

# Correlation analysis of liver tumor-associated genes with liver regeneration

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## INTRODUCTION

The liver is an organ with considerable regenerative capacity<sup>[1]</sup>. After partial hepatectomy (PH)<sup>[2]</sup>, about 95% of quiescent hepatocytes re-enter synchronously into the cell cycle to replenish the missing hepatocytes<sup>[3,4]</sup>. Whereas excessive liver mass is regulated by apoptosis<sup>[5]</sup>, this process is called liver regeneration (LR)<sup>[3]</sup>. The regeneration process, which according to cellular physiological and biochemical activities is divided into the following parts: initiation (0.5-4 h after PH), transition from G0 to G1 (4-6 h after PH), cell proliferation (6-66 h after PH), and cell differentiation and reorganization of the structure-function (72-168 h after PH)<sup>[6]</sup>, or according to time course, into forepart (0.5-4 h after PH), prophase (6-12 h after PH), metaphase (16-66 h after PH) and anaphase (72-168 h after PH), involves various physiological and biochemical activities such as cell activation, de-differentiation, proliferation and its regulation, re-differentiation, and rebuilding of the structure and function<sup>[7,8]</sup>. Actually, some biological activities in LR including cell proliferation and growth are also observed in liver tumor (LT). It is usually thought that tumorigenesis is mainly ascribed to the anomalous activation of the genes having positive effects on LT cell proliferation, growth, invasion and LT angiogenesis, as well as the genes suppressing LT cell apoptosis, and/or inactivation of the inhibitory genes related to LT cell proliferation, growth, invasion and LT angiogenesis<sup>[9]</sup>, and the promotive genes of LT cell differentiation and apoptosis. To elucidate the intrinsic differences between the two events at transcriptional level, we checked the expression profiles of above genes in regenerating livers following 2/3 hepatectomy utilizing the Rat Genome 230 2.0 Array containing 249 LT-associated genes, and primarily analyzed their expression changes and actions in LR, as well as their relevance with LR.

## Abstract

**AIM:** To study at transcriptional level the similarities and differences of the physiological and biochemical activities between liver tumor (LT) and regenerating liver cells.

**METHODS:** LT-associated genes and their expression changes in LT were obtained from databases and scientific articles, and their expression profiles in rat liver regeneration (LR) were detected using Rat Genome 230 2.0 array. Subsequently their expression changes in LT and LR were compared and analyzed.

**RESULTS:** One hundred and twenty one LT-associated genes were found to be LR-associated. Thirty four genes were up-regulated, and 14 genes were down-regulated in both LT and regenerating liver; 20 genes up-regulated in LT were down-regulated in regenerating liver; 21 up-regulated genes and 16 down-regulated genes in LT were up-regulated at some time points and down-regulated at others during LR.

**CONCLUSION:** Results suggested that apoptosis activity suppressed in LT was still active in regenerating liver, and there are lots of similarities and differences between the LT and regenerating liver at the aspects of cell growth, proliferation, differentiation, migration and angiogenesis.

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**Key words:** Partial hepatectomy; Rat Genome 230 2.0 Array; Apoptosis; Liver regeneration-associated gene; Liver tumor-associated gene

## MATERIALS AND METHODS

### Regenerating liver preparation

Healthy Sprague-Dawley rats weighing 200-250 g were

obtained from the Animal Center of Henan Normal University. The 276 rats were separated into 46 groups randomly, 23 hepatectomized groups and 23 sham-operation (SO) groups, and each group included 6 rats. PH was performed according to Higgins and Anderson<sup>[2]</sup>, by which the left and middle lobes of liver were removed. Rats were killed by cervical vertebra dislocation at 0, 0.5, 1, 2, 4, 6, 8, 12, 16, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 96, 120, 144 and 168 h after PH and the regenerating livers were observed at corresponding time point. The livers were rinsed three times in PBS at 4°C, and then total 1-2 g livers (100-200 mg livers from middle parts of right lobe of each sample, 6 samples per group) were gathered and mixed together, then stored at -80°C. The SO group was the same as hepatectomized group except the liver lobes were not removed. The laws of animal protection of China were enforced strictly.

### RNA isolation and purification

Total RNA was isolated from frozen livers according to the manual of Trizol reagent (Invitrogen Corporation, Carlsbad, California, USA)<sup>[10]</sup> and then purified base on the guide of RNeasy mini kit (Qiagen, Inc, Valencia, CA, USA)<sup>[11]</sup>. Total RNA samples were checked to exhibit a 2:1 ratio of 28S rRNA to 18S rRNA intensities by agarose electrophoresis (180 V, 0.5 h). Total RNA concentration and purity were estimated by optical density measurements at 260/280 nm<sup>[12]</sup>.

### cDNA, cRNA synthesis and purification

One to eight gram total RNA as template was used for cDNA synthesis. cDNA purification was based on the way established by Affymetrix<sup>[13]</sup>. cRNA labeled with biotin was synthesized using cDNA as the template, and cDNA and cRNA were purified according to the purification procedure of GeneChip Analysis<sup>[13]</sup>. Measurement of cDNA, cRNA concentration and purity were the same as above.

