VERSITA

CELLULAR & MOLECULAR BIOLOGY LETTERS
http://www.cmbl.org.pl

Received: 20 November 2013
Final form accepted: 04 March 2014
Published online: March 2014

Volume 19 (2014) pp 181–200
DOI: 10.2478/s11658-014-0188-2

Short communication

STUDY ON THE ACTIVITY OF THE SIGNALING PATHWAYS REGULATING HEPATOCYTES FROM G0 PHASE INTO G1 PHASE DURING RAT LIVER REGENERATION

MENGHUA LI[1, 2], XIAOCHUN ZHOU[1, 2], JINXIN MEI[1, 2], XIAOFANG GENG[1, 2], YUN ZHOU[1, 2], WEIMIN ZHANG[2] and CUNSHUAN XU[1, 2, *]

[1]College of Life Science, Henan Normal University, Xinxiang 453007, P. R. China, [2]Key Laboratory for Cell Differentiation Regulation, Xinxiang 453007, P. R. China

Abstract: Under normal physiological conditions, the majority of hepatocytes are in the functional state (G0 phase). After injury or liver partial hepatectomy (PH), hepatocytes are rapidly activated to divide. To understand the mechanism underlying hepatocyte G0/G1 transition during rat liver regeneration, we used the Rat Genome 230 2.0 Array to determine the expression changes of genes, then searched the GO and NCBI databases for genes associated with the G0/G1 transition, and QIAGEN and KEGG databases for the G0/G1 transition signaling pathways. We used expression profile function (*Et*) to calculate the activity level of the known G0/G1 transition signal pathways, and Ingenuity Pathway Analysis 9.0 (IPA) to determine the interactions among these signaling pathways. The results of our study show that the activity of the signaling pathways of HGF, IL-10 mediated by p38MAPK, IL-6 mediated by STAT3, and JAK/STAT mediated by Ras/ERK and STAT3 are significantly increased during the priming phase (2–6 h after PH) of rat liver regeneration. This leads us to conclude that during rat liver regeneration, the HGF, IL-10, IL-6 and JAK/STAT signaling pathways play a major role in promoting hepatocyte G0/G1 transition in the regenerating liver.

Keywords: Rat liver regeneration, Signal transduction, Hepatocyte G0/G1 transition, HGF, IL-10, IL-6, JAK/STAT, p38MAPK, Ras/ERK, STAT3, Ingenuity pathway analysis 9.0 (IPA)

* Author for correspondence. Email: cellkeylab@126.com, phone: +086 373 3326001, fax: +086 373 3326524

Abbreviations used: ERK – extracellular regulated protein kinases; HGF – hepatocyte growth factor; IL-6 – interleukin-6; IL-10 – interleukin-10; JAK – Janus kinase; JNK – c-Jun NH2-terminal kinase; MAPK – mitogen-activated protein kinase; NF-κB – nuclear factor κB; STAT3 – signal transducer and activator of transcription

INTRODUCTION

The liver is an important organ with an enormous capacity to regenerate [1]. Hepatocytes (HCs) are the main functional cell populations in the liver. HCs make up 70–80% of the hepatic mass and 65% of the total hepatic cell number [2, 3]. They are involved in numerous physiological activities including the activation/expression of a series of cytokines and growth factors [4]. Under normal physiological conditions, the majority of adult liver HCs are in the functional state [5, 6], about a split phase per twenty thousand liver cells [7]. However, when injured or during a partial hepatectomy (PH), HCs can rapidly enter the cell cycle together with other liver cells to compensate for lost liver tissues until the function of the liver is restored [8, 9].

It has been well documented that DNA replication of HCs begins 12 h after PH in rats, and that the first wave of DNA synthesis occurs at 24 h, with a smaller peak at 36–66 h. The process of liver regeneration is generally complete by day 7. Therefore, the points in which the liver regeneration process is involved can be divided into 3 phases: 2–6 h, when HCs are activated and G0/G1 transition occurs (priming phase); 12–72 h, when cell proliferation takes place (proliferative phase); and 120–168 h, when liver regeneration terminates (terminal phase) [10].

In addition, liver regeneration involves a sequence of orchestrated events, including the activation and expression of a series of cytokines and growth factors, accompanied by cell proliferation, cell differentiation and dedifferentiation, and tissue structure reconstruction [11]. Here, we mainly studied the transition of HCs from G0 to G1 phase in rat liver regeneration induced by PH. This transition involves many factors and regulated by various signaling pathways [12].

In general, there are 30 signaling pathways that regulate G0/G1 transition. Among them, HGF signaling mediated by STAT3, JNK, and ERK can regulate G0/G1 transition through the activation of transcription factors including STAT3, c-JUN, c-FOS, ATF2, ETS, and ELK1 [13]. IL-10 signaling mediated by STAT3, JNK, NF-κB, and p38MAPK can regulate G0/G1 transition by activating the transcription factors of NF-κB and STAT3 as well as c-JUN, c-FOS, and ELK-1 [14]. IL-6 signaling mediated by JNK, NF-κB, ERK, and STAT3 can regulate G0/G1 transition by activating the transcription factors of NF-IL-6, STAT3, ELK-1, c-JUN, c-FOS, and NF-κB [15–20]. JAK/STAT signaling mediated by ERK, STAT3, and PI3K/AKT can regulate G0/G1 transition by activating transcription factor STATs [21]. Multiple signaling molecules and transcription factors are shared among the signaling pathways, meaning there are numerous cross-interactions among these pathways.

In order to understand the type, quantity, timing, pattern, and mode of the signaling pathways involved in HC G0/G1 transition during rat liver regeneration, we first detected genes associated with liver regeneration using Rat Genome 230 2.0 Array. We then searched the GO and NCBI databases for genes associated

with G0/G1 transition, and QIAGEN and KEGG databases for the G0/G1 transition signaling pathways. Finally, we used expression profile function (*Et*) to calculate the activity level of the known G0/G1 transition signal transduction pathways, and Ingenuity Pathway Analysis 9.0 (IPA) to determine interactions among these signaling pathways. The results of our study show that the signaling activities of HGF, IL-10 mediated by p38MAPK, IL-6 mediated by STAT3, and JAK/STAT mediated by Ras/ERK and STAT3 are significantly increased during the priming phase (2–6 h after PH), which suggests that they play important roles in the regulation of HC G0/G1 transition during rat liver regeneration.

MATERIALS AND METHODS

Isolation and identification of HCs from rat regenerating liver

Male adult Sprague-Dawley rats, weighing 230 ± 20 g, from the Animal Center of Henan Normal University were used. The rats had free access to water and food and were kept at 21 ± 2ºC with relative humidity at 60 ± 10%, illumination 12 h/day (8 a.m. – 8 p.m.). A total of 114 rats were randomly divided into 19 groups with six rats per group: nine PH groups, nine SO (sham operation) groups, and one control group.

The rats in the PH groups underwent two-thirds hepatectomy in accordance with the method of Higgins and Anderson [22]. After PH, the abdominal cavity was reopened to collect liver tissues at 2, 6, 12, 24, 30, 36, 72, 120, and 168 h. HCs were then isolated using two-step collagenase perfusion and percoll density gradient centrifugation [2, 23]. The isolated HCs were identified via immuno-cytochemistry using two marker proteins, ALB and G6P [24]. Cell viability was measured using the Trypan blue exclusion method [25]. The purity and viability of the HCs were both greater than 95%. All of the experiments were carried out in accordance with the Current Animal Protection Law of China.

Rat Genome 230 2.0 microarray detection and data analysis

Total RNA was extracted and purified following the protocols previously described [26]. Briefly, biotin-labeled cRNA was prepared using GeneChip IVT kit according to the manufacturer's instructions [27]. The hybridization was carried automatically using a GeneChip Fluidics Station 450 (Affymetrix Inc). The results were scanned and converted into signal values using a GeneChip scanner 3000 (Affymetrix Inc). The signal values were normalized according to the manufacturer's instructions.

The p-values were determined using Affymetrix GCOS 2.0 software [28]. If the p-value of a gene is < 0.05, the gene is present (P), < 0.065 is marginal (M), and > 0.065 is absent (A). A gene was considered significantly changed if the ratio of its PH value relative to that for the control was ≥ 3 or ≤ 0.33.

