Expression patterns and action analysis of genes associated with the responses to physical stimuli during rat liver regeneration☆

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Abstract

Objective. This project is to study the responses to physical stimuli in regenerating liver at transcriptional level. *Methods*. The genes associated with the responses to physical stimuli were obtained by collecting the data of databases and referring to thesis, and the gene expression changes during LR were checked by the Rat Genome 230 2.0 Array. *Results*. It was found that 120 genes were associated with liver regeneration. The initial and total expressing gene numbers occurring in initiation phase of liver regeneration (0.5 – 4 hours after PH), G0/G1 transition (4 – 6 hours after PH), cell proliferation (6 – 66 hours after PH), cell differentiation and structure-function reconstruction (66 – 168 hours after PH) were 52, 11, 66, 2 and 52, 37, 115, 78, respectively, illustrating that the associated genes were mainly triggered in the early phase of LR, 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 in 41, 20, 33, 19 and 7 genes, respectively, and the total times of their up- and down-regulation expression were 431 and 296, respectively, demonstrating that the expression of the major genes were increased, and that of the minutes ority decreased. According to time relevance, they were 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 31 types, displaying that the cellular physiological and biochemical activities were diverse and intricate during liver regeneration. *Conclusion*. The responses to physical stimuli including visible light, ultraviolet, X-ray, sonic wave, osmotic pressure and proton gradient are enhanced mainly in the forepart and prophase of liver regeneration, in which 120 genes associated with liver regeneration play important roles. [Life Science Journal. 2007;4(1):52 – 60] (ISSN: 1097 – 8135).

Keywords: partial hepatectomy; Rat Genome 230 2.0 Array; responses to physical stimuli; genes; liver regeneration

1 Introduction

When organism is stimulated by various physical factors, such as squeezing, press, sonic wave, radiation, osmotic pressure, potential difference and so on, the cells can transmit these extracellular stimuli into intracellular signal by ligand-receptor binding or special channel in the cell membrane[1, 2], and trigger the responses to the stimuli in organism and cells[3,4]. Usually, these responses contain three phases[1 – 9]: perception of stimuli, signal transduction and reaction. In brief, the sonic wave, osmotic pressure and electrochemical proton gradient are converted by mechanosensitive ion channels into electric or chemical signal[8,9], then stimulate many of physiological and biochemical changes including cell proliferation, the regeneration of damaged tissue, gene mutation, cell transformation and so on[10 – 12]. And the stimuli, such as visible light, ultraviolet (UV) and X-ray are mainly converted into electric or chemical signal by ligand-receptor binding[8] or rhodopsin-mediated phototransduction, then regulate the above activities[13].

Partial hepatectomy (PH)[14], a violent physical stimuli resulting in tissue injury, can cause the responses to physical stimuli and induce liver regeneration (LR)[15,16]. Generally, based on the physiological activities of cell, the regeneration process is classified into 4 phases: 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)[16]. According to time course, it is divided into 4 phases including forepart (0.5 – 4 hours after PH), prophase (6 – 12 hours after PH), metaphase (16 – 66 hours after PH), and anaphase (66 – 168 hours after PH)[17], which are regulated by

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many factors including the physical factors[18]. Previously, the relationship between the response to chemical stimuli and the LR had been studied[19]. In order to study the response to physical stimuli during LR at transcriptional level[20, 21], the expression changes of genes in regenerating liver after PH were detected by Rat Genome 230 2.0 Array[22] containing 227 physical stimuli-associated genes, and it was identified that 120 genes were associated with LR[23]. And expression changes, patterns and action of them were primarily 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 (Xinxiang, China). The rats were separated into two groups at random and each group included 6 rats (male:female = 1:1). PH was performed according to Higgins and Anderson[14], 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 liver tissues 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, and six samples of each group were gathered and mixed together to 1 – 2 g (0.1 – 0.2 g × 6) total liver tissue, then 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 liver tissue according to the manual of Trizol kit (Invitrogen Corporation, Carlsbad, California, USA)[24] and then purified base on the guide of RNeasy mini kit (Qiagen, Inc, Valencia, CA, USA)[25]. Total RNA samples were checked to exhibit a 2:1 ratio of 28S 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[26].

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 then purified[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 Array 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., 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[22].

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[22].

