# Expression patterns and action analysis of genes associated with the responses to fear, wound and pain during rat liver regeneration☆

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**Abstract**

*Objective*. The aim of this investigation was to study the responses to fear, wound and pain after partial hepatectomy (PH) at transcriptional level. *Methods*. The genes associated with the responses to fear, wound and pain were obtained by collecting the data of databases and referring to thesis. Their expression changes during liver regeneration (LR) were checked by Rat Genome 230 2.0 Array. *Results*. It was found that the mRNA level of 22, 117 and 14 genes involved in the responses to fear, wound and pain respectively were significantly changed during LR. The initial and total expressed gene numbers at the four phases of LR, i.e. the initiation (0.5 – 4 hours after PH), the transition from G0 to G1 (4 – 6 hours after PH), the cell proliferation (6 – 66 hours after PH), the cell differentiation and structure-function reorganization (66 – 168 hours after PH) were 70, 15, 65, 6 and 70, 51, 133, 99, respectively, demonstrating the associated genes were mainly triggered at the early phase, and worked at different phases. Based on their expression similarity, the genes were classified into 5 groups: only up-, predominantly up-, only down-, predominantly down-, up- and down-regulation, involving in 55, 19, 44, 18 and 5 genes, respectively, and the total times of their up- and down-regulation expression were 600 and 343 respectively, demonstrating that the expression of most genes was increased, whereas the few was declined. Time relevance and expression patterns of the genes were respectively sorted into 13 and 25 groups, displaying that the cellular physiological and biochemical activities were staggered, diverse and complex during LR. *Conclusion*. The responses to fear and pain were increased mainly in the early phase and prophase during LR, and wound in the early phase, prophase and later phase, and 141 genes associated with LR play an important role in these responses. [Life Science Journal. 2007;4(1):61 – 70] (ISSN: 1097 – 8135).

**Keywords**: partial hepatectomy; Rat Genome 230 2.0 Array; responses to fear, wound and pain; genes; liver regeneration

## 1 Introduction

When prokaryote and eukaryote undergo distinct stimuli such as heat[1,2], cold[3], change of osmotic pressure[4], water deprivation[5], drug[6], toxicant[7], oxidation[8], unfolded protein[9], pathogen infection[10, 11], fear[12], wound[13], pain[14], hypoxia[15], ischemia[16], nutritional deficiency[17], hormonprivia[18], starvation[19] and so on, the relevant stress protein (SP) genes are activated to protect organisms against these harmful stimuli. Highly conservative SPs in structure and function (e.g. heat shock protein 70 family) can be nearly induced by all stimuli although other SPs generated by different stress responses are diverse. Moreover, phenomenon that the stress response activated by one stimulus can increase cellular tolerance to another stimulus implies functional cross of SPs induced by different stimuli[20].

Rat liver has strong capacity for regeneration[21, 22]. The remnant hepatocytes compensate the lost liver tissue by proliferating after partial hepatectomy (PH)[23], which is called liver regeneration (LR)[24]. According to the cellular physiological activities, the regeneration process is usually categorized into four stages: initiation phase (0.5 – 4 hours after PH), transition from G0 to G1 (4 – 6 hours after PH), cell proliferation (6 – 66 hours after PH), cell differentiation and reorganization of the structure-function (66 – 168 hours after PH)[24]. According to time course, it is classified into four phases including forepart (0.5 – 4 hours after PH), prophase (6 – 12 hours after PH), metaphase (16 – 66 hours after PH), and anaphase (72 – 168 hours after PH)[22]. In addition, as a harmful stimulus, PH can in-

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duce the responses to fear, wound and pain that involve more than 300 genes. Moreover, there exist gene-gene interactions. It is almost impossible to clarify the action of genes associated with the responses to fear, wound and pain during LR at transcriptional level unless high-throughput gene expression arrays are used[24-26]. So, we used the Rat Genome 230 2.0 Array containing 38 genes involved in fear response, 217 genes in wound and 26 genes in pain to detect the gene expression changes after PH. 141 genes were found to be associated with LR. Meanwhile, their expression character, patterns and actions in regenerating liver were further analyzed.

## 2 Materials and Methods

### 2.1 Regenerating liver preparation

Healthy Sprague-Dawley rats weighing 200 - 250 g were obtained from the Animal Center of Henan Normal University. The rats were separated into two groups at random and each group included 6 rats (male: female = 1:1). Partial hepatectomy (PH) was performed according to Higgins and Anderson[21], the left and middle lobes of liver were removed. Rats were killed by cervical vertebra dislocation at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 54, 66, 72, 120, 144 and 168 hours after PH and the regenerating livers were observed at corresponding time point. The livers were rinsed three times in PBS at 4 ℃. Then 100 - 200 mg of liver tissues from the middle regions of the right lobe (0.1 - 0.2 g × 6 samples, per group) were gathered and stored at - 80 ℃. The sham-operation (SO) group was the same as PH ones except the liver lobes unremoved. The laws of animal protection of China were enforced strictly.

