Expression Patterns and Action Analysis of Genes Associated with the Responses to Ischemia, Hypoxia and Starvation during Rat Liver Regeneration

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Abstract: Objective. The aim of this project was to study the responses to ischemia, hypoxia and starvation after partial hepatectomy (PH) at transcriptional level. **Methods.** The genes associated with the responses to ischemia, hypoxia and starvation were obtained by collecting the data of databases and referring to theses. Their expression changes in regenerating liver were checked by the Rat Genome 230 2.0 Array. **Results.** It was found that 120, 65 and 23 genes respectively associated to the responses to ischemia, hypoxia, starvation were associated with liver regeneration (LR). The initial and total expressing gene numbers occurring in initiation of LR (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 structure-function reorganization (66 – 168 hours after PH) were 54, 11, 34, 3 and 54, 49, 70, 49 respectively, which illustrated that the genes were mainly triggered in the initial phase, and worked at different phases. According to their expression similarity, these genes were classified into 5 types including 63 only up-, 26 predominantly up-, 63 only down-, 16 predominantly down-, and 8 up/down-regulated genes, and the total times of their up- and down-regulated expression were 639 and 372, demonstrating that the expression of major genes was enhanced during LR, while the minority attenuated. According to the time relevance, they were classified into 14 groups, demonstrating that the cellular physiological and biochemical activities were staggered during LR. According to gene expression patterns, they were classified into 24 types, showing that the activities mentioned above were diverse and complicated during LR. **Conclusion.** The response to ischemia was mainly enhanced in the prophase, and hypoxia enhanced almost during the LR, while the response to starvation was nearly no change during LR, in which 176 genes associated with LR played an important role. [Life Science Journal. 2006;3(4):1 – 11] (ISSN: 1097 – 8135).

Keywords: partial hepatectomy; Rat Genome 230 2.0 Array; responses to ischemia, hypoxia and starvation; liver regeneration; gene

Abbreviations: GCOS: GeneChip operating software; LR: liver regeneration; NAP: normalization analysis program; PH: partial hepatectomy; SO: sham operation; SP: stress protein

1 Introduction

When the living things are stimulated by heat[1,2], cold[3], osmotic pressure change[4], water deprivation[5], drug[6], toxicant[7], oxidation[8], unfolded protein[9], pathogen infection[10, 11], fear[12], wounding[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. The stress response to one stimulus can usually increase cell tolerance to another stimulus. It implies stress proteins induced by different stimuli have functional cross[20]. The remnant hepatocytes compensate the hepatectomized liver tissue by proliferation and the structure-function reconstruction after partial hepatectomy (PH)[21], which is called liver regeneration (LR)[22, 23]. According to the cellular physiological activities, the process is usually categorized into four stages[24] including 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, PH can induce body's responses to hurtful stimulus, such as ischemia, hypoxia and starvation, in which involve nearly 350 genes. These genes exist the interaction. It is almost impossible to clarify the action of genes associated with ischemia, hypoxia and starvation at transcriptional

level during LR unless high-throughput gene expression profiles[25, 26]. So we used the Rat Genome 230 2.0 Array[27,28] containing 207 genes associated with the response to ischemia, 96 genes to hypoxia and 38 genes to starvation to detect gene expression changes in LR after PH. 176 genes were found to be associated with LR[29]. And their expression characters, patterns and actions during LR 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 randomly, hepatectomized group and sham-operation (SO) group. Each group included 6 rats (male: female = 1:1). PH was performed according to Higgins and Anderson[21], by which 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 livers from middle parts of right lobe of each group (total 1 – 2 g livers, 0.1 – 0.2 g × 6 samples, per group) were gathered and mixed together, then stored at −80 ℃. The SO group was the same as hepatectomized group except the liver lobes were 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 Reagent (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

1 – 8 μg total RNA as template was used for cDNA synthesis. cDNA purification was 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 according to the purification procedure of GeneChip Analysis[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 at 94 ℃ for 35 minutes 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 at 45 ℃ for 16 hours on a rotary mixer at 60 rpm. The microarray was washed and stained by GeneChip fluidics station 450 (Affymetrix Inc., Santa Clara, CA, USA). The chips were scanned by GeneChip Scan 3000 (Affymetrix Inc., Santa Clara, CA, 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 from the microarray analysis, each analysis was performed three times. Results with a total ratio were maximal (R^m) and that the average 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 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 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, Microsoft Excel software[28, 33, 34].

