

## Identification and Characterization of 177 Unreported Genes Associated with Liver Regeneration

Cunshuan Xu<sup>1\*</sup>, Cuifang Chang<sup>1</sup>, Jinyun Yuan<sup>1</sup>, Hongpeng Han<sup>1</sup>, Kejin Yang<sup>1</sup>, Lifeng Zhao<sup>1</sup>, Wenqiang Li<sup>3</sup>, Yuchang Li<sup>3</sup>, Huiyong Zhang<sup>3</sup>, Salman Rahman<sup>2</sup>, and Jingbo Zhang<sup>3</sup>

<sup>1</sup> College of Life Sciences, Henan Normal University, Xinxiang 453007, China; <sup>2</sup> Haemophilia Research Center, London University, London SE17EH, United Kingdom; <sup>3</sup> State Key Laboratory of Cell Differentiation and Regulation of Province and Ministry, Xinxiang 453007, China.

The mammalian liver has a very strong regeneration capacity after partial hepatectomy (PH). To further learn the genes participating in the liver regeneration (LR), 551 cDNAs selected from subtracted cDNA libraries of the regenerating rat liver were screened by microarray, and their expression profiles were studied by cluster and generalization analyses. Among them, 177 genes were identified unreported and up- or down-regulated more than twofold at one or more time points after PH, of which 62 genes were down-regulated to less than 0.5; 99 genes were up-regulated to 2–10 folds, and 16 genes were either up- or down-regulated at different time points during LR. By using BLAST and GENSCAN, these genes were located on responsible chromosomes with 131 genes on the long arms of the chromosomes. The cluster and generalization analyses showed that the gene expression profiles are similar in 2 and 4, 12 and 16, 96 and 144 h respectively after PH, suggesting that the actions of the genes expressed in the same profiles are similar, and those expressed in different profiles have less similarity. However, the types, characteristics and functions of the 177 genes remain to be further studied.

**Key words:** Partial hepatectomy (PH), subtracted cDNA libraries, complementary DNA microarray, liver regeneration (LR), cluster analysis

### Introduction

In the healthy adult rat liver, liver is a quiescent organ with >90% of the cells present in the G<sub>0</sub> stage of the cell cycle, and their division index is very low (about 1/100600; ref. 1–3). However, adult hepatocytes have enormous ability to proliferate in response to liver injury. After 70% partial hepatectomy (PH), hepatocytes in remained liver enter the cell cycle in a highly synchronized manner and undergo 1 to 2 times of cell division, then re-differentiate and rebuild the structure and function of the liver (4, 5). In the different phases of the liver regeneration (LR), the physiological and biochemical actions of different kinds of liver cells are different, and the categories and amounts of the expressed genes in the regenerating liver are various (6, 7). It means that PH leads to an orchestrated regenerative response, activating a cascade of cell signaling events, which are necessary for cell cycle progression of hepatocytes and liver regeneration (8).

It has been suggested that liver regeneration must be involved in numerous genes (9, 10). For this, we applied suppressive subtractive hybridization, large-scale gene expression analysis, complementary DNA microarrays and bioinformatics to confirm how many genes are involved in liver regeneration after PH (11, 12).

### Results

#### The genes expressed in liver regeneration

3,205 expressed sequence tags (ESTs) that were expressed highly and specifically in the regenerating rat liver after PH were screened by suppression subtractive hybridization (SSH). 551 of them were selected to make cDNA microarray, of which 177 genes were identified unreported and up- or down-regulated more than twofold at one or more time points. Accord-

\* Corresponding author.

E-mail: xucs@x263.net.cn

ing to their expression characteristics at different time points during LR, the genes were categorized into six groups: (1) expressed in the immediate early phase (IEP, 2–4 h after PH), of which expression of 23 genes were altered. Among them, 15 were up-regulated, 5 down, and 3 showed either up or down at different time points; (2) expressed in the early phase (EP, 4–8 h after PH), of which expression of 38 genes were altered. Among them, 17 were up-regulated, 18 down, and 3 either up or down at different time points; (3) expressed in the intermediate phase (IP, 12–24 h after PH), of which expression of 65 genes were altered. Among them, 39 were up-regulated, 22 down, and 4 either up or down at different time points; (4) expressed in the early-late phase (ELP, 24–36 h after PH), of which expression of 20 genes were altered. Among them, 12 were up-regulated, 3 down, and 5 either up or down at different time points; (5) expressed in the late phase (LP, 48–72 h after PH), of which expression of 27 genes were altered. Among them, 16

were up-regulated and 10 down, and 1 either up or down at different time points; (6) expressed in the terminal phase (TP, 96–144 h after PH), of which expression of 4 genes were altered. Among them, 4 were down-regulated (Figure 1). These results showed that 62 genes were down-regulated, 99 genes up to 2–10 folds, and 16 genes either up or down at different time points during LR.

### Chromosome location of the genes expressed in liver regeneration

The chromosome location of the 177 genes was analyzed by using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) and GENSCAN (<http://genes.mit.edu/GENSCAN.html/>) (Table 1). These results showed that 131 genes were located on the long arms of the chromosomes, 25 genes were on the short arms, while the chromosome location of 21 genes was not clear.