### 2.2 RNA isolation and purification

Total RNA was isolated from frozen livers according to the manual of Trizol kit (Invitrogen Corporation, Carlsbad, California, USA)[30] and then purified base on the guide of RNeasy mini kit (Qiagen, Inc, Valencia, CA, USA)[31]. Total RNA samples were checked to exhibit a 2:1 ratio of 28S rRNA to 18S rRNA intensities by agarose electrophoresis (180 V, 0.5 hour). Total RNA concentration and purity were estimated by optical density measurements at 260/280 nm[32].

### 2.3 cDNA, cRNA synthesis and purification

As template, 1 - 8 μg total RNA was used for cDNA synthesis. cDNA purification was proceeded based on the way established by Affymetrix[27]. cRNA labeled with biotin was synthesized using cDNA as the template, and cDNA and cRNA were purified followed by the GeneChip analysis purification procedure[27]. Measurement of cDNA, cRNA concentration and purity were the same as above.

### 2.4 cRNA fragmentation and microarray detection

15 μl (1 μg/μl) cRNA incubated with 5 × fragmentation buffer was digested into 35 - 200 bp fragments at 94 ℃ for 35 minutes. Rat Genome 230 2.0 microarray produced by Affymetrix was prehybridized, then the hybridization buffer was added at 45 ℃, 60 rpm for 16 hours. 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[28].

### 2.5 Microarray data analysis

The normalized signal values, signal detections (P, A, M) and experiment/control (Ri) were obtained by quantifying and normalizing the signal values using GCOS (GeneChip operating software) 1.2[28].

### 2.6 Normalization of the microarray data

To minimize error, each sample at each time point during LR was analyzed three times by Rat Genome 230 2.0 microarray. Results with a total ratio were maximal ($R^m$) and the average of three housekeeping genes (β-actin, hexokinase and glyseraldehyde-3-phosphate dehydrogenase) approached 1.0 ($R^h$) was taken as a reference. The modified data were generated by applying a correction factor ($R^m/R^h$) multiplying the ratio of every gene in $R^h$ at each time point. To remove spurious gene expression changes resulting from errors in the microarray analysis, the gene expression profiles at 0 - 4 hours, 6 - 12 hours and 12 - 24 hours after PH were reorganized by normalization analysis program (NAP) software according to the cell cycle progression of the regenerating hepatocytes. Data statistics and cluster analysis were done using GeneMath, GeneSpring, Microsoft Excel software[28, 33, 34].

### 2.7 Identification of genes associated with LR

Firstly, the nomenclature of the above three physiological responses obtained from the GENEONTOLOGY database (www.geneontology.org) was input into the databases at NCBI (www.ncbi.nlm.nih.gov) and RGD (rgd.mcw.edu) to identify the rat, mouse and human genes associated with the physiological responses. And the genes associated with the above responses were collated according to maps of biological pathways embodied by GENMAPP (www.genmapp.org), KEGG (www.genome.jp/keg/pathway.html#amino) and BIOCARTA (www.biocart a.com/genes/index.asp). Secondly, the results of these analysis were codified, and then compared with the results from mouse and human searches to identify human and mouse genes which are different from those of rat. These genes (human and mouse genes differed from those of rat) were compared with the analysis output of the Rat Genome 230 2.0 Array. Those genes which showed more than twofold changes in expression level, observed as meaningful expression changes[35], were referred to as rat homologous or rat specific genes associated with the responses to fear, wound and pain under evaluation. Genes, which displayed reproducible results with three independent analysis with the chip and which showed

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

## 3 Results

### 3.1 Expression changes of the genes associated with the responses to fear, wound and pain during LR

According to the NCBI, GENMAPP, KEGG, BIOCARTA and RGD databases, the responses to fear, wound and pain involved in 40, 251 and 26 genes respectively, with separately 38, 217 and 26 genes contained in Rat Genome 230 2.0 Array. Among them, 22, 117 and 14 genes displayed meaningful changes in expression at least at one time point after PH, showed significant or extremely significant differences in expression when PH was compared with SO, and were repeatable in three Rat Genome 230 2.0 Array. The results suggested that the genes were associated with LR. Abundance change in up-regulation ranged from 2 to 128 folds of control, and down-regulation from 2 to 10 (Table 1). The analysis indicated that 55 genes were up-regulated, 44 genes down-, 42 genes up/down- during LR. Total up- and down-regulated frequencies were 600 and 343, respectively (Figure 1A). At the initiation stage of LR (0.5 – 4 hours after PH), 42 genes displayed up-regulation, 26 genes down-regulation, 2 genes up/down- regulation; at the transition phase from G0 to G1 (4 – 6 hours after PH), 36 genes revealed up-regulation, 14 genes down- regulation, 1 gene up/down-regulation; at cell proliferation phase (6 – 66 hours after PH), 60 genes showed up-regulation, 43 genes down-regulation, 30 genes up/down-regulation; at cell differentiation and reorganization of the structure-function stage (66 – 168 hours after PH), 52 genes were up-regulated, 33 genes down-regulated, 14 genes up/down-regulated (Figure 1B).