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 whose 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 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[22, 28, 29].

2.7 Identification of genes associated with LR

Firstly, the nomenclature of six kinds of physical stimuli mentioned above were adopted from the GENEONTOLOGY database (www.geneontology.org), and 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 responses to physical stimuli. According to maps of biological pathways embodied by GENMAPP (www.genmapp.org), KEGG (www.genome.jp/kegg/pathway.html) 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 human and mouse 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 more than twofold change in expression level, observed as meaningful expression changes[23], were referred to as rat homologous or rat specific genes associated with the responses to physical stimuli 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 ($P \leqslant 0.01$) be-

tween PH and SO, were referred to as associated with LR.

3 Results

3.1 Expression changes of the genes associated with the responses to physical stimuli during LR

According to the data of databases at NCBI, GENMAPP, KEGG, BIOCARTA and RGD, 316 genes were associated with blood coagulation. In which, 226 genes were contained in the Rat Genome 230 2.0 Array. Among them, the expression of 120 genes displayed meaningful changes at least at one time point after PH, showed significant or extremely significant differences in expression when comparing PH with SO, and displayed reproducible results with three detections with Rat Genome 230 2.0 Array, suggesting that the genes were associated with LR. Range of up-regulation was 2 – 60 times higher than control, and down-regulation was 2 – 16 (Table 1). The analysis indicated that 41 genes were up-regulated, 33 genes down-, and 46 genes up/down- during LR. The total up- and down-regulated times were 431 and 296, respectively (Figure 1A). According to the gene expression changes during liver regeneration, at the initiation phase (0.5 – 4 hours after PH), 33 genes displayed up-regulation, 18 genes down, and 2 genes up/down; at the transition phase from G0 to G1 (4 – 6 hours after PH), 28 genes up, and 11 genes down; at the cell proliferation phase (6 – 66 hours after PH), 49 genes up, 39 genes down, and 28 genes up/down; at cell differentiation and structure-function reorganization phase (66 – 168 hours after PH), 37 genes up, and 31 genes down, and 12 genes up/down (Figure 1B).

Table 1. Expression abundance of 120 response to physical stimuli-associated genes during rat LR