### cRNA fragmentation and microarray detection

Fifty  $\mu$ L (1  $\mu$ g/ $\mu$ L) cRNA incubated with 5  $\times$  fragmentation buffer at 94°C for 35 min was digested into 35-200 bp fragments. The hybridization buffer prepared according to the way Affymetrix provided was added to the prehybridized Rat Genome 230 2.0 array produced by Affymetrix, then hybridization was carried out at 45°C for 16 h on a rotary mixer at 60 rpm. The microarray was washed and stained by GeneChip fluidics station 450 (Affymetrix Inc., Santa Clara, CA, USA). The chips were scanned by GeneChip Scan 3000 (Affymetrix Inc., Santa Clara, CA, USA), and the signal values of gene expression were observed<sup>[14]</sup>.

### Microarray data analysis

The normalized signal values, signal detections (P, A, M) and experiment/control (Ri) were obtained by quantifying and normalizing the signal values using GCOS (GeneChip operating software) 1.2<sup>[14]</sup>.

### Normalization of the microarray data

To minimize the technical error from the microarray

**Table 1 Primer and probe sequences used to validate the microarray analysis by quantitative RT-PCR**

Genes	Primer sequences	Tm	Amplified products
$\beta$ -actin	FP: CCTGGCACCCAGCACAAAT	58°C	221 bp
	RP: GCTGATCCACATCTGCTGGAA	58°C	
	Probe: ATCAAGATCATTTGCTCCTCCIGAGCGC	68°C	
jun	FP: TGCAAAGATGGAACGACCTT	58°C	76 bp
	RP: GCCGTAGGCGCCACTCT	59°C	
	Probe: TACGACGATGCCCTCAACGCCTC	68°C	
myc	FP: CCCCTAGTGTGTCATGAAGAG	59°C	95 bp
	RP: TCCACAGACACCACATCAATTC	58°C	
	Probe: CACCAGCAGCGACTCTGAAGAAGAACA	68°C	
tp53	FP: ATGAGGCCTTGGAAATTAAGGAT	58°C	98 bp
	RP: CGTAGACTGGCCCTTCTTGGT	59°C	
	Probe: CAGGGCTCACTCCAGCTACCCGAA	68°C	

FP: forward primer; RP: reverse primer.

analysis, each sample was hybridized three times to the gene chips. The average value of three measurements was normalized, and statistics and cluster analyses were conducted on these values with GeneMath, GeneSpring (Silicon Genetics, San Carlos, CA) and Microsoft Excel Software (Microsoft, Redmond, WA)<sup>[14-16]</sup>.

### Verification of array results by RT-PCR

Primer and probe sequences were designed by primer express 2.0 software according to mRNA sequences of three target genes jun, myc, tp53 and internal control  $\beta$ -actin gene (GenBank number: BC078738, NM\_012603, AY009504 and NM\_031144) and synthesized by Shanghai GeneCore BioTechnologies Co. Ltd (Table 1).

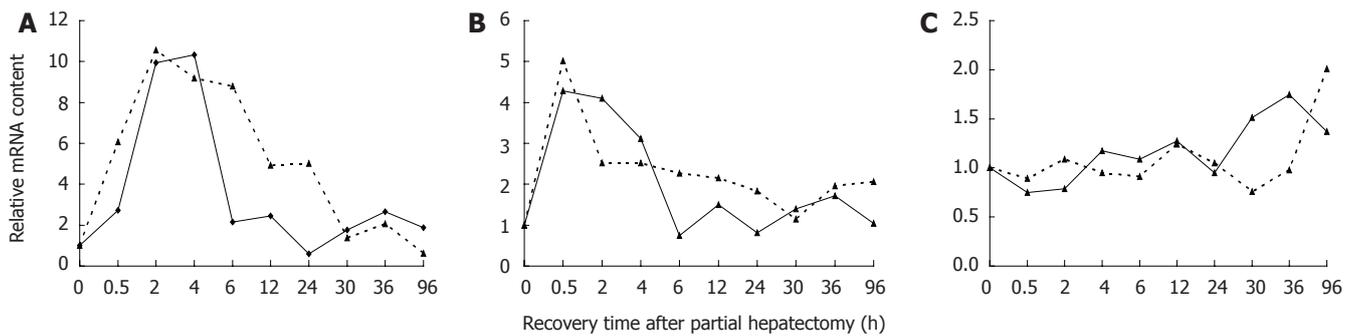
### Identification of genes associated with LR

Nomenclatures such as LT, hepatoma, hepatocellular carcinoma, hepatocarcinogenesis, cholangiocarcinoma and so on were input into the databases at NCBI (www.ncbi.nlm.nih.gov) and RGD (rgd.mcw.edu) to identify rat, mouse and human genes associated with LT. Then these LT-associated genes were reconfirmed through literature searches of the pertinent articles. Besides the rat genes, other genes, that are now thought existing in mouse and/or human and showed a greater than two-fold change in the rat regenerating livers, were referred to as rat homologous genes. Genes that displayed reproducible results with three independent analyses using Rat Genome 230 2.0 array and that showed a greater than two-fold change in expression at least at one time point as a significant difference ( $P \leq 0.05$ ) or an extremely significant difference ( $P \leq 0.01$ ) between PH and SO, were included as being associated with LR.