**Table 1.** Expression abundance of 141 genes associated with the responses to fear, wound and pain during rat LR

| Gene Abbr. | Accosiated to others | Fold difference | Gene Abbr. | Accosiated to others | Fold difference | Gene Abbr. | Accosiated to others | Fold difference | Gene Abbr. | Accosiated to others | Fold difference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1 Fear** | | | *Ccl2 | | 128.0 | Irf1 | | 0.3 | Scn3a | | 0.5, 2.8 |
| Als2 | | 0.5 | *Ccl20 | | 8.0 | Itgb3 | | 0.2 | Scube1 | | 3.2 |
| Bdnf | 3 | 0.4, 2.6 | Ccl4 | | 0.2, 3.0 | Klrc2 | | 0.4, 2.3 | Sele | | 12.9 |
| Camkk2 | | 0.5, 7.6 | Ccr1 | | 0.4, 27.9 | *Kng1 | | 2.1 | Serpina5 | | 0.1, 7.8 |
| Cnr1 | | 0.1 | *Cd36 | | 0.1 | Ltb4r | | 0.5, 8.7 | *Serpind1 | | 0.1 |
| Creb1 | 3 | 0.5 | Cd3d | | 0.4, 4.0 | *Lyz | | 0.4, 3.7 | Serpine1 | | 16.7 |
| Drd2 | 3 | 8.6 | Cklf1 | | 8.3 | Mbp | | 0.4 | Sod2 | | 5.6 |
| *Egr1 | 2, 3 | 18.6 | Coch | | 0.2, 2.3 | Mcpt6 | | 0.2 | *Spn | | 0.2, 4.0 |
| Fmr1 | | 0.4, 2.5 | Copeb | | 10.6 | Mif | | 3.2 | Tac1 | 3 | 0.2 |
| *Fos | 3 | 28.4 | *Cramp | | 0.4, 2.8 | Msn | | 5.0 | Tap1 | | 2.2 |
| Gap43 | 2 | 0.3 | Crcp | | 0.3 | Mug1 | | 0.1, 3.5 | *Tcrb | | 0.2 |
| Grik2 | | 0.4, 2.4 | *Crp | | 0.5 | *Ncf1 | | 0.2, 3.7 | Tcrg | | 0.3 |
| Mapk1 | 3 | 2.7 | *Csf2 | | 0.3 | Nfkb1 | 1 | 0.4, 2.3 | Tff1 | | 0.1 |
| Neurod2 | | 3.2 | Ctgf | | 13.9 | Ninj1 | | 5.3 | Tff3 | | 0.3 |
| Nfkb1 | 2 | 0.4, 2.3 | Ctsb | | 3.6 | Ninj2 | | 2.6 | Tfpi | | 4.4 |
| Nr3c1 | | 4.7 | Ctse | | 0.4 | Ocil | | 0.1, 9.1 | Tfpi2 | | 0.4 |
| Reln | | 0.3 | Cybb | | 2.5 | Oldlr1 | | 0.3, 6.5 | Thbd | | 9.6 |
| Sgk | | 6.5 | Cysltr1 | | 0.1, 2.1 | P2ry12 | | 0.1 | Tm4sf11 | | 0.4, 4.3 |
| Siat8b | | 0.1 | Dad1 | | 0.2, 2.5 | P2y12 | | 0.2 | Tm4sf3 | | 3.6 |
| Slc6a4 | | 0.0 | Ddt | | 4.4 | Pap | | 66.2 | *Tm4sf4 | | 2.0 |
| Snca | | 0.2 | Edg3 | | 4.0 | Pawr | | 0.3 | *Tnf | 3 | 3.2 |
| Stmn1 | | 0.2, 15.9 | *Egr1 | 1, 3 | 18.6 | Pecam1 | | 3.5 | Tnfrsf4 | | 0.3, 2.3 |
| Vdac3 | | 6.0 | F10 | | 0.5 | Plat | | 0.4, 4.9 | Umod | | 0.4, 3.0 |
| **2 Wound** | | | *F2 | | 0.3 | Plg | | 2.1 | Znf148 | | 3.6 |
| *A2m | | 0.4, 46.2 | *F2rl2 | | 0.2 | Plp | | 0.1 | **3 Pain** | | |
| Abhd2 | | 0.4 | F5 | | 0.5 | *Plscr1 | | 7.5 | Bdnf | 1 | 0.4, 2.6 |
| Adam15 | | 14.0 | Gap43 | 1 | 0.3 | *Ppbp | | 0.1, 2.1 | Creb1 | 1 | 0.5 |
| Ager | | 0.4 | Gdnf | | 0.4 | Prdx5 | | 0.5 | Cyp19a1 | | 0.2, 6.5 |
| Alox5 | | 0.2, 2.5 | Gfap | | 0.3, 2.6 | Prkca | | 4.6 | Drd2 | 1 | 8.6 |
| Alox5ap | | 4.9 | Ggtla1 | | 4.0 | Proc | | 0.3 | Edn1 | | 0.4, 2.6 |
| Anxa2 | | 4.5 | Hdac7a | | 0.5, 4.3 | Procr | | 6.5 | *Egr1 | 1, 2 | 18.6 |
| Art2b | | 0.3 | Hnf4a | | 0.1, 4.5 | Pros1 | | 2.1 | *Fos | 1 | 28.4 |
| Atrn | | 4.4 | Hoxb13 | | 0.5, 3.5 | Prrx2 | | 3.6 | Gja4 | | 3.8 |
| B4galt1 | | 3.1 | Hrh1 | | 0.5, 9.9 | Ptafr | | 7.1 | Il1b | | 0.4 |
| Bcl10 | | 2.3 | *Ifng | | 6.5 | Ptger3 | | 0.2 | Mapk1 | 1 | 2.7 |
| C1qr1 | | 5.5 | Ighe | | 0.2, 2.5 | *Ptprc | | 0.1, 3.0 | Ptgs2 | | 0.1, 2.1 |
| C3 | | 0.2 | *Il1r1 | | 0.5 | Rab27a | | 3.4 | Tac1 | 2 | 0.2 |
| *C5r1 | | 0.4, 2.6 | Il2ra | | 0.3, 4.3 | S100a8 | | 6.5 | *Tnf | 2 | 3.2 |
| Ccl17 | | 0.1 | Il4 | | 0.1, 2.6 | S100a9 | | 4.9 | Trpv1 | | 0.3 |

