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VIRAL HEPATITIS

Expression patterns and action analysis of genes associated with hepatitis virus infection during rat liver regeneration

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Abstract

AIM: To study the action of hepatitis virus infection-associated genes at transcription level during liver regeneration (LR).

METHODS: Hepatitis virus infection-associated genes were obtained by collecting the data from databases and retrieving the correlated articles, and their expression changes in the regenerating rat liver were detected with the rat genome 230 2.0 array.

RESULTS: Eighty-eight genes were found to be associated with liver regeneration. The number of genes initially and totally expressed during initial LR [0.5-4 h after partial hepatectomy (PH)], transition from G0 to G1 (4-6 h after PH), cell proliferation (6-66 h after PH), cell differentiation and reorganization of structure-function (66-168 h after PH) was 37, 8, 48, 3 and 37, 26, 80, 57, respectively, indicating that the genes were mainly triggered at the early stage of LR (0.5-4 h after PH), and worked at different phases. These genes were classified into 5 types according to their expression similarity, namely 37 up-regulated, 9 predominantly up-regulated, 34 down-regulated, 6 predominantly down-regulated and 2 up/down-regulated genes. Their total up- and down-regulation frequencies were 359 and 149 during LR, indicating that the expression of most genes was enhanced, while the expression of a small number of genes was attenuated during LR. According to time relevance, they were classified into 12 groups (0.5 and 1 h, 2 and 4 h, 6 h, 8 and 12 h, 16 and 96 h, 18 and 24 h, 30 and 42 h, 36 and 48 h, 54 and 60 h, 66 and 72 h, 120 and 144 h, 168 h), demonstrating that the cellular physiological and biochemical activities during LR were fluctuated. According to expression changes of the genes, their expression patterns were classified into 23 types, suggesting that the cellular physiological and biochemical activities during LR were diverse and complicated.

CONCLUSION: The anti-virus infection capacity of regenerating liver can be enhanced and 88 genes play an important role in LR.

Key words: Partial hepatectomy; Rat genome 230 2.0 array; Hepatitis virus infection; Genes associated with liver regeneration

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INTRODUCTION

The liver can regenerate and precisely regulate its size. Hepatocytes maintain the ability to proliferate in response to hepatectomy, liver damage caused by viruses or chemicals, liver cell death, *etc*[1,2]. The remaining liver may compensate for the lost hepatic tissue in which the growth situation of regenerating liver can be accurately detected, and liver regeneration (LR) may stop at a proper time point[3,4]. The regeneration process is usually categorized based on hepatic physiological biochemical activities into four stages: initiation [0.5-4 h after partial hepatectomy (PH)], transition from G0 to G1 (0.5-4 h after PH), cell proliferation (6-66 h after PH), cell redifferentiation and reorganization of the architecture-function (66-168 h after PH)[5]. In this process, the cellular physiological and biochemical activities and the gene expression situations have a very sweeping change.

Hepatitis viruses causing hepatic injury and liver diseases[6] include hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis E virus (HEV)[7]. HAV and HEV do not result in chronic hepatic inflammation because of their self-limited infection, whereas HBV, HCV and HDV cause both acute and chronic hepatic inflammation, and finally

result in chronic hepatitis, cirrhosis or hepatoma[8-10]. In infection, viruses first bind specifically to receptors, then complete their replication cycle by many processes such as adsorbing, penetrating, shelling, biosynthesis, assembling and release. Viruses interact with cells, and alter the gene expression of host cells. The process during which the viruses are cleaned by the host immune system results in changes in physiology and pathology of the host[11,12]. It has been reported that liver tissues with hepatitis may appear inflammation, necrosis or hyperplasia, with which over 200 genes are associated[9,13].

Since hepatitis virus infection-associated genes have a wide variety of kinds and complicated functions, it is almost impossible to give insights into their action during liver regeneration at transcriptional level unless high-throughput gene expression profile analysis is performed[14-16]. Therefore, we used the rat genome 230 2.0 array containing 193 genes associated with hepatitis virus infection to detect the gene expression changes in the regenerating liver after 2/3 hepatectomy. A total of 88 genes were found to be associated with LR, and their expression changes, patterns and actions during liver regeneration were primarily analyzed.