Gene Abbr.	associated to	Fold difference	Gene Abbr.	associated to	Fold difference	Gene Abbr.	associated to	Fold difference	Gene Abbr.	associated to	Fold difference
Adcyap1r1	1	0.4, 2.3	Kit	1, 2, 3	0.4	Vax2	1, 2, 3	0.3	Pres	4	0.1, 2.2
Bhlhb2	1	4.1	Lum	1, 2, 3	0.1, 10.3	Cd151	1, 2, 3, 4	0.2, 4.3	Ptpn11	4	0.5
Bhlhb3	1	0.1, 2.5	Mapk12	1, 2, 3	0.2, 2.4	Eml2	1, 2, 3, 4	3.7	Rad54l	4	0.3, 2.3
Gng8	1	0.1	Mertk	1, 2, 3	0.4	Fech	2	2.2	Rpgr	4	0.1, 2.8
Rho	1	0.2, 2.0	Myc	1, 2, 3	19.7	Fen1	2	0.2, 2.7	Slc19a2	4	0.2, 2.5
Rhob	1	8.1	Myoc	1, 2, 3	0.5	Hmgn1	2	3	Snai2	4	0.1, 3.2
Zcwcc2	1	4.6	Nnmt	1, 2, 3	0.3, 9.4	Msh6	2	2.7	Sox21	4	0.5, 12.1
*Abcc6	1, 2, 3	0.4	Nphp1	1, 2, 3	0.5, 2	Rev1l	2	2.2	Thrb	4	0.2
Adra1b	1, 2, 3	0.4	Oat	1, 2, 3	5.2	Tp53	2, 3	2.9	Timm8a	4	3.3
Aipl1	1, 2, 3	0.1, 2.0	Opn1sw	1, 2, 3	0.4, 5.3	Atp6V1b1	4	4	Timm8b	4	0.4, 2.4
App	1, 2, 3	6.4	Opn4	1, 2, 3	0.2, 2.5	Cdkn2d	4	3.3	Timm9	4	0.3, 2.7
Arr3	1, 2, 3	7	Pax6	1, 2, 3	0.1, 2.1	Chrna9	4	0.1	Tnfrsf11a	4	8.6
Bcl2L1	1, 2, 3	0.4, 2.1	Pdc	1, 2, 3	0.4, 17.1	Cldn14	4	0.3	Tub	4	0.4, 2.1
Cds1	1, 2, 3	0.3	Pde6g	1, 2, 3	0.2	Cntn5	4	0.4, 7.0	Ush2a	4	0.3, 4.9
Chm	1, 2, 3	0.1	Pitpn	1, 2, 3	3.4	Coch	4	0.2, 2.3	Vit	4	0.3
Clns1a	1, 2, 3	4.8	Ppm1d	1, 2, 3	6.8	Col11a1	4	0.3, 3.1	Wfs1	4	0.2
Col18a1	1, 2, 3	3.1	Ppt	1, 2, 3	0.5	Col1a2	4	3	Accn1	4, 5, 6	0.3, 10.1
Crybb1	1, 2, 3	3.2	Prom1	1, 2, 3	0.4	Col2a1	4	0.3, 3.8	Accn3	4, 5, 6	4,0.5
Crybb3	1, 2, 3	0.2	Rax	1, 2, 3	4.8	Diap1	4	0.4	Ccl7	4, 5, 6	22.6
Crygd	1, 2, 3	8	Rcvrn	1, 2, 3	21.1	Dspp	4	3.4	Dnah1	4, 5, 6	0.1
Cyp1b1	1, 2, 3	3.5	Rgr	1, 2, 3	6.1	Ercc5	4	2.4	Etv1	4, 5, 6	0.3, 3.9
Fbn1	1, 2, 3	2.8	Rp1H	1, 2, 3	0.2, 59.7	Gata3	4	0.4	Gfi1	4, 5, 6	0.2, 2.4
Fyn	1, 2, 3	0.4	Slc24a1	1, 2, 3	0.4, 3.24	Jag2	4	0.2	Itgb2	4, 5, 6	0.5
Gnat1	1, 2, 3	18.5	Slc24a2	1, 2, 3	0.2	Kcne1	4	0.5, 9.8	Mpz	4, 5, 6	8, 0.4
Gngt2	1, 2, 3	2.8	Sord	1, 2, 3	3.3	Kcne1l	4	0.2	Nos3	4, 5, 6	0.3, 2.1
Grk1	1, 2, 3	5.2	Syngap1	1, 2, 3	0.4, 2.1	Mgp	4	2.3	Prrxl1	4, 5, 6	0.1, 2.5
Grm8	1, 2, 3	0.4	*Timp3	1, 2, 3	0.5	Mtap1a	4	0.5	Prx	4, 5, 6	2.1
Guca1a	1, 2, 3	0.4, 5	Trpc3	1, 2, 3	0.3, 2.6	Nog	4	0.3, 3.3	Ptk2	4, 5, 6	8.9
Gucy2d	1, 2, 3	0.4, 5.3	Tulp1	1, 2, 3	0.4, 2.9	Phyh	4	2.3	Slc14a2	5	0.4
Impg1	1, 2, 3	0.4, 4.0	Unc119	1, 2, 3	0.3	Pmp22	4	2.2	Trpv1	5, 6	0.3

*Reported genes associated with LR; 1. Response to visible light; 2. Response to UV; 3. Response to X-ray; 4. Response to sonic wave; 5. Response to osmotic pressure; 6. Response to the electrochemical proton gradient.

