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• BASIC RESEARCH •

Gene expression differences of regenerating rat liver in a short interval successive partial hepatectomy

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Abstract

AIM: To identify the genes expressed differentially in the regenerating rat liver in a short interval successive partial hepatectomy (SISPH), and to analyze their expression profiles.

METHODS: Five hundred and fifty-one elements selected from subtractive cDNA libraries were conformed to a cDNA microarray (cDNA chip). An extensive gene expression analysis following 0-36-72-96-144 h SISPH was performed by microarray.

RESULTS: Two hundred and sixteen elements were identified either up- or down-regulated more than 2-fold at one or more time points of SISPH. By cluster analysis and generalization analysis, 8 kinds of ramose gene expression clusters were generated in the SISPH. Of the 216 elements, 111 were up-regulated and 105 down-regulated. Except 99 unreported genes, 117 reported genes were categorized into 22 groups based on their biological functions. Comparison of the gene expression in SISPH with that after partial hepatectomy (PH) disclosed that 56 genes were specially altered in SISPH, and 160 genes were simultaneously up-regulated or down-regulated in SISPH and after PH, but in various amount and at different time points.

CONCLUSION: Genes expressed consistently are far less than that intermittently; the genes strikingly increased are much less than that increased only 2-5 fold; the expression trends of most genes in SISPH and in PH are similar, but the expression of 56 genes is specifically altered in SISPH. Microarray combined with suppressive subtractive hybridization can in a large scale effectively identify the genes related to liver regeneration.

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INTRODUCTION

In the liver regeneration (LR) after partial hepatectomy (PH), a great deal of genes is involved, and varied in the different phases of LR[1-5]. Peak of DNA synthesis appears at 24 h and two small peaks occur at 36 h and 48 h after PH[6]. Despite numerous related papers, the molecular mechanism of LR has not been thoroughly elucidated[7-16]. To explore the hepatic regeneration mechanism, a 0-36-72-96-144 h short interval successive partial hepatectomy (SISPH) model was established in 2001, and has been proved an important tool for studying specific gene expression at various crucial points of LR[17-19]. To uncover unknown differential display genes relevant to LR, the method of subtractive suppression hybridization (SSH) was used, and a bulk of up-regulated and down-regulated expressed sequence tags (ESTs) in the regenerating rat liver of 0-36-72-96-144 h SISPH were obtained. With development of cDNA microarray technology, genomewide expression of thousands of genes can be simultaneously analyzed facilitating differential expression monitoring of a large number of activated or suppressed genes under various biological conditions. To further display their expression variation in the LR, an in-house cDNA microarray was successfully performed to identify gene expression profiles in regenerating liver following the SISPH. Relevant information was achieved by data analysis of Microsoft Excel and GeneSpring.

MATERIALS AND METHODS

Short interval successive partial hepatectomy of rats

Male and female Sprague-Dawley (SD) rats, aged 10-12 wk and weighing 200-220 g, were raised in Experimental Animal Center of Henan Normal University. According to Xu *et al*., lobule external sinister and lobus centralis sinister, lobus dexter, lobus centralis, and lobus caudatus were removed subsequently at four time points of 0, 36, 72, 96 h of 0-36-72-96-144 h SISPH[20].

Sample preparation and RNA extraction

The removed liver lobes were rinsed in cold 1×PBS and immersed in -80 ℃ refrigerator for RNA and protein extraction. Total RNA was isolated from frozen liver lobes 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 were examined by ultraviolet spectrometer and denaturing formaldehyde agarose electrophoresis stained by ethidium bromide (EB).

Subtracted cDNA library construction and screening

cDNA subtractive libraries were generated from total RNA by PCR-Select TM cDNA Subtraction kit (Clontech) following the manufacturer's instruction. Briefly, total RNA was reverse transcribed to double cDNA strands and digested with restriction enzymes, followed by subtractive hybridization with drivers and testers. Finally with suppression PCR, differential expression sequence tags were performed to construct

subtractive cDNA library, which was cloned into T-vector (Promega) and screened by PCR with nest primer 1 and 2.

Sequence analysis

The base sequence assay of ESTs was carried out according to the current protocols in molecular biology. All sequences were determined for both strands. Comparison analysis of the selected sequences was conducted with the DNAman and the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) GenBank database.

cDNA microarray construction

cDNA fragments amplified by polymerase chain reaction (PCR) with nested PCR primer 1 and primer 2, and purified by NaAc/isopropanol were spotted onto glass slides (Biostar) with the help of ProSys-5 510A spotting machine according to designed project. Then the gene chips were ready by hydrating, blocking and drying. Totally 1 152 elements (double spot chip) including 50 control systems (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).

Fluorescence-labeled cDNA preparation

RNA isolated from rat livers before SISPH served as a reference for all cDNA microarray analyses. Total denatured RNA was reverse 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 for 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 an overnight (16-18 h) incubation at 42 ℃. The slides were then washed twice with 2×SSC containing 5 g/L SDS for 5 min at room temperature, once with 0.2×SSC containing 5 g/L SDS at 60 ℃ for 10 min, and finally with 0.2×SSC at 60 ℃ for 10 min. After that, the slices were photographed. Hybridized images were scanned by a fluorescence laser scanning device, Gene Pix 4 000 A (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); and (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 were replaced by 200 when it was <200. When Ri (Ri=cy5/cy3) was between 0.1 and 10, Ri was taken logarithms base natural to generate Ri'[log (Ri)] and ND was taken by EXP (R) (averaged Ri'). The modified cy3* was generated when ND was multiplied by cy3, and was replaced by 200 when it was <200. The ratio was expressed as 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).

RESULTS

Category and expression changes of genes related to rat liver regeneration

Among the tested 551 genes, 216 were identified to be altered by more than 2-fold in intensity at least at one time point in the 0-36-72-96-144 h SISPH. Of the 216 identified genes, 111 were up-regulated and 106 were down-regulated. Ninety-nine of these 216 genes were unreported genes and the other 117 were reported, of which quite a few genes had not been reported to be involved in LR. Based on the functions and the time points at which they showed maximum up- or down-regulation, those reported genes were respectively involved in stress response, glycometabolism, fat and stearoyl metabolism, oxidation and reduction response, regulation-proteins, glycoproteins, lipid-proteins, nucleolar proteins, receptors, factors, hemoglobins, immunological proteins, chaperonins, cytoskeletons, marker proteins, amino acid enzymes, proteolytic enzymes, proteinase inhibitors, phosphorylases, phosphatases, synthases and transferases (Table 1).