## RESULTS

### Comparison between the quantitative RT-PCR results and the microarray results

The quantitative RT-PCR results of three chosen genes jun, myc and tp53 at 0, 0.5, 2, 4, 6, 12, 24, 30, 36 and 96 h after partial hepatectomy (PH) were compared with Rat Genome 230 2.0 Array results (Figure 1) in order to verify validity of this chip. According to quantitative RT-PCR



**Figure 1** Comparison of relative mRNA levels in regenerating liver detected by Affymetrix Rat Genome 230 2.0 microarray and real-time PCR analysis **A:** myc; **B:** jun; **C:** tp53; Real line represents quantitative real time PCR results; broken line indicates Rat Genome 230 2.0 microarray results.

results, myc was up-regulated at 0.5-12 and 30-96 h after PH with the highest point of 10.33 folds higher than control at 4 h; jun expression was significantly up-regulated at 0.5-4 h after PH, showing the greatest abundance of 4.28-fold of control at 0.5 h; and tp53 was up-regulated at 96 h after PH. The result of RT-PCR suggested that expression profiles of these three genes were basically similar to that of array, which indicated that Rat Genome 230 2.0 Array had great reliability.

#### Expression changes of the associated-genes in LT and LR

Among 252 genes associated with LT obtained by searching the related data in databases such as NCBI, RGD *etc.*, 249 genes were contained in the Rat Genome 230 2.0 Array. 121 of 249 genes yielded meaningful expression changes on at least single time point after PH, showed significant or extremely significant difference between PH and SO, and displayed reproducible results with three independent analyses with Rat Genome 230 2.0 Array, suggesting that these genes were associated with LR. The data listed below indicated that expression trends of 48 genes in LT was similar to that in LR, whereas expression of 34 genes in the former underwent opposite trend comparing with in the latter, and expression changes of 39 genes in LT were similar to that in some time point of LR. Specifically, the same trend towards up regulation of 34 genes and down regulation of 14 genes were exhibited in both LT and LR; 20 up-regulated genes in LT showed down-regulation during LR, and 14 down-regulated genes in LT revealed up-regulation during LR; 23 up-regulated genes and 16 down-regulated genes in LT were up-regulated at some time points and down-regulated at others during LR (Table 2).

#### The relationship of LT-associated genes with LR

According to function feature and expression profiles of total 121 LT-associated genes in LR, they were divided into six classes and twenty-nine subclasses (Figure 2), and their expression changes in LR were present. Genes up-regulated in both LT and regenerating liver include nine cell proliferation-associated genes (1), four cell growth-associated genes (2), one apoptosis-associated gene (3), nine cell migration-associated genes (4), three angiogenesis-associated genes (5), and eight genes involved in other biological processes (6); Genes down-

regulated in both LT and regenerating liver include six cell proliferation-associated genes (7), three apoptosis-associated genes (8), two differentiation-associated genes (9), and three genes with other functions (10); Genes down-regulated in LT but up-regulated in LR include two cell proliferation-associated genes (11), four cell growth-associated genes (12), three apoptosis-associated gene (13), and another five genes having other biological activities (14); Genes up-regulated in LT but down-regulated in LR include six cell proliferation-associated genes (15), two cell growth-associated genes (16), one apoptosis-associated gene (17), three cell migration-associated gene (18), three angiogenesis-associated genes (19), and five genes with other functions (20); Genes up-regulated in LT but up-regulated at some time points and down-regulated at others in LR include four cell proliferation-associated genes (21), three cell growth-associated genes (22), two telomerase-associated genes (23), five cell migration-associated genes (24), and nine genes participating in other actions (25). Genes down-regulated in LT but up-regulated at some time points and down-regulated at others during LR include two cell proliferation-associated genes (26), six cell growth-associated genes (27), one apoptosis-associated gene (28), and seven genes related to biological events differed from the above-mentioned actions (29).

