Analysis of the function of the genes associated with eight kinds of liver diseases with oligonucleotide microarray during rat liver regeneration

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Abstract

Objective. The aim of the study was at transcriptional level to research the roles of the genes associated with eight kinds of liver diseases including alcoholic liver diseases, fatty liver, hepatic cirrhosis, liver vascular diseases, hepato-megaly, acute liver necrosis, hepatic encephalopathy, and hepatopulmonary syndrome during liver regeneration (LR).

Methods. The associated genes of the above-mentioned liver diseases were obtained by collecting the data of databases and referring to thesis, and the gene expression changes in the rat regenerating liver were checked by the Rat Genome 230 2.0 Array.

Results. 70 genes were found to be associated with LR. The initial and total expressing gene number occurring in initiation (0.5 – 4 h after PH), G0/G1 transition (4 – 6 h after PH), cell proliferation (6 – 66 h after PH), cell differentiation and structure-function reconstruction phase (72 – 168 h after PH) was 36, 11, 29, 3 and 36, 32, 63, 49, respectively, illustrating that the associated genes mainly were triggered at the initial stage of LR and worked at different phases. According to their expression similarity, these genes were classified into 5 types including only up, predominantly up, only down, predominantly down, and equally up and down-regulated, respectively involving 27, 7, 22, 11 and 3 genes; and the total times of their up and down-expression were 293 and 155 respectively, demonstrating that expression of the major genes was enhanced during LR, while minority weakened. According to time relevance, they were classified into 14 groups, showing that the cellular physiological and biochemical activities during LR were staggered. According to gene expression patterns, they were classified into 23 types, indicating the cellular physiological and biochemical activities during LR were diverse and complicated. Conclusion. Fat assimilation was enhanced between 18 – 48 h; alcohol degradation was decreased between 6 – 24 h after PH; cell reproduction was promoted in metaphase. [Life Science Journal. 2007; 4(3): 34 – 41] (ISSN: 1097 – 8135).

Keywords: partial hepatectomy; Rat Genome 230 2.0 Array; genes; liver diseases; liver regeneration

1 Introduction

After partial hepatectomy (PH)¹ or liver injury², the remnant hepatocytes rapidly proliferate to compensate the lost liver tissue, which is called liver regeneration (LR). The regenerating process, usually categorized based on hepatic physiological activities into four stages: initiation phase (0.5 – 4 h after PH), G0/G1 transition(4 – 6 h after PH), cell proliferation (6 – 66 h after PH), cell differentiation and reorganization of the structure-function (72 –168 h after PH)³, involves many physiological and biochemical events such as cell activation, de-differentiation, proliferation and re-differentiation⁴, and are regulated by many factors including cytokines, hormones etc⁵.

The liver is an important metabolism organ in body⁶. Some liver diseases will follow immediately once the metabolic function of liver doesn’t work well⁷. For example, alcohol liver disease is caused by the alcohol metabolism obstacle⁸. Fatty liver is characterized by fatty
excessive deposit in liver cell induced by various factor or diseases\cite{9}. Hepatic cirrhosis appears liver deformation and sclerosis as the result of dispersed hepatocyte necrosis, a mass of connective tissue proliferation and hepatocytes tuberous regeneration subsequently\cite{10}. Liver vascular diseases are an type of thrombotic, obliterator and inflammatory pathology occurring in liver and/or arteries and veins around the liver\cite{11}. Hepatomegaly is marked by pathological hypertrophy of liver mainly caused by microorganisms infection\cite{12}, metabolic disturbance of fat\cite{13}. Acute liver necrosis, a sort of acute hepatitis, is characterized by massive liver tissue necrosis and sharply deteriorative liver function\cite{14}. Hepatic encephalopathy (HE) appears the maladjustment of brain function caused by chronic hepatic injury\cite{15}. Hepatopulmonary syndrome (HPS) is characterized by a combination of liver disease with intrapulmonary vasodilatation, artery oxygenation abnormality and hypoxemia\cite{16}.