2.7 Identification of genes associated with LR

Firstly, the nomenclature of three kinds of physiological responses mentioned above was adopted from the GENEONTOLOGY database (www.geneontology.org), and inputted 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. 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 the biological process were collated. The results of this

analysis were codified, and compared with the results obtained for mouse and human searches in order to identify human and mouse genes which are different from those of rat. Comparing 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 observed as meaningful expression changes[29], were referred to as rat homologous or rat specific genes associated with the responses to ischemia, hypoxia and starvation under evaluation. Genes, which displayed reproducible results with three independent analyses with the chip and which showed a greater 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 response to ischemia, hypoxia and starvation during LR

According to the data of databases at NCBI, GENEMAP, KEGG BIOCARTA and RGD, the responses to ischemia, hypoxia and starvation involved 225, 117 and 41 genes respectively, in which 207, 96 and 38 genes were contained in the Rat Genome 230 2.0 Array separately. Among them, 176 genes revealed meaningful changes in expression at least at one time point after PH, showed significant or extremely significant differences in expression when comparing PH with SO, and were repeatable in three detections by Rat Genome 230 2.0 array. The results suggested that the genes were associated with LR. Up-regulation ranged from 2 to 257 times higher than the control, and down-regulation did 2 – 17 (Table 1). The analysis indicated that 63 genes were up-regulated, 63 genes down-, 50 genes up/down- during LR. Total up- and down-regulated genes were 639 and 372, respectively (Figure 1A). At the initiation stage of LR (0.5 – 4 hours after PH), 54 genes were up-regulated, 29 genes down-, 2 genes up/down-; at the transition phase from G0 to G1 (4 – 6 hours after PH), 49 genes were up-, 13 genes down-; at cell proliferation period (6 – 66 hours after PH), 70 genes were up-, 93 genes down-, 32 genes up/down-; at cell differentiation and reorganization of the structure-function stage (66 – 168 hours after PH), 49 genes were up-regulation, 56 genes down-, 9 genes up/down- (Figure 1B).

Table 1. Expression abundance of 176 ischemia, hypoxia and starvation response-associated genes 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 Ischemia			Hspe1		0.2	*Tert	2	5.3,0.3	Mmp9	1	0.5,9.5
Adm	2	8.0	Icam1	2	3.0	*Tff3		0.3	Myc		19.7
Adora2a		0.5	Ikbkb		0.3	*Tgfb1	2,3	4.0	Nfkb1	1	0.4,2.3
Ager		0.4	Ikbkg		0.4	*Thbd		9.6	Nol3		2.6
AGT		5.0	Il1b		0.4	Tlr2		10.6	Nos3	1	0.3,2.1
Agtr1a		0.4	Il6		0.3,6.1	Tlr4		0.5	Npm1		0.5,2.8
Agtr2		0.4	Kcnk2		0.4	Tnf		3.2	Nr4a2		0.4,7.1
Akt1	2,3	4.0	Kcnk4		0.1,2.0	Tnfrsf10b		4.9	Pdlim1		0.5,3.2
Alox5		0.2,2.5	*Kdr		0.4,2.4	Tp53	2	2.9	Peg3		0.4
*Ang1		0.4,58.2	Lcn2		0.5,257.2	Txnip		2.9	Plau		0.4,3.0
Angpt1	2	0.2,9.2	Lgals3		5.7	Ucp3		2.2,0.5	Plaur		13.9
Angptl4	3	3.2	Mag		0.4,2.3	Ung		0.4	Prkaa1	3	7.5
Apoe		0.1	Mapk1	2	2.7	Vegfa	2	4.5,0.1	*Psen2	1	0.2
*Ascl1		0.1,2.2	Mapk8		0.5,19.7	Vhl	2	2.0	Ptgs1	1	3.4
Atm	2,3	0.3	Mapt		0.4	Zfp162		0.5	Ptgs2	1	0.1,2.1
Bcl2	2,3	0.3	Met		0.4,2.3	**2 Hypoxia**			Serpine1	1	16.7
BCL2L1	2	0.4,2.1	Mmp9	2	0.5,9.5	Ace		0.5	Sesn2		4.3
Bdkrb2		0.4	Mtap1a		0.5	Adm	1	8.0	Slc2a1		0.2
Bdnf		0.4,26	*Mtap2		0.4,3.6	Akt1	1,3	3.9	Slc2a4		0.1
Birc4		5.0	Mthfr		0.4,3.7	Angpt1	1	9.2,0.2	Sod2	1	5.6
Camk2a		0.5	Nedd9		0.5	App		6.4	Stat5a		0.2
*Casp12		0.4,2.6	Nes		0.2,4.6	Arnt2		6.8,0.4	Tert	1	0.3,5.3
Ccnd1		7.5	Nfkb1	2	0.4,2.3	Atm	1,3	0.3	Tgfb1	1,3	4.0
Chuk		0.3	Nos3	2	0.3,2.1	Atp1b1		6.7	Th		0.4
Cirbp	2	0.3	*Ntf3		0.1	Bcl2	1,3	0.3	Tp53	1	2.9
*Clu		3.0	P2rx7		0.4,2.5	Bcl2l1	1	2.1,0.4	Trib3		4.9
Cnr1		0.1	Parg		4.8	Birc2		2.8	Vegfa	1	0.1,4.5