## DISCUSSION

Generally, cell proliferation and growth was done in both LT and LR, but the former are malignant, and the latter are controlled stringently. According to our data, proliferation-promoting genes *pcna*, *ccne1*, *cdk4*, *ahr*, *wee1*, *ccna2*, *pin1*, *nek6* and *smo*<sup>[17-22]</sup> were up-regulated in both LT and LR, and proliferation-inhibiting genes *creb3l3*, *pten*, *kit*, *gjb1*, *tff1* and *csda*<sup>[23-28]</sup>, were down-regulated in both, indicating that these genes promote cell proliferation in the two events. Notably, the abundance of *CCNA2* mRNA in LT was approximately five-fold higher than that in normal liver<sup>[17]</sup>, and it reached its peak with 45 folds of control at 66 h after PH, which might be associated with an increased proportion of regenerated hepatocytes. Growth-promoting genes *hspb1*, *grn*, *tgfb1* and *serpine1*<sup>[29-31]</sup>, whose expression levels were elevated in LT, were up-regulated at metaphase of LR. Among these four genes, *serpine1* having the highest expression

**Table 2 Expression abundance of 121 liver tumor-associated genes during liver regeneration**

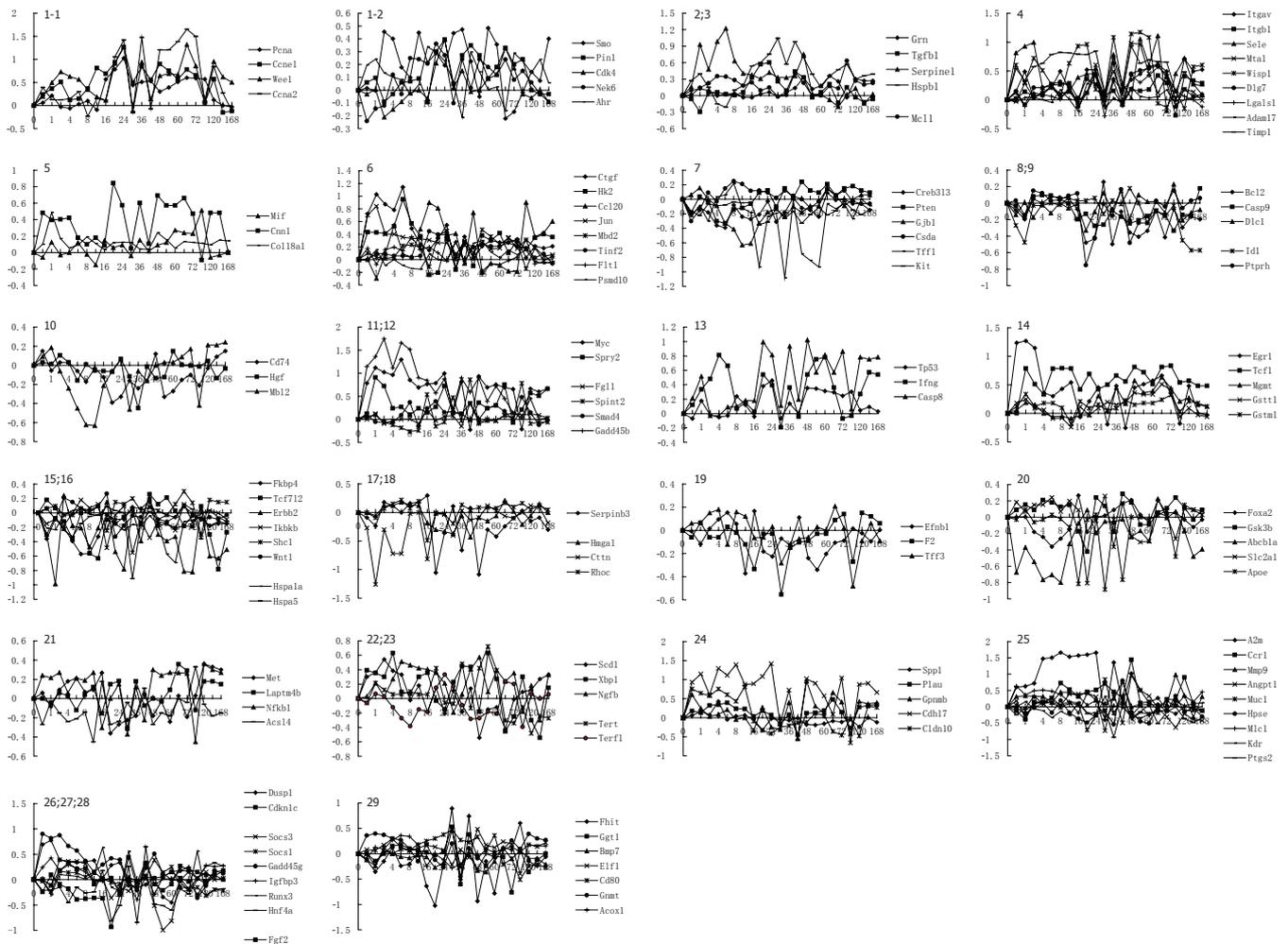
Name	Gene Abbr.	Associated to	Fold difference	Comparison	
				LT.	RRL.
<b>The same in gene expression trend</b>					
Cyclin A2	*Ccna2	2	45.1	↑	↑
WEE1 homolog	Wee1	2	20.9	↑	↑
Cyclin E1	Ccne1	2	18.5	↑	↑
Proliferating cell nuclear antigen	Pcna	2	10.6	↑	↑
Smoothened homolog	Smo	1,2	3	↑	↑
Protein NIMA-interacting 1	Pin1	2	2.5	↑	↑
Cyclin-dependent kinase 4	Cdk4	2	2.5	↑	↑
NIMA (never in mitosis gene a)-related kinase 6	Nek6	2	2.3	↑	↑
Aryl-hydrocarbon receptor	Ahr	1	2.2	↑	↑
Serpin peptidase inhibitor, clade E, member 1	Serpine1	2	16.7	↑	↑
Heat shock 27 kDa protein 1	Hspb1	2	11	↑	↑
Transforming growth factor, beta 1	Tgfb1	2	4	↑	↑
Granulin	Grn	2	2.3	↑	↑
Myeloid cell leukemia sequence 1	Mc11	2,3	4.3	↑	↑
WNT1 inducible signaling pathway protein 1	Wisp1	2,3	14.9	↑	↑
Selectin E	Sele	2	12.9	↑	↑
Metastasis associated 1	Mta1	2	9.6	↑	↑
TIMP metalloproteinase inhibitor 1	Timp1	2	8.6	↑	↑
Integrin, alpha V	Itgav	2	5.2	↑	↑
