
| Daf1      | 0.2                             | B4gal1    | 1.2                             | 3.1       | Fili                             | 1.2       | 4.9                             | 1.2, 3.6  |
| Hrg       | 0.2                             | Bdkrb2    | 1.2                             | 0.4       | G7r                              | 1.2       | 3.9                             | 1.2, 3.2  |
| Il1b      | 0.2                             | C1qaA     | 1.2                             | 0.3       | Gepx                             | 1.2       | 0.3, 2.6                         | 1.2, 0.4  |
| T16       | 0.3, 6.1                        | C1qlr1    | 1.2                             | 5.5       | Gna12                            | 1.2       | 2.5                             | 3.4, 1.2  |
| Lma1      | 1.0                             | C3        | 1.2                             | 0.2       | Gnaq                             | 1.2       | 2.5                             | 0.5, 2.0  |
| Masp1     | 0.3                             | C3arin1   | 1.2                             | 2.3       | Hgf                              | 1.2       | 0.4                             | 0.4, 2.0  |
| Mb2b      | 0.2                             | C4a       | 1.2                             | 0.5       | Htr4a                            | 1.2       | 0.1, 4.5                         | 0.2, 2.5  |
| Nfk2      | 0.4                             | C4bpa     | 1.2                             | 2.0       | Hsot2                            | 1.2       | 0.1, 2.6                         | 0.2, 0.5  |
| P9gdr     | 0.3, 2.0                        | C5rl      | 1.2                             | 0.4, 2.6  | Hrg3                             | 1.2       | 0.2                             | 0.4, 6.8  |
| P9ger2    | 0.5                             | C6d6      | 1.2                             | 0.1       | Klkb1                            | 1.2       | 0.4                             | 0.4, 2.6  |
| Ptgs1     | 0.3                             | Cfl       | 1.2                             | 6.4       | Kngl                             | 1.2       | 2.1                             | 0.5, 4.3  |
| Ptgs2     | 0.1, 2.1                        | Cica2     | 1.2                             | 0.5       | Kptn                             | 1.2       | 0.2                             | 3.4       |
| Prrs1     | 0.1                             | Cica3     | 1.2                             | 0.2       | Mmrl1                            | 1.2       | 0.1                             | 0.2, 1.0  |
| Prrs2     | 0.4, 39.4                       | Cica4     | 1.2                             | 0.3, 5.7  | P2rx1                            | 1.2       | 9.7                             | 2.3       |
| Serpin1   | 0.1                             | Crl       | 1.2                             | 6.0       | P2ry12                           | 1.2       | 0.1                             | 0.3, 2.0  |
| Tf        | 2.7                             | Crry      | 1.2                             | 2.4       | P2ry12                           | 1.2       | 0.2                             | 3.5       |
| Tpr1      | 4.4                             | Edf1      | 1.2                             | 0.2, 4.6  | Palpe4                           | 1.2       | 0.3, 2.1                         | 2.1       |
| Tps2      | 0.4                             | Epm2f     | 1.2                             | 0.5, 2.4  | Pll                              | 1.2       | 0.4, 4.9                         | 0.7, 1.78 |
| Tp33      | 2.9                             | Entpd2    | 1.2                             | 0.4       | Pll                              | 1.2       | 0.4, 3.0                         | 0.5       |
| Anxa2     | 1.2                             | F10       | 1.2                             | 0.5       | Pslur                             | 1.2       | 1.3, 9                           | 0.0       |
| Anxa3     | 1.2                             | F2        | 1.2                             | 0.3       | Pll                              | 1.2       | 2.1                             | 2.0       |
| Anxa4     | 2.0                             | F2h2l     | 1.2                             | 0.2       | Pll                              | 1.2       | 7.5                             | 2.0       |
| Anxa5     | 2.3                             | F3        | 1.2                             | 0.1, 2.0  | Plep1                            | 1.2       | 0.1, 2.1                         | 0.7       |
| Anxa7     | 2.0                             | F5        | 1.2                             | 0.5       | Rab11fip1                         | 1.2       | 0.3, 8.6                         | 2.0       |

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

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, 15 and 9, 14 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-,

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).
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
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 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, \(i l 6\) 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\(^{[29]}\). \(i f\) 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. \(p r s s 2\) 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. \(a n x a 7\) 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 factor\(^{[30-33]}\). 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, \(t h b d\) 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\(^{[46]}\). \(a 2 m\) 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\(^{[47]}\). \(p l s r 1\) 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. \(a p p\) was up-regulated at multiple phases after PH, and reached a peak at 168 h, which was 3.1 times higher than the control. \(s e r p i n e 1\) 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. plate 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.