**Table 1 Chromosome Location and Expression of the Genes Related with Liver Regeneration**

Chr.	Genes in Chr.			The expressed genes in LR after PH																	
	No.	Total	long arm	short arm	2-4 h (IEP)			4-8 h (EP)			12-24 h (IP)			24-36 h (ELP)			48-72 h (LP)			96-144 h (TP)	
				up	down	both	up	down	both	up	down	both	up	down	both	up	down	both	up	down	both
1	19	16	3	1			3	4		3	4	1	1			1					1
2	15	15		1	1				3	5	3	1			1						
3	8	6	2						1	2	1		2					1			1
4	8	8		1				1	1	3			1					1			
5	8	8							1	6	1										
6	6	6		1					1	1					1		1				1
7	8	8		2	1	1				2					1		1				
8	7	7								1	2		1				3				
9	8	8					2	1	2	1			1					1			
10	11	11		1			2	1		3	2				1						1
11	5	4	1						2		1		1				1				
12	2	2		1					1												
13	9	5	4		2			1		1			1	1	1	1	1				
14	3	1	2							2	1										
15	6	2	4						3	1				1			1				
16	4	3	1	2						1	1										
17	2	0	2								1		1								
18	5	2	3	1				1									3				
19	8	6	2	1		1				2	1		2						1		
20	3	2	1							1	1		1								
X	11	11				1		2		2	2	1	1		2						
Y																					
N/A	21	N/A	N/A	4			1	3		3			1	1			3	4			1
total	177	131	25	15	5	3	17	18	3	39	22	4	12	3	5	16	10	1			4

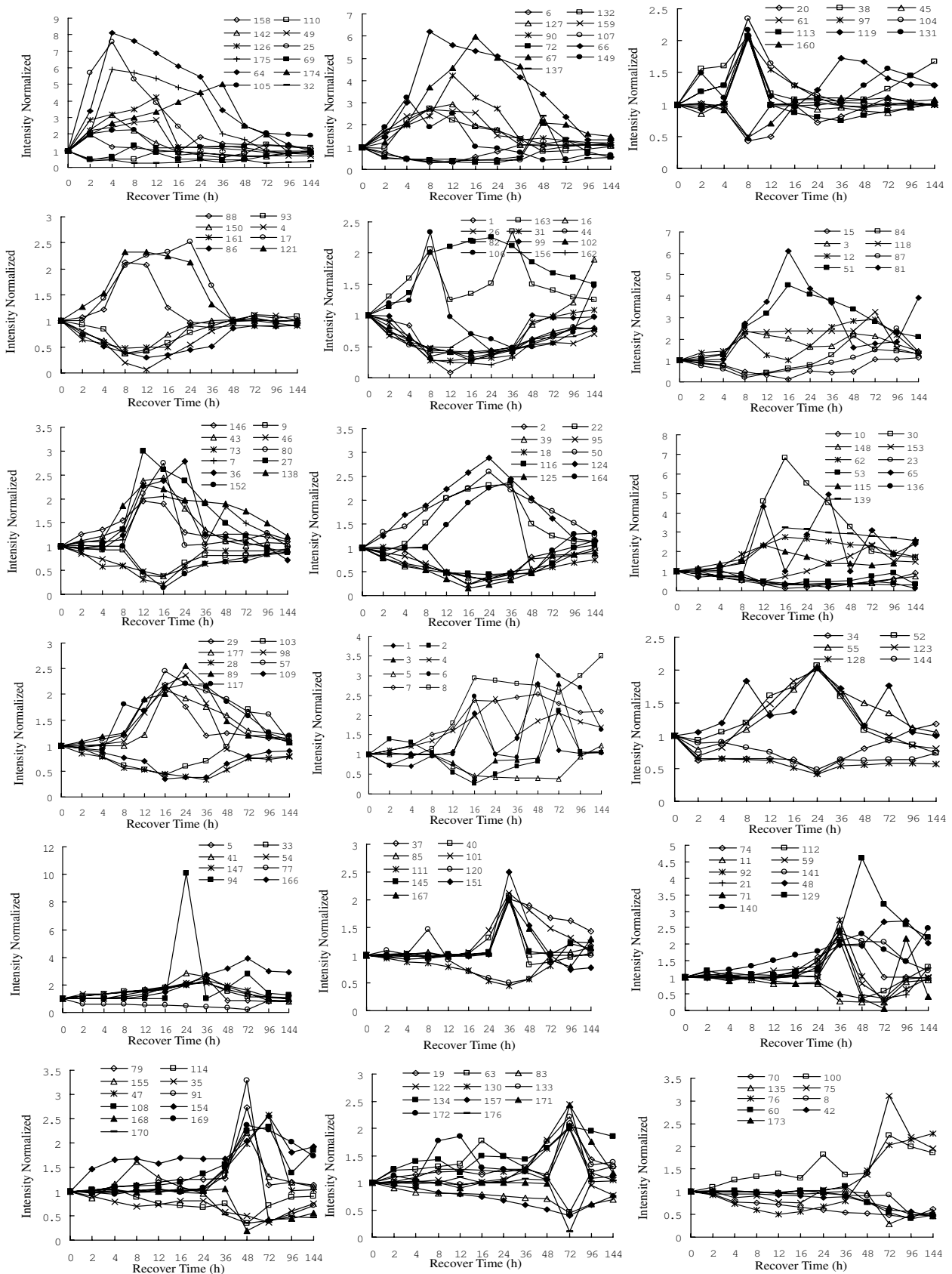


Fig. 1 Genes expressed at different time points of liver regeneration.

## Structure of the expressed genes in liver regeneration

The structure of the 177 genes was analyzed by using BLAST and GENSCAN (Table 2). These results

showed that 52 genes were made up of  $\geq 10$  exons, 102 genes  $\leq 9$  exons, while the structure of 23 genes was unclear.