Both PH and the above-mentioned liver diseases can lead to hepatic compensatory hyperplasia more or less\cite{2,17}. Studying the relevance between the physiological and biochemical activities during LR and that of these liver diseases will contribute to disclosure of the molecular mechanism of LR and establishment of the treatment and prevention methods of liver diseases\cite{18}. Therefore, Rat Genome 230 2.0 Array containing 113 genes associated with eight liver diseases was employed to check the gene expression changes in regenerating liver after 2/3 hepatectomy, and 70 of them were identified to be associated with LR. The expression profiling, patterns and actions of them during LR were primarily analyzed.

2 Materials and Methods

2.1 Regenerating liver preparation

256 healthy Sprague-Dawley rats (200 – 250 g) were obtained from the Animal Center of Henan Normal University and were divided into 44 groups randomly: 22 PH groups and 22 sham operation (SO) groups. Each group included 6 rats. The rats in PH group were subjected to an operation removing 70% of their liver, as described by Higgins et al\cite{19}. The SO group was subjected to the same procedure as the PH group but without liver removal. The rats were killed by cervical dislocation in 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 post PHx, respectively, and their livers were instantly removed. The procured livers were immediately washed three times with a cold washing buffer (0.01 M/L PBS). About 100 – 200 mg liver tissues were pooled from the middle parts of right lobe of each rat on ice. Liver tissues of six rats for each group (total mass: 0.1 – 0.2 g × 6 = 1 – 2 g ) were gathered and mixed, and then stored at – 80 °C until use. The laws of animal protection of China were enforced strictly.

2.2 RNA isolation and purification

Total RNA was isolated from frozen livers according to the manual of Trizol reagent (Invitrogen Corporation, Carlsbad, California, USA)\cite{20} and then purified base on the guide of RNeasy mini kit (Qiagen, Inc, Valencia, CA, USA)\cite{21}. 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\cite{22}.

2.3 cDNA, cRNA synthesis and purification

1 – 8 μg total RNA as template was used for cDNA synthesis. cDNA purification was based on the way established by Affymetrix\cite{23}. 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\cite{23}. Measurement of cDNA, cRNA concentration and purity were the same as above.

2.4 cRNA fragmentation and microarray detection

15 μl (1 μg/μl) cRNA incubated with 5 × 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 microarray 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\cite{24}.

2.5 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 GeneChip operating software (GCOS) 1.2\cite{24}.

2.6 Normalization of the microarray data

To minimize the technical error from the microarray analysis, each sample was hybridized three times to the gene chips. The average value of three measurements was normalized, the expression change was at least twofold and statistics were conducted on these values with Gen-
3 Results

3.1 General description of the expression of the genes associated with eight kinds of liver diseases during LR

According to the data of databases at NCBI, GENMAPP, KEGG and BIOCARTA, 118 genes were associated with liver diseases. In which, 113 genes were contained in the Rat Genome 230 2.0 Array. Among them, 70 genes revealed meaningful expression changes at least at one time point after PH, showed significant difference or extremely significant difference in expression when comparing PH with SO and displayed reproducible results with three analysis with Rat Genome 230 2.0 Array detection, suggesting that the genes were associated with LR. The analysis indicated that 27 genes were up, 22 genes down, and 21 genes up/down-regulated in regenerating liver. The range of up-regulation was from 2 to 45 times higher than control, and that of down-regulation was 2 – 12.5 folds (Table 1).