*Reported genes associated with LR; Associated to others: involved in other responses

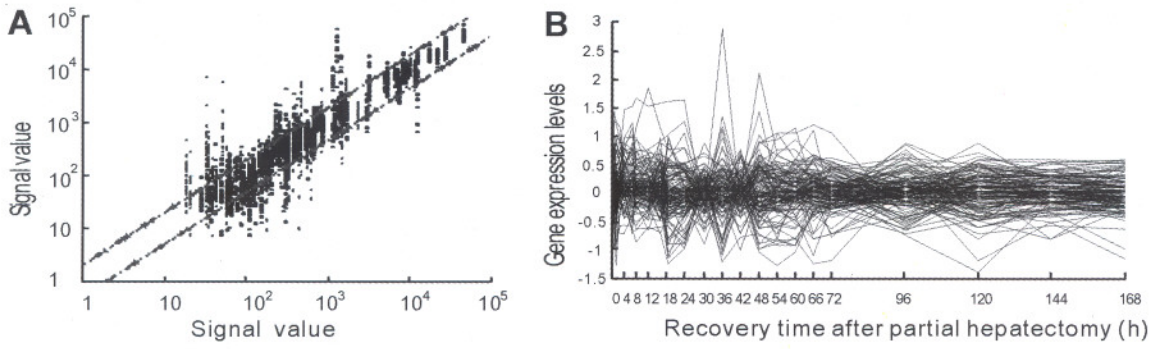


**Figure 1.** Expression frequency, abundance and changes of 141 genes associated with the responses to fear, wound and pain during rat LR. Detection data of Rat Genome 230 2.0 Array were analyzed and graphed by Microsoft Excel. A. Gene expression frequency. The dots above bias represent the genes up-regulated more than two folds, and total times of up-regulation were 600; those under bias down-regulated more than two folds, and that of down-regulation were 343; and the ones between biases no-sense alterative; B. Gene expression abundance and changes. 97 genes were 2 – 128 folds up-regulated, and 86 genes 2 – 10 folds down-regulated.

### 3.2 Initiation expression time of the genes associated with the responses to fear, wound and pain during LR

At each time point of LR, the numbers of initial up, down and total up, down-regulated genes were in sequence: both 17 and 7 at 0.5 hour; 10, 14 and 26, 19 at 1 hour; 9, 2 and 30, 6 at 2 hours; 6, 5 and 34, 9 at 4 hours; 3, 1 and 30, 11 at 6 hours; 0, 0 and 30, 7 at 8 hours; 2, 3 and 31, 11 at 12 hours; 11, 8 and 37, 14 at 16 hours; 8, 12 and 32, 27 at 18 hours; 1, 2 and 31, 22 at 24 hours; 2, 3 and 19, 16 at 30 hours; 0, 2 and 30, 21 at 36 hours; 0, 2 and 19, 12 at 42 hours; 2, 2 and 42, 29 at 48 hours; 1, 0 and 27, 21 at 54 hours; 0, 0 and 27, 16 at 60 hours; 0, 0 and 24, 16 at 66 hours; 0, 0 and 20, 11 at 72 hours; 2, 1 and 23, 18 at 96 hours; 1, 2 and 31, 23 at 120 hours; 0, 0 and 22, 12 at 144 hours; 0, 0 and 18, 15 at 168 hours (Figure 2). On the whole, gene expression changes span the whole LR, with 600 times in up-regulation and 343 times in down-regulation, respectively. The initially up-regulated genes were predominant in the forepart and prophase, and the down-regulated in the metaphase, whereas there was almost no initial expression in the anaphase.