**Table 2 Structure and Expression Differences of the Genes Related with Liver Regeneration**

No.	Gene description	Chromosome location	Section	ORF	Extron	Genebank number of CDS	Genebank number of EST	Hours after PH	Fold difference
1	BAC CH230-7A22	1	95964-195965	2151	E5	AC095402	AA818122	8-36	0.1
2	LRRP Ab1-346	1p11	35486309-35586309	2421	E11	AY325159	CD052213	12-36	0.3
3	BAC CH230-329D3	1p12	18210650-18310650	6129	E14	AC112406	BQ401082	8-72	2.4
4	BAC CH230-372C24	1q12	72808754-73167679	2262	E4	AC127734	AW917420	8-16	0.1
5	BAC CH230-203P11	1q12	68519037-68619038	309	E2	AY310139	CB816795	24-36	2.5
6	LRRP Ab2-440	1q21	78488239-78946203	N/A	N/A	N/A	N/A	4-12	0.3
7	LRRP Aa2-028	1q21	95484635-95584636	2514	E17	AY325132	CD052171	12-24	2.1
8	RIKEN cDNA 4930408O21	1q33	173267794-173367795	936	E5	XM_219288	CB964404	96-144	0.5
9	MGC5178	1q33	179502812-179602813	738	E4	XM_215073	BE095889	12-16	0.4
10	LRRP Aa2-174	1q34	185927807-186027808	2865	E19	AY325165	CD670576	12-72	0.1
11	LRRP Cc1-38	1q35	185945986-186045987	2865	E19	AY325244	BE098106	36-72	0.2
12	LRRP Ab1-108	1q37	201292805-20139280	972	E6	AY325138	CD052183	8-96	2.9
13	LRRP Cc1-9	1q37	201346364-201446365	1695	E16	AY325242	CB751508	16-144	2.5
14	LRRP Ab2-051	1q41	213006712-213106713	1851	E2	AY325175	CD670497	16-72	0.3, 2.1
15	LRRPAb2-132	1q41	215148584-215248584	1038	E6	AY325192	CD670514	8-48	0.1
16	D930042H13	1q42	214871327-214971327	966	E8	NM_172442	CD670570	8-36	0.3
17	LRRP Ac2-067	1q43	230720781-230820781	762	E5	AY321330	CB964401	8-24	2.5
18	LRRP Ab2-001	1q43	230945845-231045845	1116	E8	AY325174	CD670489	12-48	0.2
19	LOC119392	1q53	255352678-255452678	756	E4	XM_215260	BI285953	72	2.1
20	KIAA1376	2q11	7086551-7186552	1245	E8	XM_226239	BQ200976	8	0.4
21	CTD-2328C19	2q12	23663004-23763004	279	E3	AC091858	CF110743	36-96	0.4, 2.5
22	LRRP Cb1-739	2q12	28737280-28837281	780	E1	AY325235	CF110557	12-36	
23	BAC CH230-206C20	2q16	53411577-53511577	1638	E4	AC106505	CF384944	12-144	0.3
24	RP23-35D4	2q16	54688414-54788414	384	E3	AC127371	CB717217	16-72	0.4, 2.8
25	LRRP Ac2-061	2q21	71157011-71257011	2253	E10	AY321329	CB923478	2-16	7.6
26	LRRP Ab2-225	2q22	77263574-77363574	738	E5	AY325197	CD670526	8-36	0.3
27	RP23-100C5	2q23	88129644-88229644	438	E3	AL731707	BC668831	12-24	3.0
28	AW558171	2q31	147145999-147245999	2220	E6	XM_227217	CA507527	16-36	0.3
29	BAC CH230-155H4	2q32	162097483-162197484	5064	E22	AC124926	CB964413	16	2.2
30	LRRP Ab1-216	2q33	63860581-170111343	1512	E9	AY325153	CF384935	12-72	6.8
31	LRRP Ac2-125	2q34	180815021-180915022	2640	E17	AY321335	CD373009	8-36	0.3
32	129/SvJ BAC, citb585c7	2q34	188600812-188700813	228	E2	AF532116	BF282779	2-144	0.2
33	LRRP Ac2-269	2q34	196174105-196274105	1047	E10	AY321348	CB964402	24-36	2.2
34	LRRP Ab2-389	2q42	223465222-223565223	1266	E8	AY325204	CD670536	24	0.4
35	LRRP Aa2-258	3p11	15074963-15174963	570	E4	AY325167	CD670581	48-72	0.4
36	U48828	3p13	1148327-1248327	573	E2	XM_238608	CD052214	12-24	2.8
37	LOC311304	3q34	94195369-94295370	510	E1	XM_242120	CD052158	36	2.0
38	LRRP Aa2-296	3q35	107505568-107605569	624	E4	AY325169	CD670583	8	2.0
39	LRRP Ab2-305	3q41	133947234-134047235	1173	E10	AY325201	CD670531	12-36	0.4
40	LRRP Ab2-390	3q41	135162327-135262328	732	E7	AY325205	CD670552	36	2.1
41	RP23-32O9	3q41	138490626-138590626	471	E4	AL845325	CB839862	24-36	2.8
42	LRRP Ac2-282	3q43	164743271-164843271	660	E4	AY321349	CB964409	144	0.5
43	RIKEN cDNA 1600012F09	4q21	39532872-39632872	675	E1	XM_231479	CB751510	12-16	2.4
44	RIKEN cDNA 2310036D22	4q21	43050065-43150066	1047	E7	XM_231500	CB732821	8-36	0.4
45	LRRP Ab2-098	4q22	65863105-65963106	1188	E4	AY325190	CD670511	8	
46	LRRP Ab2-427	4q23	69538536-69638536	2601	E15	AY325210	CD670543	12-16	2.0
47	LRRP Aa1076	4q24	85692114-85792114	2547	E17	AY318959	CD670558	48-72	2.6

