• BASIC RESEARCH •

Identification of expressed genes in regenerating rat liver in 0-4-8-12 h short interval successive partial hepatectomy

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Abstract

AIM: To identify the genes differentially expressed in the regenerating rat liver of 0-4-8-12 h short interval successive partial hepatectomy (SISPH) and to analyze their expression profiles.

METHODS: Five hundred and fifty-one elements screened from subtractive cDNA libraries were made into a cDNA microarray (cDNA chip). Extensive gene expression analysis following 0-4-8-12 h SISPH was conducted by microarray.

RESULTS: One hundred and eighty-three elements were selected, which were either up- or down-regulated more than 2-fold at one or more time points after SISPH. Cluster analysis and generalization analysis showed that there were five distinct temporal patterns of gene expression. Eighty-six genes were unreported, associated with liver regeneration (LR).

CONCLUSION: Microarray analysis shows that the down regulated genes are much more than the up-regulated ones in SISPH; the numbers of genes expressed consistently are fewer than that expressed immediately; the genes expressed in high abundance are much fewer than that increased 2-5-fold. The comparison of SISPH with partial hepatectomy (PH) shows that the expression trends of most genes in SISPH and in PH are similar, but the expression of 43 genes is specifically altered in SISPH.

Key words: SISPH; Liver regeneration

INTRODUCTION

Liver has a capacity to regain original mass after partial hepatectomy (PH)[1-6]. It was confirmed that a great deal of genes participate in liver regeneration (LR), and the genes expressed in the different phases of LR were various, and that hepatocytes progressing from G_0 phase to G_1 phase occurred in 2-6 h after PH[7,8]. Despite numerous related papers, it is still not thoroughly elucidated how many genes participate in LR and what its molecular mechanism is[9-15]. To get sight into the mechanism of LR, we established the short interval successive partial hepatectomy (SISPH) model[16], which could offer the useful materials for studying specific gene expression at various crucial points of LR[17,18]. To seek some novel differential display genes responsible for LR, the method of subtracted suppression hybridization was used to obtain a bulk of up-regulated and down-regulated expression sequence tags (ESTs) in the regenerating rat liver. With development of cDNA microarray technology, genomewide expression of thousands and thousands of activated or suppressed genes can be simultaneously analyzed under various biological conditions[19-21]. To further explore the genes participating in LR, the present study successfully identified the gene expression profile in regenerating liver following 0-4-8-12 h SISPH by microarray, and some important information was achieved by analyzing the data using Microsoft Excel and GeneSpring.

MATERIALS AND METHODS

Rat model of SISPH

Male and female Sprague-Dawley (SD) rats, aged 10-12 wk, weighting 200-220 g, were raised in Experimental Animal Center of Henan Normal University (HNU). According to Xu et al, lobus external sinister, lobus centralis sinister, lobus centralis and lobus dexter were removed one by one at four different time points of 0, 4, 8, 12 h, which is named as 0-4-8-12 h SISPH.

Sample preparation and RNA isolation

The removed liver lobus was rinsed in cold 1×PBS and

immersed and stored in -80 ℃ refrigerator for RNA and protein extraction. Total RNA was isolated from frozen liver lobus according to the manual of TRIzol kit of Invitrogen. In brief, 50-100 mg liver tissue was homogenized in 1 mL TRIzol reagent containing phenol and guanidinium isothiocyanate/cationic detergent, followed by phenol-chloroform extraction and isopropyl alcohol precipitation. The quantity and integrity of total RNA was examined by ultraviolet spectrometer and denaturing formaldehyde agarose electrophoresis stained by ethidium bromide.

Subtracted cDNA library construction and screening
cDNA subtracted libraries were generated from total RNA by PCR-Select™ cDNA Subtraction kit (Clontech) following the manufacturer's instruction. Briefly, total RNA was transcribed into double cDNA strands and digested with restriction enzymes, followed by subtracted hybridization with drivers and testers. Finally with suppression polymerase chain reaction (PCR), differential ESTs were used to construct subtracted cDNA library. The latter was cloned into T/A vector and screened by PCR with nested primer 1 and 2.

cDNA microarray construction
cDNA fragments amplified by PCR with nested PCR primers 1 and 2 and purified by NaAc/isopropyl alcohol were spotted onto glass slides (Biostar) with the help of ProSys-5510A spotting machine following designed project. Then the gene chips were ready by hydration and blockage and drying. Totally 1 152 elements (double spot chip) including 50 control system (8 negative control, 12 blank control, 30 internal control) and 551 target genes to be studied comprised 8 submatrixes (12*12) occupying 9 mm×18 mm (Biostar). Then the gene chips were ready by hydration, blockage and drying.

Fluorescence-labeled cDNA preparation
RNA isolated from rat livers before SISPH was ready for a reference for all cDNA microarray analyses. Totally denatured RNA was reversely transcribed with cy3-conjugated dCTP (control group) and cy5-conjugated dCTP (test group) (Amersham-Pharmacia Biotech) using MMLV reverse transcriptase (Promega) with olig (dT) primer. After bath incubation for 2 h, labeled buffer I and II were subsequently added to the reaction. The control group and test group were mingled together symmetrically and stored in the dark until use.

Hybridization and scanning
The glass slices were prehybridized at 42 ℃ for 5-6 h in hybridization buffer containing freshly cooked shared salmon sperm DNA. The labeled denatured probe was hybridized against cDNA microarrays with overnight (16-18 h) incubation at 42 ℃. The slides were then washed twice with 2× SSC containing 0.5% SDS for 5 min at room temperature, once with 0.2× SSC containing 0.5% SDS at 60 ℃ for 10 min, and finally with 0.2× SSC at 60 ℃ for 10 min. The slices were exposed to photographer. Hybridized images were scanned by a fluorescence laser scanning device, Gene Pix 4000A (Axon Instruments, Inc., Foster City, CA).

At least two hybridizations were performed at each time point. In addition, a semiquantitative inspection of the hybridization results was performed for (1) green signal (down-regulation); (2) yellow signal (no obvious regulation); (3) red signal (up-regulation).