MATERIALS AND METHODS

Regenerating liver preparation

Healthy SD rats weighing 200-250 g were obtained from the Animal Center of Henan Normal University. The rats were divided into groups at random, 6 rats in each group (Male: Female = 1:1). Partial hepatectomy (PH) was performed as previously described[17], the left and middle lobes of liver were removed. The rats were killed by cervical vertebra dislocation at 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 points. The livers were rinsed three times in PBS at 4℃, then 100-200 mg liver tissue was cut from the middle of right lobe. Six samples were taken from each group and mixed into 1-2 g (0.1-0.2 g × 6) liver tissue, then stored at -80℃. The sham-operation (SO) groups underwent the same PH without removal of the liver lobes. The animal protection laws in China were strictly followed.

RNA isolation and purification

Total RNA was isolated from frozen livers according to the manual of Trizol kit (Invittrogen)[18] and then purified based on the guide of RNeasy mini kit (Qiagen)[19]. Total RNA sample was checked to exhibit a 2:1 ratio of 28S to 18S rRNA intensities by agarose electrophoresis (180V, 0.5 h). Total RNA concentration and purity were estimated by optical density measurements at 260/280 nm[20].

cDNA, cRNA synthesis and purification

As a template, 1-8 μg total RNA was used for cDNA synthesis. cDNA and cRNA synthesis was carried out as previously described[21]. cRNA labeled with biotin was synthesized using 12 μL synthesized cDNA as a template, cDNA and cRNA were purifid[21]. Measurement of concentration, purity and quality of cDNA and cRNA was performed as previously reported[20].

RNA fragmentation and microarray detection

Fifteen microliters (1 μg/μL) cRNA incubated with 5 × fragmentation buffer at 94℃ for 35 min was digested into 35-200 bp fragments. Rat genome 230 2.0 microarry produced by Affymetrix was prehybridized, then the hybridization buffer was centrifuged at 60 r/min for 16 h at 45℃. The microarray was washed and stained with GeneChip fluidics station 450 (Affymetrix Inc., USA). The chips were scanned by GeneChip scan 3000 (Affymetrix Inc., USA), and the signal values of gene expression were observed[22].

Microarray data analysis

The normalized signal values, signal detections (P, A, M) and experiment/control (Ri) were obtained by quantifing and normalizing the signal values using GCOS1.2[22].

Normalisation of microarray data

To minimize errors in the microarray analysis, each analysis was performed three times by rat genome 230 2.0 microarray. Results with a maximal total ratio (R^m) and the average of three housekeeping genes (β-actin, hexokinase and glyseraldehyde-3-phosphate dehydrogenase) approached to 1.0 (R^h) were taken as a reference. Modified data were generated by applying a correction factor (R^m/R^h) multiplying the ratio of every gene in R^h at each time point. To remove spurious gene expression changes resulting from errors in the microarray analysis, the gene expression profiles at 0-4 h, 6-12 h and 12-24 h after PH were reorganized by normalization analysis program (NAP) software according to the cell cycle progression of regenerating hepatocytes. Data statistics and cluster analysis were done using GeneMath, GeneSpring, Microsoft Excel softwares[22-24].

Identification of genes associated with liver regeneration

Firstly, the nomenclature of structure and activity of hepatitis virus (e.g. hepatitis B virus) were adopted from the NCBI (http://www.ncbi.nlm.nih.gov/) and GENEONTOLOGY database (www.geneontology.org/), and input into the databases at AmiGO (http://www.godatabase.org/), NCBI (www.ncbi.nlm.nih.gov/), RGD (rgd.mcw.edu/), MGI (http://www.informatics.jax.org/), UniProt (http://www.pir.uniprot.org/) to identify the rat, mouse and human genes associated with hepatitis virus infection. Then the genes associated with hepatitis virus infection were collated. The results of this analysis were codified, and compared with the results obtained for mouse and human searches in order to identify human genes which are different from those of rats. Compared these genes with the analysis output of the Rat Genome 230 2.0 array, those genes which showed a greater than twofold change in expression level, observed as meaningful expression changes[25], were referred to as rat homologous genes or rat specific genes associated with hepatitis virus infection. Genes, which displayed reproducible results with three independent analyses with the chip and showed a greater than twofold change in expression level in at least one time point during liver regeneration with significant difference ($0.01 \leqslant P < 0.05$) or extremely significant difference (P

Table 1 Expression abundance of 88 hepatitis virus infection-associated genes during LR