The *F*-test was used to analyze the significance of gene expression difference between the PH and SO groups. At least at one time point during liver regeneration, the genes found to be significantly changed with $p < 0.05$ were

defined as genes associated with liver regeneration. To minimize the experimental operation and microarray errors, three repetitions were performed at each time point, and the average value of three independent assays was used for the statistical analyses.

Real-time fluorescent quantitative polymerase chain reaction (qRT-PCR)

To validate the reliability of microarray data, the expression level of 3 HC genes: *TRIM24*, *AFP* and *AGT* were examined using RT-PCR. *β-ACTIN* (NM_031144) was included as an internal control. The primers were designed using Primer Express 5.0 software. mRNA was prepared from the purified rat liver cells using the reverse transcription kit (Promega) to obtain the first-strand cDNA. The quantitative PCR was performed under the following conditions: 2 min at 95ºC, followed with 40 cycles for 15 s at 95ºC, 15 s at 60ºC, and 30 s at 72ºC. Each sample was performed in triplicate. Standard curves were generated from five repeated ten-fold serial dilutions of cDNA, and the copy numbers of the target genes in every milliliter of the sample were calculated according to the standard curve.

Determination of genes associated with liver regeneration related to G0/G1 transition or signaling pathways

To determine how many genes associated with liver regeneration participated in HC G0/G1 transition, we searched for G0/G1 transition-related genes in the GO, NCBI, and IPA databases. The term "G0/G1 transition" was entered into website GO and NCBI, or into "Functions and Diseases" of IPA. The collected genes were then uploaded onto IPA and analyzed by "Canonical Pathway" to obtain cell G0/G1 transition signaling pathways. The genes defined by Rat Genome 230 2.0 microarray analyses as being associated with liver regeneration were compared with the G0/G1 transition-related genes or G0/G1 transition signaling-related genes to obtain the genes related to G0/G1 transition or G0/G1 transition signaling.

The calculation of G0 / G1 transition activity coefficient -log (p-value)

Genes related to G0/G1 transition signaling were uploaded to the IPA software "Dataset Files" column, and the physiological activity coefficient –log(p-value) was calculated through "Comparison Analyses" in the IPA software. The *F*-test highlights differences in the –log(p-value) between the PH and SO groups. When the activity of a pathway is significantly ($p \leq 0.05$) or very significantly ($p \leq 0.01$) different between PH and SO, it indicates that this pathway regulates HC G0/G1 transition during rat liver regeneration.

Analysis of gene synergy (*Et*)

Liver regeneration is a complex and highly ordered process involving numerous signaling pathways and factors. It is extremely difficult to determine the functions of signaling genes through the signaling networks. We have established a mathematical model (*Et*) [29] to analyze the synergy between

genes. In brief, the ratio values of G0/G1 transition signaling pathway-related and G0/G1 transition-related genes were converted into Log ratio values, then the log ratio values for these genes were put into the formula of *Et* to calculate the gene synergy. The *Et* values of the control are defined as 0. When the *Et* value is greater than 0 and the *Et* of a PH group was greater than that of the SO group, physiological activity has increased. When *Et* value is less than 0 and the PH group value was less than that of the SO group, physiological activity has decreased.

Analysis of the correlation between G0/G1 transition signaling transduction activity and hepatocyte G0/G1 transition

To identify G0/G1 transition signaling pathways, we input the known G0/G1 transition signaling pathways into "Pathways and tox lists" of the IPA software to get their compositions, functions, and relationships, and their mode of activation of transcription factors. To determine the activity level of each signaling pathway and its branches, we used the *Et* to analyze the signaling activity as described in the Materials and Methods section.

To confirm the interaction relationships between G0/G1 signaling pathways and HC G0/G1 transition, transcription factors of G0/G1 transition signaling pathways constructed above were input into TRED and Lymph TF-DB [30, 31] to find their downstream target genes. Then the *Et* value of G0/G1 transition activity was calculated according to the section "Analysis of gene synergy (*Et*)" above. Finally, we matched the signaling activity and G0/G1 transition activity to confirm the correlation between G0/G1 transition signaling and liver cell G0/G1 transition.

RESULTS

Isolation and identification of HCs

As an example, here we present the morphology and vitality of hepatocytes isolated from rats 0, 6, 24, 72, and 168 h after PH. The purity and viability of the HCs were greater than 95% (Fig. 1).

Consistency of qRT-PCR and microarray results

In order to determine the validity of the results obtained using the microarray analysis, three randomly selected genes were subjected to quantitative RT-PCR analysis. RT-PCR and Rat Genome 230 2.0 Array showed that the expression trends of these genes, including *TRIM24*, *AFP* and *AGT*, were generally consistent, suggesting that the array check results were reliable (Fig. 2). The RT-PCR results for other genes were published elsewhere [32].

Signal transduction activity of HC G0/G1 transition signaling pathways in rat liver regeneration

From the "Ingenuity Canonical Pathways" of IPA and the QIAGEN website, we found that G0/G1 transition is regulated by 30 signaling pathways. The value of gene expression abundance detected by Rat Genome 230 2.0 was input into IPA

software to calculate the –log(p-value). The results of the IPA software comparison analysis and F-test indicated that: the G0/G1 transition activities in HCs regulated by HGF, IL-10, and IL-6 signaling pathways in the PH group and those of the SO group were significantly different ($p \leq 0.05$); the G0/G1transition activities regulated by JAK /STAT signaling pathways in the PH group and those of the SO group were very significantly different ($p \leq 0.01$) and the results

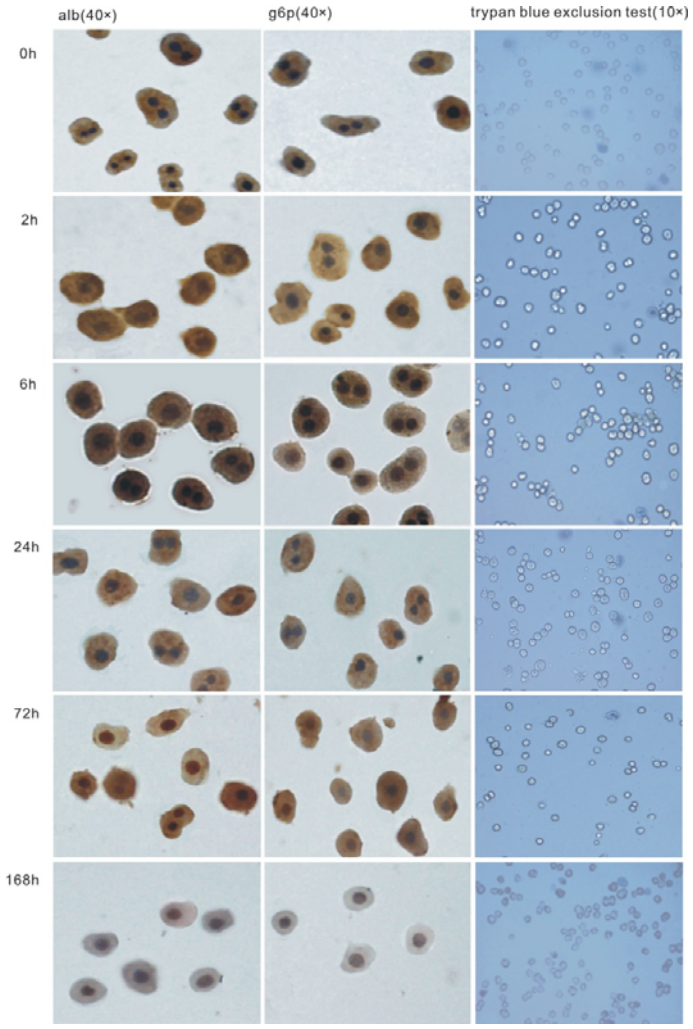

Fig. 1. Images from ALB, G6P immunostaining, and Trypan blue exclusion tests of HCs isolated from rat liver 0, 6, 24, 72, and 168 h after partial hepatectomy. A few purified hepatocytes were taken and fixed with 4% paraformaldehyde in PBS, and then smeared onto polylysine-coated glass slides. After the cell suspension dried, a peroxidase block step was performed. Then the sections were incubated separately with a 1:200 dilution (V/V) of primary pAb and then with a 1:2000 (V/V) diluted secondary antibody. HCs were stained with 0.4% Trypan blue.