Data analysis
The cy3 and cy5 signal intensities were quantified by Gene Pix Pro 3.0 software (Axon Instruments, Inc., Foster City, CA). Subsequently, we normalized the obtained numerical data with classical linear regression techniques. In brief, quantified cy3 and cy5 signal intensities were obtained when foreground signal intensities were deducted by background signal intensities and cy5 signal intensities was replaced by 200 when it was <200. When R_i (R_i = cy5/cy3) was between 0.1 and 10, R_i was taken as logarithms base natural to generate R_i'[log (R_i)] and ND was taken by EXP (R) (averaged R_i'). The modified cy3* was generated by ND multiplying cy3 and was replaced by 200 when it was <200. The ratio was expressed by cy5/cy3*. Therefore, we selected genes whose ratio was more than 2 or less than 0.5 representing a 2-fold difference in expression level.

To analyze the selected gene expression data, we applied κ-means cluster analysis, and performed GeneMaths hierarchical clustering to appraise the number of groups. Whole analyses were executed with Microsoft Excel (Microsoft, Redmond, WA) and GeneSpring (Silicon Genetics, San Carlos, CA).

Chromosome location and functional prediction of the novel ESTs
All the clones of ESTs were sequenced by TaKaLa and blasted in NCBI. The unreported ESTs were searched at http://www.ncbi.nlm.nih.gov/genomeguide/rat/index.html/ for gene location in chromosome and gene corresponding whole genome shotgun. In virtue of rat genome database, electronic cloning and chromosome location of the unreported ESTs representing novel cDNA full-length were performed successfully (data not shown). By delivering the clones sequences to http://genes.mit.edu/GENSCAN.html/, we acquired corresponding coding domain sequences (CDS) supported by the ESTs. Compared with known proteins by BLASTP (http://www.ncbi.nlm.nih.gov/BLAST/), their function and accession number were achieved.

RESULTS
Category of genes identified in the 0-4-8-12 h SISPH
One hundred and eighty-three elements altered by more than 2-fold intensity at least at one time point in the 0-4-8-12 h SISPH were identified and selected, of which, 77 were up regulated and 106 were down regulated. Eighty-six of them belonged to the unreported novel genes, and the other 97 were reported, of which quite a few genes had been previously reported to be involved in LR. Following the functions of the reported genes and the time points at which they attained maximum up or down-regulation, they were respectively categorized into 21 groups: the genes involved in stress response, in glycometabolism, in stearoyl metabolism,

in oxidative and reductive response, genes encoding regulation proteins, glycoproteins, lipid-proteins, nucleolar proteins, receptors, factors, hemoglobins, immunological proteises, cytoskelets, marker proteins, amino acid enzymes, proteolytic enzymes, proteinase inhibitors, phosphorylase, phosphatases, synthases and transferases (Table 1).

Table 1 Genes related to LR altered in SISPH

No.	Gene description	Fold difference	No.	Gene description	Fold difference
	Unreported genes		62	LRRPAb2-132	0.2
1	CH230-206C20	0.3	63	MGC10946	2.8
2	CH230-403C20	0.5	64	MGC38937	2.4
3	CH230-329D3	2.2	65	MGC5178	0.4
4	CH230-372C24	0.1	66	mKIAA0665	2.4
5	CH230-7A22	0.1	67	RIKEN 1300002A08	0.2
6	CH230-11N5	2.1	68	RIKEN 1600027G01	0.3
7	CH230-4L11	3.5	69	RP11-281N10	0.3
8	CH230-211F21	0.4	70	RP11-586K2	2.6
9	CH230-155H3	0.5	71	RP23-100C5	2.0
10	CG31759-PA	2.9	72	RP23-165H7	0.5
11	Citb585c7	0.2	73	RP23-235O1	0.4
12	D930042H13	0.3	74	RP23-28G130	2.3
13	DNA segment of Chr 1 (Wsu94)	3.8	75	RP23-32O9	2.3
14	DNA segment of Chr 17 (Wsu94)	2.0	76	RP23-417P22	0.3
15	KIAA0205	0.4	77	RP23-476D16	1.6
16	KIAA1376	0.4	78	RP23-480P21	2.9
17	LOC303588	2.0	79	RP23-92K11	2.9
18	LOC333273	2.9	80	RP24-347B22	0.3
19	LRRP Cc1-27	0.3	81	Rp32-28p17	0.3
20	LRRP Aa2-028	3.1	82	U48828	2.4
21	LRRP Aa2-111	0.2	83	Adult male liver cDNA	0.1
22	LRRP Aa2-174	0.3	84	Mouse mRNA	0.4
23	LRRP Aa2-258	0.5	85	Open reading frame 31	0.5
24	LRRP Ab1-021	9.1	**86**	**13 d embryo liver cDNA**	**3.6**
25	LRRP Ab1-022	0.5		Stress response	
26	LRRP Ab1-114	3.5	87	Acute-phase protein alpha-1-inhibitor 3	0.3
27	LRRP Ab1-216	3.5	88	alpha-1 major acute phase protein prepeptide	6.8
28	LRRP Ab1-334	4.4	89	Angiotensinogen (Agt)	11.5
29	LRRP Ab2-001	0.3	90	Kininogen	2.5
30	LRRP Ab2-034	0.3	91	T-kininogen (Kng)	5.0
31	LRRP Ab2-037	0.5		Glycometabolism	
32	LRRP Ab2-051	2.1	92	alpha enolase (Enolase 1)	2.7
33	LRRP Ab2-131	0.3	93	Isocitrate dehydrogenase 1 (Idh1)	0.1
34	LRRP Ab2-132	0.4	94	3-Phosphoglycerate dehydrogenase	2.2
35	LRRP Ab2-225	0.3		Fatty and stearoyl metabolism	
36	LRRP Ab2-232	0.2	95	Bile acid CoA ligase	2.0
37	LRRP Ab2-255	0.2	96	Malonyl-CoA decarboxylase	0.4
38	LRRP Ab2-371	0.1	97	Methylmalonate semialdehyde dehydrogenase	0.4
39	LRRP Ab2-389	0.5	98	P450 cholesterol 7-alpha-hydroxylase (P450 VII)	0.1
40	LRRP Ab2-402	0.2	99	NAD (P) dependent steroid dehydrogenase	0.1
41	LRRP Ab2-416	0.3	100	3-alpha-hydroxysteroid dehydrogenase	0.1
42	LRRP Ab2-417	0.2		Oxidation and reduction	
43	LRRP Ab2-427	0.5	101	Acyl-coA oxidase	0.5
44	LRRP Ab2-440	0.3	102	Alcohol dehydrogenase (ADH)	0.1
45	LRRP Ac1-060	0.3	103	Cytochrome b5 (Cyb5)	0.3
46	LRRP Ac1-158	2.1	104	Cytochrome P450	0.3
47	LRRP Ac1-177	7.3	105	Cytochrome P450 15-beta gene (Cyp2c12)	0.4
48	LRRP Ac1-233	5.6	106	Cytochrome P450 2E1	0.2
49	LRRP Ac1-873	4.6	107	Cytochrome P450, 2c39 (Cyp2c39)	0.3
50	LRRP Ac2-061	6.4	108	Cytochrome P450 (PNCN inducible, Cyp3A1)	0.4
51	LRRP Ac2-067	2.6	109	Flavin-containing monooxygenase 1 (Fmo1)	0.1
52	LRRP Ac2-143	2.0	110	Peroxisomal sarcosine oxidase (PSO)	0.1
53	LRRP Ac2-210	2.3	111	Plasma selenoprotein P1 (Sepp1)	0.4
54	LRRP Ba1-647	3.3	112	Small subunit precursor of RuBPCase	0.1
55	LRRP Bm403207	7.7		Regulation proteins	
56	LRRP Cb1-727	0.4	113	G-protein beta polypeptide 2-like 1 (Gnb2l1)	2.1
57	LRRP Cb1-739	2.2	114	II-Protein with tetratricopeptide repeats 3	0.2
58	LRRP Cc1-27	0.3	115	RAKb	0.2
59	LRRP Cc1-38	0.1		Glycoproteins	
60	LRRP Cc1-9	3.5	116	apha-1-B glycoprotein (A1bg)	0.3
61	LRRP zbs559	3.5			