Gene expression differences at various time points of the 0-36-72-96-144 h SISPH

The gene expression profiles at different time points were generalized at 36, 36-72, 36-96, 36-144, 72, 72-96, 72-144, 96, 96-144, 144 h, and it was found that at 36 h of SISPH, 17 genes were up-regulated and 2 were down-regulated; at the time points of 36 h and 72 h of SISPH, 3 genes were up-regulated and 3 down-regulated; at the time points of 36 h and 96 h of SISPH, only 2 genes were up-regulated; at the time points of 36 h and 144 h of SISPH, 32 genes were up-regulated and 23 genes down-regulated, which is the largest group at all time points of SISPH; at 72 h of SISPH, 13 genes were down-regulated and 12 up-regulated. At the time points of 72 h and 96 h of SISPH, 5 genes were down-regulated and 4 up-regulated; at the time points of 72 h and 144 h of SISPH, 14 genes were up-regulated and 21 down-regulated; at 96 h of SISPH, one gene were up-regulated and 6 down-regulated; at the time points of 96 h and 144 h of SISPH, 10 genes were down-regulated and 7 up-regulated; at 144 h of SISPH, 11 genes were up-regulated and 31 down-regulated. Briefly, the LR of 0-36-72-96-144 h SISPH involved 216 elements, of which, 111 were up-regulated and 105 were down-regulated (Figure 1).

Gene expression level in the regenerating rat liver of 0-36-72-96-144 h SISPH

According to the up-regulated and down-regulated intensity of genes in the 0-36-72-96-144 h SISPH, we categorized the genes into 3 groups: (1) 105 genes were down-regulated by less than 50%; (2) 93 genes were up-regulated by 2-5 fold; (3) 18 genes were strongly up-regulated by more than 5-fold (Figure 2).

Hierarchical cluster analysis of genes expressed in the liver regeneration

The expression profile of the 216 genes altered by more than 2-fold in intensity at least at one time point in the 0-36-72-96-144 h SISPH was emanative to the last time point, indicating that at 144 h of SISPH, the liver regeneration has not been completed yet (Figure 3A). We undertook hierarchical clustering of 5 time points (0, 36, 72, 96 and 144 h) of SISPH using GeneSpring software and discovered that gene expression profiles had no similarity at the four time points (Figure 3B).

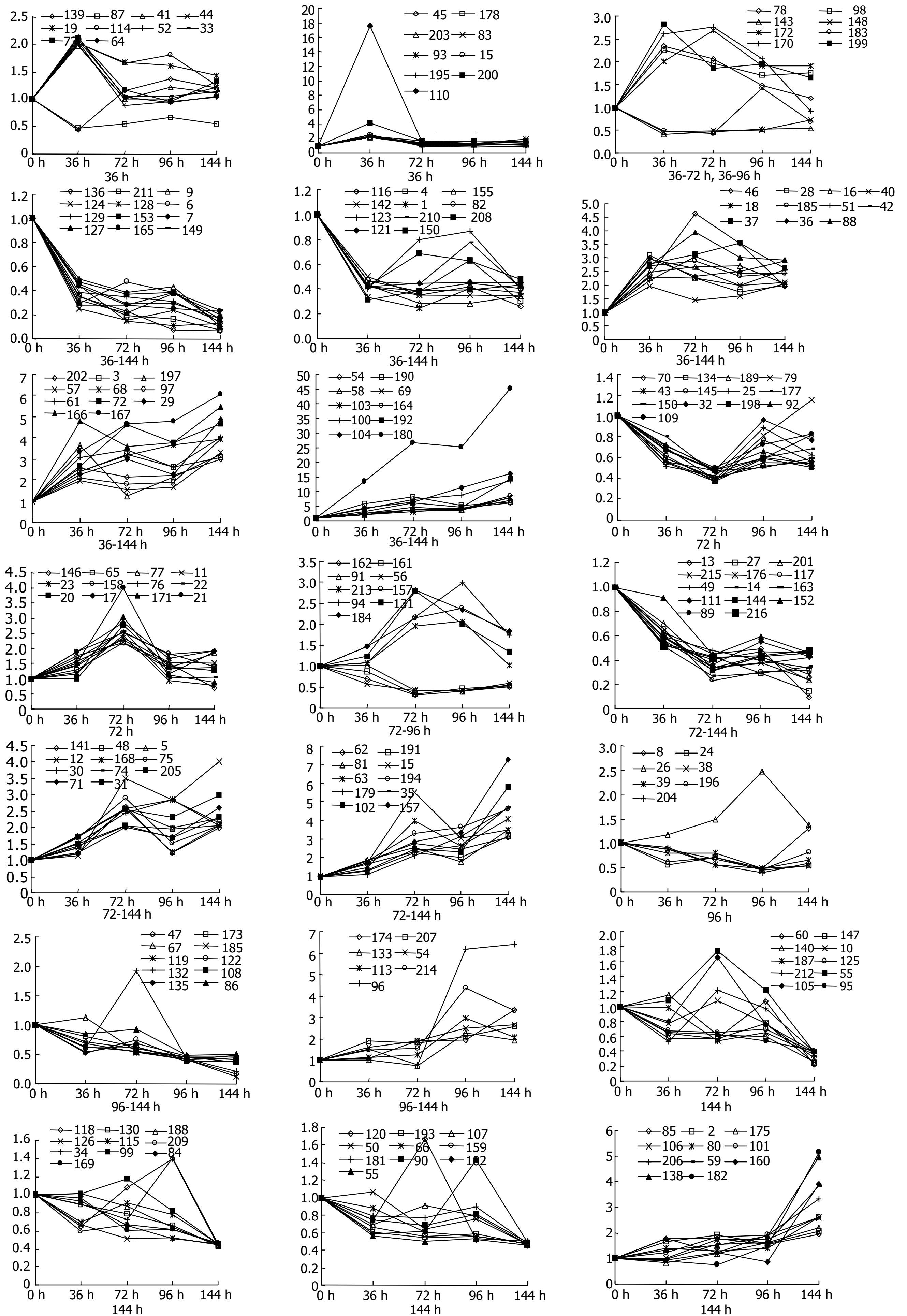

Figure 1 Gene expression differences in the regenerating rat liver of 0-36-72-96-144 h SISPH.