Table 2 Continued

No.	Gene description	Chromo- some location	Section	ORF	Extron	Genebank number of CDS	Genebank number of EST	Hours after PH	Fold difference
48	FLJ20356	4q27	85510415-85610415	333	E4	AL731692	BQ196472	36-144	2.7
49	MGC10946	4q44	186152493-186252493	519	E7	XM_232511	BQ079186	2-12	2.9
50	KIAA1230	4q44	191055672-191155673	2082	E24	XM_232536	CB964405	12-48	2.6
51	BAC CH230-4L11	5	15031-115032	612	E2	AC094527	CF384931	8-144	4.5
52	NM-031706	5q11	1448097-1548098	690	E3	XM_237702	CF111181	24	2.0
53	RP11-281N10	5q11	2740845-2840845	396	E3	AC104021	AI454500	12-144	0.3
54	LRRP Aa1027	5q22	65424631-65524631	408	E2	AY318964	CD670556	24-36	2.1
55	LRRP Cc1-6	5q32	126596899-126696899	1176	E11	AY325240	CB545374	24	2.1
56	RP23-476D16	5q35	150869854-150969854	N/A	N/A	N/A	CF599486	16-72	2.4
57	LRRP Ab1-210	5q36	168544019-168644019	768	E9	AY325152	CD052200	16-36	2.5
58	LRRP Cc2-27	5q36	187026189-187126190	3381	E25	AY325247	BE112912	16-144	2.5
59	LRRP Ac1-060	6q13	23824388-23924388	14232	E30	AY318958	CD670557	36-72	0.4, 2.3
60	LRRP Ac2-300	6q14	35606159-35706160	1131	E3	AY321351	CB964416	96-144	0.4
61	RP23-165H7	6q21	65371357-65471358	3738	E2	AC114002	CB923455	8	0.5
62	LRRP Ab1-334	6q31	113914082-114014083	999	E4	AY325158	CD052211	12-96	2.7
63	LRRP Ab1-046	6q32	123903841-124003841	1389	E5	AY325135	CD052177	72	0.5
64	LRRP Ab1-021	6q32	124234183-124410449	1836	E9	AY298742	CB751516	2-72	8.1
65	LRRP Ac1874	7q21	63885461-63985462	2349	E6	AY310161	CB923479	12-144	4.9
66	LRRP Ab1-217	7q22	63865596-63965597	1512	E9	AY325153	AI059690	4-72	6.2
67	LRRP Ac1873	7q22	63882040-63982041	2349	E6	AY310161	AA925421	4-72	6.0
68	LRRP Ac2-224	7q31	88895265-88995265	372	E3	AY321343	CB964375	16-48	2.8
69	LRRP Ab1-152	7q32	88976262-89076263	489	E4	AY325143	CD052192	2-48	0.4
70	LRRP Ac1-149	7q34	128444080-128544081	1023	E11	AY321320	CF384947	72-96	0.4
71	LRRP Cc1-27	7q35	128445472-128545473	1203	E11	AY325130	CD052168	36-144	0.1, 2.1
72	LRRP Cc1-28	7q35	128445721-128545721	1303	E11	AY325243	CB784604	4-48	0.3, 2.1
73	LRRP Ab2-232	8q22	46442272-46542273	3258	E19	AY325198	CD670527	12-16	0.2
74	LRRP Ab2-079	8q23	52227696-52327697	1440	E10	AY325176	CD670506	36-48	2.0
75	LRRP Ab2-095	8q31	98122497-98222497	1896	E11	AY325189	CD670510	72-144	3.1
76	LRRP Cc1-8	8q32	112340140-112440140	2940	E23	AY325241	CF110684	72-144	2.3
77	LRRP Ab2-417	8q32	112353973-112453974	2940	E23	AY325214	CD670542	24-72	0.2
78	RIKEN cDNA 1110061A24	8q32	117805127-117905127	N/A	N/A	N/A	BM386879	16-144	3.5
79	RIKEN cDNA 2810051A14	8q32	122548903-122648903	552	E4	XM_236668	CF110981	48	2.7
80	DNA segment (WSU 94)	9q12	17887320-17987320	1044	E9	NM_145353	CB964340	12-16	2.7
81	DNA segment (WSU 40)	9q21	40291508-40391508	1176	E11	XM_237046	CB733329	8-144	6.1
82	LRRP Ac2-193	9q21	43447192-43547192	384	E3	AY325222	CB964364	8-36	2.3
83	BAC CH230-211F21	9q22	50485632-50585632	2697	E6	AC111460	CD670562	72	0.4
84	LRRP Ab2-131	9q22	51455369-51555370	3498	E34	AY325191	CD670513	8-48	0.3, 2.2
85	LRRP Ab1-119	9q33	92287968-92387969	738	E3	AY325140	CD052186	36	2.1
86	rp32-28p17	9q34	95678996-95778996	2433	E13	AC092530	CB721103	8-24	0.3
87	LRRP Aa2-111	9q38	133283544-133383545	1692	E11	AY325162	CD670573	8-96	0.2, 2.5
88	LRRP Ac1158	10q11	2351441-2451441	1623	E10	AY310144	CB839838	8-12	2.2
89	mKIAA0665	10q12	13595294-13695294	2604	E11	XM_220262	CA945827	16-36	2.6
90	LRRP Ac1233	10q21	64598506-64698507	1422	E4	AY310149	CB839860	4-24	4.2
91	LRRP Ac1177	10q22	37322101-37422102	189	E2	AY310147	CB839846	48-72	0.4, 3.3
92	Open reading frame 31	10q24	60880930-60980931	498	E4	XM_220705	CB964395	36-72	0.3, 2.7
93	LRRP Cb1-727	10q24	61101964-61201964	2049	E17	AY325234	CD052159	8-12	0.4
94	LRRP Aa1018	10q24	62936624-63036624	1473	E8	AY318963	CD670555	24-72	10.1
95	BAC CH230-403C20	10q26	69406193-69506194	345	E2	AC118722	CD670533	12-36	0.9
96	LRRP Ab2-371	10q31	88515183-88615183	4923	E25	AY325211	CD670534	16-72	0.4
97	LOC303588	10q32+1	92219141-92319142	699	E3	XM_239346	CB839873	8	2.0
98	LRRP Ac2-210	10q32+1	94976696-95076697	663	E5	AY321338	CB964370	16-24	2.4
99	LRRP Aa2-066	11p11	7832409-7932409	1662	E10	AY325170	CD670567	8-36	0.4
100	LRRP Ab2-379	11q11	23912514-24012514	1608	E12	AY325203	CD670534	72-96	2.2

