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

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**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 $G_0$ 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*).

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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-

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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 | short | 2-4 h (IEP) | | | 4-8 h (EP) | | | 12-24 h (IP) | | | 24-36 h (ELP) | | | 48-72 h (LP) | | | 96-144 h (TP) | | |
| | | arm | arm | 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 | |

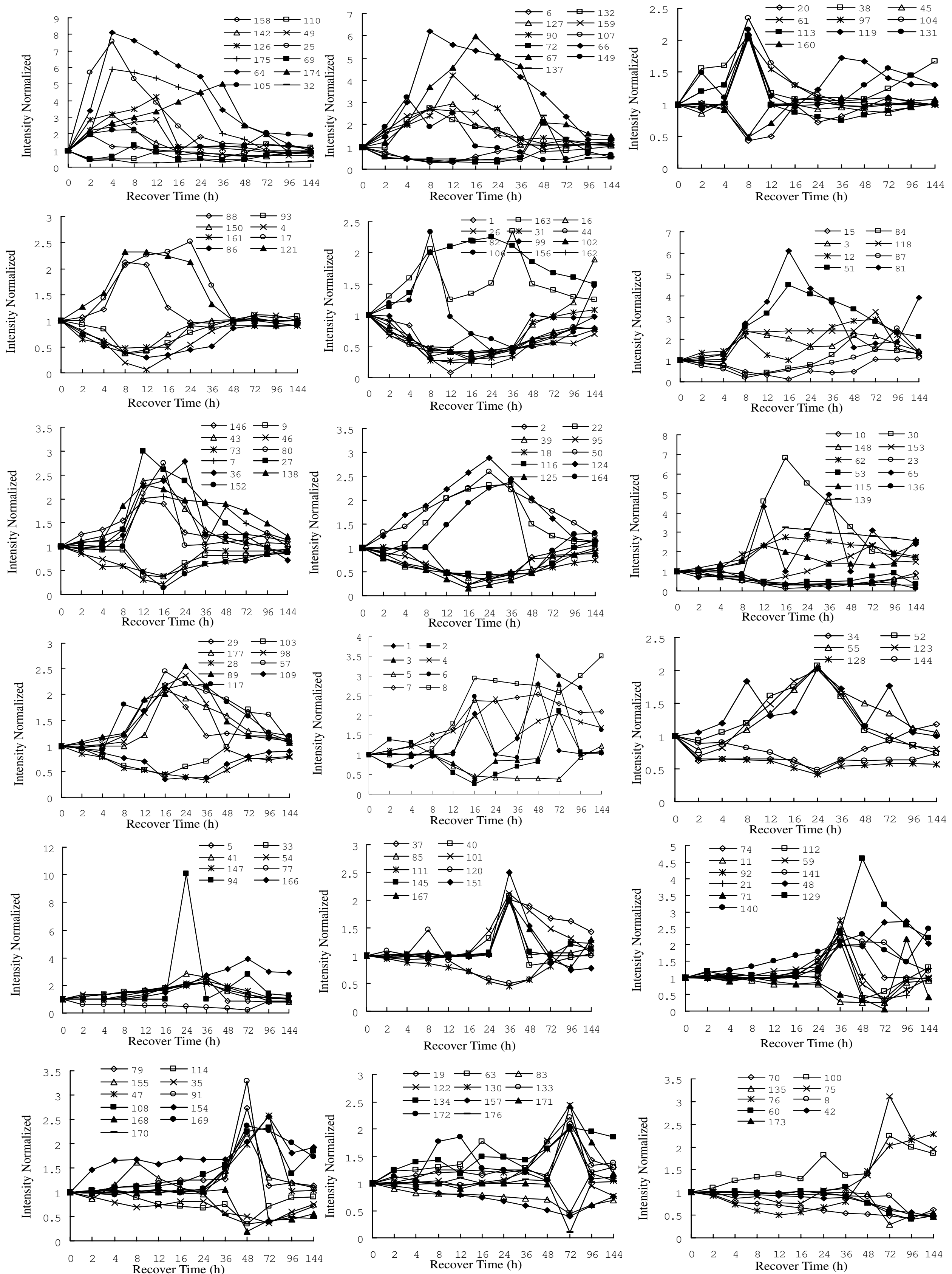


**Fig. 1** Genes expressed at different time points of liver regeneration.

## 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 ≥10 extrons, 102 genes ≤9 extrons, 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 | Chromosome 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 | Chromosome 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 | RP23-23501 | 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