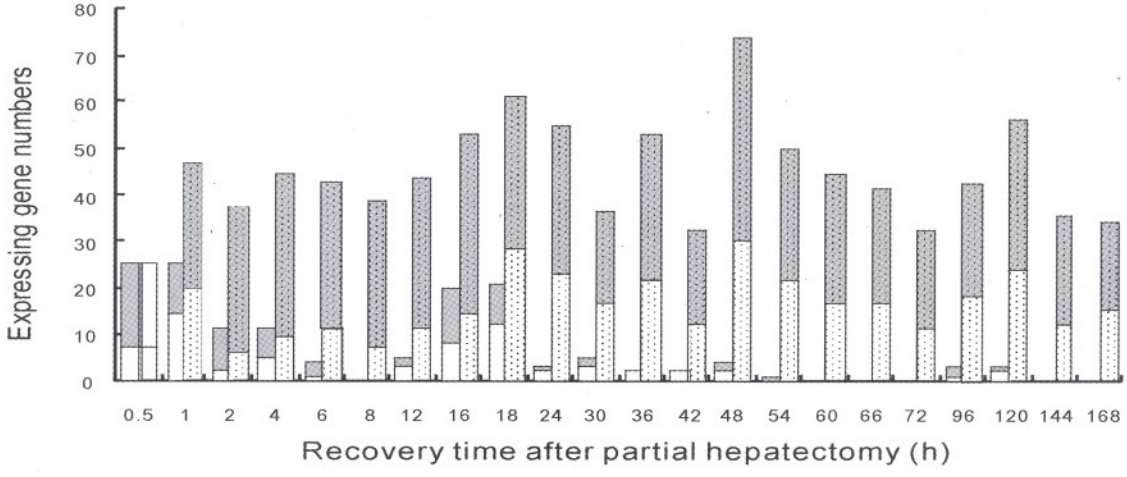


**Figure 2.** The initial and total expression profiles of 141 genes associated with the responses to fear, wound and pain at each time point of LR. Grey bars: Up-regulated genes; White bars: Down-regulated genes. Blank bars indicate initially expressed genes, in which up-regulation genes are predominant in the forepart and prophase, and the down-regulation in the metaphase, whereas very few in the anaphase. Dotted bars indicate the totally expressed genes, in which some genes are up regulation and others down during LR.

### 3.3 Expression similarity and time relevance of the genes associated with the responses to fear, wound and pain during LR

141 genes mentioned above during LR could be characterized based on their similarity in expression as following: only up-, predominantly up-, only down-, predominantly down-, and up-/down-regulated, involved in 55, 19, 44, 18 and 5 genes, respectively

(Figure 3). 141 genes could also be classified based on time relevance into 13 groups including 0.5 and 168h, 1 and 2 hours, 4 hours, 6 and 8 hours, 12 and 16 hours, 18 and 120 hours, 24 and 30 hours, 36 and 48 hours, 42 hours, 54 hours, 60 and 66 hours, 72 and 96 hours, 144 hours, in which the up- and down-regulated gene numbers were 35 and 22; 56 and 25; 34 and 9; 64 and 20; 68 and 25; 62 and 45; 49 and 37; 72 and 50; 19 and 12; 27 and 21; 44 and 27; 45 and 30; 22 and 12, respectively (Figure 3). The up-regulation genes were mainly associated with inflammation response, structure-function reconstruction of nervous tissue, oxidation resisting, revascularization and anti-coagulation. The down genes were mostly with immune response and pro-coagulation.

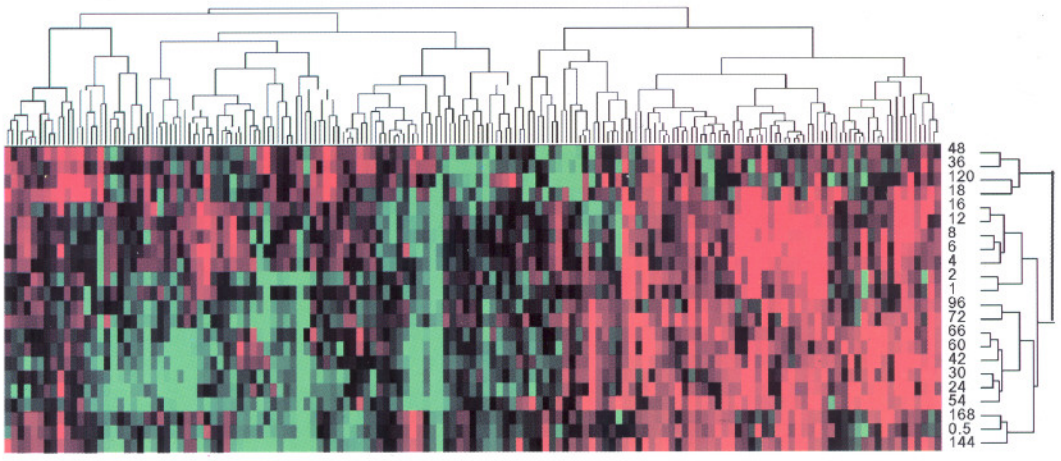


**Figure 3.** Expression similarity and time relevance cluster of 141 genes associated with the responses to fear, wound and pain during LR. Detection data of Rat Genome 230 2.0 Array were analyzed by H-clustering. Red represents up-regulation genes mainly associated with inflammation response, structure-function reconstruction of nervous tissue, oxidation resisting, revascularization and anti-coagulation; Green represents the down ones mostly associated with alcohol degradation; Black: No-sense change in expression. 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.