Gene Abbr.	Involved in others	Fold difference	Gene Abbr.	Involved in others	Fold difference	Gene Abbr.	Involved in others	Fold difference	Gene Abbr.	Involved in others	Fold difference
1 Virus life cycle and virion			Ifng		6.5	Bcl2		0.3	Rgd1306332		0.3
Abce1		2.7	Il1b	3	0.4	Casp8		10.6	Serpinf2		0.2
Ccl2	3	128.0	Il1r1		0.5	Ccl2		128.0	Snrpd1		4.3
Ccl4		0.2, 3.0	Il4		2.6	Cyp17a1		0.3	Tap1		2.2
Ctbp2		1.4	Ilf3		4.0	Cyp2d6		0.3	Tgfb1		4.0
Ctse		2.0	Mapk8		19.7	Ddx3x		0.4	Tlr2		10.6
Gfi1		0.2, 2.4	Mbl2		0.2	Dffa		0.3, 2.8	Tnf	2	3.2
Hbxip	2	2.0	Mmp9		9.5	E2f1		21.2	Tp53	2	2.9
Hipk2		0.2, 2.8	Nfkb1		2.3	Eif4a1		4.1	Vapa		28.8
Hrmt1l2		0.5, 2.3	Nr4a1		7.5	Ephx1		0.4, 2.8	Vipr1		2.3
Npap60		1.3	Nrf1		2.4	Hm13		0.1	Wnt1		0.5
Oprk1		1.6	Pcna		10.6	Ifna1		13.0	Ywhaz		2.3
Ppia		2.6	Ptgs2	3	2.1	Ikbkb		0.3	4 Involved in other hepatitis		
Tnip1		0.5	Ptk2b		3.6	Il1b	2	0.4	virus		
Ubp1		0.5	Rfx1		7.9	Irf1		0.3	Adarb1		0.4
Wwp1		3.9	Serpinb3		0.1	Ltbr		0.4	Ambp		5.1
2 Hepatitis B virus			Shc1		0.5	Mapk1		2.7	Btbd1		0.5
Creb1		0.5	Tcf1		6.8	Mbp		0.4	Copg		0.1
Ddb2		2.8	Timp1		8.6	Nfkbib		11.8	Copg2		2.8
Egr2		6.8	Timp3		0.5	Nolc1		3.7	Dap3		5.5
Esr1		6.1	Tnf	3	3.2	Pitx1		4.6	Havcr1		16.0
Foxa2		0.4	Tp53	3	2.9	Ptbp2		2.2	Nd1		0.4
Hbxip		0.5	Xpo1		3.2	Pten		0.5	Snrpn		7.3
Hnf4a		4.5	3 Hepatitis C virus			Ptgs2	2	0.1, 2.1	Sos1		0.4, 14.9
Hspa5		0.1	Apoe		0.1	Rb1		2.6			

Involved in others: associated with other type of hepatitis virus infection liver diseases.

$\leqslant$ 0.01) between PH and SO, were referred to as associated with liver regeneration.

RESULTS

Expression changes of hepatitis virus infection-associated genes during LR

According to the data from AmiGO, NCBI, RGD, MGI and UniProt databases, 204 genes were associated with hepatitis virus infection, and 193 of the above 204 genes were contained in rat genome 230 2.0 array. Among them, 88 genes revealed meaningful changes in expression at least at one time point after partial hepatectomy (PH), and showed significant or extreme significant differences in expression when compared with sham operation (SO), and were reproducible in three detections by rat genome 230 2.0 array, suggesting that the genes were associated with LR (Table 1). The analysis indicated that 37 genes were up regulated, 34 genes down-regulated, and 17 genes up/down-regulated during LR. Total expression frequencies of up- and down-regulated genes were 359 and 149, respectively (Figure 1A). The expression patterns varied with the phases in regenerating liver. At the initiation stage of LR (0.5-4 h after PH), 20 genes displayed up-regulation, 14 genes down-, 2 genes up/down-regulation; at the transition phase from G0 to G1 (4-6 h after PH), 15 genes revealed up-, 11 genes down-regulation; at cell proliferation phase (6-66 h after PH), 40 genes showed up-, 26 genes down-regulation, 12 genes up/down-regulation; at cell differentiation and structure-function reorganization stage (66-168 h after PH), 35 genes displayed up-, 17 genes down-, 5 genes up/down-regulation (Figure 1B).