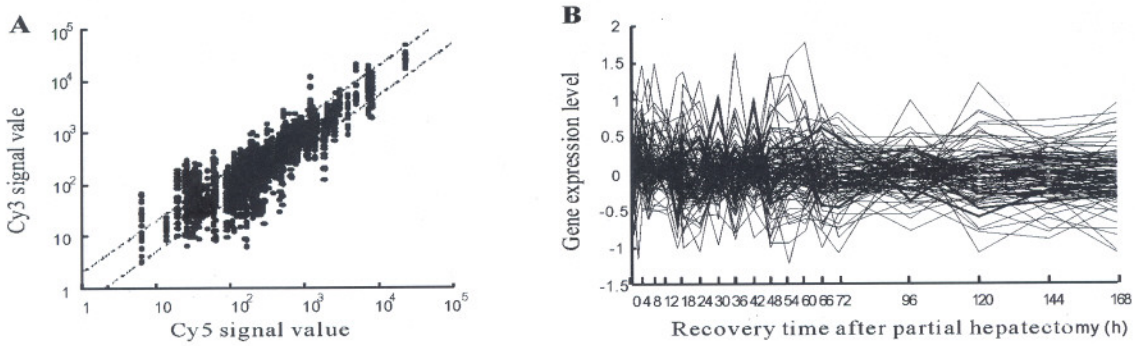

Figure 1. Expression frequency, abundance and changes of 120 physical stimuli-associated genes during rat LR. Detection data of Rat Genome 230 2.0 Array were analyzed and graphed by Microsoft Excel. A. Gene expression frequency. The dots above bias represent the genes up-regulated more than two folds, and total times of up-regulation were 431; those under bias down-regulated more than two folds, and that of down-regulation were 296; and the ones between biases no-sense alterative; B. Gene expression abundance and changes, in which 87 genes were 2.0 − 60 folds up-regulated, and 79 genes 2 − 16 folds down-.

3.2 Initiation expression time of the genes associated with the responses to physical stimuli during LR

At each time point of liver regeneration, the numbers of initial up-, down-regulated and total up-, down-regulated genes were in sequence: both 10 and 6 at 0.5 hour; 10, 11 and 17, 14 at 1 hours; 9, 1 and 23, 3 at 2 hours; 4, 1 and 23, 4 at 4 hours; 3, 3 and 16, 9 at 6 hours; 1, 0 and 20, 5 at 8 hours; 2, 1 and 22, 7 at 12 hours; 7, 5 and 18, 11 at 16 hours; 9, 12 and 23, 25 at 18 hours; 3, 3 and 25, 28 at 24 hours; 5, 3 and 16, 12 at 30 hours; 1, 1 and 16, 14 at 36 hours; 2, 2 and 20, 10 at 42 hours; 0, 1 and 24, 22 at 48 hours; 1, 0 and 27, 10 at 54 hours; 1, 0 and 25, 16 at 60 hours; 0, 0 and 26, 15 at 66 hours; 0, 0 and 19, 19 at 72 hours; 1, 0 and 21, 11 at 96 hours; 0, 1 and 15, 20 at 120 hours; 0, 0 and 13, 17 at 144 hours; 0, 0 and 12, 18 at 168 hours (Figure 2). Generally, gene expression changes occurred during the whole liver regeneration, and the up- and down-regulation times were 431 and 296, respectively. The initially up-regulated genes were predominantly expressed in the forepart, and the number of the initially up- and down- genes were similar in the prophase and metaphase, while few genes were initially expressed in the anaphase.

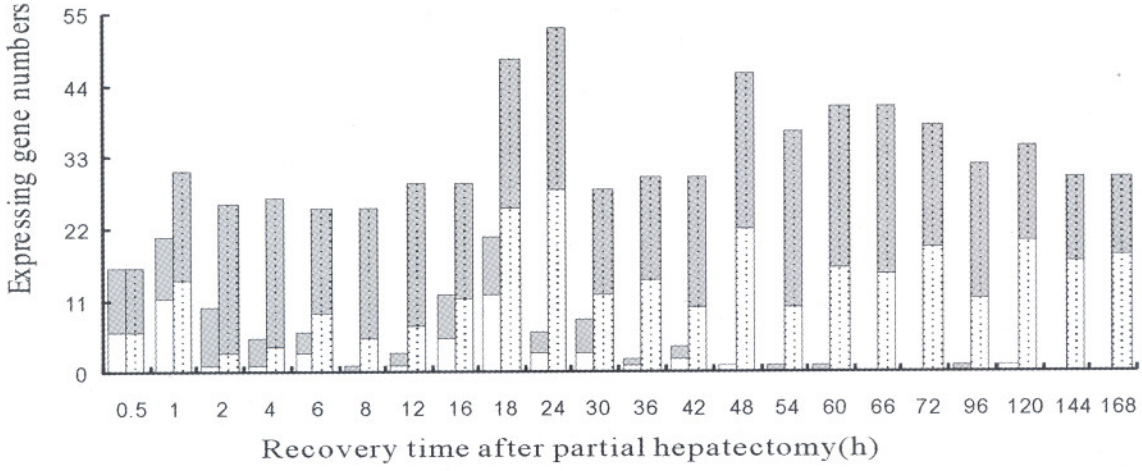

Figure 2. The initial and total expression profiles of 120 physical stimuli-associated genes at each time point of LR. Grey bars: Up-regulated gene; White bars: Down-regulated. Blank bars represent initial expressing genes, in which up-regulation genes are predominant in the forepart, and the up- and down- are similar in the prophase and metaphase, and very few in the anaphase. Dotted bars represent the total expressing genes, in which some genes are up-regulation, and the others down- during LR.