117 Fibronectin 1 (Fn1)	3.0	
118 Fibrinogen, gamma polypeptide (Fgg)	8.0	
119 Myelin-associated glycoprotein (L-MAG)	8.4	
120 UDP-glucuronosyltransferase 2B3 (Udpgt)	0.4	
121 TRAM1	0.3	
Lipid-proteins		
122 Adipose differentiation-related protein	4.6	
123 Solute carrier family 20, mem 1 (Slc20a1)	0.4	
Nucleolar proteins		
124 Damage-specific DNA binding protein 1 (Ddb1)	0.5	
125 Nuclear protein 1 (Nupr1)	2.9	
126 Nucleolar protein family A, mem 2	2.9	
127 ORF2 endonuclease and Rnase H	0.5	
128 RNase A family 4	0.3	
Receptors		
129 ATP-binding cassette, sub-family B	0.5	
130 ATP-binding cassette, sub-family C	0.1	
131 Interleukin 1 receptor, type I (Il1r1)	7.3	
132 Nuclear receptor subfamily 0, member 2 (Nr0b2)	0.4	
Factors		
133 Amphoterin	0.5	
134 Angiogenin	0.4	
135 Eukaryotic translation initiation factor 4A1	3.5	
136 Early growth response factor 1 (Egr1)	4.3	
137 Neuropeptide Y (Npy)	11.3	
138 NF-E2-related factor 2 (Nfe2l2)	0.3	
139 Pre-B-cell colony-enhancing factor (Pbef)	5.3	
140 ADP-ribosylation factor 3 (Arf3)	0.8	
141 Angiopoietin-like 3	0.3	
Hemoglobins		
142 Hemoglobin, alpha 1 (Hba1)	2.4	
143 Hemoglobin beta chain (Hbb)	2.5	
Immunological proteises		
144 Achaete-scute complex homolog-like 1 (Ascl1)	0.4	
145 Complement component 5 (C5)	19.4	
146 Complement component 6 (C6)	0.4	
147 Immunoglobulin C kappa gene	0.3	
148 JE/MCP-1	2.5	
Cytoskelets		
149 Actin -beta	2.3	
150 Actin gamma	5.1	

151 Keratin 8 (Krt8)	2.8	
Marker proteins		
152 alpha globin	2.4	
153 Amyloid a-5 protein	53.9	
154 Subchromosomal transferable fragment	0.4	
Amino acid enzymes		
155 Arginase 1 (Arg1)	6.4	
156 Argininosuccinate lyase (Asl)	5.6	
157 Cytosolic aspartate aminotransferase	6.8	
158 2-Hydroxyphytanoyl-CoA lyase (Hpcl2)	0.3	
Proteolytic enzymes		
159 Cathepsin C (Ctsc)	0.4	
160 Cathepsin D (Ctsd)	0.5	
161 Coagulation factor 2 (F2)	0.5	
162 Neutrophil collagenase (Mmp8)	0.2	
Proteinase inhibitors		
163 alpha-2-macroglobulin (A2m)	5.1	
164 alpha-trypsin inhibitor heavy chain	0.4	
165 Contrapsin-like protease inhibitor related protein	6.1	
166 Leuserpin-2 (Serpind1)	0.3	
167 Serine protease inhibitor 1	5.6	
Phosphorylase		
168 CDK103	1.2	
169 Purine-nucleoside phosphorylase	0.3	
Phosphatases		
170 Phosphatidylserine-specific phospholipase A1	4.9	
171 Pyrophosphatase/phosphodiesterase 1 (Enpp1)	3.1	
172 Protein phosphatase 1 (GL-subunit)	0.2	
Synthase		
173 Carbamyl phosphate synthetase I	2.5	
174 Fatty acid elongase 1 (rELO1)	0.1	
175 Glutamyl-prolyl-tRNA synthetase (Eprs)	2.2	
176 RNA cyclase	2.0	
Transferases		
177 Glutathione *S*-transferase, alpha 1 (Gsta1)	0.3	
178 Glutathione *S*-transferase, type 3 (Yb3) (Gstm3)	0.5	
179 Glutathione *S*-transferase Y(b) subunit	0.5	
180 Serine hydroxymethyl transferase 1	0.4	
181 Sialyltransferase 1 (Siat1)	0.2	
182 Sulfotransferase K2	0.2	
183 UDP-glucuronosyltransferase 2, mem 5 (Ugt2b5)	0.5	