### 3.4 Expression patterns of the genes associated with the responses to fear, wound and pain during LR

Above 141 genes during LR might be categorized according to the expression changes into 25 patterns: (1) up-regulation at one time point, at 6, 16, 30, 48, 54, 66, 96, 120 hours after PH (Figure 4A), 10 genes involved; (2) up at two time points, at 16 and 42 hours, 16 and 96 hours, 48 and 120 hours (Figure 4B), 3 genes involved; (3) up at three time points (Figure 4B), 3 genes involved; (4) up at four time points (Figure 4C), 5 genes involved; (5) up at one phase, at 1 – 48 hours, 18 – 24 hours (Figure 4D), 2 genes involved; (6) up at two time points/one phase (Figure 4D), 4 genes involved; (7) up at three time points/one phase (Figure 4E), 3 genes involved; (8) up at one time point/two phases (Figure 4E), 3 genes involved; (9) up at two time points/two phases (Figure 4F), 4 genes involved; (10) up at three time points/two phases (Figure 4G), 5 genes involved; (11) up at two time points/three phases (Figure 4H), 5 genes involved; (12) up at one time point/four phases (Figure 4H), 3 genes involved; (13) at more time points/phases (Figure 4I), 5 genes involved; (14) down at one time point, at 6, 16, 24, 36, 42, 48, 96, 120 hours (Figure 4J), 10 genes involved; (15) down at two time points, at 0.5 and 18 hours, 6 and 12 hours, 16 and 30 hours, 18 and 48 hours, 18 and 54 hours, 24 and 54 hours, 30 and 42 hours, 30 and 48 hours, 30 and 96 hours, 36 and 48 hours, 48 and 60 hours (Figure 4K), 11 genes involved; (16) down at three time points (Figure 4L), 2 genes involved; (17) down at four time points (Figure 4L), 4 genes involved; (18) down at one phase, at 6 – 12 hours, 120 – 168 hours (Figure 4M), 2 genes involved; (19) down at one time point/one phase, at 1 and 120 – 144 hours, 36 and 12 – 24 hours, 48 and 18 – 24 hours, 120 and 18 – 24 hours (Figure 4M), 4 genes involved; (20) down at one time point/two phases (Figure 4N), 2 genes involved; (21) down at two time points/two phases (Figure 4N), 2 genes involved; (22) down at more time points or phases (Figure 4O), 7 genes involved; (23) first up and then down (Figure 4P), 9 genes involved; (24) first down and then up (Figure 4Q), 6 genes involved; (25) up/down mixed (Figure 4R), 27 genes involved.