3.3 Expression similarity and time revelance of the genes associated with the responses to physical stimuli during LR

120 genes mentioned above could be characterized based on their similarity in expression as following: only up-, predominantly up-, only down-, predominantly down-, up/down-regulated, involved in 41, 20, 33, 19 and 7 genes, respectively (Figure 3). According to time

relevance, they were classified into 15 groups, including 0.5 and 1 hour, 2 hours, 4 and 8 hours, 6 hours, 12 hours, 16 hours, 18 hours, 24 and 48 hours, 30 and 42 hours, 36 hours, 54 and 60 hours, 66 and 72 hours, 96 hours, 120 hours, 144 and 168 hours, and the up- and down-regulated times were 37 and 20, 23 and 3, 43 and 9, 16 and 9, 22 and 7, 18 and 11, 23 and 25, 49 and 50, 36 and 22, 16 and 14, 52 and 26, 45 and 34, 21 and 11, 15 and 20, 25 and 35, respectively (Figure 3). The up-regulation expression genes were chiefly associated with the responses to visible light and sonic wave, and the down- mostly with conduction of the physical stimuli.

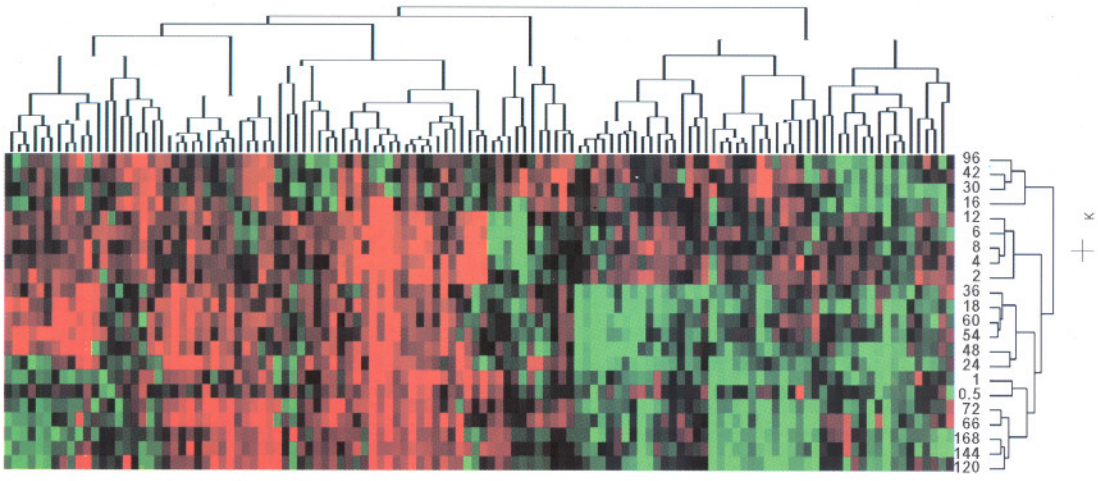

Figure 3. Expression similarity and time relevance clusters of 120 physical stimuli-associated genes during LR. Detection data of Rat Genome 230 2.0 Array were analyzed by H-clustering. Red represents up-regulation genes chiefly associated with promoting blood coagulation; Green represents down-regulation ones mainly associated with inhibiting blood coagulation; Black: No-sense in expression change. The upper and right trees respectively show function and time series clusters, by which the above genes were classified into 5 and 15 groups separately.