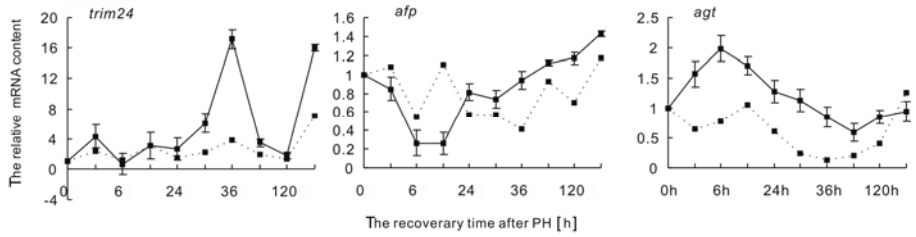

Fig. 2. Comparison of relative mRNA level detected with Affymetrix Rat Genome 230 2.0 arrays (solid lines) and real-time PCR (dotted lines). The vertical axis indicates the relative mRNA level; the horizontal axis indicates the recovery time points after partial hepatectomy. An error bar based on standard deviation from three different repetitions was added to confirm the reliability of chip results.

for the rest of the signaling pathways were not significantly different between the PH and SO groups (Table 1). The –log (p-value) calculated using the "Comparison Analyses" in the IPA software and the *F*-test highlighted the differences between the PH and SO groups. The signaling of HGF, IL-10, IL-6, and JAK/STAT were considered to regulate the G0/G1 transition of HCs.

The expression change of genes related to G0/G1 transition signaling pathways in the priming phase during rat liver regeneration

The results of the expression change of genes related to G0/G1 transition signaling pathways detected by Rat Genome 230 2.0 Array showed that 29 genes were upregulated, three genes were downregulated, and one gene was up- or downregulated in the priming phase during rat liver regeneration (Table 2A). The HGF signaling pathway included 76 genes: 14 genes were related to rat liver regeneration (*MAP3K6*, *MAP3K8*, *ELF*, etc.), 13 genes were upregulated, and *MRAS* was downregulated (Table 2B). The IL-10 signaling pathway included 41 genes: 12 genes were related to rat liver regeneration (*IL1R2*, *IL1RN*, *SOCS3*, etc.), 11 genes were upregulated, and *MAP2K6* was downregulated (Table 2C). The IL-6 signaling pathway included 88 genes: 20 genes were related to rat liver regeneration (*A2M*, *IL1R2*, *IL1RN*, etc.), 18 genes were upregulated, and *MAP2K6* and *MRAS* were downregulated (Table 2D). The JAK/STAT signaling pathway included 58 genes: 10 genes were related to rat liver regeneration (*FOS*, *SOCS3*, *STAT3*, etc.), 7 genes were upregulated, *PIAS3* and *MRAS* were downregulated, and *CISH* was up- or downregulated (Table 2E).

The expression changes of HC G0/G1 transition-related genes regulated by G0/G1 transition pathways in the priming phase during rat liver regeneration

The NCBI library showed that 1081 genes are related to G0/G1 transition (May 21, 2013). Rat Genome 230 2.0 contained 818 of the 1081 genes and 144 of these genes were significantly altered during the priming phase. Of these, 127 genes were upregulated, including *A2M*, *GUCY2C*, and *TGM1*; 15 genes were downregulated, such as *ABCC2*, *ACLY*, *CEBPA*, and *AKR1B7*; and one gene (*CYP2C12*) was up- or downregulated (Table 3). Those genes were regulated by the

HGF, IL-10, IL-6 and JAK/STAT signaling pathways, involving the transcription factors STAT3, ELK1, c-JUN, c-FOS, NF-κB, NFIL6, SP1, ETS, and STAT.

Table 1. HC G0/G1 transition signaling pathways in the priming phase during rat liver regeneration.

Signaling pathways	Liver regeneration-related genes			*F*-test results
	UR	DR	UR/DR	
Pancreatic adenocarcinoma signaling	15	3	0	0.2901
Small cell lung cancer signaling	5	2	0	0.5152
p53 signaling	14	1	1	0.9756
HGF signaling	25	4	0	0.0471*
Ovarian cancer signaling	21	5	0	0.4979
Prostate cancer signaling	7	3	0	0.8267
Wnt/β-catenin signaling	30	2	0	0.8909
Melanoma signaling	8	3	0	0.4473
PTEN signaling	17	5	1	0.5252
PI3K/AKT signaling	18	3	0	0.6615
Estrogen-dependent breast cancer signaling	13	5	0	0.7937
IL-10 signaling	17	2	0	0.0282*
GM-CsF signaling	6	3	1	0.2766
Renal cell carcinoma signaling	9	4	0	0.5463
JAK/STAT signaling	13	5	1	0.0071**
HER-2 signaling in breast cancer	8	4	0	0.9536
PDGF signaling	10	1	0	0.6608
IL-6 signaling	30	5	0	0.0419*
Acute phase response signaling	25	4	0	0.4348
Oncostatin M signaling	4	1	0	0.3737
NF-κB signaling	33	5	1	0.3404
iNOs signaling	12	2	0	0.3420
PPAR signaling	16	2	1	0.4222
Role of NANOG in mammalian embryonic stem cell pluripotency	18	3	1	0.1196
IL-17 signaling	18	5	0	0.3037
Role of Wnt/Gsk-3β signaling in the pathogenesis of influenza	11	1	0	0.4793
Interferon signaling	3	2	0	0.6866
Telomerase signaling	19	6	0	0.8333
Role of IL-17F in allergic inflammatory airway diseases	6	0	0	0.7913
Renin-angiotensin signaling	31	3	0	0.9774

*Indicates a significant difference between the PH and SO groups ($p \leq 0.05$), **Indicates a very significant difference between the PH and SO groups ($p \leq 0.01$).

Table 2. The expression changes of genes related to G0/G1 transition signaling pathways in the priming phase during rat liver regeneration. Data are presented as the log ratio of PH groups vs. the control group. Dark gray fields indicate fold-change values ≥ 3, light gray fields indicate fold-change values ≤ 0.33, and white fields indicate fold-change values between 0.33 and 3.

	Genes	Recovery time (h) after PH			
		HC		Liver tissue	
		2h	6h	2h	6h
A	A2M	**9.65**	**41.30**	2.73	**4.21**
	BCL2L1	**3.46**	1.38	1.90	1.33
	CCKBR	**3.54**	**3.95**	1.28	1.28
	CDKN1A	**3.40**	2.62	**3.61**	2.38
	CDKN2A	1.40	**5.45**	1.29	1.36
	CISH	**3.68**	**0.28**	**6.11**	**3.26**
	ELF5	**5.64**	**6.04**	1.32	2.81
	ETS2	2.93	**3.41**	1.50	1.72
	FOS	**5.02**	1.12	1.74	2.22
	HGF	1.37	**3.23**	**3.86**	**3.88**
	HSPB1	**3.63**	**3.27**	0.84	0.55
	HSPB3	**3.26**	2.33	0.42	1.63
	HSPB7	2.74	**5.97**	**3.56**	0.82
	IL1R1	**4.31**	**4.00**	**3.30**	2.98
	IL1R2	**33.59**	**21.03**	**23.69**	**19.01**
	IL1RL1	1.68	**5.00**	**3.47**	**3.19**
	IL1RN	**20.57**	**7.29**	**10.96**	**12.21**
	JUN	**3.29**	2.68	2.77	2.04
	LBP	2.86	**5.25**	2.22	**3.60**
	MAP2K6	**0.13**	**0.30**	0.84	1.59
	MAP3K6	**9.77**	**18.63**	**3.05**	2.72
	MAP3K8	**14.44**	2.28	**3.43**	1.47
	MAPK10	**3.52**	2.16	2.33	2.93
	MAPK11	1.09	**3.43**	1.35	1.09
	MAPK13	**3.31**	2.52	0.69	0.83
	MRAS	**0.32**	0.88	0.95	0.85
	PIAS3	**0.30**	0.76	0.53	0.90
	PRKCQ	**3.14**	1.07	2.44	**3.15**
	PRKCZ	**4.11**	0.71	0.54	**4.83**
	PTPN1	**3.37**	2.46	**4.34**	2.82
	SOCS3	**7.22**	**3.87**	**4.45**	2.92
	STAT3	**3.90**	**4.01**	2.13	2.67
	TNF	1.71	**4.98**	**4.17**	**9.12**