Gene expression differences at the various time points of the 0-4-8-12 h SISPH
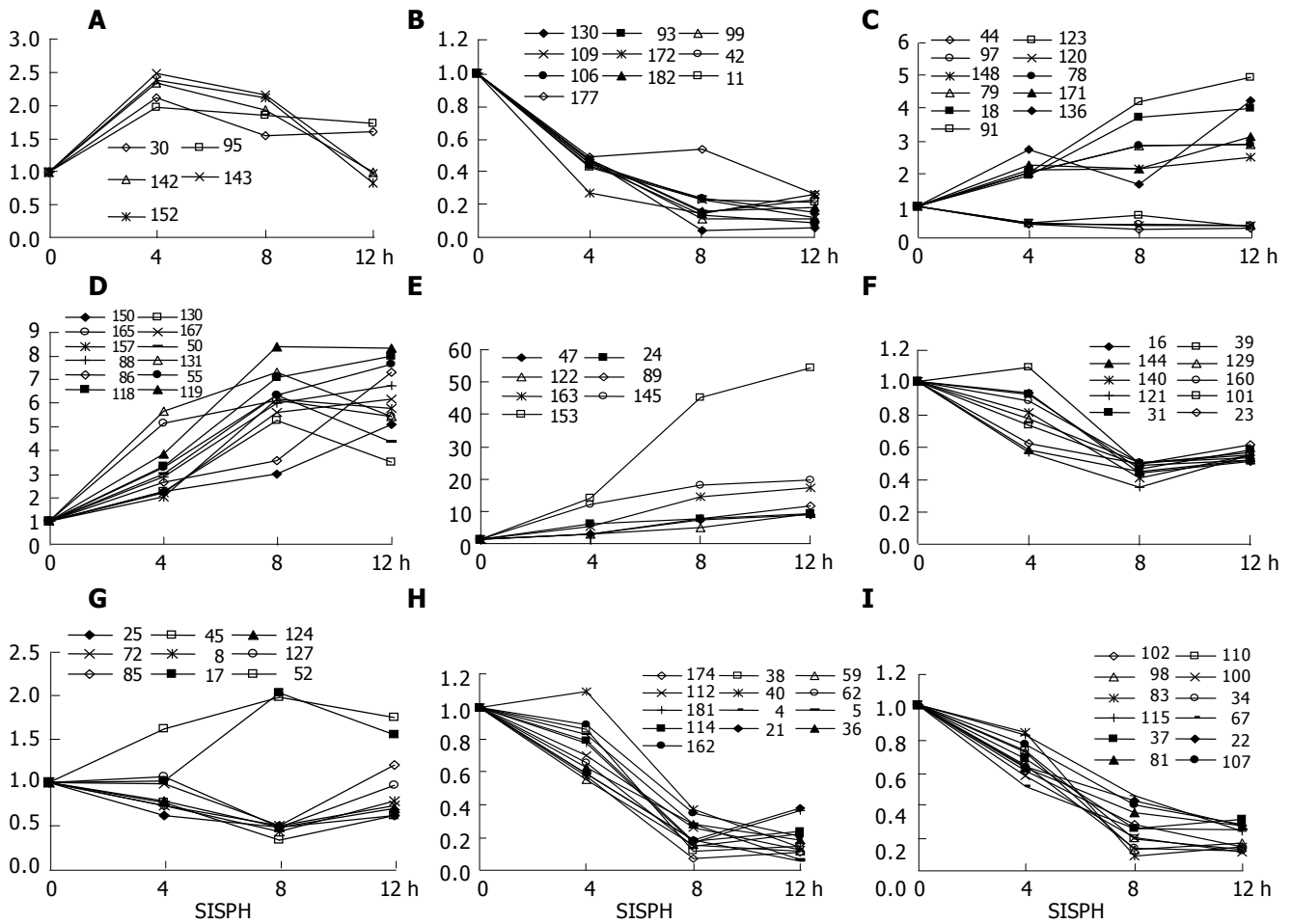
The gene expression differences induced or suppressed at 4, 4-8, 4-12, 8, 8-12 h of SISPH are shown in Figure 1. At 4 h of SISPH, 5 genes expressed up over 2-fold (Figure 1A); at 4-8 h of SISPH, 2 genes expressed up over 2-fold (Figure 1A); at 4-12 h of SISPH, 29 genes expressed up (Figures 1B-E), of which 10 genes up over 5-fold (Figure 1B), and 7 genes up over 10-fold (Figure 1C), and 10 genes expressed down (Figures 1D and E); at 8 h of SISPH, 2 genes expressed up (Figure 1G) and 7 genes down (Figure 1F); at 8-12 h of SISPH, 25 genes expressed down (Figures 1H and I).

Gene expression level in the 0-4-8-12 h SISPH

According to the up- and down-regulated density of genes in the 0-4-8-12 h SISPH, we categorized them into three groups: (1) 106 genes were down-regulated by less than 50% (Figure 2A); (2) 54 genes were up-regulated by 2-5-fold (Figure 2B); (3) 23 genes were strongly up-regulated by more than 5-fold (Figure 2C).

Hierarchical cluster analysis of genes expressed in the 0-4-8-12 h SISPH

The expression profile of the 183 genes altered by more than a 2-fold intensity at least at one time point in the 0-4-8-12 h SISPH was emanative to the last time point, which indicated that LR at 12 h has not been completed yet, quite active on the contrary (Figure 3A). We undertook hierarchical clustering of four time points 0, 4, 8 and 12 h of SISPH using GeneSpring software and discovered that gene expression profiles had no similarity between the four time points (Figure 3B). To facilitate the visualization and interpretation of the gene expression program represented in this very large body of data, we used the method of κ-means to order genes on the basis of similarities in their expression patterns and displayed the results in a compact graphical format, generating seven kinds of ramose gene expression clusters (Figure 3C). We then categorized the 183 elements into five distinct temporal induction or suppression patterns following immediate induction, late induction, consistent induction, late suppression, consistent suppression (Figure 3D).