Table 2 Continued

No.	Gene description	Chromo- some location	Section	ORF	Extron	Genebank number of CDS	Genebank number of EST	Hours after PH	Fold difference
101	LRRP Ab2-093	11q11	26552957-26652957	1167	E7	AY325177	CD670509	36	2.1
102	LRRP Ab2-416	11q12	45489717-45589717	570	E5	AY325209	CD670541	8-36	0.3
103	rA4 (LOC288276)	11q21	63323657-63423658	2760	E21	XM_213658	CB767025	16	0.4
104	LRRP Ac2-223	12q12	27587564-27687565	2427	E16	AY321342	CB964374	8	2.4
105	LRRP Ab2-034	12q16	46993775-47093776	2796	E13	AY325182	CD670494	2-96	2.3
106	LRRP Ab2-196	13	67592-167593	3048	E8	AY325196	CB964372	8-36	0.5, 2.3
107	LRRP Ba2-692	13	68685-168685	3048	E8	AY325232	CD052172	4-36	0.6
108	LRRP Da2-35	13p11	25648312-25748313	837	E6	AY325258	CB577376	48-72	2.3
109	AI255964	13p13	1495219-1595220	723	E1	XM_222300	CF384936	16-36	0.4
110	LRRP Ab2-142	13q13	44304354-44404355	762	E5	AY325206	CD670515	2-4	0.5
111	LRRP Ac2-120	13q22	70689772-70789772	6309	E23	AY321333	CB964337	36	0.5
112	LRRP Da1-10	13q22	76465392-76565393	1203	E7	AY325249	CF413592	36-48	0.4, 2.1
113	LRRP Da2-20	13q26	100465676-100565677	663	E7	AY325255	AW533083	8	2.1
114	BAC CH230-329A5	13q27	102124785-102224785	3315	E8	AC136091	CF111118	48	0.3
115	MGC38937	14p11	35837306-35937307	2598	E4	XM_223356	N/A	12-144	2.6
116	RP23-480P21	14p22	12545360-12645361	747	E6	AC121829	CF110914	12-48	0.4
117	Ab2-450	14q21	70474767-70574767	4350	E25	AY325216	CD670545	16-36	2.2
118	LRRP Ac2-143	15p11	48449846-48549847	1770	E10	AY321337	CB964347	8-72	3.3
119	LRRP Ab2-008	15p12	34809952-34909952	5052	E27	AY325180	CD670488	8	2.1
120	LRRP Bm403207	15p12	36022558-36122558	1278	E2	AY325260	CB569793	36	0.5
121	RP11-586K2	15p14	18968447-19068448	327	E3	AC090797	CB964336	8-24	2.3
122	LRRP Aa1114	15q12	66624623-66724624	744	E6	AY318960	CD670559	72	2.5
123	RIKEN cDNA 3100001N19	15q12	67179494-67279494	1065	E2	XM_224414	CB316157	24	2.0
124	CG31759-PA	16p16	1873071-1973071	2118	E6	XM_224583	BQ204949	12-48	2.9
125	RIKEN cDNA 1600027G01	16q11	46571222-46671222	1671	E12	XM_224872	CB964376	12-48	0.2
126	LRRP Ab1-114	16q12+5	70088524-70188524	1017	E7	AY325139	CD052184	2-12	4.2
127	LOC333273	16q12+5	74592385-74692386	7596	E24	XM_289530	CB751507	4-16	2.9
128	LRRP Aa2-020	17p12	31648226-31748227	510	E5	AY325171	CD670565	24	0.4
129	BAC CH230-11N5	17p12	33653050-33753051	3654	E3	AC097745	CB839875	36-144	4.6
130	LRRP Da1-6	18p11	33359680-33459680	7246	E36	AY325252	BE115795	72	2.0
131	LRRP Ab1-331	18p11	35993431-36093432	456	E3	AY325157	CD052210	8	2.2
132	LRRP Ac2-032	18p12	19873342-19973343	411	E4	AY321326	CB923461	4-12	2.7
133	LRRP Ac2-256	18q12+1	54367673-54467674	1968	E14	AY321347	CB964394	72	2.2
134	LRRP Ab2-143	18q12+3	73779132-73879133	1041	E4	AY325193	CD670515	72	2.0
135	LRRP Ab1-196	19	61817-161818	249	E2	AY325149	CD052196	72-96	0.3
136	BAC CH230-186B23	19p12	11378776-11478777	6966	E17	AC099101	CF384939	12-144	0.1
137	RIKEN cDNA 1300002A08	19q11	26425206-26525206	2421	E14	BC049090	CD670580	4-96	0.3, 2.4
138	RP23-28G13	19q11	27619446-27719447	648	E5	AL611926	CB964359	12-24	2.3
139	LRRP Ba1-647	19q12	37558273-37558273	1146	E6	AY325231	CF110667	12-144	3.2
140	LRRP Ac2-202	19q12	39540703-39640704	1506	E11	AY321341	CB964365	36-144	2.5
141	LRRP Da2-4	19q12	45067705-45167705	1485	E12	AY325261	BQ192775	36-72	2.4
142	LRRP zbs559	19q12	49398632-49498633	1251	E8	AY310156	CF108941	2-8	3.1
143	LRRP Da1-24	20p12	4989596-5089596	3351	E28	AY325253	CF110992	16-96	3.5
144	LRRP Aa2-166	20q11	30100395-30200395	846	E7	AY325164	CD670575	24	0.5
145	LRRP Ab2-018	20q12	45555921-45655921	411	E3	AY325181	CD670492	36	2.0
146	LRRP Ab1-287	Xq11	363389-463389	435	E1	AY325156	CD052208	12	2.0
147	LRRP Ab2-183	Xq14	29669669-29769670	1347	E12	AY325195	CD670520	24-48	2.3
148	BAC CH230-155H3	Xq22	51955598-52055598	963	E1	AC124926	AW917419	12-72	0.3
149	LRRP Ab2-401	Xq31	70622246-70722246	633	E7	AY325208	CB803870	4-72	0.4, 3.2
150	KIAA0205	Xq31	71048564-71148564	807	E2	XM_228902	BE115518	8-12	0.4
151	Ab2-404	Xq31	64588093-64688094	4353	E8	AY325213	CD670539	36	2.5
152	LRRP Ab2-402	Xq33	70623188-70723189	633	E7	AY325208	CD670538	12-24	0.1
153	BAC CH230-404C20	Xq33	86798373-86898373	3987	E9	AC118772	CB923463	12-72	0.5, 2.4