Cts1		2.0	*Pcna		10.6	Bnip3		0.4	Vh1	1	2.0
Cybb		2.5	Pla2g2a		11.3	Camk2d		2.1	**3 Starvation**		
Daf1		0.2	Plat		0.4,4.9	Capn2		2.1	Aco1		0.5
Ddit3	2	0.5,2.7	Pln		0.3	Casp1		3.0	Akt1	1,2	3.9
Diablo		2.6	*Pon1		0.4	Casp9		0.5	Angptl4	1	3.2
E2f1		21.2	Pon2		0.5	Ccl2		128.0	Atm	1,2	0.3
Edn1	2	0.4,2.6	Prkaa2	3	0.2,5.3	*Cirbp	1	0.3	Bcl2	1,2	0.3
Eif2s1		2.4	*Prss15		2.4	Creb1		0.5	Casp8		10.6
Endog		4.6	Psen2	2	0.2	Cyp19a1		6.5,0.2	Cck		0.5
Epor		0.4	Pspn		0.3,2.1	Ddit3	1	2.7,0.5	Ccne1		18.5
F2		0.3	Pten		0.5	Ddit4		3.0,0.2	Fads1		0.1
F2rl2		0.2	Ptgs2	2	0.1,2.1	Drd2		8.6	Ghrl		4.0
F5		0.5	Ptk2		8.9	Edn1	1	2.6,0.4	Impact		0.4
Fgf2	2	0.5,2.1	Rela		0.5	Ednra		0.5	Mc4r		0.1
Fos	2	28.4	Serpine1	2	16.7	Egln1		4.4	Mcl1		4.3
Fut4		0.5,2.2	Sgk		6.5	Egr1		18.6	Mcts1		0.2
Fyn		0.5	Shc1		0.5	Fgf2	1	2.1,0.5	Ppara		0.3
Gfap		0.3,2.6	Shh		0.5,2.8	Fos	1	28.4	Ppargc1a		0.2,2.5
Grasp		2.8	Slc23a2		0.2	Hif3a		0.3	Prkaa1	2	7.5
Grin2a		2.4	Slc6a11		6.0	Hyou1		0.3	Prkaa2	1	0.2,5.3
Hdh		2.3	*Slc8a1		0.4,5.7	Icam1	1	3.0	Retn		0.2,2.2
Hgf		0.4	Slc8a3		0.2,2.2	Igf1r		0.4	Rpl11		6.5
Hrh3		0.2	Sod2	2	5.6	Itgb1		2.6	Slc38a3		5.3
Hspa1a		0.2	Sstr2		0.4,4.9	Jun		6.9	Tgfb1	1,2	4.0
Hspa1b		0.3,3.4	Stat4		0.4,4.0	Map2k1		9.1	Trim24		0.1
Hspb8		4.7	Tac1		0.2	Mapk1	1	2.7			