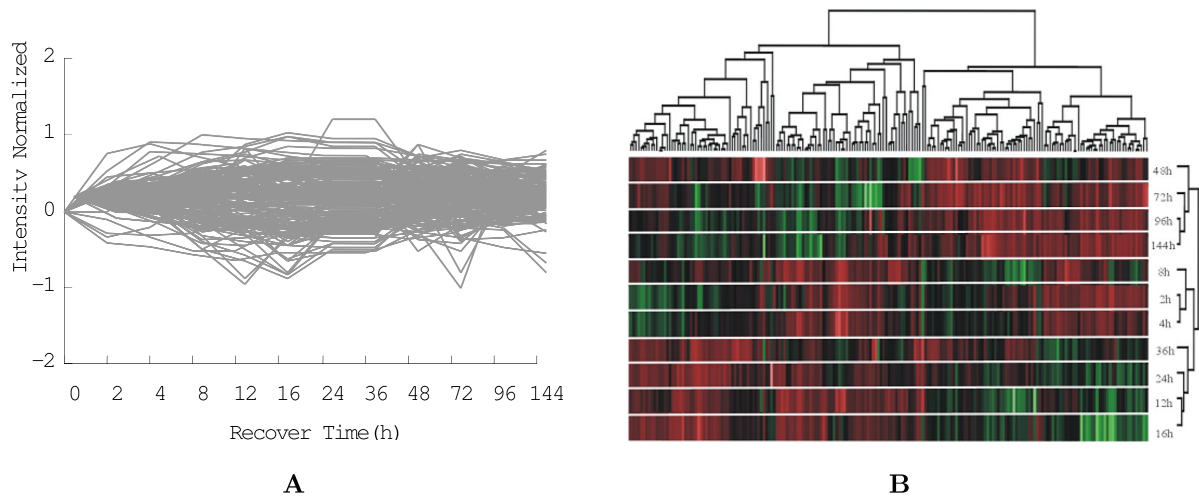


**Fig. 2** Hierarchical cluster analysis of 177 genes. **A.** Cluster of distribution trend. 177 genes differing with more than twofold intensity at least one time point of liver regeneration were identified; **B.** Cluster of hierarchical relativity at eleven time points.

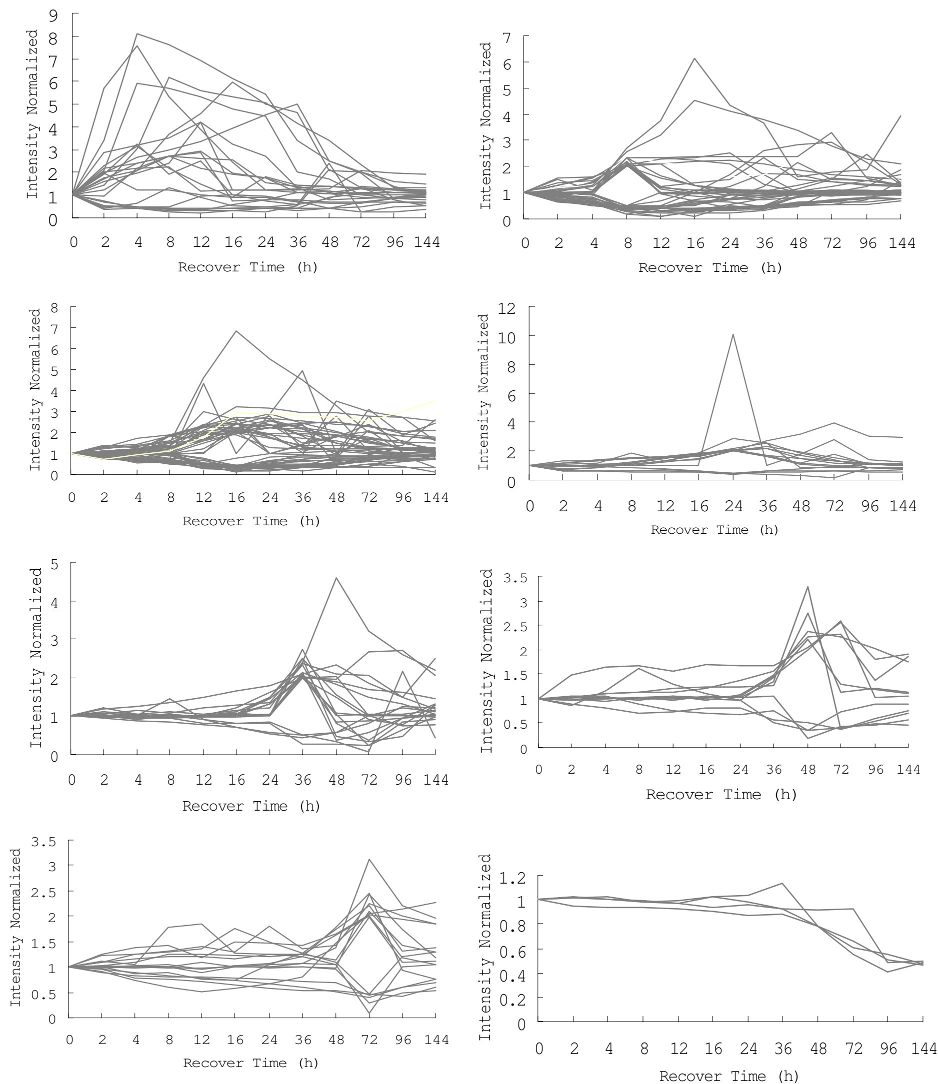


**Fig. 3** The 177 genes expressed at different time points after PH are categorized into 8 patterns based on their similarity, that is, 2 and 4, 8, 12 and 16, 24, 36, 48, 72, 96 and 144 h.

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 Higgens 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℃ 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 electrophesis stained by ethidium bromide (EB).

## Subtracted cDNA library construction and screening

The subtracted cDNA library was generated from total RNA by PCR-Select$^{TM}$ cDNA subtraction kit (Clontech, Palo Alto, USA) following the manufactory'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 submatrixes (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℃ 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℃. 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℃ 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. 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, Ri was taken logarithms to generate Ri′ [log (Ri)] and ND was taken by EXP (R) (averaged Ri′). 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.

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