Initial and total number of hepatitis virus infection-associated genes in expression at each time point of LR

At each time point of LR, the number of initially up-, down-regulated and totally up-, down-regulated genes was 13 and 5 at 0.5 h; 6, 5 and 15, 7 at 1 h; 3, 2 and 13, 3 at 2 h; 0, 3 and 13, 6 at 4 h; 2, 3 and 13, 7 at 6 h; 0, 0 and 12, 4 at 8 h; 2, 1 and 16, 3 at 12 h; 8, 5 and 21, 7 at 16 h; 5, 9 and 24, 12 at 18 h; 0, 2 and 23, 11 at 24 h; 3, 0 and 13, 7 at 30 h; 0, 0 and 17, 9 at 36 h; 0, 0 and 14, 5 at 42 h; 3, 3 and 23, 11 at 48 h; 1, 1 and 20, 10 at 54 h; 0, 0 and 19, 7 at 60 h; 0, 0 and 21, 4 at 66 h; 0, 0 and 13, 5 at 72 h; 0, 2 and 13, 10 at 96 h; 1, 0 and 15, 3 at 120 h; 0, 0 and 14, 6 at 144 h; 0, 0 and 14, 7 at 168 h (Figure 2).

Expression similarity and time relevance of hepatitis virus infection-associated genes during LR

A total of 88 genes during LR could be divided into as following: 37 up-regulated, 9 predominantly up-regulated, 34 down-regulated, 6 predominantly down-regulated, and 2 up/down-regulated genes based on their similarity in expression (Figure 3). These 88 genes during liver regeneration could also be classified based on time relevance into 12 groups (0.5 and 1 h, 2 and 4 h, 6 h, 8 and 12 h, 16 and 96 h, 18 and 24 h, 30 and 42 h, 36 and 48 h, 54 and 60 h, 66 and 72 h, 120 and 144 h, 168 h) in which the number

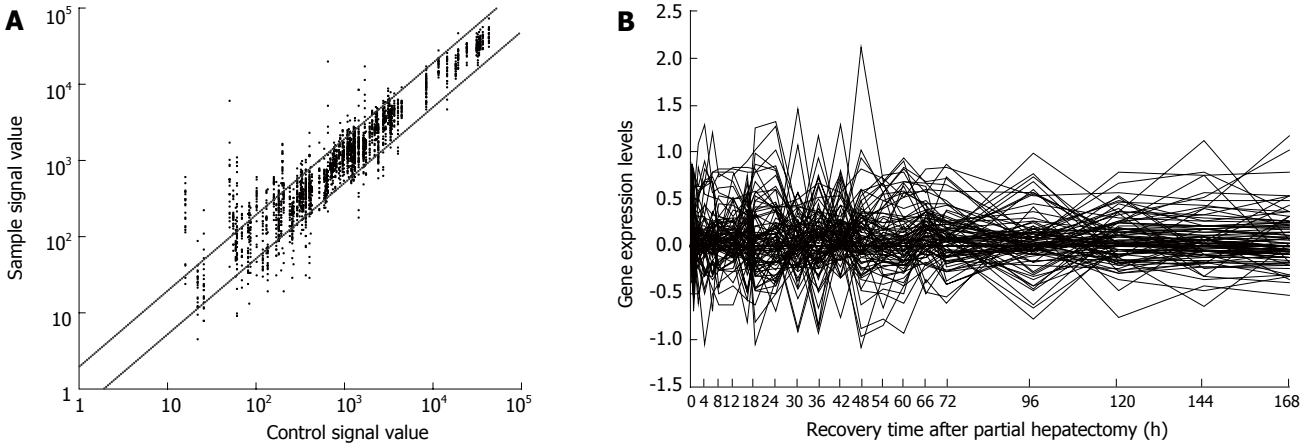

Figure 1 Expression files of 88 hepatitis virus infection-associated genes in rat liver regeneration. **A**: The abundance and frequency of gene expression, each point represents the signal value of one gene at the corresponding time point. The dots above bias indicate that the genes are more than two-fold up-expressed, the dots under bias indicate that the genes are more than two-fold down-expressed, and the dots between biases indicate that the genes have no alteration in expression. The more far genes are from the bias, the longer the time the greater the gene change; **B**: Expression changes in genes associated with LR.