**Table 2** *Continued*

No.	Gene description	Chromosome location	Section	ORF	Extron	Genebank number of CDS	Genebank number of EST	Hours after PH	Fold difference
154	LRRP Da2-19	Xq34	89848255-89948255	1032	E3	AY325254	CB325852	48-72	2.6
155	RP24-347B22	Xq35	95493296-95593296	1641	E7	AC122009	CF384946	48	2.2
156	LRRP Ac1-163	Xq37	114620155-114720156	2847	E8	AY321321	CB839841	8-36	0.4
157	LRRP Ab2-057	N/A	40208673-40308673	1527	E8	AY318962	CD670560	72	0.4
158	LRRG Ac2019	N/A	N/A	N/A	N/A	N/A	CF384941	2	2.0
159	12 days embryo cDNA	N/A	N/A	N/A	N/A	N/A	CD670493	2-72	5.0
160	13 days embryo liver cDNA	N/A	N/A	N/A	N/A	N/A	CB839861	2-36	5.9
161	Liver cDNA	N/A	N/A	N/A	N/A	N/A	CD052216	72	0.1
162	Testis cDNA	N/A	N/A	N/A	N/A	N/A	CB964382	16	2.1
163	RP23-92K11	N/A	N/A	N/A	N/A	N/A	CD052178	4-16	2.7
164	RIKEN cDNA 4833439L19	N/A	N/A	N/A	N/A	N/A	CD670574	8	0.5
165	Hippocampus cDNA	N/A	N/A	N/A	N/A	N/A	CD670502	8-16	0.5
166	RP23-235O1	N/A	N/A	N/A	N/A	N/A	CB315158	8-36	0.2
167	RP23-195K1	N/A	N/A	N/A	N/A	N/A	AA900787	8-36	2.4
168	RP24-176A1	N/A	N/A	N/A	N/A	N/A	CD670517	12-48	0.5, 2.4
169	RIKEN cDNA 2700060E02	N/A	N/A	N/A	N/A	N/A	BM386943	24	2.0
170	RP23-195K1	N/A	N/A	N/A	N/A	N/A	AA900787	24-144	4.0
171	KIAA0433	N/A	N/A	N/A	N/A	N/A	N/A	36	2.0
172	RP23-417P22	N/A	N/A	N/A	N/A	N/A	CF384932	48-96	0.2
173	LRRP Ac2-019	N/A	N/A	N/A	N/A	N/A	CF384941	48-96	2.4
174	RP23-235O1	N/A	N/A	N/A	N/A	N/A	CB315158	48-144	0.4
175	LRRG Ab2052	N/A	N/A	N/A	N/A	N/A	CD670498	72	2.4
176	RP24-155I9	N/A	N/A	N/A	N/A	N/A	CB923489	72	2.0
177	RIKEN cDNA 2310045J23	N/A	N/A	N/A	N/A	N/A	CB751520	144	0.5

### Cluster analysis of genes expressed differently in liver regeneration

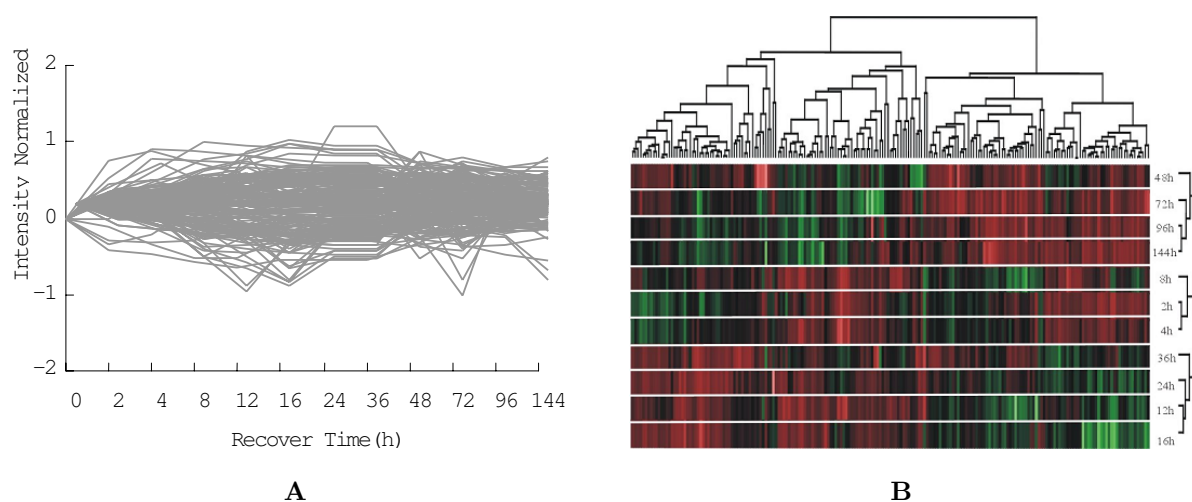
Cluster analysis was done to facilitate the visualization and interpretation of the gene expression program represented in this very large body of data. The results showed that the distribution trend of the 177 genes is as follows: the genes altered in the beginning phase of LR are more than those in the other phases; the genes up-regulated in LR are more than those down-regulated; the expression folds of up-regulated genes are higher than the suppression folds of the down-regulated; and the expression changes of the down-regulated genes are more complex than those of the up-regulated (Figure 2A). On the basis of similarities in their expression patterns and display results in a compact graphical format, 18 kinds of ramose gene expression clusters are generated (Figure 2B).