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

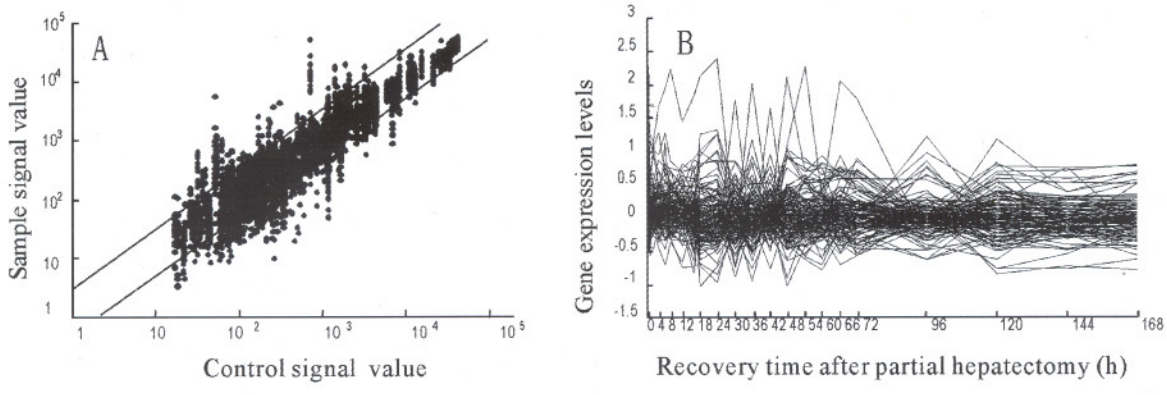

Figure 1. Expression frequency, abundance and changes of 176 ischemia, hypoxia and starvation response-associated genes during rat LR. Detection data of Rat Genome 230 2.0 Array were analyzed and graphed by Microsoft Excel. A. Gene expression frequency. The dots above bias indicated the genes up-regulated more than two folds, and total times of up-regulation were 639; those under bias indicated the genes down-regulated more than two folds, and that of down-regulation were 372; and the ones between biases no-sense alterative; B. Gene expression abundance and changes. 113 genes were 2 – 257 folds up-regulated, and 113 genes 2 – 17 folds down-regulated.

3.2 Initial expression time of the genes associated with the responses to ischemia, hypoxia and starvation during LR

At each time point of LR, the numbers of initial up-, down-regulated and total up-, down-regulated genes were in sequence both 21 and 7 at 0.5 hour; 13, 15 and 26, 19 at 1st hour; 12, 0 and 35, 3 at 2nd hour; 8, 7 and 42, 8 at 4th hour; 3, 3 and 37, 10 at 6th hour; 1, 2 and 35, 6 at 8th hour; 0, 5 and 32, 9 at 12th hour; 8, 8 and 30, 16 at 16th hour; 8, 14 and 37, 34 at 18th hour; 2, 2 and 38, 26 at 24th hour; 7, 6 and 24, 23 at 30th hour; 0, 4 and 28, 24 at 36th hour; 1, 1 and 27, 9 at 42nd hour; 2, 4 and 41, 31 at 48th hour; 0, 2 and 27, 19 at 54th hour; 1, 1 and 31, 14 at 60th hour; 1, 0 and 26, 18 at 66th hour; 0, 2 and 22, 20 at 72nd hour; 1, 3 and 19, 15 at 96th hour; 1, 1 and 27, 25 at 120th hour; 0, 0 and 20, 16 at 144th hour; 0, 0 and 16, 20 at 168th hour (Figure 2). Wholly, gene expression changes occurred during the whole LR, with the up- and down-regulation times 639 and 372, respectively.

The initially up-regulated genes were predominantly expressed in the forepart, and the down- in the prophase and metaphase, whereas there were only few initial expressions in the anaphase.

3.3 Expression similarity and time relevance of the genes associated with the responses to ischemia, hypoxia and starvation during LR

176 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 63, 26, 63, 16 and 8 genes, respectively (Figure 3). They could also be classified based on time relevance to 14 groups including 0.5th hour and 168th hour, 1st and 2nd hour, 4th hour, 6th and 8th hour, 12th and 16th hour, 18th and 120th hour, 24th and 30th hour, 36th and 48th hour, 42nd hour, 54th hour, 60th and 66th hour, 72nd and 96th hour, 144th hour, in which the up- and down-regulated gene numbers were

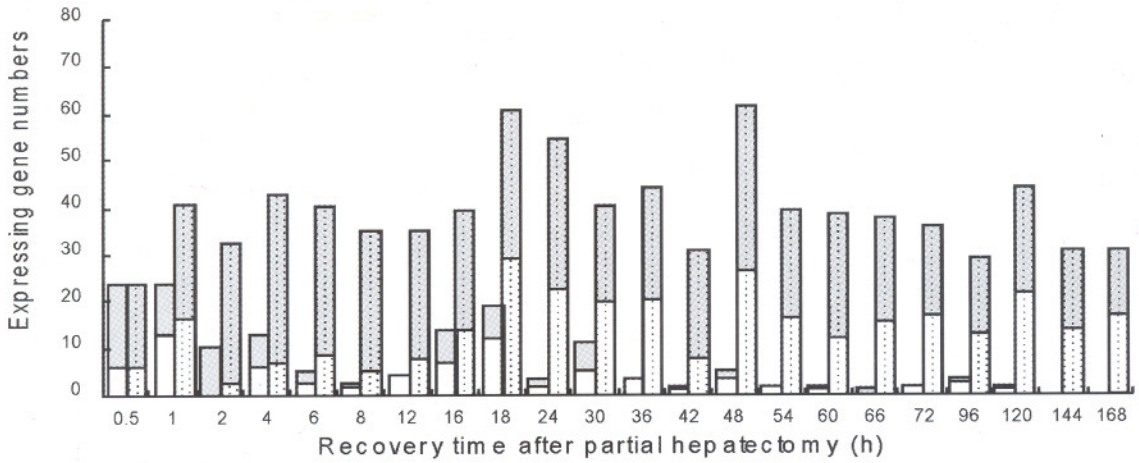

Figure 2. The initial and total expression profiles of 176 genes associated with the responses to ischemia, hypoxia and starvation at each time point of LR. Grey bars: Up-regulated genes; White bars: Down-regulated genes. Blank bars represent initially expressed genes, in which up-regulation genes are predominant in the forepart, and the down- in the prophase and metaphase, whereas only few in the anaphase. Dotted bars represent the totally expressed genes, in which some genes are up-regulation and others down-regulation during LR.