Discs, large homolog 7	Dlg7	2	4.3	↑	↑
Lectin, galactoside-binding, soluble, 1	Lgals1	2	3.7	↑	↑
ADAM metalloproteinase domain 17	Adam17	2	2.7	↑	↑
Integrin, beta 1	Itgb1	2	2.6	↑	↑
Calponin 1, basic, smooth muscle	Cnn1	2	7	↑	↑
Macrophage migration inhibitory factor	Mif	2	3.2	↑	↑
Collagen, type XVIII, alpha 1	Col18a1	2	3.1	↑	↑
Connective tissue growth factor	*Ctgf	2	13.9	↑	↑
Hexokinase 2	Hk2	2	8.9	↑	↑
Chemokine (C-C motif) ligand 20	Ccl20	2	8	↑	↑
v-jun sarcoma virus 17 oncogene homolog	*Jun	2	6.9	↑	↑
Methyl-CpG binding domain protein 2	Mbd2	2	3	↑	↑
TERF1 (TRF1)-interacting nuclear factor 2	Tinf2	1	2.8	↑	↑
FMS-like tyrosine kinase 1	*Flt1	2	2.3	↑	↑
Proteasome 26S subunit, non-ATPase, 10	Psm10	2	2	↑	↑
Phosphatase and tensin homolog	Pten	1,2	0.5	↓	↓
Cold shock domain protein A	Csda	1	0.5	↓	↓
cAMP responsive element binding protein 3-like 3	Creb3l3	1	0.4	↓	↓
v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	Kit	2	0.4	↓	↓
Gap junction protein, beta 1, 32 kDa	Gjb1	1,2	0.2	↓	↓
Trefoil factor 1	Tff1	3	0.1	↓	↓
Caspase 9, apoptosis-related cysteine peptidase	Casp9	2	0.5	↓	↓
Deleted in liver cancer 1	Dlc1	1,2	0.5	↓	↓
B-cell CLL/lymphoma 2	Bcl2	2	0.3	↓	↓
Inhibitor of DNA binding 1	Id1	1,2	0.3	↓	↓
Protein tyrosine phosphatase, receptor type, H	Ptprh	2	0.2	↓	↓
CD74 molecule, major histocompatibility complex, class II invariant chain	Cd74	2	0.4	↓	↓
Hepatocyte growth factor	*Hgf	1,2	0.4	↓	↓
Mannose-binding lectin (protein C) 2, soluble	Mbl2	2	0.2	↓	↓
<b>The contrary in gene expression trend</b>					
Myelocytomatosis oncogene	Myc	1,2	19.7	↓	↑
Sprouty homolog 2	Spry2	2	8.1	↓	↑
Growth arrest and DNA-damage-inducible, beta	Gadd45b	2	55.7	↓	↑
Serine peptidase inhibitor, Kunitz type, 2	Spint2	2	7.2	↓	↑
MAD homolog 4	Smad4	1,2	3	↓	↑
Fibrinogen-like 1	Fgl1	2	2.2	↓	↑
Caspase 8, apoptosis-related cysteine peptidase	Casp8	2	10.6	↓	↑
Interferon gamma	Ifng	1,2	6.5	↓	↑
Tumor protein p53	Tp53	1,2,3	2.9	↓	↑
Early growth response 1	*Egr1	2	18.6	↓	↑
Transcription factor 1, hepatic	Tcf1	2	6.8	↓	↑
O-6-methylguanine-DNA methyltransferase	Mgmt	2	4.3	↓	↑
Glutathione S-transferase theta 1	Gstt1	2	3.2	↓	↑