Cluster analysis of genes expressed at 12 time points after PH showed that the 177 genes are categorized into 8 patterns of gene expression based on the similarity, that is, 2 and 4, 8, 12 and 16, 24, 36, 48, 72, 96 and 144 h, and are placed in a major branch of the dendrogram (Figure 3).

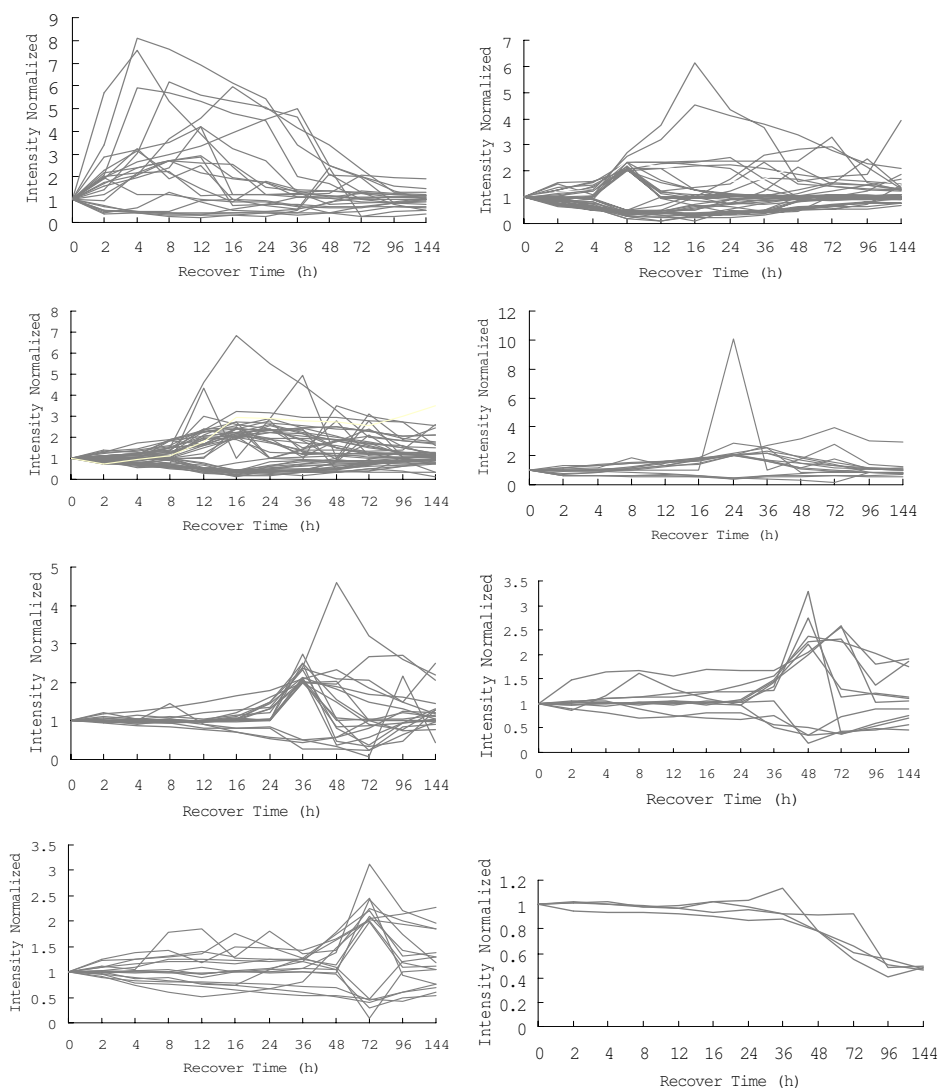
### Discussion

In this study, 177 unreported genes were identified by microarray to be associated with the rat liver regeneration. It shows that a large number of genes related with LR remain to be found and studied. It was found by the analysis of chromosome location of genes that 131 genes were located on the long arms of the chromosomes and 25 genes were on the short arms. This is responsible for the structure of chromosomes. In the 177 genes, expression of 61 genes was altered in the intermediate phase of LR. It means that the progress of S phase of cell cycle is involved in lots of genes. It was confirmed that 99 genes were up-regulated in LR and 62 genes down, suggesting that the number of the activated genes were more than that of the suppressed ones.

Following the cluster analysis, the 177 genes related with rat liver regeneration were categorized into 18 distinct temporal patterns of induction, and based on the similarity, the 177 genes showed 8 expression profiles, that is, 2 and 4, 8, 12 and 16, 24, 36, 48, 72, 96 and 144 h, indicating that the genes expressed in



**Fig. 2** Hierarchical cluster analysis of 177 genes. **A.** Cluster of distribution trend. 177 genes differing with more than twofold intensity at least one time point of liver regeneration were identified; **B.** Cluster of hierarchical relativeity at eleven time points.



**Fig. 3** The 177 genes expressed at different time points after PH are categorized into 8 patterns based on their similarity, that is, 2 and 4, 8, 12 and 16, 24, 36, 48, 72, 96 and 144 h.



the same time share common expression profiles, and the metabolism and physiology of the cells with common gene expression profiles are similar. On the other hand, the genes expressed in different times have no common expression profiles, and the metabolism and physiology of the cells with different gene expression profiles are not similar. However, the types and characteristics of these genes are still unclear, and their functions remain to be further studied.

## Materials and Methods

### Partial hepatectomy of rats and RNA isolation

200±20 g healthy adult SD (Sprague Dawley) rats were obtained from the experimental animal center of Henan Normal University. Following the method of Higgins and Anderson (6), 70% of the total rat liver was removed, which was performed under sterile conditions (7). The regenerating livers of four rats (male: female = 1:1) were taken at 2, 4, 8, 12, 16, 24, 36, 48, 72, 96 and 144 h respectively after PH. The taken livers were rinsed in cold PBS and immersed in -80°C refrigerator for RNA extraction. Total RNA was isolated from frozen livers according to the manual of Trizol kit of Invitrogen. In brief, 50–100 mg liver 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 (EB).