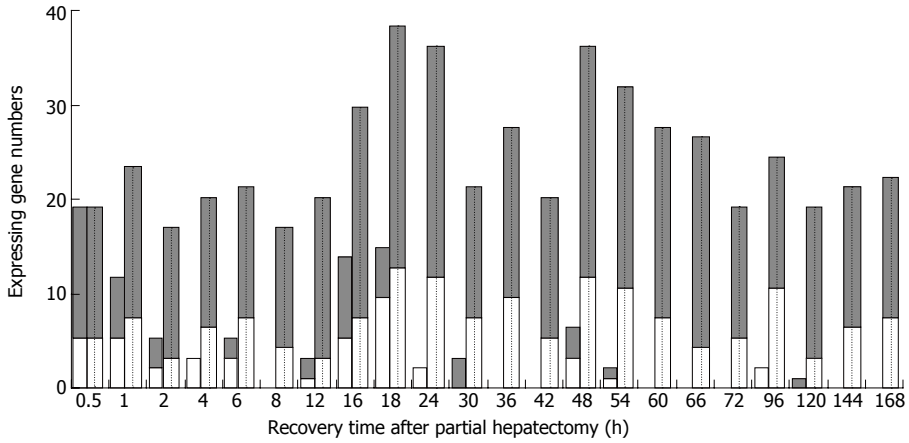

Figure 2 The initial and total expression profiles of 88 hepatitis virus infection-associated genes at each time point of liver regeneration. Blank bars: the number of initially expressed genes; Dotted bars: the number of totally expressed genes; Grey bars: up-regulated genes; White bars: down-regulated genes.

of up- and down-regulated genes was 28 and 12, 26 and 9, 13 and 7, 28 and 7, 34 and 17, 47 and 23, 27 and 12, 40 and 20, 39 and 17, 34 and 9, 29 and 9, 14 and 7 (Figure 3).

Expression patterns of hepatitis virus infection-associated genes during LR

The expression patterns of 88 genes during liver regeneration might be categorized into 23 types according to the expression changes: (1) up-regulated expression at one time point, i.e. 30, 48, 120 h after PH (Figure 4A-C and E) with 4 genes involved; (2) up-regulated expression at two time points, i.e. 30 and 42 h, 16 and 42 h, 16 and 96 h, 18 and 54 h (Figure 4A and B) with 4 genes involved; (3) up-regulated expression at multiple time points (Figure 4A and B) with 5 genes involved; (4) up-regulated expression at one phase, i.e. 1-168 h, 16-96 h (Figure 4B and E) with 2 genes involved; (5) up-regulation expression at two phases, i.e. 16-24 and 42-48 h (Figure 4B) with 1 gene involved; (6) up-regulated expression at multiple phases (Figure 4E) with 1 gene involved; (7) up-regulated expression at one time point/one phase, i.e. 54 and 144-168 h (Figure 4C) with 1 gene involved; (8) up-regulated expression at one time point/two phases (Figure 4B-D) with 4 genes involved; (9) up-regulated expression at one time point/multiple phases (Figure 4E and J) with 2 genes involved; (10) up-regulated expression at two time points/one phase (Figure 4B, D and E) with 3 genes involved; (11) up-regulated expression at two time points/two phases (Figure 4B and D) with 2 genes involved; (12) up-regulated expression at two time points/multiple phases (Figure 4B, D and E) with 3 genes involved; (13) up-regulated expression at multiple time points/one phase (Figure 4A and C) with 2 genes involved; (14) up-regulated expression at multiple time points/two phases (Figure 4C, D and E) with 3 genes involved; (15) down-regulated expression at one time point, i.e. 2, 4, 6, 16, 18, 24, 48 , 96h (Figure 4F, G and H) with 14 genes involved; (16) down-regulated expression at two time points, i.e. 0.5 and 4 h, 16 and 96 h, 18 and 54 h (Figure 4G, H and I) with 3 genes involved; (17) down-regulated expression at multiple time points (Figure 4G, H and I) with 6 genes involved; (18) down-regulated expression at one phase, i.e. 4-6 h, 18-24 h, 54-60 h (Figure 4G and H) with 3 genes involved; (19) down-regulated expression at one time point and one phase, i.e. 1 and 144-168 h, 48 and 18-24 h, 30 and 2-8 h (Figure 4H, I and J) with 3 genes involved; (20) down-regulated expression at one time point/two phases (Figure 4I) with 1 gene involved; (21) down-regulated expression at two time points/one phase