3.2 Expression changes of the genes associated with eight kinds of liver diseases during LR

At each time point of LR, the numbers of initially up, down-regulated and totally up, down-regulated gene were in sequence: both 12 and 4 at 0.5 h; 7, 4 and 15, 6 at 1 h; 4, 0 and 15, 1 at 2 h; 3, 4 and 19, 7 at 4 h; 1, 3 and 18, 7 at 6 h; 0, 3 and 15, 9 at 8 h; 0, 1 and 9, 7 at 12 h; 2, 2 and 10, 9 at 16 h; 2, 5 and 15, 19 at 18 h; 2, 1 and 18, 16 at 24 h; 1, 1 and 6, 3 at 30 h; 0, 2 and 11, 13 at 36 h; 1, 0 and 14, 1 at 42 h; 1, 0 and 17, 11 at 48 h; 0, 0 and 14, 8 at 54 h; 0, 1 and 11, 8 at 60 h; 0, 0 and 16, 3 at 66 h; 0, 1 and 13, 6 at 72 h; 1, 0 and 12, 3 at 96 h; 1, 0 and 15, 6 at 120 h; 0, 0 and 10, 3 at 144 h; 0, 0 and 8, 5 at 168 h. In the respect of the initial expression of the above 70 genes, 38 and 32 genes were initially up-regulated and down-regulated during LR, respectively. A detailed introduction is as follows: at the initiation stage (0.5 – 4 h after PH), the G0/G1 transition phase (4 – 6 h after PH), cell proliferation period (6 – 66 h after PH), cell differentiation and the structure-function reorganization stage of LR (72 – 168 h after PH), the number of initially up and initially down-regulated genes were 26 and 12, 4 and 7, 10 and 19, 2 and 1. The whole situation of the genes expression was that the total frequencies of up and down-regulated expression were respectively 293 and 155. Specifically, at the above-mentioned four phases of LR, the number of times of up-regulation and down-regulation was separately 61 and 18, 37 and 14, 174 and 114, 58 and 23 (Figure 1).

3.3 Expression similarity and time relevance of the genes associated with eight kinds of liver diseases during LR

70 genes mentioned above during LR could be characterized based on their similarity in expression as follow: only up-, predominantly up-, only down-, predominantly down-, and up/down-regulated, involving 27, 6, 22, 10 and 5 genes, respectively (Figure 2). They could also be classified based on time relevance into 14 groups including 0.5 and 2 h, 1 h, 4 and 6 h, 8 and 12 h, 16 h, 18 and 24 h, 30 and 42 h, 36 h, 48 h, 54 and 60 h, 66 and 72 h, 96 h, 120 h, 144 and 168 h, in which the times of up- and down-regulation genes were respectively 27 and 5, 15 and 6, 37 and 14, 24 and 16, 10 and 9, 33 and 35, 20 and 4, 11 and 13, 17 and 11, 25 and 16, 18 and 8, 25 and 9, 15 and 6, 18 and 8 (Figure 2).

3.4 Expression patterns of the genes associated with eight kinds of liver diseases during LR

70 genes mentioned above during LR might be categorized according to the changes in expression into 23 types of patterns: (1) up-regulation at one time point, i.e. at 42,
Chen, et al, Analysis of the genes associated with eight kinds of liver diseases during rat liver regeneration

Table 1. Expression abundance of 70 eight kinds of liver diseases-associated genes during rat LR

<table>
<thead>
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<th>Name</th>
<th>Abbr.</th>
<th>Involved in others</th>
<th>Fold difference</th>
<th>Name</th>
<th>Abbr.</th>
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*Reported genes associated with LR; Involved in others: involved in other liver diseases