Figure 1 Gene expression differences in 0-4-8-12 SISPH. **A**: 4 h, 4-8 h; **B-E**: 4-12 h; **F** and **G**: 8 h; **H** and **I**: 8-12 h.

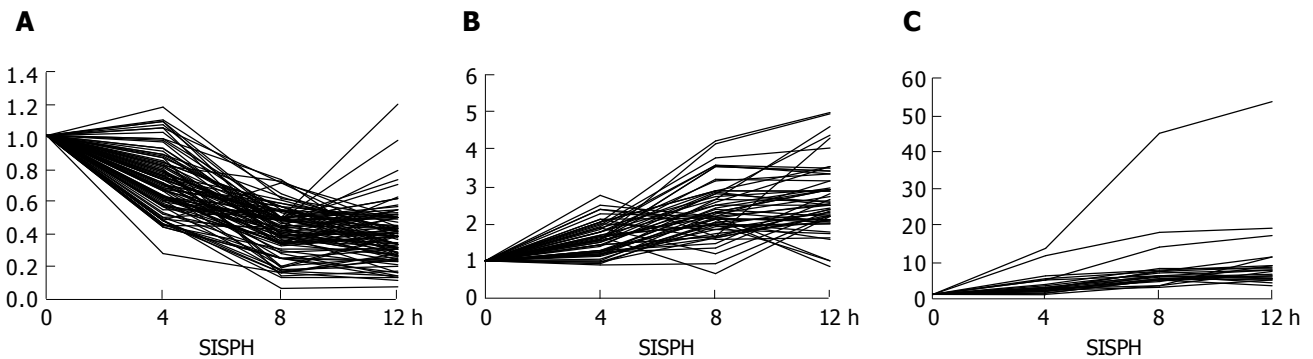### Comparison of gene expression in SISPH with that in PH

Comparing gene expression profile in SISPH with that in PH, we discovered that 43 genes were specially induced by SISPH, and the expression of 140 genes were altered simultaneously in both SISPH and PH, but the time points of their expression and extent of up- and down-regulation were different (Table 2).

DISCUSSION

This study found that hemoglobin alpha and beta were immediately induced to synthesize in the residual liver at 4 h in SISPH and after PH and returned to normal level there after, and that the activity peak of bile acid CoA ligase appeared at the same time, indicating a role of hemoglobin and amino acids transport in early phase of LR[22,23].

Thirty-six genes were up-regulated and reached a maximum level at 8 h in SISPH, and then were down regulated, in which 18 were unreported new genes and 18 were reported genes, with little knowledge about their relationship with LR. These results showed that their expression products could be necessary for the initiation of S phase. Myelin-associated glycoprotein-binding activity of novel sulfated GM1b, high-affinity ligands for neural siglecs

Figure 2 Expression level of genes in the 0-4-8-12 h SISPH. **A**: Down-regulated genes; **B**: up-regulated genes; **C**: strong up-regulated genes.
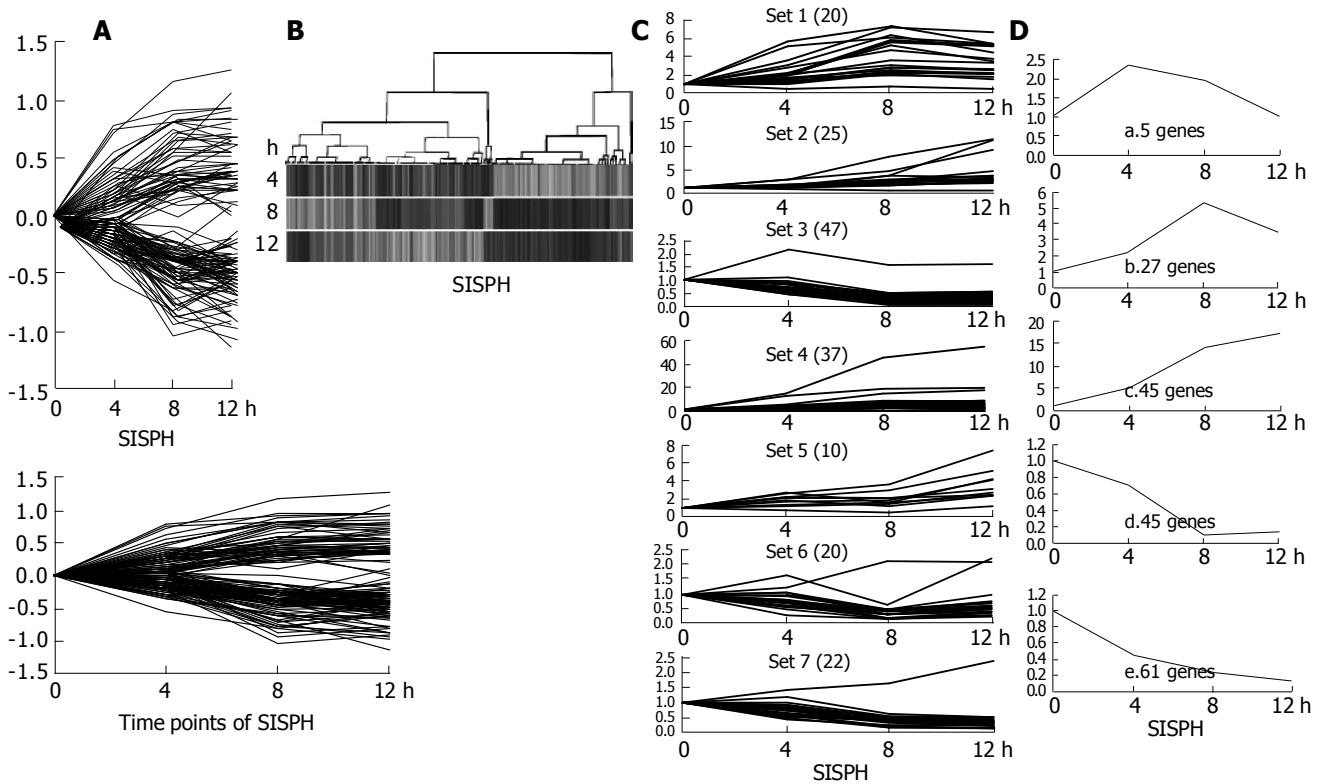
Figure 3 Cluster analysis of 185 elements. **A**: The difference of their intensity was identified more than 2-fold at least at one time point; **B**: A hierarchical clustering of five time points indicated that the genes in these time points hardly had a common expression profile; **C**: The k-means method was used and these genes were classified into seven clusters; **D**: Five distinct temporal patterns were designated. a. Immediate induction; b. Late induction; c. Consistent induction; d. Late suppression; e. Consistent suppression.