	Genes	Recovery time (h) after PH			
		HC		Liver tissue	
		2h	6h	2h	6h
B	HGF Signaling				
	CDKN1A	**3.40**	2.62	**3.61**	2.38
	CDKN2A	1.40	**5.45**	1.29	1.36
	ELF5	**5.64**	**6.04**	1.32	2.81
	ETS2	2.93	**3.41**	1.50	1.72
	FOS	**5.02**	1.12	1.74	2.22
	HGF	1.37	**3.23**	**3.86**	**3.88**
	JUN	**3.29**	2.68	2.77	2.04
	MAP3K6	**9.77**	**18.63**	**3.05**	2.72
	MAP3K8	**14.44**	2.28	**3.43**	1.47
	MAPK10	**3.52**	2.16	2.33	2.93
	MRAS	**0.32**	0.88	0.95	0.85
	PRKCQ	**3.14**	1.07	2.44	**3.15**
	PRKCZ	**4.11**	0.71	0.54	**4.83**
	STAT3	**3.90**	**4.01**	2.13	2.67
C	IL-10 Signaling				
	FOS	**5.02**	1.12	1.74	2.22
	IL1R1	**4.31**	**4.00**	**3.30**	2.98
	IL1R2	**33.59**	**21.03**	**23.69**	**19.01**
	IL1RL1	1.68	**5.00**	**3.47**	**3.19**
	IL1RN	**20.57**	**7.29**	**10.96**	**12.21**
	JUN	**3.29**	2.68	2.77	2.04
	LBP	2.86	**5.25**	2.22	**3.60**
	MAP2K6	**0.13**	**0.30**	0.84	1.59
	MAPK11	1.09	**3.43**	1.35	1.09
	MAPK13	**3.31**	2.52	0.69	0.83
	SOCS3	**7.22**	**3.87**	**4.45**	2.92
	STAT3	**3.90**	**4.01**	2.13	2.67
D	IL-6 Signaling				
	A2M	**9.65**	**41.30**	2.73	**4.21**

	Genes	Recovery time (h) after PH			
		HC		Liver tissue	
		2h	6h	2h	6h
	FOS	**5.02**	1.12	1.74	2.22
	HSPB1	**3.63**	**3.27**	0.84	0.55
	HSPB3	**3.26**	2.33	0.42	1.63
	HSPB7	2.74	**5.97**	**3.56**	0.82
	IL1R1	**4.31**	**4.00**	**3.30**	2.98
	IL1R2	**33.59**	**21.03**	**23.69**	**19.01**
	IL1RL1	1.68	**5.00**	**3.47**	**3.19**
	IL1RN	**20.57**	**7.29**	**10.96**	**12.21**
	JUN	**3.29**	2.68	2.77	2.04
	LBP	2.86	**5.25**	2.22	**3.60**
	MAP2K6	**0.13**	**0.30**	0.84	1.59
	MAPK10	**3.52**	2.16	2.33	2.93
	MAPK10	**3.52**	2.16	2.33	2.93
	MAPK11	1.09	**3.43**	1.35	1.09
	MAPK13	**3.31**	2.52	0.69	0.83
	MRAS	**0.32**	0.88	0.95	0.85
	SOCS3	**7.22**	**3.87**	**4.45**	2.92
	STAT3	**3.90**	**4.01**	2.13	2.67
	TNF	1.71	**4.98**	**4.17**	**9.12**
E	JAK/STAT Signaling				
	BCL2L1	**3.46**	1.38	1.90	1.33
	CCKBR	**3.54**	**3.95**	1.28	1.28
	CDKN1A	**3.40**	2.62	**3.61**	2.38
	CISH	**3.68**	**0.28**	**6.11**	**3.26**
	FOS	**5.02**	1.12	1.74	2.22
	MRAS	**0.32**	0.88	0.95	0.85
	PIAS3	**0.30**	0.76	0.53	0.90
	PTPN1	**3.37**	2.46	**4.34**	2.82
	SOCS3	**7.22**	**3.87**	**4.45**	2.92
	STAT3	**3.90**	**4.01**	2.13	2.67

Table 3. The expression changes of G0/G1 transition-related genes regulated by G0/G1 transition pathways in the priming phase in rat regenerating HCs and liver tissue after PH. Data are presented as the log ratio of the PH groups vs. the control group. Dark gray fields indicate fold-change values ≥ 3, light gray fields indicate fold-change values ≤ 0.33, and white fields indicate fold-change values between 0.33 and 3. The numerals from 1 to 9 in the "TF" column sequentially represent transcription factors STAT3, ELK1, c-JUN, c-FOS, NF-κB, NFIL6, MYC, ETS, and STAT. The symbols a-d in the "signaling" column stand for HGF, IL-10, IL-6, and JAK/STAT signaling, respectively.

Genes	TF	Signaling	Recovery time (h) after PH			
			HC		Liver tissue	
			2h	6h	2h	6h
ANGPT2	8	a	**3.65**	2.92	1.19	1.17
B3GALT4	8	a	**0.31**	0.55	0.82	0.91
F13A1	8	a	**3.16**	**3.67**	1.91	**3.15**
JUNB	8	a	**8.32**	**4.32**	**4.23**	**3.06**
KDR	8	a	**0.22**	0.33	0.55	0.43
MYL7	8	a	**3.30**	**3.41**	**3.38**	1.14
NCDN	8	a	**5.10**	**6.18**	2.05	**5.36**
NELL1	8	a	2.50	**3.96**	0.81	1.36
NNAT	8	a	1.84	**4.81**	2.94	**3.51**
NOS3	8	a	**0.24**	0.87	0.72	0.89
NTRK1	8	a	1.30	**3.76**	2.12	1.59
PECAM1	8	a	2.73	**4.18**	1.13	1.24
POR	8	a	**3.33**	2.68	1.71	1.70
SH2D1A	8	a	**3.39**	**5.90**	1.06	**4.26**
THBD	8	a	**6.28**	**4.23**	**5.87**	**5.98**
VCAM1	8	a	**4.91**	**3.30**	2.36	2.67
ACLY	7	b	1.41	**0.26**	0.93	0.38
ADRA1D	7	b	**3.31**	1.10	**0.27**	0.44
AKR1B7	7	b	**85.21**	**0.15**	**0.06**	0.33
BACH1	7	b	**7.30**	1.06	**5.42**	2.09
BST1	7	b	2.67	**5.00**	**11.58**	**20.35**
BTK	7	b	**5.99**	**5.61**	1.24	1.20
BUB1B	7	b	**4.32**	1.10	0.54	1.42
CDH5	7	b	**3.75**	**4.21**	1.06	0.96
CKMT1	7	b	2.98	**3.88**	2.09	1.83
CLDN19	7	b	**0.28**	0.49	**0.26**	0.88
COL11A1	7	b	**5.84**	2.93	**3.82**	2.45
CPT1A	7	b	2.56	**3.72**	2.26	2.95
CPT1B	7	b	1.64	**3.58**	1.06	1.89
CRABP2	7	b	**3.74**	**4.26**	1.77	1.63
CTGF	7	b	**12.74**	**3.61**	**8.03**	**4.02**

Genes	TF	Signaling	Recovery time(h) after PH			
			HC		Liver tissue	
			2h	6h	2h	6h
BCL3	5 7	b	**4.35**	**3.20**	2.96	1.96
ABCC2	6	c	1.25	**0.33**	0.83	0.37
ALOX5AP	6	c	**7.88**	**11.45**	1.55	1.50
CCNE1	6	c	**3.81**	**4.10**	0.93	1.04
CEBPA	6	c	0.84	**0.22**	1.09	1.05
CRH	6	c	**6.88**	**7.16**	2.14	1.85
GADD45G	6	c	**3.60**	2.21	**15.70**	**7.71**
HMGCS1	6	c	0.77	**0.16**	0.68	1.06
IL24	6	c	**4.57**	**3.86**	2.09	2.40
PDGFRA	6	c	2.39	**3.70**	0.97	1.21
SH2D3C	6	c	**3.44**	**3.55**	1.29	1.44
SPINK1	6	c	**5.56**	**12.12**	**8.51**	**5.81**
SST	6	c	**0.20**	1.16	0.92	0.56
ALOX15	9	d	**4.36**	**4.36**	2.50	0.67
BCL6	9	d	2.92	**8.08**	1.31	1.34
GATA3	9	d	2.51	**12.77**	1.72	**8.01**
IL1R1	9	d	**4.31**	**4.00**	**3.30**	2.98
SELE	9	d	1.69	**3.31**	**9.48**	1.94
SERPINA3N	9	d	2.17	**3.14**	1.46	1.61
SHH	9	d	1.42	**3.17**	1.13	0.99
ABCC1	7 8	ab	**6.86**	**4.80**	1.19	1.62
CCNB1	7 8	ab	**3.43**	1.18	0.77	0.86
CYP11A1	7 8	ab	2.96	**5.31**	**0.11**	0.83
Ets2	7 8	ab	2.93	**3.41**	1.50	1.72
Kit	7 8	ab	0.92	**3.21**	0.58	1.05
Krt18	7 8	ab	**3.30**	**3.27**	1.81	**3.73**
PKD1	7 8	ab	2.66	**4.62**	1.52	2.32
SPP1	7 8	ab	**5.17**	**3.25**	2.09	**8.19**
THY1	7 8	ab	2.22	**7.92**	2.88	2.25
CSF3R	6 8	ac	**5.77**	**5.12**	2.74	**4.67**
HGF	6 8	ac	1.37	**3.23**	**3.86**	**3.88**