3.4 Expression patterns of the genes associated with the responses to physical stimuli during LR

120 genes mentioned above were categorized into 31 patterns, according to the changes in their expression. (1) up-regulation at one time point, i.e. 1, 12, 24, 30, 42, 60, 96 hours after PH (Figure 4A), 8 genes involved; (2) up at two time points, i.e. 0.5 and 4 hours (Figure 4B), 1 gene involved; (3) up at three time points (Figure 4B), 2 genes involved; (4) up at multiple time points (Figure 4B), 1 gene involved; (5) up at one phase, i.e. 0.5 – 12 hours or 6 – 12 hours (Figure 4C), 2 genes involved; (6) up at two phases, i.e. 30 – 42 and 60 – 96 hours (Figure 4C), 1 gene involved; (7) up at multiple time phases (Figure 4C), 1 gene involved; (8)up at one time point/phase, i.e. 24 and 66 – 72 hours, 66 and 18 – 24 hours, 18 and 48 – 60 hours (Figure 4D), 3 genes involved; (9) up at one time point/two phases (Figure 4D), 4 genes involved; (10) up at one time point/three phases (Figure 4E), 5 genes involved; (11) up at one time point/multiple phases (Figure 4E), 1 gene involved; (12) up at two time points/one phase (Figure 4F), 3 genes involved; (13) up at two time points/phases (Figure 4F), 1 gene involved; (14) up at three time points/one phase (Figure 4F), 1 gene involved; (15) up at three time points/two phases (Figure 4G), 3 genes involved; (16) up at multiple time points/phases (Figure 4G), 4 genes involved; (17) down at one time point, i.e. 6, 16, 18, 30, 36, 42, 48 hours (Figure 4H1-H2), 10 genes involved; (18) down at two time points, i.e. 24 and 36 hours (Figure 4I), 1 gene involved; (19) down at three time points (Figure 4I), 2 genes involved; (20) down at multiple time points (Figure 4I), 2 genes involved; (21) down at one phase, i.e. 4 – 18 hours, 6 – 12 hours, 18 – 24 hours, 24 – 30 hours (Figure 4J), 4 gene involved; (22) down at two phases, i.e. 16 – 48 and 60 – 120 hours, 18 – 24 and 48 – 60 hours (Figure 4J), 2 genes involved; (23) down at one time point/phase, i.e. 48 and 18 – 24 hours (Figure 4K), 2 genes involved; (24) down at one time point/two phases (Figure 4K), 3 genes involved; (25) down at two time points/one phase (Figure 4L), 3 genes involved; (26) down at two time points/phases (Figure 4L), 2 genes involved; (27) down at three time points/one phase (Figure 4L), 1 gene involved; (28) down at three time points/two phases (Figure 4L), 1 gene involved; (29)

predominantly up (Figure 4M1 – 4M3), 20 genes involved; (30) predominantly down (Figure 4N1 – 4N3), 19 genes involved; (31) similarly up/down (Figure 4O), 7 genes involved.