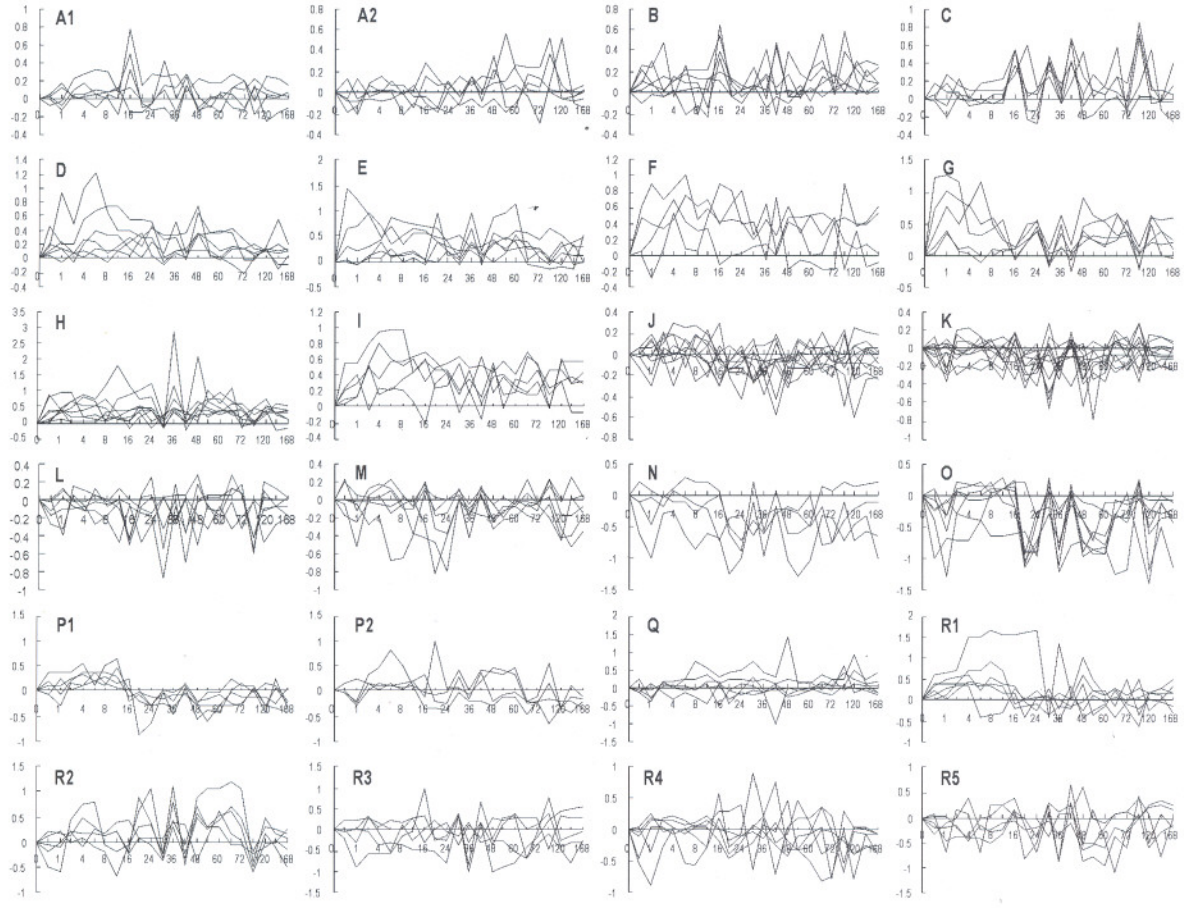


**Figure 4.** Twenty-five expression patterns of 141 genes associated with the responses to fear, wound and pain during LR. Expression patterns were obtained by the analysis of detection data of Rat Genome 230 2.0 Array with Microsoft Excel. A－I. 55 up-regulated genes; J－O. 44 down-regulated genes; P－R. 42 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.

## 4 Discussion

PH serves as an inducement to physiological responses, such as fear, wound and pain and so on, which result from direct or indirect interaction between various proteins. Among the proteins associated with fear response, five kinds of proteins including calcium/calmodulin-dependent protein kinase kinase 2 (CAMKK2) are associated with emotion, memory and fear[36]. Six kinds of proteins including dopamine receptor 2 (DRD2) are related to regulation of neurotransmission and anxiety[37]. Four kinds of proteins including early growth response 1(EGR1) regulate apoptosis[38]. Four kinds of proteins including cannabinoid receptor 1 (CNR1) participate in neuron activation and nerve regeneration, and prevent the neurodegenerative process from occurring[39]. The meaningful expression changes of these genes are same or similar at some time points, then different at other points during LR, speculating that they co-regulate the response to fear. Among them, *egr*1 was up-regulated during almost the whole LR, especially significantly in the forepart, and reached a peak at 1 hour that is 18.6 folds of control, which was consistent with the results reported by Mueller *et al*[40]. *camkk*2 was up at 18－24, 36 and 48 hours after PH, and reached a peak at 18 hours that was 7.6 folds of control. *drd*2 was mainly up at 0.5－18 and 48－60 hours, and reached a peak at 2 hours that was 8.6 folds of control. It is assumed that the genes mentioned above play important roles in fear response during LR.

Among the proteins associated with response to wound, six kinds of proteins including thrombomodulin (THBD) restrain coagulation[41]. Five proteins including coagulation factor 5 (F5) are concerned with coagulation[42]. Seven proteins including selectin E (SELE) accelerate inflammation[43]. Fourteen proteins including alpha-2-macroglobulin (a2M) are concerned with inflammation[44]. Four proteins including pancreatitis-associated protein (PAP) suppress inflammation[45]. Fif-