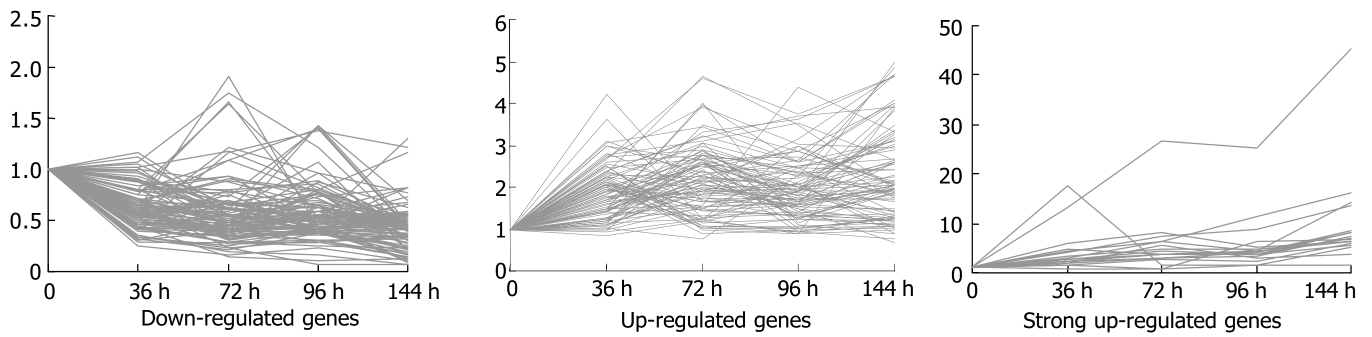

Figure 2 Expression level of genes in the regenerating rat liver of 0-36-72-96-144 h SISPH.

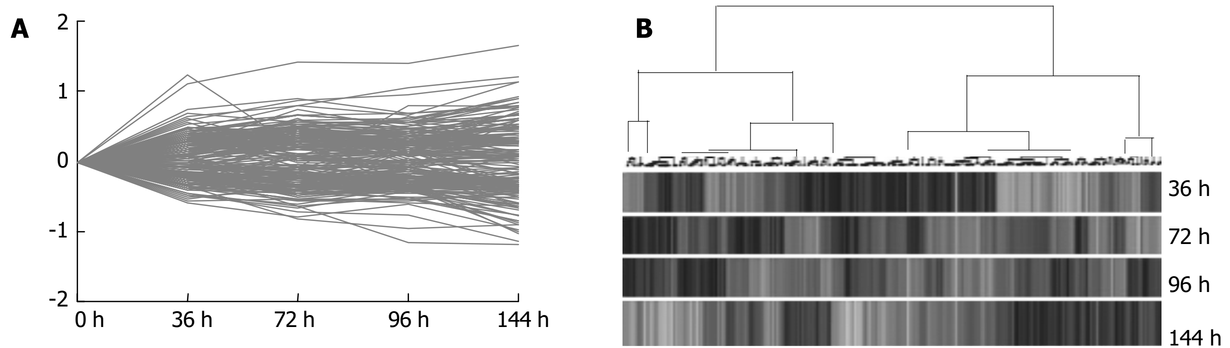

Figure 3 Cluster analysis of 216 elements. A: The difference of their intensity was identified more than two-fold at least at one time point. B: A hierarchical clustering of five time points indicated that the genes at these time points hardly had a common expression profile.

Figure 4 Cluster analysis of gene expression profiles identified by cDNA microarray. These genes were classified into 8 clusters by the κ-means method.

Table 1 The genes related to liver regeneration altered in 0-36-72-96-144h SISPH (*genes specially altered in SISPH)