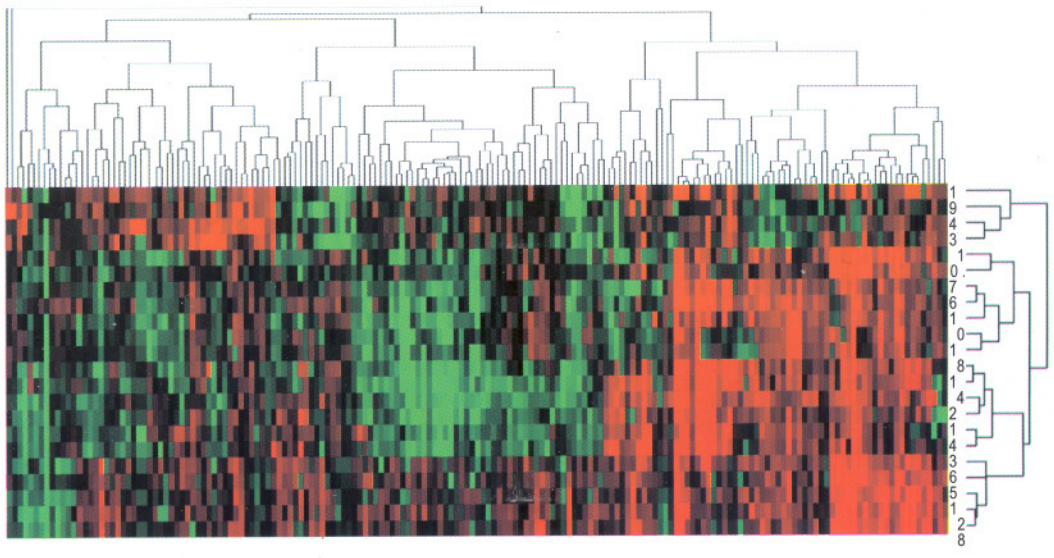

Figure 3. Expression similarity and time relevance cluster of 176 genes associated with the responses to ischemia, hypoxia and starvation during LR. Detection data of Rat Genome 230 2.0 Array were analyzed by H-clustering. Red represents up-regulation genes mainly associated with energy metabolism, vascular repair and apoptosis-promoting; Green represents down- ones mostly associated with anticoagulation; Black: No-sense in expression change. The upper and right trees respectively show expression similarity and time series cluster, by which the above genes were classified into 5 and 14 groups separately.

respectively 50th and 26th, 35th and 3rd, 79th and 18th, 35th and 6th hour, 32nd and 9th, 30th and 16th, 75th and 60th, 51th and 32nd, 69th and 55th, 58th and 33rd, 48th and 38th, 19th and 15th, 27th and 25th, 36th and 36th (Figure 3). The up-regulation genes were mainly associated with energy metabolism, vascular repair and apoptosis-promoting. The down- genes were mostly anticoagulation-associated genes.

3.4 Expression patterns of the genes associated with the responses to ischemia, hypoxia and starvation during LR

176 genes mentioned above during LR might be categorized according to the changes in expression into 24 types of patterns: (1) 11 genes up-regulated at one time point, i. e. 2nd, 6th, 16th, 30th, 42nd, 48th, 60th, 66th, 96th, 120th hour after PH (Figure 4A); (2) 9 genes up-regulated at two time points, i. e. 16th and 42nd hour, 16tg and 96th hour, 24th and 36th hour, 30th and 42nd hour, 30th and 96th hour, 48th and 120th hour (Figure 4B); (3) 2 genes up-regulated at three time points (Figure 4B); (4) 3 genes up-regulated at one phase, i. e. 1st－48th hour, 4th－8th hour, 16th－96th hour (Figure 4C); (5) 2 genes up-regulated at two phases (Figure 4C); (6) 3 genes up-regulated at one time point/one phase (Figure 4C); (7) 6 genes up-regulated at two time points/one phase (Figure 4D); (8) 8 genes up-regulated at one time point/two phases (Figure 4E); (9) 5 genes up-regulated at two time points/two phases (Figure 4D); (10) 4 genes up-regulated at three time points/two phases (Figure 4E); (11) 2 genes up-regulated at one time point/three phases (Figure 4F); (12) 2 genes up at two time points/ three phases (Figure 4F); (13) 3 genes up-regulated at multiple time points/ multiple phases (Figure 4G); (14) 24 genes down-regulated at one time point, i. e. 4, 6, 8, 12, 18, 30, 36, 42, 48, 54, 60, 72, 96 hours (Figure 4H); (15) 11 genes down-regulated at two points in time, i. e. 1 and 168 h, 1 and 72 h, 30 and 96 h, 16 and 30 h, 18 and 48 h, 30 and 48 h, 16 and 30 h, 18 and 54 h, 120 and 168 h, 36 and 48 hours , 48 and 60 hours (Figure 4I); (16) 7 genes down-regulated at three time points (Figure 4J); (17) 6 genes down-regulated at four time points (Figure 4K); (18) 2 genes down-regulated at one time point/one phase (Figure 4L); (19) 2 genes down-regulated at two time points/one phase (Figure 4L); (20) 4 genes down-regulated at two time points/two phases (Figure 4L); (21) 7 genes down at multiple time points/ multiple phases (Figure 4M); (22) 15 genes first up-regulated and then down-regulated (Figure 4N); (23) 8 genes first down-regulated and then up-regulated (Figure 4O); (24) 27 genes up/down mixed (Figure 4P).