REFERENCES

1. Østebød B, Bjerkild E. Sources of tissue factor. Semin Thromb Hemost 2006; 32: 11-23
2. Machado FR, Silva E. Coagulation and sepsis. Endocr Metab Immune Disord Drug Targets 2006; 6: 175-182
3. Celikel R, McClintock RA, Roberts JR, Mendolicchio GL, Ware J, Varughese KJ, Ruggeri ZM. Modulation of alpha-thrombin function by distinct interactions with platelet glycoprotein Ib alpha. Science 2003; 301: 218-221
4. Khrenov AV, Ananyeva NM, Saenko EL. Role of the B domain in proteolytic inactivation of activated coagulation factor VIII by activated protein C and activated factor X. Blood Coagul Fibrinolysis 2006; 17: 379-388
5. Wu SY, Wang ZY, Dong NZ, Zhang W, Bai X, Ruan CG. [A novel genetic defect in a Chinese family with inherited coagulation factor XII deficiency]. Zhonghua Xueyexue Zazhi 2006; 27: 145-149
6. Higgins GM, Anderson RM. Experimental pathology of the liver I. Restoration of the liver of the white rat following partial surgical removal. Arch Pathol 1931; 12: 186-202
7. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. Hepatology 2006; 43: S45-S53
8. Michalopoulos GK, DeFrances M. Liver regeneration. Adv Biochem Eng Biotechnol 2005; 93: 101-134
9. Taub R. Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol 2004; 5: 836-847
10. Xu CS, Chang CF, Yuan JY, Li WQ, Han HP, Yang KJ, Zhao LF, Li YC, Zhang HY, Rahman S, Zhang JB. Expressed genes in regenerating rat liver after partial hepatectomy. World J Gastroenterol 2005; 11: 2952-2940
11. Okada K, Ueshima S, Imano M, Kataoka K, Matsuo O. The regulation of liver regeneration by the plasmin/alpha 2-antiplasmin system. J Hepatol 2004; 40: 110-116
12. Dransfeld O, Gehrmann T, Köhler K, Kircheis G, Holneicher C, Tiedje MA, Wietzke T, Moller S. Oligonucleotide microarray analysis of differential transporter regulation in the regenerating rat liver. Liver Int 2005; 25: 1243-1258
13. Knapp JH, Gehar MA, Forman MS, Valsamakis A. Comparison of automated and manual nucleic acid extraction methods for detection of enterovirus RNA. J Clin Microbiol 2003; 41: 3532-3536
14. Nuys S, Van Mellaert L, Lambin P, Arné J. Efficient isolation of total RNA from Clostridium without DNA contamination. J Microbiol Methods 2001; 44: 235-238
15. Arkin A, Ross J, McAdams HH. Stochastic kinetic analysis of developmental pathway bifurcation in phage lambda-infected Escherichia coli cells. Genetics 1998; 149: 1633-1648
16. Li L, Roden J, Shapiro BE, Wold BJ, Bhatia S, Forman SJ, Bahta R. Reproducibility, fidelity, and discriminant validity of mRNA amplification for microarray analysis from primary hematopoietic cells. J Mol Diag 2005; 7: 48-56
17. Hood L. Leroy Hood expounds the principles, practice and future of systems biology. Drug Discov Today 2003; 8: 436-438
18. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 1998; 95: 14863-14868
19. Werner T. Cluster analysis and promoter modelling as bioinformatics tools for the identification of target genes from expression array data. Pharmacogenomics 2001; 2: 25-36
20. Yue H,Eastman PS, Wang BB, Minor J, Doctolero MH, Nuttall RL, Stack R, Becker JW, Montgomery JR, Vainer M, Johnston R. An evaluation of the performance of cDNA microarrays for detecting changes in global mRNA expression. Nucleic Acids Res 2001; 29: E41-E41
21. Gomez K, McVey JH. Tissue factor initiated blood coagulation. Front Biosci 2006; 11: 1349-1359
22. Mannila MN, Ericsson P, Ericsson CG, Hamsten A, Silveira E. Epistatic and pleiotropic effects of polymorphisms in the fibrinogen and coagulation factor XIII genes on plasma fibrinogen concentration, fibrin gel structure and risk of myocardial infarction. Thromb Haemost 2006; 95: 420-427
23. Shan J, Baguinon M, Zheng L, Krishnamoorthi R. Expression, refolding, and activation of the catalytic domain of human blood coagulation factor XII. Protein Expr Purif 2003; 27: 143-149
24. Shrader WD, Kolesnikov A, Burgess-Henry J, Rai R, Hendrix J, Hu H, Torkelson S, Tong Y, Young WB, Katz BA, Yu C, Tang J, Cabuslay R, Sanford E, Janc JW, Sprenger PA. Factor Vlla inhibitors: gaining selectivity within the trypsin family. Biorg Med Chem Lett 2006; 16: 1596-1600
25. de Pont AC, Moons AH, de Jonge E, Meijers JC, Vlasuk GP, J, McVey JH. Tissue factor initiated blood coagulation. Thromb Haemost 2004; 2: 65-70
26. Park JE, Lee DH, Lee J, Park SG, Kim NS, Park BC, Cho S. Annexin A5 is a potential angiogenic mediator. Biochem Biophys Res Commun 2005; 337: 1283-1287
27. Peterson EA, Sutherland MR, Nesheim ME, Prydzial EL. Thrombin induces endothelial cell-surface exposure of the plasminogen receptor annexin 2. J Cell Sci 2005; 116; 2399-2408
28. Battistelli S, Vittianna A, Cappelli R, Stefanoni M, Roviello F. Protein S in cancer patients with non-metastatic solid tumours. Eur J Surg Oncol 2005; 31: 798-802
29. Fulop AK, Pocisk E, Brozzi M, Karaberyos C, Kiss A, Novak I, Szalai C, Dobozy O, Falus A. Hepatic regeneration induces transient acute phase reaction: systemic elevation of acute phase reactants and soluble cytokine receptors. Cell Biol Int 2001; 25: 585-592
30. Sims JP, Wiedmer T. Unraveling the mysteries of phospholipid scrambling. Thromb Haemost 2001; 86: 266-275
31. Matsuo K, Nakanishi N, Smitth JR, Lupski JR. Isolation of a paralog of the Doyne honeycomb retinal dystrophy gene from the multiple retina dysplasia critical region on 11q13. Hum Genet 2000; 106: 66-72
32. Pouvoir-Quintard VB, Bianchina EP, Calmel-Tareau C, Tagat M, Le Bonniec BF. Thrombin-activable factor X re-establishes coagulation and the interleukin-10 response in human endotoxemia. J Thromb Haemost 2004; 2: 65-70
33. Matsumura Y, Nakajima T, Kikutani M, Matsuzaki M, Ohno S, Inoue T, Ikeda T, Yamasaki K, Ueda T, Higashi M, Higashimoto K. Expression of cDNA microarrays for detecting changes in global mRNA expression. Nucleic Acids Res 2001; 29: E41-E41
beta-1,4-galactosyltransferase I causes the congenital disorder of glycosylation type IId. *J Clin Invest* 2002; 109: 725-733