No.	Gene description	Fold difference
	Unreported genes	
1	AW558171	0.2
2	CG31759-PA	2.0
*3	CH230-11N5	3.2
4	CH230-155H3	0.3
5	CH230-155H3	2.6
6	CH230-186B23	0.1
7	CH230-206C20	0.2
8	CH230-329A5	0.5
9	CH230-372C24	0.1
10	CH230-403C20	0.3
*11	CH230-404C20	2.4
12	CH230-4L11	4.0
13	CH230-7A22	0.1
14	Citb585c7	0.3
*15	CTD-2328C19	2.5
*16	FLJ20356	2.7
17	KIAA1230	2.8
18	LOC119392	2.3
*19	LOC311304	2.0
*20	LRRP Aa1-018	2.8
21	LRRP Aa1027	4.0
*22	LRRP Aa1-076	2.6
*23	LRRP Aa1-114	2.4
24	LRRP Aa2-020	0.5
25	LRRP Aa2-066	0.4
*26	LRRP Aa2-111	2.5
27	LRRP Aa2-174	0.1
28	LRRP Aa2-296	3.1
29	LRRP Ab1-021	4.9
30	LRRP Ab1-046	2.9
31	LRRP Ab1-108	3.0
32	LRRP Ab1-114	3.4
*33	LRRP Ab1-119	2.1
34	LRRP Ab1-152	0.5
35	LRRP Ab1-216	4.7
36	LRRP Ab1-331	3.0
37	LRRP Ab1-334	3.6
38	LRRP Ab2-001	0.5
39	LRRP Ab2-001	0.5
40	LRRP Ab2-008	2.1
*41	LRRP Ab2-018	2.0
42	LRRP Ab2-034	3.0
*43	LRRP Ab2-057	0.4
*44	LRRP Ab2-079	2.0
*45	LRRP Ab2-093	2.1
46	LRRP Ab2-095	4.6
47	LRRP Ab2-132	0.4
48	LRRP Ab2-143	2.0
49	LRRP Ab2-225	0.3
*50	LRRP Ab2-255	0.5
51	LRRP Ab2-379	3.1
*52	LRRP Ab2-390	2.1
53	LRRP Ab2-402	0.4
54	LRRP Ac1-060	2.7
55	LRRP Ac1-163	0.5
56	LRRP Ac1177	0.4
57	LRRP Ac1-233	3.3
58	LRRP Ac1873	7.1
59	LRRP Ac2-061	3.8
60	LRRP Ac2-125	0.2
61	LRRP Ac2-143	4.0
62	LRRP Ac2-193	3.1
63	LRRP Ac2-202	4.1
64	LRRP Ac2-223	2.1
*65	LRRP Ac2-256	2.2
*66	LRRP Ac2-282	0.5
*67	LRRP Ac2-300	0.4
68	LRRP Ba1-647	3.9
69	LRRP Bm403207	7.9
70	LRRP Cc1-27	0.4
71	LRRP Cc1-8	2.6
72	LRRP Cc1-9	4.7
*73	LRRP Da1-10	2.1
74	LRRP Da1-24	2.8
75	LRRP Da1-6	2.9
*76	LRRP Da2-19	2.6
*77	LRRP Da2-35	2.3
*78	LRRP Da2-4	2.3
79	LRRP zbs559	0.4
*80	MGC38937	2.6
81	RIKEN 1110061A24	3.5
82	RIKEN 1300002A08	0.4
*83	RIKEN 1500012D08	2.2
*84	RIKEN 2310045J23	0.5
85	RIKEN 2810051A14	2.0
*86	RIKEN 4930408O21	0.5
87	RP11-281N10	0.5
88	RP23-195K1	3.9
89	RP23-235O1	0.4
90	RP23-35D4	0.5
91	RP23-417P22	0.4
92	RP23-480P21	0.5
	Chaperonins	
*168	DnaJ (Hsp40), subfamily B, mem 11 (Dnajb11)	2.6
*169	TCP-1 containing cytosolic chaperonin (CCT)	0.5
	Cytoskelets	
170	Actin gamma	2.7
*171	Actin beta (Actb)	3.0
172	Clathrin, heavy polypeptide (Hc) (Cltc)	2.7
173	Karyopherin (importin) alpha 2	0.4
*174	Mutant beta-actin (beta-actin)	3.4
*175	Ribosomal protein S12 (Rps12)	2.2
	Marker proteins	
176	ATP-binding cassette, sub-family C	0.3
*177	CD164 antigen (Cd164)	0.4
*178	CD44 antigen (Cd44)	2.2
179	Pregnancy-zone protein (Pzp)	4.7
180	Serum amyloid a-5 protein	45.1
181	Subchromosomal transferable fragment 4	0.5
	Amino acid enzymes	
182	Cytosolic aspartate aminotransferase	5.1
*183	Phenylalanine hydroxylase (Pah)	0.4
*184	Tissue-type transglutaminase (Tgm2)	2.8
185	2-hydroxyphytanoyl-CoA lyase (Hpcl2)	0.4
	Proteolytic enzymes	
*186	Alpha/beta hydrolase domain containing protein 1	2.9
187	Cathepsin C (Ctsc)	0.4
*188	Proteasome (macropain subunit, beta type 6 Psmb6)	0.4
	Proteinase inhibitors	
189	Alpha-1 microglobulin/bikunin (Ambp)	0.4
190	Alpha-2-macroglobulin (A2m)	8.1
*93	RP24-176A1	2.4
94	RP24-347B22	3.0
95	RP32-28p17	0.4
96	Adult male liver cDNA	6.4
97	DNA segment of Chr 1	3.9
98	12 d embryo liver cDNA	2.2
99	13 d embryo liver cDNA	0.5
	Stress response	
100	Alpha-1 major acute phase protein prepeptide	13.6
101	Petaxin	2.6
102	Angiotensinogen (Agt)	5.8
103	Kininogen	8.0
104	T-kininogen	16.1
	Glycometabolism	
105	Aldolase B	0.4
*106	C-reactive protein	2.6
107	Glycerol 3-phosphate dehydrogenase (Gpd3)	0.5
108	Isocitrate dehydrogenase 1 (Idh1)	0.4
*109	Maize aldolase	0.5