4 Discussion

The responses to ischemia, hypoxia and starvation are instinctive reaction of self-regulation and adaptation of the organism. Lots of proteins are associated with them. In the response to ischemia, three proteins including adrenomedullin (ADM) inhibit ischemia[35]. Five proteins including icrotubule-associated protein 2 (MTAP2) prevent or repair ischemic damnification[36]. Three proteins including angiotensinogen serpin inhibitor clade A member 8 (AGT) regulate blood pressure[37]. Interleukin 6 (IL6) relates to maintaining blood balance[38]. Poly ADP-ribose glycohydrolase (PARG) resists the inflammation caused by ischemia/reperfusion[39]. Uncoupling protein 3 (UCP3) enhances the endurance of cells to local ischemia[40]. Lectin galactose binding soluble 3 (LGALS3) relates to hematopoiesis and regulation of haematocyte number[41]. Adrenergic receptor α 2a (ADRA2A) can restrain noradrenalin excretion[42]. Endothelin receptor type A (EDNRA) produces noradrenalin and hypertension Ⅱ[43]. Eight proteins including protein tyrosine kinase 2 (PTK2) relate to maintaining the normal function and development of blood vessel[44]. Paraoxonase 2 (PON2) can cause the disease of coronary artery through induction of atherosis[45]. 5, 10-methylenetetrahydrofolate reductase (MTHFR) inhibits coronary artery disease[46]. Adenosine monophosphate-activated protein kinase α2 catalytic subunit (PRKAA2) can sustain the supply and demand balance of the cell energy[47]. Tachykinin 1 (TAC1) inhibits the ischemic transmission of nervous excitement[48]. 70 kDa heat shock protein 1B (HSPA1B) participates in activities of cell resisting adversity[49]. E2F transcription factor 1 (E2F1) promotes cell growth by gene transcription and regulating signal conduction pathway[49]. Thrombomodulin (THBD) resists ischemia[50]. Lipocalin 2 (LCN2) restrains production of red cell[51]. Three proteins including angiopoietin 1 (ANGPT1) promote coagulation[52]. Four proteins including proliferating cell nuclear antigen (PCNA) protect the cell or tissue under ischemia[53]. Three proteins including histamine receptor H3 (HRH3) can prick up damage[54]. Seven proteins including diablo homolog (DIABLO) promote apoptosis[55]. Ataxia telangiectasia mutated homolog (ATM) induce apoptosis[56]. Baculoviral IAP repeat-containing 4 (BIRC4) and 70kDa heat shock protein 1A (HSPA1A) suppress apopto-