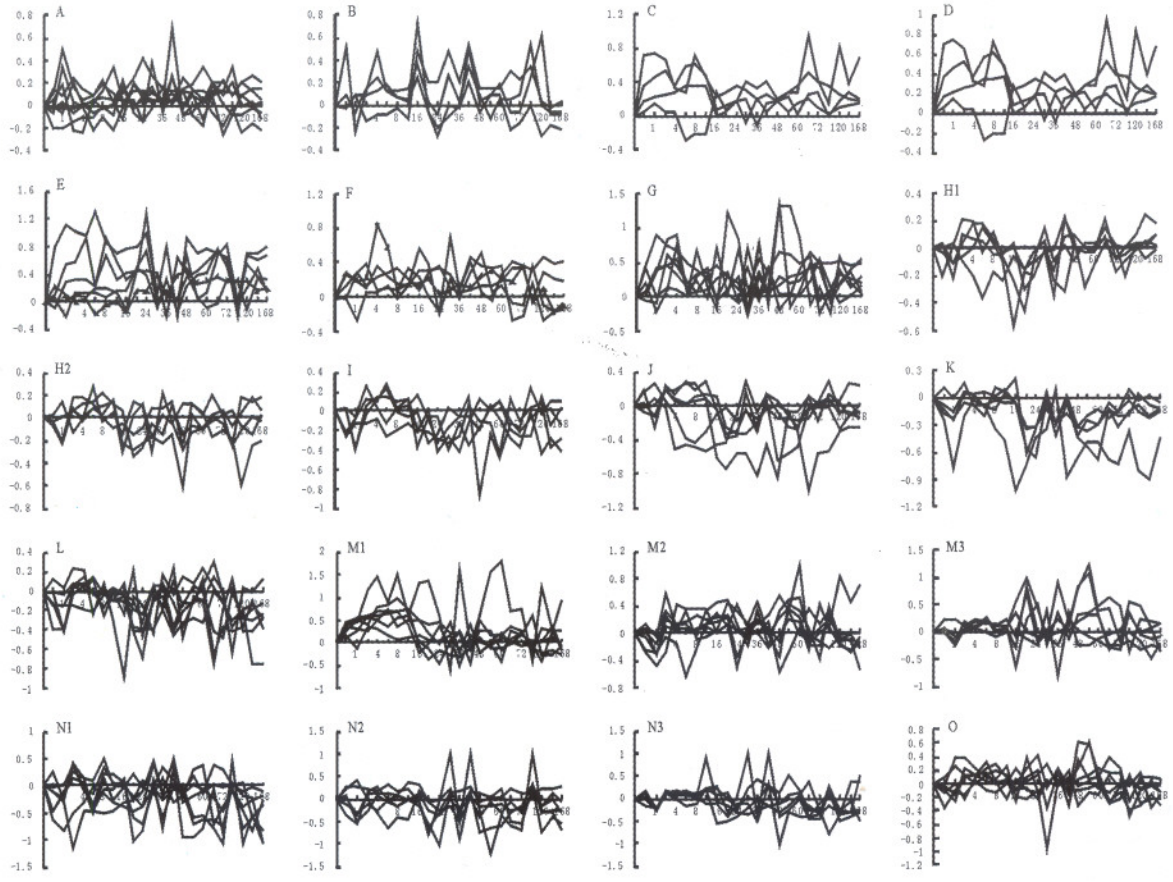

Figure 4. Thirty-one expression patterns of 120 physical stimuli-associated genes during LR. Expression patterns were obtained by the analysis of detection data of Rat Genome 230 2.0 Array with Microsoft Excel. A – G. 27 up-regulated genes; H – L. 22 down-regulated genes; M – O. 21 up/down-regulated genes. X-axis represents recovery time after PH; Y-axis shows logarithm ratio of the signal values of genes at each time point to control.

4 Discussion

Organisms can response to all kinds of environmental stimuli including physical stimuli in instinct, which is closely linked with organisms' survival[19]. The proteins associated with the response to visible light are stated as follows. Nine proteins including guanine nucleotide binding protein (G protein) γ transducing activity polypeptide 2 (GNGT2) are associated with visual signal transduction by G-protein coupled receptor protein signaling pathway[30]. Eight proteins including retinitis pigmentosa 1 homolog (RP1H) accelerate the rhodopsin- mediated phototransduction by conducting signal cascade[31]. Six proteins including myelocytomatosis viral oncogene homolog (MYC) have an anti-radiation effect by accelerating the cell's stress response to radiation[32]. Twelve proteins including ornithine aminotransferase (OAT) promote the signal conduction of retinitis and maintain normal visual perception, and the mutation of the corresponding genes can cause retinitis pigmentosa and other eye diseases[33]. Opsin 1 short-wave-sensitive (OPN1SW), an component of rhodopsin, is involved in transduction of the visual signal[34]. Solute carrier family 24 member 1 (SLC24A1) can transmit the visual signal by transporting sodium, kalium, calcium ion[35]. Guanylate cyclase activator 1a (GUCA1A) relates to the rods-mediated conduction of the visual signal[36]. Rhodopsin (RHO) is response for perception of light stimuli[37]. Basic helix-loop-helix domain containing class B2 (BHLHB2) adjusts photoperiod by light perception[38]. Crystallin γ D (CRYGD) is related to the formation of lens[39]. Transient receptor potential cation channel subfamily C member 3 (TRPC3) can convert light stimuli into electric sig-