*110	3-phosphoglycerate dehydrogenase	17.6
	Fatty and stearoyl metabolism	
111	Malonyl-CoA decarboxylase	0.4
112	NAD(P) dependent steroid hydrogenase	0.5
113	P450 cholesterol 7-α-hydroxylase (P450 VII)	3.0
114	Prostaglandin D2 synthase 2 (Ptgds2)	2.0
*115	Retinol dehydrogenase 11	0.5
116	3-alpha-hydroxysteroid dehydrogenase	0.3
	Oxidation and reduction response	
117	Acyl-coA oxidase	0.2
118	Alcohol dehydrogenase (ADH)	0.4
119	Cytochrom P450 15-beta (Cyp2c12)	0.1
120	Cytochrome b	0.5
121	Cytochrome b5 (Cyb5)	0.4
122	Cytochrome P450	0.2
123	Cytochrome P450 (PNCN inducible, Cyp3A1)	0.4
124	Cytochrome P450 2E1	0.1
125	Cytochrome P450, 2c39 (Cyp2c39)	0.4
*126	CytochromeP450, 2b19 (Cyp2b15)	0.4
127	CytP450 arachidonic acid epoxygenase (cyp 2C23)	0.2
128	Flavin-containing monooxygenase 1 (Fmo1)	0.1
129	Paraoxonase 1 (Pon1)	0.1
*130	Peroxiredoxin 1 (Prdx1)	0.4
*131	Plasma selenoprotein P1 (Sepp1)	2.8
132	Selenium-dependent glutathione peroxidase	0.2
	Regulation-proteins	
133	II-protein with tetratricopeptide repeats 3	2.3
*134	Glu-Pro dipeptide repeat protein	0.4
135	RAKb	0.5
	Glycoproteins	
136	Alpha-1-B glycoprotein (A1bg)	0.1
137	Fibrinogen, gamma polypeptide (Fgg)	7.2
138	Fibronectin 1 (Fn1)	5.0
139	Histidine-rich glycoprotein (Hrg)	0.4
140	Myelin-associated glycoprotein (L-MAG)	0.3
141	TRAM1	2.6
142	UDP-glucuronosyltransferase 2B3 (Udpgt)	0.3
	Lipid-proteins	
143	Apolipoprotein C-I (Apoc1)	0.4
144	Apolipoprotein C-II	0.3
145	Apolipoprotein C-III	0.4
146	C57BL/6J	2.2
147	Fatty acid binding protein 1 (Fabp1)	0.2
148	Plasma retinol-binding protein (PRBP)	0.5
149	Transthyretin-related protein (TTN)	0.2
	Nucleolar proteins	
150	RNase A family 4	0.4
	Receptors	
151	Cocoa protein	5.5
*152	Golgi SNAP receptor member 1 (Gosr1)	0.4
153	Nuclear receptor subfamily 0, mem 2 (Nr0b2)	0.2
154	Type I interleukin 1 receptor (Il1r1)	6.2
	Factors	
155	Angiogenin	0.3
156	Angiopoietin-like 3	0.5
157	Early growth response factor 1 (Egr1)	2.4
158	Eukaryotic translation initiation factor 4A1	2.5
159	Insulin-like growth factor I	0.5
160	Neuropeptide Y (Npy)	3.9
	Hemoglobins	
161	Hemoglobin, alpha 1 (Hba1)	0.3
162	Hemoglobin beta chain (Hbb)	0.3
	Immunological proteises	
163	Achaete-scute complex homolog-like 1 (Ascl1)	0.3
164	Complement component 5 (C5)	8.6
165	Immunoglobulin C kappa	0.2
*166	Fc-gamma receptor class III	5.5
167	JE/MCP-1	6.1
191	Alpha-1-macroglobulin	3.1
192	Contrapsin-like protease inhibitor (CPi-26)	14.1
193	Leuserpin-2 (Serpind1)	0.5
194	Serine protease inhibitor 1	4.6
	Phosphorylases	
*195	CDK103	2.5
196	CDK110	0.5
197	Mss4 protein	3.6
*198	Rho-associated kinase beta (Rock1)	0.5
199	Thymidylate kinase (dTMP kinase)	2.8
	Phosphatases	
200	Pyrophosphatase/phosphodiesterase 1(Enpp1)	4.2
201	Phosphatase 1 (GL-subunit)	0.2
202	Phosphatidylserine-specific phospholipase A1	3.0
*203	Secreted phosphoprotein 1 (Spp1)	2.2
*204	UTP-glucose-1-phosphate	0.4
	Synthases	
*205	ATPase synthase subunit 6	2.3
206	Carbamyl phosphate synthetase I	3.3
*207	Glutamyl-prolyl-tRNA synthetase (Eprs)	2.6
	Transferases	
208	Carnitine O-octanoyltransferase (Crot)	0.3
209	Glutathione S-transferase 1 (Mgst1)	0.5
*210	Glutathione S-transferase Y(b) subunit	0.4
211	Glutathione S-transferase, alpha 1 (Gsta1)	0.1
212	Glutathione S-transferase, type 3 (Yb3) (Gstm3)	0.4
213	Serine hydroxymethyl transferase 1	2.1
214	Sialyltransferase 1 (Siat1)	4.4
215	Sulfotransferase K2	0.2
216	UDP-glucuronosyltransferase 2, mem 5 (Ugt2b5)	0.4