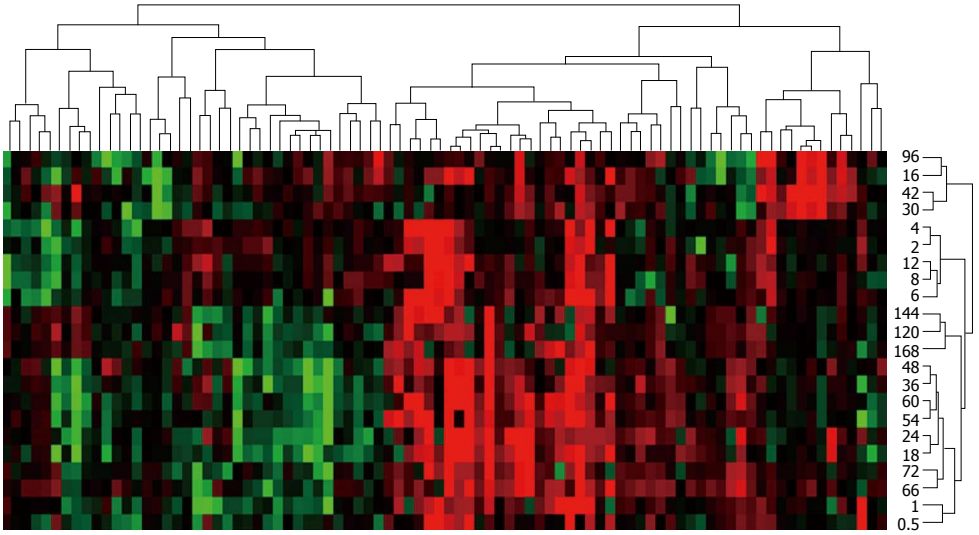

Figure 3 Expression and time series clusters of 88 hepatitis virus infection-associated genes during liver regeneration. Detection data of rat genome 230 2.0 array were analyzed by H-clustering. Red: up-regulated genes; Green: down-regulated genes; Black: no-sense genes in expression; Upper and right trees showing expression and time series clusters, respectively.

(Figure 4I) with 1 gene involved; (22) down-regulated expression at two time points/two phases (Figure 4I) with 3 genes involved; (23) up/down-regulated expression (Figure 4B, H and J) with 17 genes involved.

DISCUSSION

The role of 193 genes associated with hepatitis virus infection in liver regeneration was studied in this study. Among the 45 genes associated with hepatitis virus life cycle and virion, peptidylprolyl isomerase A (PPIA), nuclear pore associated protein (NPAP60) and opioid receptor kappa 1 (OPRκ1) are involved in viral infection[26]. Eight genes, including chemokine ligand 2 (CCL2), WW domain containing E3 ubiquitin protein ligase 1 (WWP1), chemokine ligand 3 (CCL3), C-terminal binding protein 2 (CTBP2), cathepsin E (CTSE), TNFAIP3 interacting protein 1 (TNIP1), upstream binding protein 1 (UBP1) and heterogeneous nuclear ribonucleoproteins methyltransferase-like 2 (HRMT1L2), participate in the viral genome replication and transcription[27,28]. Hbx interacting protein (HBXIP) suppresses HBV X protein activity[29]. ATP-binding cassette sub-family E member 1 (ABCE1) plays a part in viral capsid assembly[30]. Homeodomain interacting protein kinase 2 (HIPK2) relates to induction of apoptosis[31]. Growth factor independent 1 (GFI1) controls the differentiation of dendritic cells into macrophages[32]. The above genes tend to have a same or similar expression at some time points, but a different expression at other time points which may enhance inflammatory reaction and immunity of regenerating liver.