### Subtracted cDNA library construction and screening

The subtracted cDNA library was generated from total RNA by PCR-Select™ cDNA subtraction kit (Clontech, Palo Alto, USA) following the manufacturer's instruction. Briefly, total RNA was reverse-transcribed into double cDNA strands and digested with restriction enzymes, followed by subtracted hybridization with drivers and testers. Finally, with suppression PCR (polymerase chain reaction), differential expression sequence tags were performed to construct subtracted cDNA library (13). The subtracted cDNA library was cloned into T/A vector and screened by PCR with nest primers 1 and 2.

### cDNA microarray construction

551 cDNA fragments were amplified by Nested PCR. Primers 1 and 2 were purified by NaAc/isopropyl alcohol. Subsequently, they and 50 controls (8 negative, 12 void, and 30 internal) were doubly spotted onto glass slides by ProSys-5510A spotting machine following designed project and comprised 8 submatrices (48\*24) occupying 9×18 mm (BioStar, Shanghai, China). Then the gene chips were ready by hydrating, blocking and drying (13).

### Hybridization and scanning

RNA prepared from rat livers before PH was ready for 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, England) using MMLV reverse transcriptase (Promega) with olig(dT) primer. After bath incubation for 2 h, labeled buffers I and II were subsequently added to the reaction. The control group and test group were mingled together symmetrically and stored, avoiding light for application (13). The glass slices were prehybridized at 42°C for 5–6 h in hybridization buffer containing freshly cooked shared salmon sperm DNA. The labeled denatured probe was hybridized against cDNA microarray with overnight (16–18 h) incubation at 42°C. The slices were then washed twice with 2×SSC containing 0.5% SDS at room temperature for 5 min, once with 0.2×SSC containing 0.5% SDS at 60°C for 10 min, and finally with 0.2×SSC at 60°C for 10 min. The slices were exposed to photographer. Hybridized images were scanned by a fluorescence laser scanning device, Gene Pix 4000A. At last, 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. 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 intensity was replaced by 200 when it was <200. When Ri (Ri=cy5/cy3)

was between 0.1 and 10,  $R_i$  was taken logarithms to generate  $R_i'$  [ $\log(R_i)$ ] and ND was taken by EXP (R) (averaged  $R_i'$ ). The modified  $cy3^*$  was generated when taking ND multiply  $cy3$  and was replaced by 200 when it was  $<200$ . The ratio was performed by  $cy5/cy3^*$ . Therefore, we selected genes whose ratio was more than 2 or less than 0.5, representing a twofold difference in expression level. To analyze the selected gene expression data, we applied GeneMaths cluster analysis and performed hierarchical clustering to apprise the number of groups. Euclidean distance was used as the dissimilarity measure. Whole analyses were executed with Microsoft Excel and GeneSpring (Silicon Genetics, San Carlos, USA).

### Structure and chromosome location of the genes

The base sequence assay of ESTs was carried according to the current protocols in molecular biology. The EST sequences were sent to GeneBank to perform homology analysis. The accession number of the whole novel ESTs is achieved. In virtue of rat genome database (RGD), electronic cloning and chromosome location of the unreported ESTs representing unreported full-length cDNA were performed successfully. They were searched at <http://www.ncbi.nlm.nih.gov/genomeguide/rat/index.html/> for gene location in chromosome and genes corresponding with WGS (Whole Genome Shotgun). By delivering the sequences to GENSCAN, we acquired CDS (coding domain sequences) supported by the full-length cDNA. Compared with known proteins in virtue of BLASTP (<http://www.ncbi.nlm.nih.gov/BLAST/>), their functions and accession numbers were achieved.

### Acknowledgements

We thank *BioStar* for microarray.

## References

1. Michalopoulos, G.K., *et al.* 1997. Liver regeneration. *Science* 276: 60-66.
2. Fausto, N. 2000. Liver regeneration. *J. Hepatol.* 32: 19-31.
3. Zimmermann, A. 2002. Liver regeneration: the emergence of new pathways. *Med. Sci. Moint.* 8: 53-63.
4. Satyanarayana, A., *et al.* 2004. Gene expression profile at the G(1)/S transition of liver regeneration after partial hepatectomy in mice. *Cell Cycle*. In press.
5. Xu, C.S., *et al.* 2001. Effects of the short interval successive partial hepatectomy on rat survival and liver tissue structure. *Acta Zool. Sin.* 47: 659-665.
6. Higgins, G.M. and Anderson, R.M. 1931. Experimental pathology of the liver I. Restoration of the liver of white rat following partial surgical removal. *Arch. Pathol.* 12: 186-202.
7. Zhu, J.H., *et al.* 1989. Rapid isolation and primary culture of the rat hepatocytes. *J. Henan Normal Uni.* 16: 46-52.
8. Thevananther, S., *et al.* 2004. Extracellular ATP activates c-jun N-terminal kinase signaling and cell cycle progression in hepatocytes. *Hepatology* 39: 393-402.
9. Jensen, S.A. 2001. Liver gene regulation in rats following both 70 or 90% hepatectomy and endotoxin treatment. *J. Gastroenterol. Hepatol.* 16: 525-530.
10. Su, A.I., *et al.* 2002. Gene expression during the priming phase of liver regeneration after partial hepatectomy in mice. *Proc. Natl. Acad. Sci. USA* 99: 11181-11186.
11. Togo, S., *et al.* 2004. Mechanism of liver regeneration after partial hepatectomy using mouse cDNA microarray. *J. Hepatol.* 40: 464-471.
12. Xu, C.S., *et al.* 2004. Gene expression differences of regenerating rat liver in a short interval successive partial hepatectomy. *World J. Gastroenterol.* In press.
13. Yang, G.P., *et al.* 1999. Combing SSH and cDNA microarray for rapid identification of differentially expressed genes. *Nucleic Acids Res.* 27: 1517-1523.

This work was supported by National Natural Science Foundation of China (No. 30270673).