Table 2 The comparison of difference of gene expression in SISPH with that after in PH

Gene description	Fold difference	
	SISPH	PH
Unreported genes		
AW558171	0.2	0.3
CG31759-PA	2.0	2.9
CH230-155H3	0.3	0.3
CH230-155H3	2.6	2.2
CH230-186B23	0.1	0.2
CH230-206C20	0.2	0.3
CH230-329A5	0.5	0.3
CH230-372C24	0.1	0.1
CH230-403C20	0.3	0.2
CH230-4L11	4.0	4.5
CH230-7A22	0.1	0.1
Citb585c7	0.3	0.2
KIAA1230	2.8	2.6
LOC119392	2.3	2.1
LRRP Aa1027	4.0	2.1
LRRP Aa2-020	0.5	0.4
LRRP Aa2-066	0.4	0.4
LRRP Aa2-174	0.1	0.1
LRRP Aa2-296	3.1	2.1
LRRP Ab1-021	4.9	8.1
LRRP Ab1-046	2.9	0.5
LRRP Ab1-108	3.0	2.9
LRRP Ab1-114	3.4	4.2
LRRP Ab1-152	0.5	0.4
LRRP Ab1-216	4.7	6.8
LRRP Ab1-331	3.0	2.2
LRRP Ab1-334	3.6	2.7
LRRP Ab2-001	0.5	0.2
LRRP Ab2-001	0.5	0.2
LRRP Ab2-008	2.1	2.1
LRRP Ab2-034	3.0	2.3
LRRP Ab2-095	4.6	3.1
LRRP Ab2-132	0.4	0.1
LRRP Ab2-143	2.0	3.3
LRRP Ab2-225	0.3	0.3
LRRP Ab2-379	3.1	2.2
LRRP Ab2-402	0.4	0.1
LRRP Ac1-060	2.7	0.4,2.3
LRRP Ac1-163	0.5	0.4
LRRP Ac1177	0.4	0.4, 3.3
LRRP Ac1-233	3.3	4.2
LRRP Ac1873	7.1	6
LRRP Ac2-061	3.8	7.6
LRRP Ac2-125	0.2	0.3
LRRP Ac2-143	4.0	3.3
LRRP Ac2-193	3.1	2.3
LRRP Ac2-202	4.1	2.5
LRRP Ac2-223	2.1	2.4
LRRP Ba1-647	3.9	3.2
LRRP Bm403207	7.9	5.7
LRRP Cc1-27	0.4	2.2
LRRP Cc1-8	2.6	2.3
LRRP Cc1-9	4.7	2.5
LRRP Da1-24	2.8	3.5
LRRP Da1-6	2.9	2.0
LRRP zbs559	0.4	3.1
RIKEN 1110061A24	3.5	3
RIKEN 1300002A08	0.4	0.3, 2.4
RIKEN 2810051A14	2.0	2.7
RP11-281N10	0.5	0.3
RP23-195K1	3.9	2.4
RP23-235O1	0.4	0.2
RP23-35D4	0.5	0.4, 2.8
RP23-417P22	0.4	0.1
RP23-480P21	0.5	0.4
RP24-347B22	3.0	2.2
RP32-28p17	0.4	0.3
Adult male liver cDNA	6.4	0.1
DNA segment of Chr 1	3.9	6.1
13 d embryo liver cDNA	0.5	5.9
Stress response		
Alpha-1 major acute phase protein prepeptide	13.6	6.2
Petaxin	2.6	2.2
Angiotensinogen (Agt)	5.8	8.4
Kininogen	8.0	3.4
T-kininogen	16.1	5.9
Glycometabolism		
Aldolase B	0.4	0.3
Glycerol 3-phosphate dehydrogenase (Gpd3)	0.5	0.4
Isocitrate dehydrogenase 1 (Idh1)	0.4	0.3
Fatty and stearoyl metabolism		
Malonyl-CoA decarboxylase	0.4	0.3
NAD(P) dependent steroid hydrogenase	0.5	0.4
P450 cholesterol 7-α -hydroxylase (P450 VII)	3.0	0.5, 2.3
Prostaglandin D2 synthase 2 (Ptgds2)	2.0	3
3-alpha-hydroxysteroid dehydrogenase	0.3	0.2
Oxidation and reduction response		
Acyl-coA oxidase	0.2	0.4, 2.7
Alcohol dehydrogenase (ADH)	0.4	0.1, 2.4
Cytochrom P450 15-beta (Cyp2c12)	0.1	0.2
Cytochrome b	0.5	0.5
Cytochrome b5 (Cyb5)	0.4	0.2
Cytochrome P450	0.2	0.2
Cytochrome P450 (PNCN inducible, Cyp3A1)	0.4	0.2
Cytochrome P450 2E1	0.1	0.1
Cytochrome P450, 2c39 (Cyp2c39)	0.4	0.1
CytP450 arachidonic acid epoxygenase (cyp 2C23)	0.2	0.2
Flavin-containing monooxygenase 1 (Fmo1)	0.1	0.1
Paraoxonase 1 (Pon1)	0.1	0.2
Selenium-dependent glutathione peroxidase	0.2	0.4
Regulation-proteins		
II-protein with tetratricopeptide repeats 3	2.3	0.2
RAKb	0.5	0.2
Glycoproteins		
Alpha-1-B glycoprotein (A1bg)	0.1	0.1
Fibrinogen, gamma polypeptide (Fgg)	7.2	7.2
Fibronectin 1 (Fn1)	5.0	7.2
Histidine-rich glycoprotein (Hrg)	0.4	0.1
Myelin-associated glycoprotein (L-MAG)	0.3	7
TRAM1	2.6	5.1
UDP-glucuronosyltransferase 2B3 (Udpgt)	0.3	0.3
Lipid-proteins		
Apolipoprotein C-I (Apoc1)	0.4	3.3
Apolipoprotein C-II	0.3	0.3
Apolipoprotein C-III	0.4	0.5
C57BL/6J	2.2	7.3
Fatty acid binding protein 1 (Fabp1)	0.2	0.3
Plasma retinol-binding protein (PRBP)	0.5	0.4
Transthyretin-related protein (TTN)	0.2	0.3
Nucleolar proteins		
RNase A family 4	0.4	0.2
Receptors		
Cocoa protein	5.5	4.6
Nuclear receptor subfamily 0, mem 2 (Nr0b2)	0.2	0.2
Type I interleukin 1 receptor (Il1r1)	6.2	7.8
Factors		
Angiogenin	0.3	0.2
Angiopoietin-like 3	0.5	0.2
Early growth response factor 1 (Egr1)	2.4	3.6
Eukaryotic translation initiation factor 4A1	2.5	3.8
Insulin-like growth factor I	0.5	0.5
Neuropeptide Y (Npy)	3.9	18.2
Hemoglobins		
Hemoglobin, alpha 1 (Hba1)	0.3	0.3
Hemoglobin beta chain (Hbb)	0.3	0.3
Immunological proteises		
Achaete-scute complex homolog-like 1 (Ascl1)	0.3	0.4
Complement component 5 (C5)	8.6	8.8
Immunoglobulin C kappa	0.2	0.2
JE/MCP-1	6.1	4
Cytoskelets		
Actin gamma	2.7	4.7
Clathrin, heavy polypeptide (Hc) (Cltc)	2.7	3.3
Karyopherin (importin) alpha 2	0.4	0.4
Marker proteins		
ATP-binding cassette, sub-family C	0.3	0.4
Pregnancy-zone protein (Pzp)	4.7	2.4
Serum amyloid a-5 protein	45.1	90.5
Subchromosomal transferable fragment 4	0.5	0.3
Amino acid enzymes		
Cytosolic aspartate aminotransferase	5.1	5.3
2-hydroxyphytanoyl-CoA lyase (Hpcl2)	0.4	0.4
Proteolytic enzyme		
Cathepsin C (Ctsc)	0.4	0.4
Proteinase inhibitor		
Alpha-1 microglobulin/bikunin (Ambp)	0.4	2.1
Alpha-2-macroglobulin (A2m)	8.1	21.3
Alpha-1-macroglobulin	3.1	2
Contrapsin-like protease inhibitor (CPi-26)	14.1	6.6
Leuserpin-2 (Serpind1)	0.5	0.2
Serine protease inhibitor 1	4.6	5
Phosphorylases		
CDK110	0.5	0.5
Mss4 protein	3.6	2.1
Thymidylate kinase (dTMP kinase)	2.8	3
Phosphatases		
Pyrophosphatase/phosphodiesterase 1(Enpp1)	4.2	6.6
Phosphatase 1 (GL-subunit)	0.2	0.2
Phosphatidylserine-specific phospholipase A1	3.0	2.8
Synthase		
Carbamyl phosphate synthetase I	3.3	2.9
Transferases		
Carnitine O-octanoyltransferase (Crot)	0.3	0.3
Glutathione S-transferase 1 (Mgst1)	0.5	0.4
Glutathione S-transferase Y(b) subunit	0.4	0.3
Glutathione S-transferase, alpha 1 (Gsta1)	0.1	0.1
Glutathione S-transferase, type 3 (Yb3) (Gstm3)	0.4	0.3
Sialyltransferase 1 (Siat1)	4.4	2.6
Sulfotransferase K2	0.2	0.3
UDP-glucuronosyltransferase 2, mem 5 (Ugt2b5)	0.4	0.3