Of the 65 genes associated with hepatitis B virus infection, forkhead box A2 (FOXA2), interleukin 4 (IL4) and transcription factor 1 (TCF1) may restrain HBV gene replication and RNA transcription[33,34], whereas regulatory factor X 1 (RFX1) promotes RNA transcription by binding to HBV enhancer[35]. Tissue inhibitor of metalloproteinases 1, 3 (TIMP1, TIMP3) exhibits low level expression after HBV transinfection[36], but matrix metallopeptidase 9 (MMP9) displays high activity after HBV transinfection[36]. Interferon gamma (IFNγ) supresses hepatic cell proliferation[37]. CAMP responsive element binding protein 1 (CREB1), nuclear respiratory factor 1 (NRF1) and hepatocyte nuclear factor 4 alpha (HNF4α) activate HBV promoter[34,38,39]. Nine genes, such as early growth response 2 (EGR2), proliferating cell nuclear antigen (PCNA), tumor suppressor p53 (TP53), serpin peptidase inhibitor clade B member 3 (SERPINB3), prostaglandin-endoperoxide synthase 2 (PTGS2), SHC transforming protein 1 (SHC1), interleukin enhancer binding factor 3 (ILF3), nuclear factor of kappa light chain gene enhancer in B-cells 1 (NFκB1) and mitogen-activated protein kinase 8 (MAPK8), accelerate cell proliferation[40-45]. Tumor necrosis factor (TNF) and estrogen receptor 1 (ESR1) increase cell susceptibility to HBV infection[46,47]. 70kDa heat shock protein 5 (HSPA5) plays a role in anti-apoptosis[48]. Protein tyrosine kinase 2 beta (PTK2β) promotes migration and invasion of glioblastoma[49]. Exportin 1 (XPO1) takes part in HBV-induced aberrant centriole replication and abnormal mitotic spindles[50]. Mannose binding lectin 2 (MBL2) participates in HBV infection[51]. Interleukin 1 beta (IL1β) may enhance resistance to chronic diseases[52]. Interleukin 1 receptor type I (IL1R1) elevates blood-brain barrier[53]. Damage specific DNA binding protein 2 (DDB2) promotes turnover of HBV X protein[54]. Nuclear receptor subfamily 4 group A member 1 (NR4A1) enhances role of the HBx-induced Fas/FasL signaling pathway[55]. The above genes may have same or similar expression changes at some time points, but different expression changes at other time points, suggesting that they promote inflammation recovery and strengthen anti-hepatitis B virus infection ability.

Among the 90 genes associated with hepatitis C virus infection, Three genes concluding polypyrimidine-tract binding protein 2 (PTBP2), mitogen-activated protein kinase 1 (MAPK1) and interferon alpha 1 (IFNα1) play a role in suppression of hepatitis C virus replication[56-58]. Nucleolar and coiled-body phosphoprotein 1 (NOLC1), transporter 1 ATP-binding cassette sub-family B (TAP1) and eukaryotic translation initiation factor 4A1 (EIF4A1) promote protein biosynthesis[59]. Six genes including toll-like receptor (TLR2), transforming growth factor beta 1 (TGFβ1), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAζ),