nal[40]. Arrestin 3 retinal (ARR3) may bind to phosphorylated receptors to deactivate the phototransduction cascade[41]. ATP-binding cassette sub-family C member 6 (ABCC6), coupling with ATPase, accelerates the conduction of the visual signal[42]. Tissue inhibitor of metalloproteinase 3 (TIMP3) may play a role in light signal-transduction by receptor tyrosine protein kinase-mediated signal transduction pathway[43]. The meaningful expression profiles of genes mentioned above are same or similar at some points while different at others, indicating that they may co-regulate the responses to physical stimuli. Among them, *abcc*6 was down-regulated 2.3 times at 6 hours during LR, which is generally consistent with Dransfelds' result[20]. *timp*3 was down-regulated 2.1 times at 18 – 24 hours, which wasn't consistent with Dransfelds', and it needed to be further analyzed by Northern blotting[20]. *gnat*1 was up-regulated at 1 – 4, 8 – 24, 48 – 72 and 144 hours, and reached a peak at 24 hours that is 18.5 times higher than the control. *rp*1*h* was up at 0.5 – 24, 36, 48 – 72, 120 and 168 hours, having a peak at 60 hours that is 59.7 times higher than the control. *crygd* was up at 1 – 4, 18, 36 – 66, 120 and 168 hours, and reached a peak at 4 hours that is 8 fold of control. It is presumed that they play a key role in the response to visual light during LR.

The proteins associated with the responses to UV and X-ray are stated as follows. Five proteins including ferrochelatase (FECH), which can prevent and repair DNA damage caused by UV, may take a role in antiradiation[44]. Tumor protein p53 (TP53) prevents the DNA damage resulting from UV and X-ray irradiation by base-excision repair[45]. The meaningful expression profiles of genes mentioned above are same or similar at some points while different at others, indicating that they may co-regulate the responses to UV and X-ray. Among them, the highest expression abundance of fech at 1 hour after PH was 2.7 folds of control. *tp*53 was up-regulated at 18 – 24, 48 – 60 and 96 hours, and reached a peak at 24 hours that was 2.9 times higher than the control. It is presumed that they are of importance in the response to visual light stimuli during liver regeneration.

The proteins associated with the response to sonic wave are stated as following. Seven proteins including neuronal amiloride-sensitive cation channel 1 (ACCN1), serving as ion channel, transmit the signal of sound, light and other stimuli[46]. Five proteins including noggin (NOG) can perceive sonic wave, and their mutation can cause hearing loss etc[47]. Three proteins including procollagen type XI α1 (COL11A1) are the components of system of sound transduction[49]. Translocase of inner mitochondrial membrane 8 homolog a (TIMM8A) encodes a small protein known as deafness/dystonia peptide involved in perception of sound stimulus[50]. Tumor necrosis factor receptor superfamily member 11a (TNFRSF11A) promotes the transduction of sound stimulus[51]. Ets variant gene 1 (ETV1) is involved in the response to mechanical stimulus[52]. The meaningful expression profiles of genes mentioned above are same or similar at some points while different at others, indicating that they may co-regulate the response to sonic wave. Among them, *accn*1 was up-regulated at 30 and 42 hours after PH, having the highest abundance of 10.1 folds at 30 hours. *tnfrsf*11*a* was up at multiple phases, reaching a peak at 8 hours that is 8.2 times higher than the control. *etv*1 was up at 0.5 – 12 and 144 hours, and reached a peak at 12 hours that is 4-fold of control. It is supposed that they play crucial roles in the response to visual light stimuli during liver regeneration.

The proteins associated with the responses to osmotic pressure and electrochemical proton gradient are stated as follows. Solute carrier family 14 member 2 (SLC14A2) regulates the osmotic pressure of cell by transporting urea[53]. *slc*14*a*2 was down-regulated at the metaphase during liver regeneration. Transient receptor potential cation channel subfamily V member 1 (TRPV1), a cation channel, participates in the stress responses to osmotic pressure and the electrochemical proton gradient[54], and it was down only at one time point (120 hours after PH) after PH. It is speculated that the capacity of anti-osmotic pressure and the response to electrochemical proton gradient decrease in the anaphase of liver regeneration.

In conclusion, based on the experimental methods of longer time and multiple time points being adopted, the expression changes of the genes associated with the response to six kinds of physical stimuli during liver regeneration were investigated by high-throughput gene expression analysis. It was primarily proved that the regenerating liver response to physical stimuli was different in different phase; 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 proteins interaction. Therefore, the above results need to be further analyzed by the techniques, such as Northern blotting, protein chip, RNA interference, protein-interaction etc.

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