Glutathione S-transferase M1	Gstm1	2	2.2	↓	↑
Wingless-type MMTV integration site family, member 1	Wnt1	2	0.5	↑	↓
SHC (Src homology 2 domain containing)	Shc1	2	0.5	↑	↓
Transforming protein 1					
Inhibitor of kappaB kinase beta	Ikkbb	1,2	0.3	↑	↓
FK506 binding protein 4, 59 kDa	Fkbp4	2	0.3	↑	↓
Transcription factor 7-like 2	Tcf7l2	2	0.2	↑	↓
v-erb-b2 erythroblastic leukemia viral oncogene Homolog 2	ErbB2	3	0.1	↑	↓
Heat shock 70kDa protein 1A	Hspa1a	2	0.2	↑	↓
Heat shock 70kDa protein 5	Hspa5	1,2	0.1	↑	↓
High mobility group AT-hook 1	Hmga1	2	0.4	↑	↓
Ras homolog gene family, member C	Rhoc	2	0.3	↑	↓
Cortactin	Cttn	2	0.1	↑	↓
Serpin peptidase inhibitor, clade B, member 3	Serpinb3	2	0.1	↑	↓
Ephrin-B1	Efnb1	2	0.4	↑	↓
Coagulation factor II	F2	1,2	0.3	↑	↓
Trefoil factor 3	Tff3	2	0.3	↑	↓
Forkhead box A2	Foxa2	2	0.4	↑	↓
Glycogen synthase kinase 3 beta	Gsk3b	1,2	0.4	↑	↓
ATP-binding cassette, sub-family B, member 1A	*Abcb1a	1	0.2	↑	↓
Solute carrier family 2, member 1	*Slc2a1	2	0.2	↑	↓
Apolipoprotein E	*ApoE	2	0.1	↑	↓
<b>The comparable in gene expression trend</b>					
Lysosomal-associated protein transmembrane 4B	Laptm4b	2	2.3,0.5	↑	↑↓
Met proto-oncogene	*Met	1,2,3	2.3,0.4	↑	↑↓
Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	Nfkb1	2	2.3,0.4	↑	↑↓
Acyl-CoA synthetase long-chain family member 4	Acsl4	2	2.1,0.4	↑	↑↓
X-box binding protein 1	Xbp1	1	4.3,0.3	↑	↑↓
Nerve growth factor, beta polypeptide	Ngfb	2	3.7,0.5	↑	↑↓
Stearoyl-Coenzyme A desaturase 1	Scd1	1,2	3.5,0.3	↑	↑↓
Telomerase reverse transcriptase	Tert	1,2,3	5.3,0.3	↑	↑↓
Telomeric repeat binding factor (NIMA-interacting) 1	Terf1	1	2.2,0.4	↑	↑↓
Cadherin 17	Cdh17	2	26.1,0.2	↑	↑↓
Glycoprotein (transmembrane) nmb	GpnmB	2	9.2,0.3	↑	↑↓
Claudin 10	Cldn10	2	6.5,0.3	↑	↑↓
Plasminogen activator, urokinase	Plau	2	3.0,4	↑	↑↓
Secreted phosphoprotein 1	Spp1	2,3	2.7,0.5	↑	↑↓
Alpha-2-macroglobulin	*A2m	2	46.2,0.4	↑	↑↓
Chemokine (C-C motif) receptor 1	Ccr1	2	27.9,0.4	↑	↑↓
Matrix metalloproteinase 9	Mmp9	1,2	9.5,0.5	↑	↑↓
Angiopoietin 1	*Angpt1	2	9.2,0.2	↑	↑↓
Mucin 1, cell surface associated	Muc1	2,3	6.8,0.2	↑	↑↓
Heparanase	*Hpse	2	6.3,0.3	↑	↑↓
Megalencephalic leukoencephalopathy with subcortical cysts 1	Mlc1	2	4.3,0.4	↑	↑↓
Kinase insert domain protein receptor	*Kdr	2	2.4,0.4	↑	↑↓
Prostaglandin-endoperoxide synthase 2	Ptgs2	1,2,3	2.1,0.1	↑	↑↓
Dual specificity phosphatase 1	Dusp1	2	6.0,4	↓	↑↓
Cyclin-dependent kinase inhibitor 1C	Cdkn1c	2	2.8,0.1	↓	↑↓
Growth arrest and DNA-damage-inducible, gamma	Gadd45g	2	8.0,4	↓	↑↓
Hepatic nuclear factor 4, alpha	Hnf4a	2	4.5,0.1	↓	↑↓
Runt-related transcription factor 3	Runx3	1,2	4.3,0.5	↓	↑↓
Insulin-like growth factor binding protein 3	Igfbp3	1,2	2.7,0.4	↓	↑↓
Suppressor of cytokine signaling 3	*Socs3	2	2.5,0.1	↓	↑↓
Suppressor of cytokine signaling 1	*Socs1	1,2	2.4,0.5	↓	↑↓
Fibroblast growth factor 2	Fgf2	1,2	2.1,0.5	↓	↑↓
Fragile histidine triad gene	Fhit	1,2	7.8,0.1	↓	↑↓
Gamma-glutamyltransferase 1	Ggt1	2	3.4,0.2	↓	↑↓
Bone morphogenetic protein 7	Bmp7	2	3.0,4	↓	↑↓
E74-like factor 1 (ets domain transcription factor)	Elf1	2	3.0,4	↓	↑↓
CD80 molecule	Cd80	2	3.0,3	↓	↑↓
Glycine N-methyltransferase	Gnmt	2	2.5,0.4	↓	↑↓
Acyl-Coenzyme A oxidase 1, palmitoyl	Acox1	2	2.3,0.5	↓	↑↓