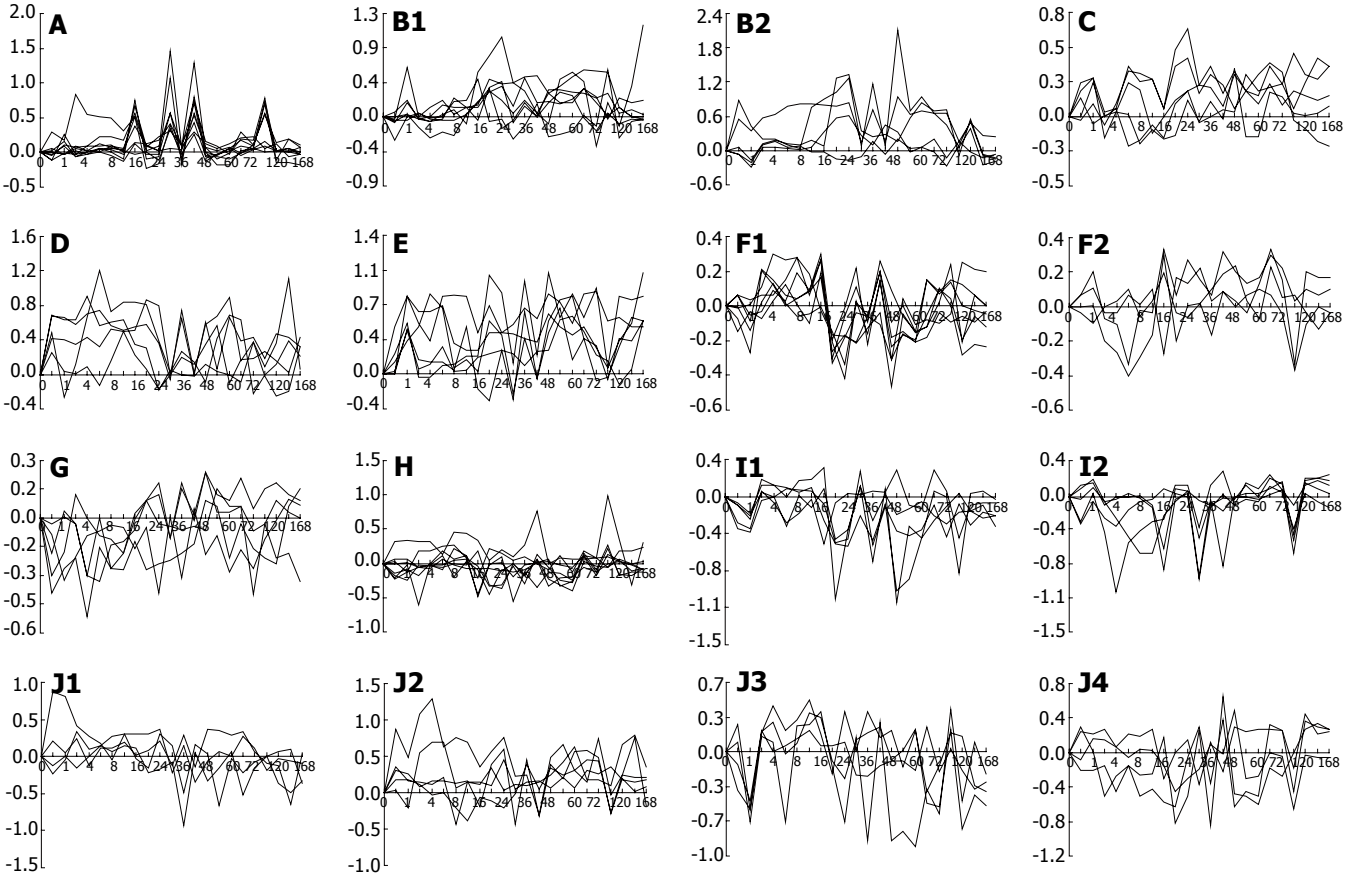

Figure 4 Expression patterns of 88 hepatitis virus infection-associated genes during liver regeneration. Expression patterns of these genes exhibit 23 types. **A-E**: up-regulated expression; **F-I**: down-regulated expression; **J**: up/down-regulated expression. X-axis represents recovery time after PH (h), Y-axis shows logarithm ratio of the signal values of genes at each time point to control.

vasoactive intestinal peptide receptor 1 (VIPR1), cytochrome P450 family 2 subfamily D polypeptide 6 (CYP2D6) and lymphotoxin B receptor (LTBR), are related to immune reponse[60,61]. VAMP-associated protein A (VAPA) accelerates HCV replication[62]. Nuclear factor of kappa light chain gene enhancer in B-cell inhibitor beta (NFκBIβ) and E2F transcription factor 1 (E2F1) have transcriptional activator activity[63]. Inhibitor of kappaB kinase beta (IKκBKβ) inhibits activation of NFκB. Small nuclear ribonucleoprotein D1 (SNRPD1) and serine peptidase inhibitor clade F member 2 (SERPINF2) can repair liver damage[64,65]. Paired-like homeodomain transcription factor 1 (PITX1) modulates interferon expression[66]. Genetic polymorphisms of cytochrome P450 family 17 subfamily alpha polypeptide 1 (CYP17α1) and epoxide hydrolase 1 (EPHX1) are closely associated with liver disease[67,68]. Four genes containing interferon regulatory factor 1 (IRF1), phosphatase and tensin homolog (PTEN), myelin basic protein (MBP) and DEAD box polypeptide 3 X-linked (DDX3X) are associated with regulation of liver disease[69-72]. Apolipoprotein E (APOE) has a role in liver protection[73]. Retinoblastoma 1 (RB1) and caspase 8 (CASP8) inhibit cell growth[74,75]. Wingless-type MMTV integration site family member 1 (WNT1) can inhibit cell apoptosis[76]. B-cell leukemia/lymphoma 2 (BCL2) is associated with infectious vasculitis[77]. Histocompatibility antigen 13 (HM13) is responsible for immunological recognition[78]. HM13 is highly expressed in human liver HL-7702 cells as HCV NS3-transactivated protein 1 (RGD1306332)[79]. DNA fragmentation factor alpha subunit (DFFα) plays a role in suppressing tumors[80]. The above genes may have the same or similar expression changes at some time points, different expression changes at other time points, thus promoting inflammatory reaction and anti-infection ability of regenerating liver.

In summary, the expression changes of hepatitis virus infection-associated genes after rat partial hepatectomy can be analyzed with high-throughput gene expression profiling. Immunological competence, abilities of anti-inflammation and anti-infection are increased during liver regeneration. Rat genome 230 2.0 array is a useful tool for analyzing hepatitis virus infection and liver disease at transcriptional level. However, DNA→ mRNA→protein is affected by many factors including protein interaction. Further study is needed to confirm the results at cell level.

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