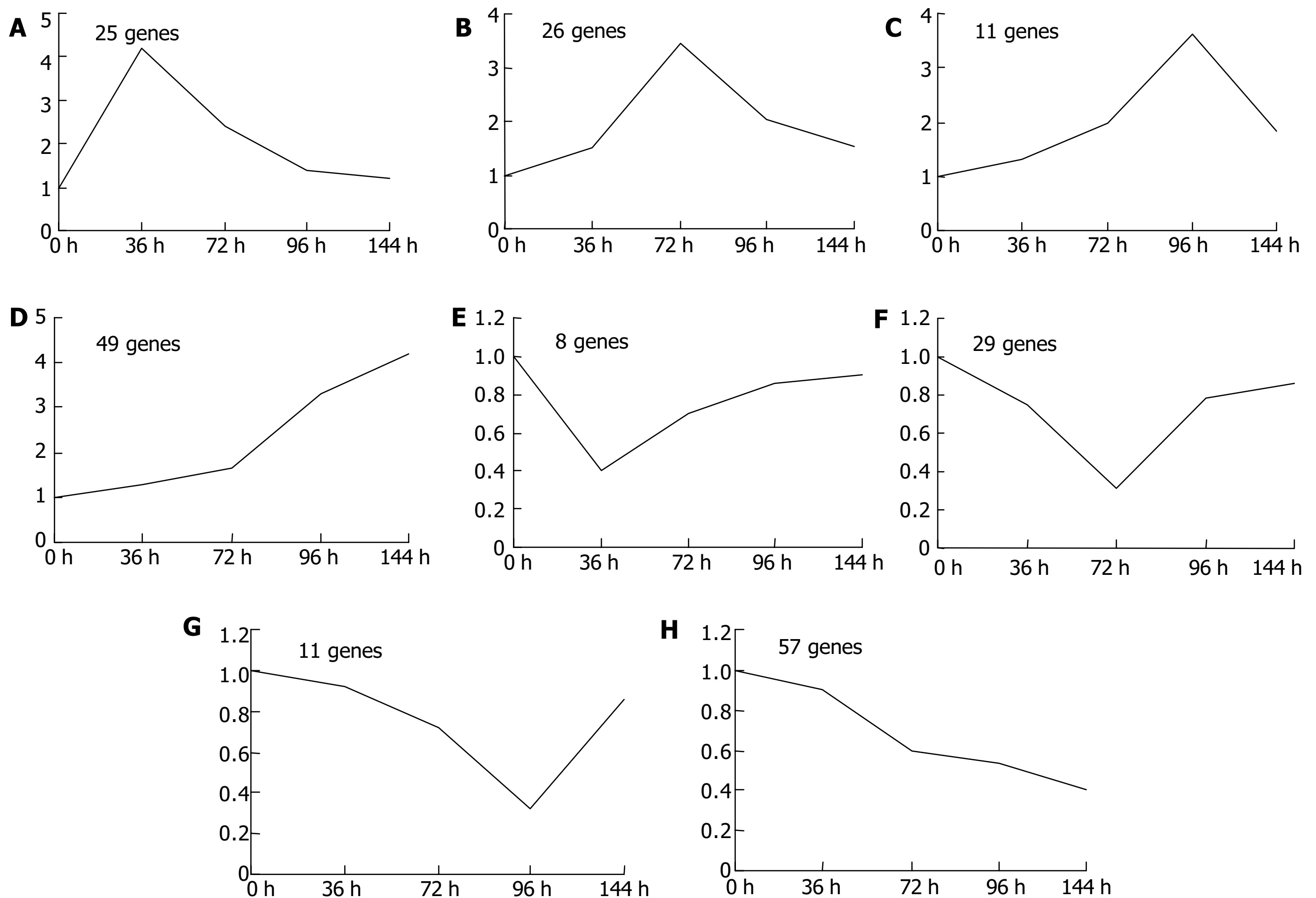

Figure 5 Category of the 216 elements. Based on the results of the cluster analysis, eight distinct temporal patterns were designated. A: Immediate induction, B: Middle induction, C: Late induction, D: Consistent induction, E: Immediate suppression, F: Middle suppression, G: Late suppression, H: Consistent suppression.

To facilitate the visualization and interpretation of the gene expression program presented 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 8 kinds of ramose gene expression clusters (Figure 4). We then categorized the selected elements into 8 distinct temporal induction or suppression patterns immediate induction, middle induction, late induction, consistent induction, immediate suppression, middle suppression, late suppression, consistent suppression (Figure 5).

Comparison of gene expression in SISPH with that after PH

Comparison of gene expression profile in SISPH with that after PH revealed that 56 genes were specially induced by SISPH, and the expression of 160 genes was altered simultaneously with the same trend in both SISPH and PH, but the time points of their expression and degree of up-regulation and down-regulation were different (Table 2).

DISCUSSION

This study found that 111 genes were up-regulated in the 0-36-72-96-144 h SISPH, suggesting that they could promote the liver growth, development and regeneration. It was also found that a large number of genes were related to positive and negative acute phase reaction to the successive hepatectomy, which suggests that these genes might regulate the balance of cell proliferation and death in the acute-phase response.

In the 25 genes up-regulated to reach the highest level at 36 h of 0-36-72-96-144 h SISPH, 20 genes were decreased immediately to control level after the peak of 36 h, but 5 kept a high level until 144 h of SISPH. Among them, 3-phosphoglycerate dehydrogenase (PGDH) was reported to catalyze the first step in serine biosynthesis and was increased in regenerating liver[21-23]. Prostaglandin D2 synthase 2 was confirmed to play an important role in reproduction as a PGD2-producing enzyme and a retinoid transporter[24,25]. Phosphoprotein 1 was involved in regulation of hepatocyte proliferation in LR[26]. The maximum expression of these genes at 36 h of SISPH showed that they could regulate hepatocyte multiplication after the peak of DNA replication in LR.

In the 27 genes up-regulated to reach the highest level at 72 h of 0-36-72-96-144 h SISPH, 12 of them declined gradually to control level at 96-144 h, and 6 did not decline until 144 h of SISPH, of which *eIF4A1* was reported to control melanoma cell proliferation by over expression[27], whose up-regulation was assumed to accelerate protein synthesis at 72 h of SISPH. Actin γ played specific roles in the growth of liver parenchymal cells in the LR of SISPH[28]. Cocoa extract could protect against early alcohol-induced liver injury in the rat[29], whose conduction at 72 h was presumed to be involved in relieving hepatocytes from alcohol damage in LR of SISPH. Alpha-2-macroglobulin (A2M) was confirmed to reduce paracrine-and autocrine-stimulated extracellular matrix synthesis by scavenging TGF-beta[30]. The successive induction of alpha 2-macroglobulin, a multifunctional binding protein with protease and cytokine scavenging properties[31], may restrain protein degradation and termination of TGF-β in LR. The increase of HSP40 at 72 h means that lots of newly synthesized proteins need to correctly

fold with help of HSP40 in LR of SISPH.

In the 11 genes up-regulated to reach the highest level at 96 h of 0-36-72-96-144 h SISPH, cytochrome P450 cholesterol 7-alpha-hydroxylase (CYP7) is confirmed to regulate the protein modeling and the mRNA level in response to multiple physiological activities, including liver cholesterol synthesis, bile acid feedback inhibition, and diurnal rhythm[32,33]. The conduction of CYP7 at 96 h is supposed to relate with cholesterol synthesis and hormone regulation in LR of SISPH.