is reported important to nerve system regeneration[24]. It may be available to repair liver or hepatocytes damage caused by SISPH. A CPi-21 encoded by contrapsin-like protease inhibitor is found to inhibit the proteolysis of Edman to glycosylation proteins[25]. Abrupt increase of CPi-21 is presumed to be beneficial to cell adherence in SISPH, in which it was severely destroyed. Aspartate amino transformation reaction catalyzed by aspartate aminotransferase is necessary for accommodated purine, indicating liver cells need a great deal of purine for DNA synthesis in SISPH and after PH. Pre-B-cell colony-enhancing factor is known up regulated in the infected fetal membranes[26], which was continuously induced in the early phase of SISPH and PH, and lately suppressed, suggesting that it is associated with recovery of damage in LR. Translation initiation factor eIF-4A1 is reportedly over expressed in human melanoma cells[27], whose abundant expression in SISPH shows that it may participate in protein synthesis in LR.

Forty-five genes were consistently up regulated and reached a maximum level at 12 h in SISPH, in which 21 were unreported new genes, and the others were reported genes, but whether they have relationship with LR is unknown yet. JE/MCP-1 is known as a CC chemokine attracting monocytes, basophils and T-lymphocytes responsible for IL-1β and TNF-α[28,29]. Its consistent induction is assumed to be involved in relinquishing inflammation caused by SISPH. It was confirmed that keratin 8 could form characteristically intermediate filaments of liver and internal epithelia, including derivative cancers, whose consistent induction may help maintain hepatocyte structural and

functional integrity following trauma after SISPH. T-kininogen is important in the acute phase response to trauma as a cysteine proteinase inhibitor[30]. IL-6 is confirmed to act as a direct hepatic mitogen and potent liver growth factor with potential clinical utility for increasing liver mass following injury[31]. The augment of T-kininogen and kininogen level in SISPH and PH may reveal that there is an increasing response of kininogen promoter to IL-6 as an LR signal. Serum amyloid α-5 protein, precursor of serum amyloid A, was successively and dramatically up-regulated for more than 50-fold in SISPH and PH, probably on account of IL-6 simulation in LR[32], indicating that they have some positive role in LR, which is worth studying. Angiotensinogen is already known to be induced by TNF-NFκB and the IL-6-STAT3 signaling pathways in PHx[33], whose level was consistently increased by 11-fold in SISPH, implying a role in avoiding excessive loss of hepatic blood in LR. The successive increase of G-protein in SISPH and PH suggests that DNA synthesis by cAMP in hepatocytes are largely activated for LR[34]. The ensuing variations of cAMP levels are supposed to modulate both growth and differentiated functions during LR of SISPH. The advanced transcription level of enolase 1 (α-enolase) is supposed to provide sufficient VEGF for LR of SISPH. Activity and marked content increase of arginase in the previous period of regenerating liver co-ordinated with other urea cycles' enzymes[35,36]. The consistent induction of glutamyl-prolyl-tRNA synthetase (Eprs) may indicate that there is a great demand for glutamyl to induce anti-toxin in LR after SISPH. NPY localized exclusively to hepatic vascular walls is conceived