96, 120 h after the rat partial hepatectomy (Figure 3A), 3 genes involved; (2) up-regulation at two time points, i.e. 1 and 72 h, 24 and 66 h (Figure 3B), 3 genes involved; (3) up-regulation at more time points (Figure 3C), 2 genes in-
(4) up-regulation at two phases, i.e. 0.5 – 42 h and 48 – 60 h, 16 – 24 h and 42 – 48 h (Figure 3D), 2 genes involved; (5) up-regulation at one time point/two phases (Figure 3E1, 3E2), 4 genes involved; (6) up-regulation at one time point/more phases (Figure 3E2), 2 genes involved; (7) up-regulation at two time points/one phase (Figure 3F), 2 genes involved; (8) up-regulation at two time points/two phases (Figure 3F), 4 genes involved; (9) up-regulation at three time points and two phases (Figure 3G), 4 genes involved; (10) up-regulation at more time points/one phase (Figure 3G), 1 gene involved; (11) down-regulation at one time point, i.e. 4, 6, 16, 36, 60 or 72 h after PH (Figure 3H), 6 genes involved; (12) down-regulation at two time points, at 30 and 48 h (Figure 3I), 1 gene involved; (13) down-regulation in more time points (Figure 3I), 4 genes involved; (14) down-regulation at one phase, i.e. 6 – 8 h (Figure 3J), 1 gene involved; (15) down-regulation at two phases, i.e. 16 – 24 and 48 – 54 h (Figure 3J), 1 gene involved; (16) down-regulation at one time point/two phases, i.e. 1 h and 4 – 24 h, 1 and 144 – 168 h, 36 and 48 – 60 h, 42 and 8 – 30 h (Figure 3K), 4 genes involved; (17) down-regulation at one time point/two phases (Figure 3K), 1 genes involved; (18) down-regulation at two time points and one phase (Figure 3L), 1 genes involved; (19) down-regulation at two time points/two phases (Figure 3L), 2 genes involved; (20) down-regulation at more time points/more phases (Figure 3L), 1 gene involved.

Figure 1. The initial and total expression profiles of 70 liver diseases-associated genes at each time point of LR. Blank bars: Initially expressing gene number; Dotted bars: Total expressing gene number; Grey-background bars: Up-regulated genes; White-background bars: Down-regulated genes. Expression change of the genes spans the whole LR. Initially up-regulated genes are predominant at 24 – 30, 42 – 48, 66 and 96 – 120 h after PH; initially down-regulated genes are overwhelmed at 6 – 18, 36, 60 and 72 h; there are no initially expressed genes at 54 and 144 – 168 h.

4 Discussion

This paper aims to study the roles of the genes associated with eight liver diseases (e.g. alcoholic liver disease, fatty liver, hepatic cirrhosis, liver vascular diseases, hepatomegaly, acute liver necrosis, HE and HPS) in LR.
Here, we mainly discuss the relationship between LR and the genes involved in the former three kinds of liver diseases. Many studies have shown that almost all ingested alcohol is metabolized in liver and consumption of excessive alcohol can lead to various liver diseases, such as alcoholic liver disease, fatty liver, hepatic cirrhosis. The biochemical mechanism of alcoholic liver disease is as following: firstly, alcohol is broken down to acetaldehyde by alcohol dehydrogenase genes \( adh4, adh7, dhrs4, dhrs9 \) and \( ltb4dh \)\footnote{28}, cytochrome gene \( cyp2d6 \)\footnote{29}, catalase gene \( sod2 \)\footnote{30} and transcription regulator gene \( hnf4a \)\footnote{31} which promote alcohol degradation; subsequently, acetaldehyde is converted to acetate by aldehyde dehydrogenase genes \( aldhl1a1, aldhl1a2 \) and \( aldhl1b1 \), whose enzymatic activities are positively regulated by \( hsd11b2 \). The results from the chip detection (see Table 1) showed that above 12 genes involved in alcohol degradation were all markedly reduced in mRNA level from 6 to 24 h time points after PH, inferring that alcohol oxidation maybe decreases in these phases.

Fatty liver is the initial and most common consequence of chronic alcohol ingestion, the process of fatty liver production is as follows: the increased acetate formation leads to generation of excessive hydrogen; the produced hydrogen in turn converts NAD to NADH, which lowers fatty acid oxidation and allows triglyceride to accumulate, causing fatty liver. Investigation has shown that fatty acid and triglyceride metabolisms involve many genes, among these genes, the lipid transporter \( atp10a \)\footnote{32} and three fat accumulation-promoting genes \( ghrl, trib3 \) and \( sreb1 \)\footnote{30,33} exhibited the observable up-regulation at post-PH 18 – 24 h and 48 h; while four fatty acid oxidation-enhancing genes \( lpin1, lpin2, creb1 \) and \( ppara \)\footnote{34,35} were meaningless or down expressed in the same periods as the above. Based on the above results, it is presumable that fat assimilation is possibly enhanced between 18 – 48 h.