In the 48 genes up-regulated to reach the highest level at 144 h of 0-36-72-96-144 h SISPH, plasma fibronectin was decreased in favor of LR impairment[34-36], its expression at 144 h indicated that fibronectin-mediated function between the cells and the extracellular matrix was active in LR of SISPH. α-1-macroglobulin, serine protease inhibitor 1, angiotensinogen (Agt), fibrinogen γ, pregnancy-zone protein (Pzp) were always up-regulated from 36 h to 144 h of 0-36-72-96-144 h SISPH, suggesting that they are necessary for inhibiting proteolysis and facilitating cell growth and connection at these time points of SISPH. α-1 major acute phase protein (alpha 1-MAP) is one of the cysteine protease inhibitors[37]. Complement component 5 can increase hepatic glycogenolysis by a prostanoid-mediated intercellular communication between Kupffer cells and hepatocytes[38]. Fc-γ receptor III is responsible for IgG-dependent cell cytotoxicity and production of several cytokines and chemokines and involved in macrophage inflammatory protein 1α (MIP-1alpha) and neutrophil influx[39-41]. JE/MCP-1 is known as a CC chemokine attracting monocytes, basophils and T-lymphocytes[42,43]. Serum amyloid A-5 (SAA-5) is a major acute-phase protein synthesized and secreted mainly by the liver[44], and is increased in response to acute inflammation in LR of SISPH. T-kininogen and kininogen are promoters to IL-6 as LR signal. These genes were always up regulated from 36 h to 144 h of 0-36-72-96-144 h SISPH, suggesting that they are necessary for relinquishing inflammation and promoting growth in whole SISPH.

This study found that 105 genes were suppressed in 0-36-72-96-144 h SISPH and a large number of them were related to energy metabolism, suggesting that they restrain LR by various paths, and that the need for energy in LR of SISPH is not as important as for other demand, which is different after PH.

Eight genes were suddenly down-regulated at 36 h after SISPH, including histidine-rich glycoprotein (HRG), apolipoprotein C-I (Apo C-I), retinol-binding protein (PRB), cytochrome P450 3A1 (Cyp3A1), RNase A family 4, carnitine O-octanoyltransferase (Crot), cytochrome b5 (Cyb5), *etc*. Histidine-rich glycoprotein (HRG) is confirmed an abundant serum exhibitive protein in diverse biological systems, whose combination with zinc could be used as an antidote for heparin[45,46]. Therefore, the down-regulation of HRG at 36 h indicated that the increased activity of heparin is essential for LR of SISPH. Apo C-I is known associated with the lipid surface of the plasma chylomicron, VLDL, and HDL subfractions, and reverse transfer from VLDL to HDL and to SBV[47], acting as a major plasma inhibitor of cholesteryl ester transfer protein and phospholipase inhibitor[48,49]. From the above evidence, a low level of Apo C-I at 36 h is supposed to facilitate lipoprotein linkage to LDL receptor, LDL receptor-related protein, and VLDL receptor, as well as fatty acid uptake of hepatocytes in LR of SISPH. Cyp3A1 enzymes belong to the most abundant subfamily of the cytochrome P-450 system that is predominantly found in the liver where they metabolize numerous drugs and endogenous substances such as oestrogens[50]. The down-regulation of cyp 3A1 suggested that the harm induced by hepatectomy was presumably distinct from that by drugs and endogenous substances in rat liver.

Twenty-nine genes were suppressed and had a minimum expression at 72 h in after SISPH. Among them, 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 (LXR)[51]. The extensive suppression of angiopoietin-like protein 3 mRNA at 72 h suggested that the activity of lipolysis of hepatocytes was very low in LR of SISPH. Acyl-CoA can play many important roles in numerous biochemical reactions, such as tricarboxylic acid cycle, glycoxylate bypass, fatty acid synthesis. The mRNA level of acyl-coA oxidase was first dropped to meet the condition and later increased to eliminate over expressed acyl-CoA in LR of SISPH.

Hpcl 2 was expressed at 96 h in SISPH, and involved in the carbon-carbon bond cleavage as peroxisomal pyrophosphate-dependent enzyme during α-oxidation of 3-methyl-branched fatty acids[52,53]. Down-regulation of Hpcl 2 can protect phytanic acid against being broken down, which may store energy during LR of SISPH. Fmo1 can lead to the decrease of cytochrome P-450[54], which was repressed at 96 h to accommodate electronic environment for hepatocyte multiplication in LR of SISPH.

Retinoic acid is known necessary for the maintenance of many lining epithelia of the body, whereas retinol dehydrogenase can catalyze the first step in retinoic acid biosynthesis[55]. Its suppression at 144 h after SISPH demonstrates that retinoic acid is not necessary in late phase of LR. In normal liver the activity of ADH is in excess, while in regenerating rat liver, the rate of ethanol elimination may be limited by the activity of alcohol dehydrogenase in SISPH[56]. Cathepsin C (Ctsc) and dipeptidyl aminopeptidase I are regarded to play an important role in protein degradation and the activation of proenzyme in rat liver[57]. The down-regulation of cathepsin C may be due to the indispensability of peptide for protein construction in LR of SISPH. Hepatectomy is reported to decrease liver cytochrome P450 levels by inducing heme oxygenase and inhibiting ALA synthase activities[58], which was inhibited at 144 h to regulate the oxidation reaction of hepatocytes in LR of SISPH. Glutathione S-transferase (GST) is a family of conjugative enzymes that catalyze neucleophilic addition of tripeptide glutathione to xenobiotics carcinogens and endogenous lipophilic compounds[59]. It was manifested that xenobiotics carcinogens and endogenous lipophilic might produce some uncertain toxic effect on LR of SISPH. Glutathione S-transferase type 3 (Yb3) mRNA was always hampered, implying that over accumulation of Yb3 could lead to contrary reaction. 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. The repression of its mRNA in SISPH indicates that long chain fatty acid was not in badly need until 144 h in LR of SISPH. Leuserpin-2 (Sperpind1) was confirmed to participate in complement activation in fibrinolysis and inflammatory response[60], which was continuously repressed in SISPH, suggesting that it can regulate inflammatory response to improve severely injured hepatocytes in LR of SISPH. Myelin-associated glycoprotein (MAG)-binding activity of novel sulfated GM1b, high-affinity ligands for neural singles is important to nervous system regeneration[61]. The repression of MAG at 144 h of SISPH may result in mild damage of hepatocytes and nerve system in late phase of LR.

In conclusion, further experiments will be done by using sham surgical rats as control, so as to confirm which genes reported in this paper are related to surgical operation, and which are really related to liver regeneration.

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