Table 2 **Comparison of difference of gene expression in SISPH with that in PH**

Gene description	SISPH	PH	Gene description	SISPH	PH
Unreported genes			Isocitrate dehydrogenase 1 (Idh1)	0.1	0.2
CH230-206C20	0.3	0.3	Fatty and stearoyl metabolism		
CH230-403C20	0.5	0.2	Bile acid CoA ligase	2.0	2.3
CH230-372C24	0.1	0.1	Malonyl-CoA decarboxylase	0.4	0.3
CH230-7A22	0.1	0.1	Methylmalonate semialdehyde dehydrogenase	0.4	0.2
CH230-11N5	2.1	4.5	3-alpha-hydroxysteroid dehydrogenase	0.1	0.2
CH230-4L11	3.5	4.5	Oxidation and reduction		
CG31759-PA	2.9	0.5	Alcohol dehydrogenase (ADH)	0.1	0.1, 2.4
D930042H13	0.3	0.3	Cytochrome b5 (Cyb5)	0.3	0.2
LRRP Cc1-27	0.3	0.3, 2.1	Cytochrome P450	0.3	0.2
LRRP Aa2-028	3.1	3.0	Cytochrome P450 15-beta gene (Cyp2c12)	0.4	0.2
LRRP Aa2-174	0.3	0.1	Cytochrome P450 2E1	0.2	0.1
LRRP Ab1-021	9.1	10.0	Cytochrome P450, 2c39 (Cyp2c39)	0.3	0.1
LRRP Ab1-022	0.5	8.1	Cytochrome P450 (PNCN inducible, Cyp3A1)	0.4	0.2
LRRP Ab1-114	3.5	4.2	Flavin-containing monooxygenase 1 (Fmo1)	0.1	0.1
LRRP Ab1-216	3.5	6.8	Peroxisomal sarcosine oxidase (PSO)	0.1	0.1
LRRP Ab1-334	4.4	2.7	Plasma selenoprotein P1 (Sepp1)	0.4	0.3
LRRP Ab2-001	0.3	0.2	Small subunit precursor of RuBPCase	0.1	0.1, 3.3
LRRP Ab2-132	0.4	0.1	Regulation proteins		
LRRP Ab2-225	0.3	0.3	G-protein beta polypeptide 2-like 1 (Gnb2l1)	2.1	2.4
LRRP Ab2-232	0.2	0.2	II-Protein with tetratricopeptide repeats 3	0.2	0.2
LRRP Ab2-371	0.1	0.4	RAKb	0.2	0.2
LRRP Ab2-402	0.2	0.1	Glycoproteins		
LRRP Ab2-417	0.2	0.2	apha-1-B glycoprotein (A1bg),	0.3	0.3
LRRP Ab2-427	0.5	0.4	Fibronectin 1 (Fn1)	3.0	7.2
LRRP Ab2-440	0.3	0.3	Fibrinogen, gamma polypeptide (Fgg)	8.0	5.1
LRRP Ac1-060	0.3	0.4, 2.3	Myelin-associated glycoprotein (L-MAG)	8.4	0.3
LRRP Ac1-177	7.3	0.5, 4.9	UDP-glucuronosyltransferase 2B3 (Udpgt)	0.4	0.3
LRRP Ac1-233	5.6	4.2	TRAM1	0.3	0.4, 3.3
LRRP Ac1-873	4.6	0.3	Lipid-proteins		
LRRP Ac2-061	6.4	7.6	Adipose differentiation-related protein	4.6	7.3
LRRP Ac2-067	2.6	2.5	Solute carrier family 20, mem1 (Slc20a1)	0.4	0.4
LRRP Ac2-143	2.0	3.3	Nucleolar proteins		
LRRP Ac2-210	2.3	2.4	Damage-specific DNA binding protein 1 (Ddb1)	0.5	0.3
LRRP Ba1-647	3.3	3.2	Nuclear protein 1 (Nupr1)	2.9	0.5, 5.1
LRRP Bm403207	7.7	2.9	Nucleolar protein family A, mem 2	2.9	3.9
LRRP Cb1-739	2.2	2.3	ORF2 endonuclease and Rnase H	0.5	0.4
LRRP Cc1-27	0.3	0.1, 2.2	RNase A family 4	0.3	0.2
LRRP Cc1-38	0.1	0.2	Receptors		
LRRP Cc1-9	3.5	3.6	ATP-binding cassette, sub-family C	0.1	0.2
LRRP zbs559	3.5	3.1	Interleukin 1 receptor, type I (Il1r1)	7.3	7.9
LRRPAb2-132	0.2	0.1	Nuclear receptor subfamily 0, member 2 (Nr0b2)	0.4	0.2
MGC10946	2.8	2.8	Factors		
MGC5178	0.4	0.4	Amphoterin	0.5	0.3
RIKEN 1300002A08	0.2	0.3, 2.4	Angiogenin	0.4	0.2
RIKEN 1600027G01	0.3	0.1	Eukaryotic translation initiation factor 4A1	3.5	3.8
RP11-281N10	0.3	0.2	Early growth response factor 1 (Egr1)	4.3	3.5
RP23-100C5	2.0	3.0	Neuropeptide Y (Npy)	11.3	18.1
RP23-235O1	0.4	0.2	NF-E2-related factor 2 (Nfe2l2)	0.3	0.4
RP23-32O9	2.3	2.8	Pre-B-cell colony-enhancing factor (Pbef)	5.3	3.3
RP23-417P22	0.3	0.1	Angiopoietin-like 3	0.3	0.2
Rp32-28p17	0.3	0.3	Hemoglobins		
U48828	2.4	0.2	Hemoglobin, alpha 1 (Hba1)	2.4	0.3
Open reading frame 31	0.5	0.3, 2.8	Hemoglobin beta chain (Hbb)	2.5	0.3
13 d embryo liver cDNA	3.6	5.9	Immunological proteises		
Stress response			Complement component 5 (C5)	19.4	16.5
alpha-1 major acute phase protein prepeptide	6.8	66.4	Immunoglobulin C kappa gene	0.3	0.3
Angiotensinogen (Agt)	11.5	8.4	JE/MCP-1	2.5	4.0
Kininogen	2.5	5.9	Cytoskelets		
T-kininogen (Kng)	5.0	0.2	Actin gamma	5.1	4.8
Glycometabolism			Keratin 8 (Krt8)	2.8	3.8
Alpha enolase (Enolase 1)	2.7	3.9			

Marker proteins			Contrapsin-like protease inhibitor related protein	6.1	6.6
Amyloid a-5 protein	53.9	90.4	Leuserpin-2 (Serpind1)	0.3	0.2
Subchromosomal transferable fragment	0.4	0.3, 2.4	Serine protease inhibitor 1	5.6	5.0
Amino acid enzymes			Phosphatases		
Arginase 1 (Arg1)	6.4	3.5	Phosphatidylserine-specific phospholipase A1	4.9	2.8
Argininosuccinate lyase (Asl)	5.6	4.9	Pyrophosphatase/phosphodiesterase 1 (Enpp1)	3.1	5.0
Cytosolic aspartate aminotransferase	6.8	7.4	Protein phosphatase 1 (GL-subunit)	0.2	0.2
2-Hydroxyphytanoyl-CoA lyase (Hpcl2)	0.3	0.4	Synthase		
Proteolytic enzymes			Carbamyl phosphate synthetase I	2.5	2.9
Cathepsin C (Ctsc)	0.4	0.4	Glutamyl-prolyl-tRNA synthetase (Eprs)	2.2	4.5
Cathepsin D (Ctsd)	0.5	0.3	RNA cyclase	2.0	0.2
Coagulation factor 2 (F2)	0.5	0.2	Transferases		
Neutrophil collagenase (Mmp8)	0.2	0.2	Glutathione *S*-transferase, alpha 1 (Gsta1)	0.3	0.1
Proteinase inhibitors			Glutathione *S*-transferase, type 3 (Yb3) (Gstm3)	0.5	0.3, 2.2
alpha-2-macroglobulin (A2m)	5.1	21.3	Glutathione *S*-transferase Y(b) subunit	0.5	0.3
alpha-trypsin inhibitor heavy chain	0.4	0.2	UDP-glucuronosyltransferase 2 , mem 5 (Ugt2b5)	0.5	0.3

and involved in more complex physiological response[37]. The ascending mRNA level in SISPH was suggested to be correlated to recover damaged vascular walls after hepatectomy. The continuous elevation of nucleolar protein family A and nuclear protein 1 (Nupr1) may ascribe to the need of DNA synthesis for nuclear reconstruction of hepatocytes. The successive induction of alpha 2-macroglobulin, as homotetrimeric protease inhibitor, binding TGF-β family, is premised to participate in restraining protein degradation in SISPH for accumulation of protein and prevention of TGF-β termination for longer LR[38]. Hemoglobin secreted by hepatocytes was replacer of hemachrome in liver without hemachrome[22]. Hemoglobin alpha and beta were always induced by SISPH according to the change in PHx, exerting the same function of hepatocytes nourishment in LR. Fibronectin and fibrinogen gamma polypeptide (Fgg) were all induced abundantly through the process of SISPH for the sake of cell adherence in LR. Compared with normal beta actin which has no obvious change in PHx, mutant beta actin was continuously increased, indicating that house keeping gene is additionally expressed for LR of SISPH.