Genes	TF	Signaling	Recovery time (h) after PH			
			HC		Liver tissue	
			2h	6h	2h	6h
CXCL1	7	b	**47.47**	**28.53**	**8.78**	**4.20**
CYP17A1	7	b	**3.26**	0.62	0.93	0.42
CYP4F5	7	b	1.76	**5.00**	**3.53**	1.63
EFEMP1	7	b	**4.15**	1.52	**8.45**	**10.93**
EGLN3	7	b	1.78	**3.05**	1.42	1.46
GLYAT	7	b	0.56	**0.12**	0.62	**0.09**
GPC3	7	b	1.66	**7.74**	**11.07**	**5.64**
GRIN2A	7	b	2.22	**3.60**	1.83	0.93
GUCY2C	7	b	**25.32**	**60.44**	0.91	**4.63**
HSD17B1	7	b	2.12	**3.50**	1.06	1.14
IGFBP1	7	b	**4.77**	1.04	2.49	1.38
KLF5	7	b	1.33	**5.78**	**5.09**	**4.13**
LDHB	7	b	2.45	**4.55**	0.85	1.44
LDLR	7	b	1.48	**0.16**	0.76	0.74
LHCGR	7	b	1.93	**3.47**	2.45	0.48
LOX	7	b	1.26	**5.68**	**33.40**	**4.64**
MADCAM1	7	b	0.54	**3.68**	1.68	**4.71**
MERTK	7	b	**3.84**	0.73	0.68	1.37
MT3	7	b	**5.38**	**5.17**	**8.37**	**5.19**
MUC5AC	7	b	**4.60**	**4.79**	2.95	**4.68**
MYH2	7	b	**11.60**	**5.08**	0.95	0.61
NF1	7	b	**4.33**	**4.50**	**3.18**	**5.02**
NQO2	7	b	0.77	**0.21**	0.69	**0.21**
ODC1	7	b	2.55	**3.44**	1.41	2.95
PKM2	7	b	**4.39**	**4.08**	1.59	**4.37**
PTGER4	7	b	**4.88**	2.06	**5.29**	**8.56**
PTH	7	b	2.61	**4.22**	**5.08**	1.48
RHOB	7	b	**13.48**	**3.08**	**8.62**	**3.69**
SERPINB2	7	b	**4.08**	**3.98**	2.62	**3.21**
SERPINE1	7	b	**16.12**	**3.33**	**43.44**	**15.93**
SGK	7	b	2.03	**4.32**	**9.94**	2.58
SLC26A2	7	b	1.98	**3.74**	**5.64**	**3.19**
SLC5A1	7	b	2.17	**4.67**	**6.61**	2.31
SPRY2	7	b	**3.94**	1.33	1.60	1.02
SREBF1	7	b	0.47	**0.12**	0.53	**0.11**
SYN2	7	b	**3.98**	**5.42**	**3.06**	**4.20**
TAGLN	7	b	**5.32**	2.54	**4.04**	**9.67**
TAL1	7	b	1.49	**4.99**	1.20	1.27

Genes	TF	Signaling	Recovery time(h) after PH			
			HC		Liver tissue	
			2h	6h	2h	6h
CDC2A	6 7	bc	**3.07**	1.03	0.77	0.70
CYP7A1	6 7	bc	**0.29**	**0.02**	0.73	**0.02**
SOD2	6 7	bc	2.31	**4.27**	1.25	2.75
ADRA2B	5 7 9	bd	1.97	**4.81**	0.79	2.61
ESR1	7 9	bd	**7.26**	2.20	**3.06**	2.22
HSPB1	7 9	bd	**3.63**	**3.27**	0.84	0.55
ACTG2	2	abc	**4.40**	**4.56**	1.00	1.35
ATP10A	3	abc	2.21	**4.70**	1.10	1.62
IL5RA	3	abc	**3.69**	2.50	**3.27**	1.23
TGM1	4	abc	**7.96**	**31.85**	**7.90**	**17.75**
F3	1 6 9	abc	**3.15**	**3.05**	**6.12**	**3.30**
PRL	2 6-8	abc	0.99	**4.50**	1.29	0.91
ACTA1	2 7	abc	**3.24**	**8.29**	0.71	**3.72**
KCNMA1	2 7	abc	**3.45**	**3.65**	2.64	2.36
PLAT	2 7	abc	**3.56**	**3.20**	1.82	1.66
EGR1	2 7 8	abc	**6.20**	0.41	2.32	0.79
MT1A	3 4 7	abc	**4.99**	**3.68**	2.29	2.68
MYOD1	3 7	abc	1.90	**15.60**	**6.48**	**10.05**
STAR	4 6 7	abc	**7.22**	2.71	**3.93**	2.17
IL12B	4 6-8	abc	2.01	**3.89**	1.12	**4.10**
NOS2	6 8 9	acd	**4.42**	1.15	1.31	2.14
SCGB1A1	6 7 9	bcd	**0.32**	0.61	1.50	1.20
JUN	1 3 7-9	abcd	**3.29**	2.68	2.77	2.04
HMOX1	1 4 7-9	abcd	**13.12**	**7.99**	1.85	**3.57**
TIMP1	1 4 7-9	abcd	1.88	**4.32**	2.72	**4.66**
A2M	1 5 6 9	abcd	**9.65**	**41.30**	2.73	**4.21**
BCL2	1 5 7-9	abcd	**3.07**	1.85	1.70	2.15
BCL2L1	1 5 9	abcd	**3.46**	1.38	1.90	1.33
CCL2	1 6 7 9	abcd	**5.95**	**6.20**	**6.29**	**7.29**
CYP2C12	1 6 9	abcd	**8.99**	**0.09**	**0.23**	0.89
DMBT1	1 6 9	abcd	1.28	**3.27**	1.10	2.53
TRH	1 6 9	abcd	1.09	**3.88**	1.13	2.61
CDKN1A	1 6-9	abcd	**3.40**	2.62	**3.61**	2.38
MYC	1 7-9	abcd	**5.43**	1.77	**7.44**	**5.44**
CEBPD	1 9	abcd	**14.03**	**5.71**	**5.71**	**4.12**
LBP	1 9	abcd	2.86	**5.25**	2.22	**3.60**
SOCS3	1 9	abcd	**7.22**	**3.87**	**4.45**	2.92
STAT3	1 9	abcd	**3.90**	**4.01**	2.13	2.67

Genes	TF	Signaling	Recovery time (h) after PH			
			HC		Liver tissue	
			2h	6h	2h	6h
WT1	7	b	2.55	**3.19**	2.20	2.04
AGT	5 7	b	1.97	**3.02**	1.57	1.99
AHRR	5 7	b	**3.49**	**12.60**	**11.68**	**6.31**

Genes	TF	Signaling	Recovery time(h) after PH			
			HC		Liver tissue	
			2h	6h	2h	6h
TLR2	1 9	abcd	**3.49**	**3.10**	1.55	2.55
TNF	1-3 6-9	abcd	1.71	**4.98**	**4.17**	**9.12**
FOS	1-4 6-9	abcd	**5.02**	1.12	1.74	2.22

The qRT-PCR validation in the expression of genes regulating G0/G1 transition

In order to determine the validity of the results, nine randomly selected G0/G1 transition-regulating genes from Table 2 (*HGF*, *JUN*, *FOS*, *IL1RN*, and *TNF*) and Table 3 (*MYC*, *CTGF*, *MED1*, and *SOX15*) were subjected to quantitative RT-PCR analysis (Fig. 3).