teen proteins including leukotriene B4 receptor (LTB4R) regulate immunoreactions to protect cells against damnification[46]. Gamma-glutamyltransferase-like activity 1 (GGTLA1) and peroxiredoxin 5 (PRDX5) can protect cells from oxidation damage[47]. Nine proteins including core promoter element binding protein (COPEB) hasten cell growth and proliferation[48]. Three kinds of proteins including serine or cysteine peptidase inhibitor clade E member 1 (SERPINE1) suppress cell proliferation[49]. Eight proteins including glial cell line derived neurotrophic factor (GDNF) can protect nerve[50]. Four proteins including nerve injury-induced protein 1 (NINJ1) can facilitate nerve regeneration[51]. Four proteins including a disintegrin and metallopeptidase domain 15 (ADAM15) play a role in angiogenesis[52]. Defender against cell death 1 (DAD1) and interleukin 4 (IL-4) prevent cells from apoptosis[53]. Four proteins including early growth response 1 (EGR1) can induce and accelerate cell apoptosis[13]. Lymphocyte antigen 68 (C1QR1) can promote cell-cell interaction and prevent cells impaired by pathogen or harmful cell fragment[54]. Coagulation factor C homolog (COCH) induces cells aggregation and mucopolysaccharide deposition[55]. Transmembrane 4 superfamily member 11 (TM4SF11) relates to formation of myelin sheath[56]. Fatty acid binding protein 5 (FABP5) concerns lipid metabolism[57]. Hepatocyte nuclear factor 4 alpha (HNF4a) is involved in embryonal liver development and regulation of gene expression in adult liver[58]. Annexin A2 (ANXA2) is associated with movement of endocytic vesicles[59]. Histamine receptor H 1 (HRH1) stimulates nitric oxide synthesis to facilitate vasodilatation[60]. Moesin (MSN) is associated with lipopolysaccharide-mediated secretion of tumor necrosis factor[61]. Cathepsin E (CTSE) restrains communion of substance between cytoplasm and lysosome[62]. Superoxide dismutase 2 (SOD2) is involved in elimination of free radicals and degradation of matrix[63]. Cytochrome b-245 beta polypeptide (CYBB) can induce reactive oxygen species produced by endothelial cells[64]. Trefoil factor 1 (TFF1) can hasten mucosal regeneration[65]. Overexpression of trefoil factor 3 (TFF3) can result in hepatoma[66]. Glial fibrillary acidic protein (GFAP) can accelerate astrocyte producing laminin[67]. Complement component 3 (C3) accelerates formation of fatty liver[68]. Tissue plasminogen activator (PLAT) is associated with toxicosis of neural excitability[69]. Homeo box B13 (HOXB13) promotes cell differentiation and repair of injury[70]. The sameness or similarity in some time points, then dissimilarity in others of meaningful expression changes of these genes during LR perhaps regulate the response to wound together. Notably, *thbd* showed up-regulation during almost the whole LR, and had the highest abundance of 9.6 times higher than control at 6 hours, which was generally in line with the result reported by Takatori *et al*[71]. *pap* was up-regulated at multiple phages after PH, especially significantly at the middle phase, and had the highest abundance of 68.6-fold at 12 hours, which was on the whole agree with the result reported by Simon *et al*[72]. *serpine*1 was up at 1 – 48 hours after PH, and reach a peak with 16.7 folds of control at 66 hours, which was in conformity with the result reported by Mueller *et al*[40]. *sele* and *c*1*qr*1 were up at multiple time points and multiple phages respectively, and showed the highest abundances of 13 folds at 66 hours and 5.5 folds at 8 hours in sequence. *a*2*m* was up significantly at metaphase, and had the highest abundance at 8 hours that was 46.2 folds of control. *ltb*4*r* was up at 0.5 – 14 hours post PH, and had the highest abundance at 8 hours that was 8.7 folds of control. *copeb* showed up-regulation during the whole LR, and had the highest abundance at 6 hours that was 10.6 folds of control. *ninj*1 was up-regulated at 15, 30, 42 and 96 hours after PH, and reached a peak at 96 hours, that was 5.3 folds than control. *adam*15 was mainly up in the metaphase, and had the highest abundance at 60 hours that was 14 folds of control. It is speculated that the genes mentioned above play crucial roles in the response to wound during LR.

Among the proteins associated with response to pain, FBJ murine osteosarcoma viral oncogene homolog (FOS) is response for the activation of neuron[73]. Tachykinin 1 (TAC1) has a role of acesodyne by repressing excitatory synaptic transmission[74]. Transient receptor potential cation channel subfamily V member 1 (TRPV1) can assuage pain and counteract inflammation[75]. Prostaglandin-endoperoxide synthase 2 (PTGS2), associating with neuron damage related to epilepsy, prevents toxic shock occurring[76]. Endothelin 1 (EDN1) and gap junction membrane channel protein alpha 4 (GJa4) are involved in conduction of impulses[15]. The sameness or similarity in some time points, then dissimilarity in others of meaningful expression changes of these genes during LR perhaps co-regulate the response to pain. Among them, fos was up at 0.5 – 30 and 42 – 48 hours post PH, especially significantly in the early phase, and had the highest abundance at 0.5 hour that was 28.4 folds of control, which was basically consistent with the result reported by Coni *et al*[77]. It is supposed that it play a key role in the response to pain.

In summary, commencing from long times (0.5 hour – 7 days after PH) and multiple time points (total 23), high-throughput gene expression analysis was used to investigate the expression changes of the genes associ-

ated with the responses to fear, wound and pain. It was primarily proved that PH can cause various physiological responses including responses to fear, wound and pain etc; that Rat Genome 230 2.0 Array was a useful tool analyzing the above responses at transcriptional level. Whereas, these processes DNA → mRNA → protein were influenced by many factors including interaction between proteins. Therefore, the expression changes and actions of genes in regenerating liver need to be further analyzed with the techniques, such as Northern blotting, protein chip, RNA interference, protein-interaction etc.

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