It was revealed that 45 genes were included in consistent suppression and late induction at 8 h time point, of which 22 are unreported genes and others are reported genes hardly known to be related to LR previously. Trypsin and its substrate are supposed to accelerate hepatocytes regeneration under growth factor, but excessive inhibition by inter alpha-trypsin inhibitor may lead to imbalance of glucose concentration in blood, therefore, there was a late induction at 8 h of SISPH to keep the balance[39]. Commonly, acyl-coA plays many important roles in numerous biochemical reactions, such as tricarboxylic acid cycle, glyoxylate bypass, fatty acid synthesis. As a result, the mRNA level of acyl-coA oxidase is assumed to have dropped to retain the level of fat and glycos vital to LR of SISPH and later ascended to eliminate over expressed acyl-coA. Sialyl is a vital constituent of ganglioside, whose sialyltransferase down-regulation may be intervened to prevent liver from successive injury by SISPH and immigrate to other functional substance in late phase. Mutant glutathione *S*-transferase (GST) mRNA was not always hampered in SISPH, which indicates that its overfull accumulation may lead to opposite reaction in LR. Fatty

binding protein is well known to transfer fat from cytoplasm to nuclear or membrane, and fatty acid elongase 1 (rELO1) catalyzes short chain fat transition to long chain fat whose repression of mRNA in SISPH manifested that long chain fat was not badly needed until 12 h in LR. The decrease of purine-nucleoside phosphorylase and phosphatase 1 (GL-subunit) in SISPH may suggest that purine-nucleoside and eser are in demand until 8 h in LR. Nrf2 was reported to lead to antioxidant reaction element to drive gene expression[29], which was suppressed and induced at 8 h, implying that antioxidant reaction was mild in LR of SISPH. Cathepsin D (aspirin endopeptidase) was discovered to participate in basal membrane dissolving, promoting tumor cell migration[40]. The continuous suppression but late induction suggests that basal membrane dissolving was not necessary in early phase of LR of SISPH. Coagulation factor 2 protease inhibitor was supposed to decrease to facilitate blood circulation for LR but at 12-h time point to increase to hamper great loss of blood by SISPH[23]. Damage-specific DNA binding protein was reported to recognize many types of DNA lesions and inducible by DNA damage agent. Its down regulation shows that hepatocytes suffer no severe DNA damage in SISPH, different from other injuries.

Sixty-one genes were consistently suppressed and reached minimum level at 12-h time point of SISPH, of which 22 were novel genes and the others were reported genes that may or may not be related to LR. The genes in the group are presumed to promote LR by various ways. A large number of them are the genes related to energy metabolism, suggesting that the demand for energy in LR of SISPH is not as important as for other demands, differing from partial hepatectomy. The mRNA level of cytochrome P450 2c39 (Cyp2c39), cytochrome P450 2E1, cytochrome b5 (Cyb5), cytochrome P450 15-beta, cytochrome P450, flavin-containing monooxygenase 1 (Fmo1), peroxisomal sarcosine oxidase, malonyl-CoA decarboxylase, methylmalonate semialdehyde dehydrogenase (Mmsdh), alcohol dehydrogenase, microsomal UDP-glucuronosyltransferase 2B3 (Udpgt) are all consistently suppressed from 0 to 12 h of SISPH. The substrate or product of these enzymes related to oxidation and reduction were in urgent need for or toxic to LR, for instance, imine is a cell toxic substance by isocitrate dehydrogenase reaction holding back LR[41]. Angiopoietin-

like protein 3 (Angptl3) is reported to activate lipolysis in adipocytes as a vascular endothelial growth factor by response to the liver X receptor[42]. The sustaining suppression of angiopoietin-like protein 3 mRNA in SISPH suggested that the activity of lipolysis of hepatocytes is very low in LR. Neutrophil collagenase specially degrading type I collagen is generated by osteoblastic progenitors, differentiated osteoblasts, osteocytes, and chondrocytes in the growth plate[43]. The decrease of neutrophil collagenase showed that collagen is in great demand for cell skeletal conformation or osteoblasts, osteocytes and chondrocytes were not distinctly harmed in LR after SISPH. Cathepsin C (dipeptidyl aminopeptidase I) in rat liver was discovered playing significant role in protein degradation and the activation of proenzyme[44]. The decreased mRNA level of cathepsin C was supposed to satisfy requisite of peptide for protein construction in SISPH. 2-Hydroxyphytanoyl-CoA lyase, a peroxisomal pyrophosphate-dependent enzyme, was involved in the carbon-carbon bond cleavage during α-oxidation of 3-methyl-branched fatty acids[45]. 2-Hydroxyphytanoyl-CoA lyase was always repressed through SISPH, implying that degradation of long and branch chain fat by α-oxidation was not important in LR. GST was reported to catalyze nucleophilic addition of tripeptide glutathione to xenobiotics carcinogens and endogenous lipophilic compounds[46]. Different kinds of GST were all reduced in SISPH, implying that xenobiotics carcinogens and endogenous lipophilic compounds may bring some uncertain toxic effect on LR. Leuserpin-2 (Sperpind1) is confirmed to participate in fibrinolysis complement activation and inflammatory response[47], which was continuously repressed in SISPH, suggesting that it can regulate inflammatory response to resume severe injured hepatocytes in LR. In previous research, selenoprotein P can defense against peroxynitrite. Its down-regulation may be the result from activation of vast peroxynitrite in SISPH. Amphoterin is believed to enhance process of extension and migration of embryonic neurons and tumor cells by binding to advanced glycation end products (RAGE)[48], whose down-regulation in SISPH makes clear that LR has not much to do with hepatocytes or other cells' migration.

ACKNOWLEDGMENTS

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