sis[57]. Serine or cysteine peptidase inhibitor clade E member 1 (Serpine 1) restrains the function of hepatocyte growth factor[58]. Five proteins including FBJ murine osteosarcoma viral oncogene homolog (FOS) maintain nerve function[59]. Three proteins including intercellular adhesion molecule 1 (Icam1) accelerates inflammatory response[60]. Solute carrier family 8 member 1 (SLC8A1) hastens transportation of Ca^{2+}[61]. Serum glucocorticoid regulated kinase (SGK) modulates the balance of Na^{+} *in vivo*[62]. Phospholamban (PLN) depresses the activity of Ca^{2+} ATPase[63]. The meaningful expression profiles of these genes are same or similar at some point while different at others, indicating that they may co-regulate response to ischemia. Among them, *fos* was up- at 0.5th－30th, 42nd－48th and 120th hour after PH, and reached a peak at 0.5th hour that was 28.4 folds of control, which was consistent with the results reported by Kawaguchi K *et al*[64]. *adm* nearly up- in all LR phase, and reached a peak at 48th and 54th hour that was 8 folds of control. *agt* was up- at 1st－24th and 144th hour after PH, and reached a peak at 8th hour that was 5 folds of control. *ptk2* was up- for many periods of time after PH, and reached a peak at 66th hour that was 8.9 folds of control. *angpt1* was up- at 12th－24th, 36th and 48th－60th hour after PH, and reached a peak at 48th hour that was 9.2 folds of control. *lcn2* was nearly up- for all the LR, and reached a peak at 24th hour that is 257.2 folds of control. *e2f1* was up- at 18th－30th, 54th－72nd and 120th hour after PH, and reached a peak at 24th hour that was 21.2 folds of control. *serpine1* was up- at 1st－48th hour after PH, and reached a peak at 6th hour that was 16.7 folds of control. It is speculated that the genes mentioned above genes play crucial roles in ischemia response during LR.

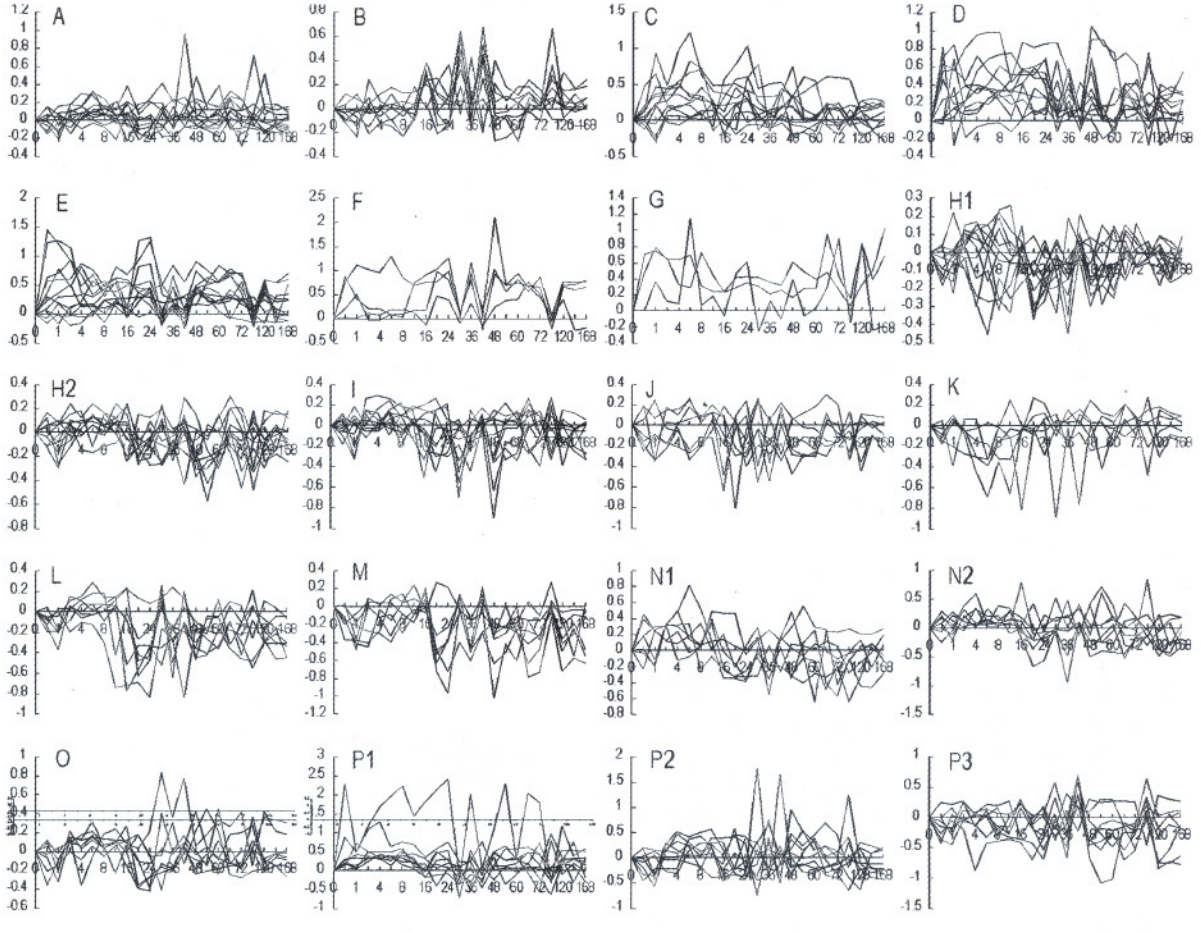

Figure 4. Twenty-five expression patterns of 176 genes associated with the responses to ischemia, hypoxia and starvation during LR. Expression patterns were obtained by the analysis of detection data of Rat Genome 230 2.0 array with Microsoft Excel. A－G: 63 up-regulation genes; H－M: 63 down-regulated genes; N－P: 50 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.