36 **Paredes N**, Xu L, Berry LR, Chan AK. The effects of chemotherapeutic agents on the regulation of thrombin on cell surfaces. *Br J Haematol* 2003; 120: 315-324

37 **Varani K**, Portaluppi F, Gessi S, Merighi S, Ongini E, Belardi-nelli L, Borea PA. Dose and time effects of caffeine intake on human platelet adenosine A(2A) receptors: functional and biochemical aspects. *Circulation* 2000; 102: 285-289

38 **Rohlena J**, Kolkman JA, Boertjes RC, Mertens K, Lenting PJ. Residues Phe342-Asn346 of activated coagulation factor IX contribute to the interaction with low density lipoprotein receptor-related protein. *J Biol Chem* 2003; 278: 9394-9401

39 **Mosnier LO**, Griffin JH. Protein C antiocoagulant activity in relation to anti-inflammatory and anti-apoptotic activities. *Front Biosci* 2006; 11: 2381-2399

40 **Sabovic M**, Salobir B, Preloznik Zupan I, Bratina P, Bojec V, Buturovic Ponikvar J. The influence of the haemodialysis procedure on platelets, coagulation and fibrinolysis. *Pathophysiol Haemost Thromb* 2005; 34: 274-278

41 **Nagai N**, Habuchi H, Esko JD, Kimata K. Stem domains of heparan sulfate 6-O-sulfotransferase are required for Golgi localization, oligomer formation and enzyme activity. *J Cell Sci* 2004; 117: 3331-3341

42 **Ji X**, Meng R, Zhou J, Ling F, Jia J. Dynamic change of coagulation and anticoagulation markers of patients with acute cerebral infarction during intravenous urokinase thrombolysis. *Neuror Res* 2006; 28: 46-49

43 **Oszajca K**, Sznajd J, Bartkowiak J. [The influence of nitric oxide on the regulation of plasminogen activator inhibitor type 1 and tissue-type plasminogen activator expression]. *Postepy Biochem* 2005; 51: 407-413

44 **Shariat-Madar Z**, Mahdi F, Warnock M, Homeister JW, Srikanth S, Krijanovski Y, Murphey LJ, Jaffa AA, Schmaier AH. Bradykinin B2 receptor knockout mice are protected from thrombosis by increased nitric oxide and prostacyclin. *Blood* 2006; 108: 192-199

45 **Yamashita T**, Sekiguchi A, Iwasaki YK, Sagara K, Hatano S, Linuma H, Aizawa T, Fu LT. Thrombomodulin and tissue factor pathway inhibitor in endothelial of rapidly paced rat atria. *Circulation* 2003; 108: 2450-2452

46 **Takatori M**, Iwabuchi S, Ro S, Murayama M, Maeyama S, Uchikoshi T, Nakano M, Ishii H. Increased serum levels and sinusoidal expression of thrombomodulin in acute liver damage. *Thromb Res* 1999; 93: 113-120

47 **Scoté M**, Masson S, Lyouni S, Hiron M, Ténière P, Lefebvre JP, Daveau M. Cytokine gene expression in liver following minor or major hepatectomy in rat. *Cytokine* 1997; 9: 859-867