Asterisks represent the reported genes associated with liver regeneration; LT: liver tumor; RRL: rat regenerating liver; 1: hepatocarcinogenesis; 2: hepatocellular carcinoma; 3: cholangiocarcinoma. ↑ represents genes up-regulated, ↓ down-regulated, and ↑↓ up-regulated at some time points and down-regulated at others during liver regeneration. Gene expression changes in liver tumors were obtained from scientific articles, and expression changes during liver regeneration were the result of microarray detection.

(16.7 folds higher than control) at 6 h following PH might explain why it played an important role in growth of the regenerated hepatocytes. Dysregulated expression

of anti-apoptosis gene bcl-2 was present in LT as well as at metaphase and anaphase of LR, and another anti-apoptotic gene mcl1<sup>[32]</sup>, whose change trend toward up-



**Figure 2** Correlation analysis of 121 liver tumor-associated genes with liver regeneration. Twenty-nine subcategories were obtained by the analysis for detection data of Rat Genome 230 2.0 array with Microsoft Excel. 1-6: 34 genes up-regulated in both liver tumor (LT) and rat regenerating liver (RRL); 7-10: 14 genes down-regulated in both LT and RRL; 11-14: 14 genes down-regulated in LT but up-regulated in RRL; 15-20: 20 genes up-regulated in LT but down-regulated in RRL; 21-25: 23 up-regulated genes in LT were up-regulated at some time points and down-regulated at others in RRL; 26-29: 16 down-regulated genes in LT were up-regulated at some time points and down-regulated at others in RRL. X-axis represents recovery time after PH (h); Y-axis shows logarithm ratio of the signal values of genes at each time point to control.

regulation in LT was identical to that in LR; and down-regulation of pro-apoptosis genes casp9 and dlc1<sup>[33,34]</sup> occurred in LT and the metaphase of LR, which supported the idea that mcl1, casp9 and dlc1 might be related with cell survival in the two events. The differentiation-related genes id1 and ptp<sup>[35,36]</sup> down-regulation in LT and at forepart, metaphase and anaphase of LR suggested that they failed to promote cell differentiation in both events. Up-regulation of enhancement of hepatoma cell migration-related genes itgav, itgb1, adam17, dlx7, sele, mta1, wisp1 and lgals1<sup>[37-43]</sup> in LT and the entire LR, especially a sustained high-level expression (12-fold higher than control) of wisp1 at 48-60 h post-PH might imply the active cell migration in both LT and LR. According to up-regulated expression pattern in LT and almost the whole LR, metalloproteinase inhibitor timp1 was supposed to perform other biological functions except cell migration in the two events. Mif inducing angiogenesis of LT<sup>[44]</sup>, cnn1 enhancing differentiation of vascular smooth muscle cells<sup>[45]</sup> and apoptosis-inhibiting gene col18a1 encoding endostatin<sup>[46]</sup> were up-regulated both in LT and in forepart of LR, which was presumably that the three genes might

co-regulate angiogenesis in LT and LR.

Study demonstrated that six pro-proliferation genes ikkbb, shc1, erbb2, fkbp4, wnt1, tcf7l2<sup>[47-51]</sup> up-regulated in LT were down-regulated during LR, at the same time, the down-regulated genes myc and spry2 possessing anti-proliferation effect<sup>[52,53]</sup> in LT were up-regulated almost during the whole LR; two growth-promoting genes hspa5 and hspa1a<sup>[29,54]</sup> and four growth inhibitory genes gadd45b, fgl1, spint2 and smad4<sup>[55-58]</sup> were respectively up-regulated and down-regulated in LT, whose expression correspondingly underwent opposite trend at metaphase of LR comparing with LT, which was supposed to be closely associated with the differences in proliferation and growth between hepatoma cells and regenerating hepatocytes. Particularly, the expression abundance of gadd45b in human hepatocellular carcinoma was fifteen-fold lower than control<sup>[55]</sup>, just the contrary, its expression reached climax (nearly 56-fold over the control) at 2 h in rat LR, demonstrating there was a significant distinction in gadd45b expression change between normal and transforming liver cells. The down-regulated pro-apoptotic genes tp53, ifng and casp8<sup>[59-61]</sup> and the up-