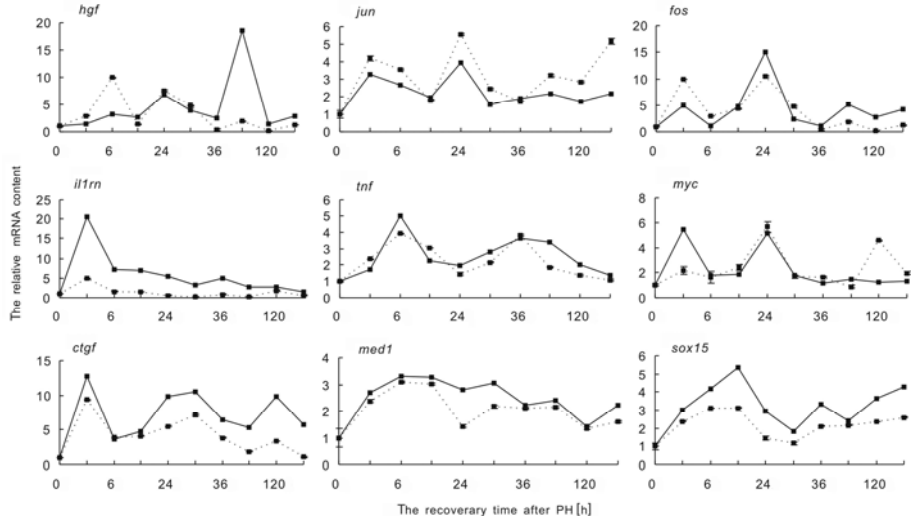

Fig. 3. Comparison of the relative mRNA levels in HCs detected by Affymetrix Rat Genome 230 2.0 Array (solid lines) and real-time PCR analysis results (dotted lines). An error bar based on the standard deviation from three different repetitions was added to confirm the reliability of PCR results. The vertical axis indicates the relative mRNA level and the horizontal axis indicates the recovery times after partial hepatectomy.

The correlation of HC G0/G1 transition activities in the priming phase during rat liver regeneration and HGF, IL-10, IL-6, and JAK/STAT signal transduction activities

The correlation of signal transduction activities and HC G0/G1 transition activities in the priming phase during rat liver regeneration was analyzed using the *Et* values. The results indicated that HGF signaling, the p38MAPK branch in IL-10 signaling, the STAT3 branch in IL-6 and JAK/STAT signaling, and the Ras/ERK branch in JAK/STAT signaling were co-related with HC G0/G1

transition. Of these, the *Et* values of signal transduction activities and HC G0/G1 transition activity regulated by HGF signaling were higher than those of the SO groups 6 h after PH, while the *Et* values of signal transduction activities and HC G0/G1 transition activity regulated by the p38MAPK branch in IL-10 signaling, the STAT3 branch in IL-6 and JAK/STAT signaling, and the Ras/ERK branch in JAK/STAT signaling were higher than those of the SO groups 2–6 h after PH. This indicates that these signaling branches enhance HC G0/G1 transition in the priming phase during rat liver regeneration (Table 4).

Table 4. The correlation of signal transduction activities and HC G0/G1 transition activities in the regenerative liver.

Recovery time (h) after PH	2 h		6 h	
Pathways and branches	Signal transduction	G0/G1 transition	Signal transduction	G0/G1 transition
HGF signaling pathway	+	~	+	+
STAT3 branch	~	+	~	+
JNK branch	~	~	~	+
ERK branch	~	~	+	~
IL6 signaling pathway	+	~	+	~
JNK branch	~	~	~	~
NF-κB branch	~	~	~	~
Ras/ERK branch	~	+	~	+
STAT3 branch	+	+	+	+
IL10 signaling pathway	~	+	~	+
STAT3 branch	~	+	~	+
JNK branch	~	~	~	~
NF-κB branch	+	~	+	~
p38MAPK branch	+	+	+	+
JAK/STAT signaling pathway	+	~	+	~
Ras/ERK branch	+	+	+	+
PI3K/AKT branch	+	~	+	~
STAT3 branch	+	+	+	+

"+" indicates that the experimental group was significantly stronger than the control; "~" indicates that there was no significant difference between the experimental group and the control.

The HC G0/G1 transition regulated by HGF, IL-10, IL-6, and JAK/STAT signaling in the priming phase during rat liver regeneration

The regulation of HC G0/G1 transition in the priming phase during rat liver regeneration was analyzed using IPA. The results indicated that the pathway of HGF signaling promoting hepatocyte G0/G1 transition is: HGF → MET → STAT3, MET → GRB2/SOS/GAB1/SHP2 → Ras → Rac1 → MEKK → MKK4/7 → JNK → AP1(cJun/cFos)/ATF2, MET → GRB2/SOS/GAB1/SHP2 → Ras → c-RAF → MEK1/2 → ERK1/2 → ETS/ELK. The pathway of IL-10 signaling promoting hepatocyte G0/G1 transition is its p38MAPK branch: IL1 → IL1R → TRAF6 →

TAK1 → MKK3/6 → p38MAPK → MYC. The pathway of IL-6 signaling promoting HC G0/G1 transition is its STAT3 branch: IL6 → GP130/IL6R → SHC/JAK2 → STAT3/NF-IL-6. The pathway of JAK/STAT signaling promoting hepatocyte G0/G1 transition is its ERK branch: IL/IF/GF → cytokine receptor → SHC/GRB2/SOS → Ras → c-Raf → MEK1/2 → ERK1/2 → STAT3/NF-IL-6 and its STAT3 branch: gastrin → CCK2R → Gaq → JAK2 → STAT3. Fig. 4 is a schematic representation of these pathways and interactions.

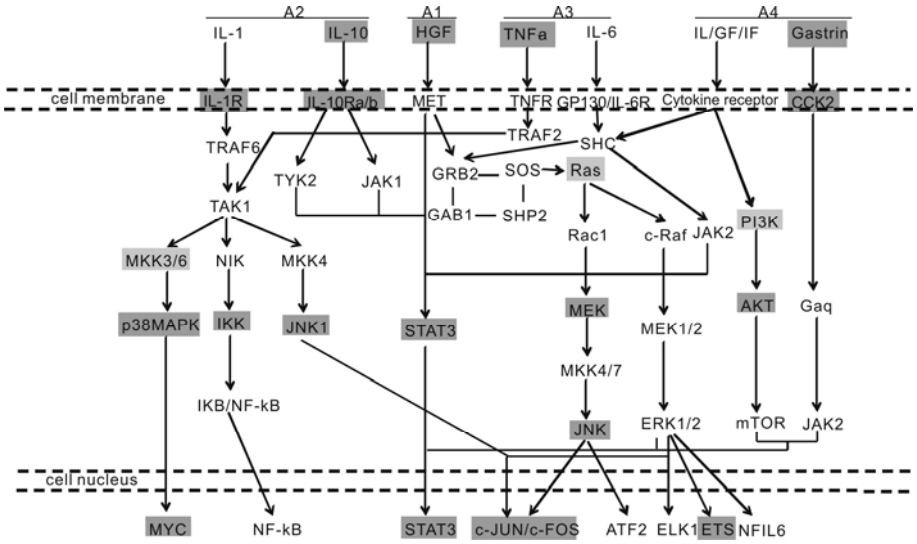

Fig. 4. Signaling regulating hepatocyte G0/G1 transition in rat liver regeneration. The numerals A1-4 sequentially represent HGF, IL-10, IL-6, and JAK/STAT signaling. Symbols in dark gray fields and light gray fields respectively represent upregulation and downregulation of genes in HCs during liver regeneration.

DISCUSSION

We used multiple approaches to identify and analyze the signaling pathways in regulation of HC G0/G1 transition during rat liver regeneration. Since some genes associated with the cell cycle can be activated in hepatocytes isolated by collagenase perfusion [33], this study also included SO groups as a control to reduce the effects of experimental operations on liver function and gene expression. The results show that HGF signaling, the STAT3 branch in IL-6 signaling, the p38MAPK branch in IL-10 signaling, and the ERK and STAT3 branches in JAK/STAT signaling play important roles in the regulation of rat HC G0/G1 transition.