Among the proteins associated with response to hypoxia, four proteins including DNA-damage-inducible transcript 4 (DDIT4) resist anoxic injury[65]. EGL nine homolog 1 (EGLN1) participates hypoxia stress[66]. Thymoma viral proto-oncogene 1 (AKT1) can raise the activities of AMP kinase to keep the heart's normal functions[66]. Five proteins including tribbles homolog 3 (TRIB3) can accelerate apoptosis under hypoxia[67]. Aryl hydrocarbon receptor nuclear translocator 2 (ARNT2) takes part in the removal of dioxins in vivo produced under hypoxia[68]. Angiotensin I converting enzyme 1 (ACE1) stimulates vasoconstriction[69]. cAMP responsive element binding protein 1 (CREB1) restrains nerve excitation[70]. Dopamine receptor 2 (DRD2) relates to spirit anxiety[71]. Four proteins including amyloid beta precursor protein (APP) play the role in nerve protection[72]. Four proteins including myelocytomatosis viral oncogene homolog avian (MYC) promote cell proliferation[73]. Chemokine ligand 2 (CCL2) prevents apoptosis[74]. The meaningful expression profiles of these genes show the sameness or similarity at some point while different at others, indicating that they may co-regulate responses to hypoxia. Among them, *trib3* was up- at 1st, 8th – 24th hour, 48th and 66th – 72nd hour after PH, and reached a peak at 48th hour that was 4.9 folds of control. *arnt2* was up- at 30th – 42nd, 60th, 72nd and 120th hour after PH, and reached a peak at 30th hour that was 6.8 folds of control. *drd2* was up- at 0.5th – 18th, 48th – 60th and 168th hour after PH, and reached a peak at 2nd hour that was 8.6 folds of control. *app* was up- at metaphase and anaphase after PH, and reached a peak at 168th hour that was 6.4 folds of control. *ccl2* was up- at 0.5th – 1st, 12th – 24th, 36th, 48th – 72nd and 120th hour after PH, and reached a peak at 48th hour that was 128 folds of control. *myc* was nearly up- all the LR, and reached a peak at 6th hour that was 19.7 folds of control. It is presumed that they play key roles in hypoxia response during LR.

Among the proteins associated with response to starvation, caspase 8 (CASP8) and myeloid cell leukemia sequence 1 (MCL1) promote apoptosis[75]. Cholecystokinin (CCK) and melanocortin 4 receptor (MC4R) stimulate digestion[76]. Five proteins including protein kinase AMP-activatedα1 catalytic subunit (PRKAA1) participate in the metabolism of carbohydrates and fats[77]. Cyclin E (CCNE1) stimulates transit from G1 into S phase. Aconitase 1 (ACO1) relates to cholesterolemia caused by excessive intake[78]. Solute carrier family 38 member 3 (SLC38A3) participates in sodium-dependent transportation of Glu, Asn and His[79]. Peroxisome proliferative activated receptor gamma coactivator 1α (PPARGC1A) can maintain temperature and stabilize metabolism[80]. The meaningful expression profiles of these genes are same or similar at some points, whereas different at others, suggesting that they may co-regulate responses to starvation. Among them, *prkaa1* was up- at 0.5th – 12th, 48th and 144th – 168th hour after PH, and reached a peak at 4th hour that was 7.5 folds of control, which was consistent with the results reported by Dransfeld *et al*[26]. *ccne1* was up at 1st – 2nd, 8th – 72nd and 120th hour after PH, and reached a peak at 24th hour that was 18.5 folds of control. *casp8* was up at 1st, 18th – 24th, 36th, 48th – 72nd and 120th – 168th hour after PH, having a peak at 48th hour that was 10.6 folds of control. It is assumed that the genes play vital roles in starvation response during LR.

In conclusion, the high-throughput gene expression analysis technique was used to investigate the expression changes of the genes associated with the responses to ischemia, hypoxia and starvation in long time range (0.5th hour – 7 days after PH) and multiple time points (total 23). It was primarily confirmed that PH can cause various physiological responses, such as hypoxia, hypoxia and starvation etc; that Rat Genome 230 2.0 Array was a useful tool analyzing the above responses-associated genes at transcriptional level. However, the processes DNA→ mRNA→ protein were influenced by many factors including protein interaction, we'll further analyze the above-mentioned results by the techniques, such as Northern blotting, protein chip, RNA interference, protein-interaction etc.

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