Both acetaldehyde and lipid peroxidation products can recruit leukocytes to produce multiple inflammatory cytokines, which will elicit a vicious circle of inflammation and loss of hepatocytes. And hepatic cirrhosis is just advanced liver disease characterized by extensive fibrosis. Many studies have shown that cirrhosis involves various physiological and biochemical processes such as cell proliferation, cell death, inflammatory response and extracellular matrix formation etc, involving multiple genes. For example, five genes \( cdk4, ceca2, cendl1, ccnel \) and \( wee1 \) participate in cell cycle progression; \( pdgfc \) has the role in phosphorylation of the protein encoded by \( rps6kb1 \) and both of them coordinately induce

\[ \text{\textit{Figure 3. Expression patterns of 70 liver diseases-associated genes during LR. These genes exhibit 23 types of expression patterns. A – G: up-regulated in expression; H – L: down-regulated; M – O: up/down-regulated mixed. X-axis represents recovery time after PH (h); Y-axis shows logarithm ratio of the signal values of genes at each time point to control.}} \]
activation and proliferation of hepatic satellite cells (HSCs); entpd2 has the negative regulatory role in bile ductular proliferation. There appeared the strikingly increased expression of the former seven genes and the reduced expression of the last one between 18 – 72 h after PH according to the chip data, suggesting that cell reproduction is promoted in metaphase of LR. Igfr1r inhibits apoptosis in hepatic fibrosis; igfbp3 can induce apoptosis and the function of hgf is just contrary to that of igfbp3. The above three genes were apparently down-regulated mainly at 36 h after PH. These results indicate that cell proliferation at metaphase in LR is not limitless but controlled by related genes. Mif is response for inflammation in cirrhosis; ptgs2 is involved in inflammatory response by catalyzing prostaglandin synthesis; ctila4 can inhibit T-celled mediated the immune response, once aberrance is susceptibility to alcoholic cirrhosis; CTLA4 can inhibit T-cell mediated the immune response, which is response for inflammation in cirrhosis; LPS inhibits apoptosis in hepatic fibrosis; TGFβ3 inhibits ECM deposition by repressing HSC activation; tlr4 enhances immunoreaction in primary biliary cirrhosis. These genes were down-regulated mainly at 48 – 60 h after PH, supposing that inflammatory response perhaps become weaker at late-metaphase of LR. Col3a1, col1a2 and col4a6 all act as the structural components of extracellular matrix (ECM); agtr1a may enhance ECM deposit; timp1 inhibits ECM degradation; lgals3 stimulates procollagen synthesis in injured liver myofibroblasts; il13ra2 accelerates collagen deposition; tgfβ2 can stimulate type IV collagen synthesis; fgf1 and fgf2 increase collagen type I synthesis in HSC; ifng prevents liver from fibrosis by accelerating collagen degradation; NR0B2-FXR (farnesoid X receptor) regulatory cascade repress the expression of type I collagen; lhx2 inhibits ECM deposition by repressing HSC activation. It is observed that the former 10 genes negatively affect the amount of ECM while the actions of the latter 3 genes are just reversed. At the same time, our study indicated that expression level of these 13 genes was all increased at 0.5 – 6 and 48 – 72 h after PH, speculating that they co-regulate the total mass of ECM proteins during LR.

In summary, this paper employs the Rat Genome 230 2.0 Array to check the expression changes of the genes associated with eight kinds of liver diseases, finding that 27 liver diseases-associated genes are up-regulated during LR, 22 genes down-regulated and 21 up/down-regulated. It was primarily approved that fat assimilation was enhanced between 18 – 48 h; alcohol degradation was decreased between 6 – 24 h after PH; cell reproduction was promoted in metaphase. However, DNA → mRNA → protein is affected by many factors including protein interaction. In future, the above results need to be further tested using the techniques including Northern blotting, protein array, RNA interference etc.

References
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