Studies have shown that HGF promotes the proliferation of hepatocytes by directly binding to its specific tyrosine kinase-type receptor, c-MET, which is expressed on the surface of hepatocytes. The binding of HGF/c-MET initiates multiple downstream signaling pathways, including the Ras-Raf-MEK, ERK1/2 [34], PI3K/ PDK1/AKT [35], and mTOR/S6 kinase pathways. Maria et al. have

shown that the expressions of proto-oncogene c-MYC, c-JUN, and c-FOS were increased in the presence of HGF, and promoted cells into the proliferative phase [13]. Research by Stolz et al. shows that the expression of c-MET increased 1–5 min and 60 min after mouse 2/3 hepatectomy, and the release of the growth factors into the cytoplasmic matrix stimulates liver cells into the cell cycle from resting [36]. We found that the expression of the receptor gene *MET* did not significantly change. We suspect that during rat liver regeneration, c-MET may work by phosphorylating its downstream proteins, such as STAT3 and GRB2. The RT-PCR results (Fig. 3) and gene synergy (*Et*) analysis showed that *HGF* (encoding HGF) was upregulated 6 h after PH, the early genes *JUN* (encoding c-JUN) and *FOS* (encoding c-FOS) were upregulated 2 h after PH, *STAT3* (encoding transcription factor STAT3) was upregulated 2–6 h after PH, and the biological activity of HGF signaling was strengthened after PH. These results suggest that the HGF signaling pathway plays an important role in promoting HC G0/G1 transition by activating and regulating the transcription factor STAT3, which directly binds to a Met docking site and leads to the expression of genes required for HGF-induced cell growth [37, 38].

IL-6 has been shown to be a growth factor for many cell types including hepatocytes. The IL-6 receptor consists of an 80-kD IL-6 binding glycoprotein, termed IL-6R or gp80, and the signal transducer gp130 [39]. Also, the IL-6-type cytokines activate tyrosine kinases in the Janus kinase (JAK) family for signal transduction [40–41]. Dimerisation of the intracellular domains of two gp130 molecules brings the receptor associated JAKs (JAK1, JAK2, and TYK) into close proximity, leading to activation via inter- or intramolecular phosphorylation and activation. Phosphorylation of the distal tyrosines of gp130 molecule can activate STAT proteins, such as STAT1 and STAT3 [42]. The evidence for the essential role of IL-6 as well as its intracellular targets in liver regeneration was further confirmed using IL-$6^{-/-}$ and TNF-$R1^{-/-}$ knockout mice [43]. In these models, liver failure occurred after hepatectomy. We found that the expression of *IL6* (encoding IL-6) was not significantly changed, while *TNF* (encoding TNF-α) was upregulated 6 h after PH, transcription factor STAT3 was upregulated 2–6 h after PH, and the biological activity of its STAT3 branch was strengthened after PH.

This may occur because TNF-α activates IL-6 expression, which in turn activates the intracellular pathways in liver cells via the gp80/gp130 receptor complex. These are essential for triggering hepatocyte proliferation. We conjectured that the STAT3 branch might be involved in promoting HC G0/G1 transition via strong activation of the transcription factors STAT3 and C/EBP/nuclear factor-interleukin 6 (NF-IL-6) with an enhanced transcription of their target genes [15–20]. Many results indicate that these factors might be involved in triggering G0/G1 phase transition of HCs after PH [43].

Studies have proved that transcription factors (e.g. STAT3, c-MYC, and NFIL6) are critical for the expression of many proteins related to G0 to G1 transition.

Cytokine receptors, such as IL-1 and IL-10 receptors, are tyrosine kinase in nature. These type receptors play important roles in hepatocyte proliferation. In general, binding of cytokine promotes its receptor dimerization, which often results in autophosphorylation of specific tyrosine residues in the cytoplasmic domain of the receptor. Once phosphorylated, these tyrosine residues attract cytosolic protein signaling factors enriched in Src-homology (SH-2 and SH-3) domains to the receptor [44–46]. Then the cytosolic kinases, such as MEK and MAPKs, are activated by phosphorylation, which permits them to translocate to the nucleus [44, 47]. Several potential nuclear targets of the MAPKs have been identified, including growth-regulatory transcription factors, such as ELK-1, c-MYC, NFIL6, and C/EBP [48]. Phosphorylation of these transcription factors regulates their transcriptional activity, and hence modulates the expression of their target genes, such as *CTGF*, *MED1*, and *SOX15*.

In our study, analysis of gene synergy (*Et*) showed that *Et* values of the p38MAPK branch in IL-10 signaling, and the ERK and STAT3 branches in JAK/STAT signaling for the PH groups were higher than those for the SO groups. Meanwhile, many genes involved in these branches (i.e., *IL1RN* encoding IL1, *IL1R1*, *IL1R2* encoding IL1R, *MAPK11*, *MAPK13* encoding p38MAPK, *JUN* encoding c-JUN, *FOS* encoding c-FOS, *MYC* encoding c-MYC, *STAT3* encoding STAT3) were also upregulated, indicating that they may promote hepatocyte G0/G1 transition of the cell cycle. These results are supported by Morello et al. who have shown that MYC is the immediate early proto-oncogene that could rapidly express after partial hepatectomy and promote cells transition from G0 to G1 phase [14], and the research of Masato et al., who showed that STAT3 and NFIL6 promote HC G0/G1 transition after phosphorylation by ERK and JAK [31]. In addition, target genes such as *CTGF*, *MED1*, and *SOX15* (Table 4) have also shown positive regulation of G0/G1 transition by Gene Ontology, which is consistent with our results in Fig. 3.

In summary, we performed biological information analysis regarding the mechanism of HC G0/G1 transition based on a large-scale analysis of gene expression profiles determined by Rat Genome 230 2.0 Array. These analyses indicated a strong positive relationship of HGF, IL-6, IL-10, and JAK/STAT signaling pathways with HCs G0/G1 transition. These results are mainly based on hepatocyte gene expression changes during rat liver regeneration. As changes of gene transcriptions may not necessarily reflect the protein synthesis process, our plan is to confirm these results in experimental investigations, such as proteomics analysis, gene addition, RNA interference, and protein interactions.

Acknowledgements. This study was supported by the National Basic Research 973 Pre-research Program of China (No.2012CB722304) and the Natural Science Foundation of Henan Province (122300410355).

REFERENCES

1. Yokoyama, Y., Nagino, M. and Nimura, Y. Mechanisms of hepatic regeneration following portal vein embolization and partial hepatectomy: a review. **World J. Surg.** 31 (2007) 367–374.
2. Vondran, F.W., Katenz, E., Schwartlander, R., Morgul, R.S., Haluk, M., Raschzok, N., Gong, X.B., Cheng, X.D., Kehr, D. and Sauer, I.M. Isolation of primary human hepatocytes after partial hepatectomy: criteria for identification of the most promising liver specimen. **Artif. Organs** 32 (2008) 205–213.
3. Estes, M.D., Do J. and Ahn, C.H. On chip cell separator using magnetic bead-based enrichment and depletion of various surface markers. **Biomed. Microdevices** 11 (2009) 509–515.
4. Michalopoulos, G.K. Liver regeneration. **J. Cell Physiol.** 213 (2007) 286–300.
5. Kountouras, J., Boura, P. and Lygidakis, N.J. Liver regeneration after hepatectomy. **Hepatogastroenterology** 48 (2001) 556–562.
6. Sell, S. The hepatocyte: heperogeneity and plasticity of liver cells. **Int. J. Biochem. Cell Biol.** 35 (2003) 267–271.
7. Zimmermann, A. Regulation of liver regeneration. **Nephrol. Dial. Transplant.** 19 (2004) iv6–iv10.
8. Fausto, N., Laird, A.D. and Webber, E.M. Liver regeneration. 2. Role of growth factors and cytokines in hepatic regeneration. **FASEB J.** 9 (1995) 1527–1536.
9. Cantz, T., Manns, M.P. and Ott, M. Stem cells in liver regeneration and therapy. **Cell Tissue Res.** 331 (2008) 271–282.
10. Xu, C.S., Chen, X.G., Chang, C.F., Wang, G.P., Wang, W.B., Zhang, L.X., Zhu, Q.S., Wang, L. and Zhang, F.C. Transcriptional profiles of biliary epithelial cells from rat regenerating liver after partial hepatectomy. **Genes Genomics** 34 (2012) 245–256.
11. Xu, C.S., Yang, Y.J., Yang, J.Y., Chen, X.G. and Wang, G.P. Analysis of the role of the integrin signaling pathway in hepatocytes during rat liver regeneration. **Cell. Mol. Biol. Lett.** 17 (2012) 274–288.
12. Michalopeulos, G.K. Liver regeneration. **J. Cell Physiol.** 213 (2007) 286–300.
13. Gómez-Lechón, M.J., Guillén, I., Ponsoda, X., Fabra, R., Trullenque, R., Nakamura, T., and Castell, J.V. Cell cycle progression proteins (cyclins), oncogene expression, and signal transduction during the proliferative response of human hepatocytes to hepatocyte growth factor. **Hepatology** 23 (1996) 1012–1019.
14. Morello, D., Fitzgerald, M.J., Babinet, C. and Fausto, N. C-myc, c-fos and c-jun regulation in the regeneration livers of normal and H-2K/c-myc transgenic mice. **Mol. Cell Biol.** 10 (1990) 3185–3193.
15. Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T. and Kishimoto, T. A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. **EMBO J.** 9 (1990) 1897–1906.