regulated apoptosis-inhibitory gene *serpinb3*<sup>[62]</sup> in LT were respectively up-regulated and down-regulated at metaphase and anaphase of LR might account for the suppression of apoptosis in LT and enhancement of apoptosis at metaphase and anaphase of LR. In addition, *casp8*, inactivated caused by frame-shift mutation in hepatocellular carcinoma<sup>[61]</sup>, was up-regulated to its highest levels (10.6 folds higher than control) at 48 h post-PH, and expression of *serpinb3* declined to the lowest point (11.4 folds lower than control) at 48 h, signifying the important regulatory effect of the two genes on liver mass. Contribution of *efnb1* and *tff3* in neovascularogenesis activity<sup>[63,64]</sup> and the crucial role for *f2* in maintenance of vascular integrity<sup>[65]</sup> were helpful for understanding the hypothesis that the three up-regulated genes in LT down-regulated at metaphase and anaphase of LR implied the control of blood-vessel growth serving as one of modulation pathways of regenerated liver mass. Three hepatoma cell migration and invasion-associated genes *hmg1*, *cttn* and *rhoc* up-regulated in LT<sup>[66,67]</sup> and down-regulated in LR possibly showed the stronger migration ability of hepatoma cells.

Four up-regulated in LT genes promoting hepatoma cell proliferation including *met*, *laptm4b*, *nfk1b* and *acs4*<sup>[68,69]</sup>, revealed down-regulation at metaphase and up-regulation at anaphase of LR, and another two inhibitory genes *dusp1* and *cdkn1c*<sup>[70]</sup> were up/down-regulated in LR, i.e. the former was up-regulated at 0.5-12 and 24 h, and down-regulated at 54-60 h, while the latter was down-regulated at 6-18 h and up-regulated at 30 and 42 h; Three up-regulated *scd1*, *xbp1* and *ngfb* genes involved in hepatoma cell growth in LT<sup>[54,71,72]</sup> were up-regulated at forepart, prophase and metaphase, and down-regulated at some time points in the late phase of LR, while another six negative regulatory genes including *socs1*, *socs3*, *gadd45g*, *igfbp3*, *runx3* and *hnf4a*<sup>[73-77]</sup> down-regulated in LT had a significant increase in expression at some time points and significant decrease at others during LR. The more complicated expression of these genes during the proliferation and growth of regenerating liver cells comparing with that of hepatoma cells concluded from the above results was presumably consistent with the further improved control mechanism upon proliferation and growth of regenerating liver cells than that of hepatoma cells. Telomerase activity of TERT was interfered by *terf1* expression product<sup>[78]</sup>, and the two were up-regulated in both LT and the metaphase of LR, while down-regulated at anaphase, indicating that the balance of quantity of the two gene products was essential for maintaining telomere stability. The up-regulated genes *spp1*, *plau* and *gpnmb* promoting hepatoma cell migration and invasion<sup>[79,80]</sup> as well as the up-regulated intercellular junction-involved *cdh17* and *cldn10* genes<sup>[47,81]</sup> were up-regulated at forepart, prophase and some time points after 16h, and down-regulated at other points after 16 h, possibly illustrating that the similar cell migration and interactions in LT occurred at forepart and prophase of LR, however the difference emerged when entering the metaphase of LR. The dysregulated gene *fgf2*<sup>[82]</sup> promoting hepatoma cell apoptosis in LT was up-regulated at 4 h post-PH, consistent with the enhanced apoptotic action of regenerating liver cell at the forepart of LR, demonstrating

that it acted as a key gene involved in the regulation of apoptosis.

In conclusion, at transcriptional level, while decrease of apoptosis occurring in liver tumors, the process was still going on in LR; as far as cell growth, proliferation, differentiation, migration and angiogenesis are concerned, not only resemblances but differences exist between LT and LR. Especially, expressions of the genes, such as *ccna2*, *serpine1*, *wisp1*, *gadd45b*, *casp8* and *serpinb3*, display marked changes in two events, so their actions deserve the further study. Of course, the process of DNA → mRNA → protein → function could be influenced by many factors including gene mutation, protein interaction etc. Therefore, the further analyses are required for confirming the above results using techniques such as gene addition, knock-out, RNAi, etc.

## COMMENTS

### Background

The liver is susceptible to tumorigenesis, and it's also an organ with strong regenerating capacity. Both hepatocarcinogenesis and LR are associated with cell proliferation and growth.

### Research frontiers

Most research works had been done on the mechanism of hepatocarcinogenesis and LR, but heretofore no research report was found in investigating the correlation between them.

### Innovations and breakthroughs

121 genes associated with both hepatocarcinogenesis and LR were found. Their expression changes in LR were analyzed by Rat Genome 230 2.0 Array, and their expression similarities and differences in hepatocarcinogenesis and LR were compared.

### Applications

Our results may provide basic and useful data for further research on both LT therapy and LR mechanism.

### Terminology

PH model, an effective surgical operation to trigger LR, was established by Higgins and Anderson in 1931. The hepatectomized rat, whose left and middle lobes of liver were removed, can recover its liver mass after about a week, so it's widely used to investigate LR mechanism.

### Peer review

This is an important work examining common genes associated with HCC and LR.

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