16. Zhong, Z., Wen, Z. and Darnell, J.E. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. **Science** 264 (1994) 95–98.
17. Trautwein, C., Caelles, C., Vander, G.P., Hunter, T., Karin, M. and Chojkier, M. Transactivation by NF-IL6/LAP is enhanced by phosphorylation of its activation domain. **Nature** 364 (1993) 544–547.
18. Cressmann, D.E., Diamond, R.H. and Taub, R. Rapid activation of the Stat3 tran-scription complex in liver regeneration. **Hepatology** 21 (1995) 1443–1449.
19. Trautwein, C., Rakemann, T., Niehof, M., Rose-John, S. and Manns, M.P. Acute-phase response factor, increased binding, and target gene transcription during liver regeneration. **Gastroenterology** 110 (1996) 1854–1862.
20. Niehof, M., Manns, M.P. and Trautwein, C. CREB controls LAP/C/EBP beta transcription. **Mol. Cell Biol.** 17 (1997) 3600–3613.
21. Fujiyoshi, M. and Ozaki, M. Molecular mechanisms of liver regeneration and protection for treatment of liver dysfunction and diseases. **J. Hepatobiliary Pancreat. Sci.** 18 (2011) 13–22.
22. Higgins, G.M. and Anderson, R.M. Experimental pathology of the liver: Restoration of the liver of the white rat following partial surgical removal. **Arch. Pathol.** 12 (1931) 186–202.
23. Grisham, J.W. Cell types in rat liver cultures: their identification and isolation. **Mol. Cell Biochem.** 54 (1983) 23–33.
24. Wang, W.B., Xie, L.F., Wang, W., Wang, L. and Xu C.S. Isolation, purity and identification of hepatocytes in rat normal liver and regenerating liver. **Henan Sci.** 26 (2008) 1492–1498.
25. Strober, W. Trypan blue exclusion test of cell viability. **Curr. Protoc. Immunol.** 2001 May Appendix 3: Appendix 3B.
26. Twigger, S.N., Smith J., Zuniga-Meyer, A. and Bromberg, S.K. Exploring phenotypic data at the rat genome database. **Curr. Protoc. Bioinformatics** (2006) Chapter 1, Unit 1.14.
27. Wang, J.Z., Du, Z., Payattakool, R., Yu, P.S. and Chen, C.F. A new method to measure the semantic similarity of GO terms. **Bioinformatics** 23 (2007) 1274–1281.
28. Guo, W., Cai, C., Wang, C., Zhao, L., Wang, L. and Zhang, T. A preliminary analysis of genome structure and composition in Gossypium hirsutum. **BMC Genomics** 9 (2008) 314.
29. Xu, C.S., Wang, G.P., Zhang, L.X., Chang, C.F., Zhi, J. and Hao, Y.P. Correlation between liver cancer occurrence and gene expression profiles in rat liver tissue. **Genet. Mol. Res.** 10 (2011) 3480–3513.
30. Jiang, C., Xuan, Z., Zhao, F. and Zhang, M.Q. TRED: a transcriptional regulatory element database, new entries and other development. **Nucleic Acids Res.** 35 (2007) 137–140.
31. Childress, P.J., Flepcher, R.L. and Perumal, N.B. LymphTF-DB: a database of transcription factors involved in lymphocyte development. **Genes Immun.** 8 (2007) 360–365.

32. Wang, G.P. and Xu, C.S. Reference gene selection for real-time RT-PCR in eight kinds of rat regenerating hepatic cells. **Mol. Biotechnol.** 46 (2010) 49–57.
33. Kost, D.P. and Michalpopulos, G.K. Effect of epidermal growth factor on the expression of protooncogenes c-myc and c-Ha-ras in short-term primary hepatocyte culture. **J. Cell. Physiol.** 144 (1990) 122–127.
34. Borowiak, M., Garratt, A.N., Wustefeld, T., Strehle, M., Trautwein, C. and Birchmeier, C. Met provides essential signals for liver regeneration. **Proc. Natl. Acad. Sci.USA** 101 (2004) 10608–10613.
35. Okano, J., Shiota, G., Matsumoto, K., Yasui, S., Kurimasa, A., Hisatome, I. Steinbergd, P. and Murawakia, Y. Hepatocyte growth factor exerts a proliferative effect on oval cells through the PI3K/AKT signaling pathway. **Biochem. Biophys. Res. Commun.** 309 (2003) 298–304.
36. Stolz, D.B., Mars, W.M., Petersen, B.E., Kim, T.H. and Michalopoulos, G.K. Growth factor signal transduction immediately after two-thirds partial hepatectomy in the rat. **Cancers Res.** 59 (1999) 3954–3960.
37. Boccaccio, C., Ando, M., Tamagnone, L., Bardelli, A., Michieli, P., Battistini, C. and Comoglio, P.M. Induction of epithelial tubules by growth factor HGF depends on the STAT pathway. **Nature** 391 (1998) 285–288.
38. Zhang, Y.W., Wang, L.M., Jove, R. and Van de Woude, G.F. Requirement of Stat3 signaling for HGF/SF-Met mediated tumorigenesis. **Oncogene** 21 (2002) 217–226.
39. Hirano, T. Interleukin 6 and its receptor: Ten years later. **Int. Rev. Immunol.** 16 (1998) 249-284.
40. Lutticken, C., Wegenka, U.M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., Harpur, A.G., Wilks, A.F., Yasukawa, K., Taga, T., Kishimoto, T., Barbieri, G., Sendtner, M., Pellegrini, S., Heinrich, C. P. and Horn, F. Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signaltransducer gp130. **Science** 263 (1994) 89–92.
41. Stahl, N., Boulton, T.G., Farruggella, T., Ip. N.Y., Davis, S., Witthuhn, B.A., Quelle, F.W., Silvennoinen, O., Barbieri, G., Pellegrini, S., Ihle, J.N. and Yancopoulos, G.D. Association and activation of Jak Tyk kinases by CNTF-LIF-OSM-IL-6 beta receptor components. **Science** 263 (1994) 92–95.
42. Gerhartz, C., Heesel, B., Sasse, J., Hemmann, U., Landgraf, C., Schneider-Mergener, J., Horn, F., Heinrich, P.C. and Graeve, L. Differential activation of acute phase response factor/STAT3 and STAT1 via the cytoplasmic domain of the interleukin 6 signal transducer gp130. I. Definition of a novel phosphotyrosine motif mediating STAT1 activation. **J. Biol. Chem.** 271 (1996) 12991–12998.
43. Streetz, K.L., Luedde, T., Manns, M.P. and Trautwein, C. Interleukin 6 and liver regeneration. **Gut** 47 (2000) 309–312.
44. Marshall, C. J. Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. **Cell** 80 (1995) 179–185.

45. Cohen, C.B., Ren, R. and Baltimore, D. Modular binding domains in signal transduction proteins. **Cell** 80 (1995) 237–248.
46. Hunter, T. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. **Cell** 80 (1995) 225–236.
47. Heldin, C.H. Dimerization of cell surface receptors in signal transduction. **Cell** 80 (1995) 213–224.
48. Hill, C.S. and Treisman, R. Transcriptional regulation by extracellular signals: mechanisms and specificity. **Cell** 80 (1995) 199–212.