

# Microarray comparison of the gene expression profiles in the adult vs. embryonic day 14 rat liver

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**Abstract.** The aim of the present study was to identify the differentially-expressed genes of embryonic day 14 (ED 14) rat liver in comparison to adult rat liver, which may provide specific information for the investigation of the hepatogenesis mechanism. The gene expression profiles of ED 14 and adult rat livers were investigated using microarray analysis (the Illumina RatRef-12 Expression BeadChip). Quantitative polymerase chain reaction (qPCR) analyses were conducted to confirm the gene expression. There were 787 genes upregulated in the embryonic liver. Based on the gene ontology classification system, which was analyzed by the database for annotation, visualization and integrated discovery software, a number of the upregulated genes were categorized into the distinct and differentially-expressed functional groups, including metabolism pathway, cell cycle, transcription, signal transduction, purine metabolism, cell structure, transportation and apoptosis. qPCR analyses confirmed the gene expression. Eleven upregulated genes were found in the ED 14 rat liver, which may provide specific information for the understanding of the molecular mechanisms that control hepatogenesis. These overexpressed genes are potential markers for identifying hepatic progenitor cells.

## Introduction

The liver is the largest internal organ that is involved in a vast number of essential biochemical functions, including metabolic, exocrine and endocrine systems. Hepatogenesis is the process of liver formation, and the liver originates

from the gut endoderm. In rats, hepatic development begins at embryonic day (ED) 9.5, as primitive epithelial cells of the foregut come into contact with the cardiac mesoderm and form the liver diverticulum. Subsequently, these cells invade the septum transversum, proliferate extensively and differentiate further (1). At ED 10.5, these cells acquire the morphological appearance of immature liver epithelial cells and are termed hepatoblasts, which are bipotential and capable of generating hepatocyte and cholangiocyte cell lineages (1,2). Germain *et al* (3) reported that hepatoblasts began to differentiate to hepatocyte and cholangiocyte cells at ED 15 in rats; however, Petkov *et al* (4) found that differentiation started at ED 16-17. Previous studies have found that certain molecular mechanisms regulate hepatogenesis, such as cardiac tissue induction and the role of the septum transversum mesenchyme, as well as the presence of endothelial cells (5-7). Several transcription factors are also involved in controlling distinct aspects of hepatogenesis, including Prox1, which is necessary for hepatoblast migration (8); homeobox factor Hex, which is essential for morphogenesis and growth of the liver bud (9); HNF4, which is required for hepatocyte differentiation and epithelial transformation of the liver (10); and GATA6, which is required for liver bud growth (11).

Hepatogenesis is an extremely intricate process, as a variety of genes are involved and a complex network is formed. However, certain mechanisms are not clear; numerous associated genes have not been found. Future studies are required to investigate how to get the endoderm reactive potency in liver development, and to examine whether the upstream and downstream activation mechanisms of the genes are involved. The interactions between the epithelial and mesenchymal cells remain unclear.

In the present study, a rat genome-wide gene expression bead chip was used to investigate the global patterns of gene expression of the rat liver at ED 14 compared to adult rat liver, which may provide specific information for the investigation of hepatogenesis mechanism. The Illumina BeadChip is a forefront chip technology, and its genome expression chip can be used for human, mouse and rat genome-wide expression studies (12,13). The rat genome microarray used could detect the expression of 21,910 genes. However, in our previous study (4), the microarray only focused on a portion of the genes expressed during rat hepatogenesis. Therefore,

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the current study involves the genome-wide analysis of differentially-expressed genes.

## Materials and methods

**Animals and treatment.** Two pregnant Sprague-Dawley rats at ED 14 (experimental group) and two adult female Sprague-Dawley rats (control group) were purchased from the Experimental Animal Center in Shandong University of Traditional Chinese Medicine (Shandong, China). The livers of the rats were removed. All the experimental animals used in the study were utilized under the protocol approved by the Institutional Animal Care and Use Committee of Weifang Medical University (Shandong, China).

**Isolation of ED 14 and adult liver cells.** The cell suspensions from the ED 14 and adult rat livers were prepared as previously reported (14). The cells were plated in gelatin-coated dishes at a density of  $15 \times 10^6$  cells per 10-cm plate. Following the removal of the hematopoietic cells (no attachment) by washing with phosphate-buffered saline, the epithelial cells were allowed to attach for 16 h.

**RNA extraction and quality assessment.** Total RNA was isolated from frozen liver cells by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. The samples were digested with DNase (Invitrogen) and eluted with 30  $\mu$ l RNase-free water. The concentration and quality of the samples were measured by using the DU-640 nucleic acid/protein analyzer (Beckman Coulter, Brea, CA, USA).

**Microarray analysis procedures.** The microarray experiments (including sample labeling, hybridization and initial data analysis) were performed at Beijing Emei Tongde Technology Development Co., Ltd. (Beijing, China). For each sample, biotinylated cRNA was prepared using an Ambion Illumina TotalPrep RNA amplification kit (Applied Biosystems, Foster City, CA, USA). Total RNA (5  $\mu$ g) was converted to double-stranded cDNA using T7-oligo (dT) primers. Subsequently, an *in vitro* transcription reaction was performed to amplify biotinylated cRNA, as described in the manufacturer's instructions (Illumina, Inc., San Diego, CA, USA). The biotinylated cRNA was hybridized to a RatRef-12 Expression BeadChip platform that contained 21,910 probes (Illumina, Inc.). Hybridization, washing and scanning were performed in accordance with the manufacturer's instructions.

The chips were scanned by the BeadArray reader (Illumina, Inc.). The microarray images were registered and extracted automatically during the scan using the manufacturer's default settings.

**Microarray data analysis.** The microarray data were analyzed with Illumina BeadStudio software (Illumina, Inc.). The average normalization method was used. The sample intensities were scaled by a factor equal to the ratio of the average intensity of the virtual sample to the average intensity of the provided sample. The background was subtracted prior to scaling. The average normalization minimizes the amount of variation resulting from constant multiplicative factors

(including scanner power). The sample intensities were scaled by a factor so that the average signal of all the samples was equal to the global average of all the sample signals. Background subtraction was performed prior to scaling, and therefore, one-half of the unexpressed targets were predicted to have negative signals.

The gene expression differences between the two groups were calculated by DiffScore. The screening criteria for the differences in gene expression were that the detection P-value of the experimental or control group was  $<0.01$  and the experimental group DiffScore value was  $>20$ .

Gene ontology (GO) analysis was performed with the GO classification system and using the database for annotation, visualization and integrated discovery software (<http://david.abcc.ncifcrf.gov/>) (15). The over-representation of the genes with altered expression within specific GO categories was determined using the one-tailed Fisher's exact probability test, which was modified by adding a jack-knifing procedure. This penalizes the significance of categories having extremely few (one or two) genes and favors more robust categories having a larger number of genes (16). A number of these upregulated genes could be categorized into distinct, differentially-expressed functional groups (including the genes that are associated with the metabolism pathway, the cell cycle, transcription, signal transduction, urine metabolism, cell structure, transportation and apoptosis).

**Quantitative polymerase chain reaction (qPCR) experiment.** To validate the microarray results, the 11 most differentially and significantly expressed genes were selected for qPCR analysis using the same RNA samples that were used for the microarray. The ABI PRISM 7700 sequence detection system (Applied Biosystems) was used to perform one-step qPCR. The results were quantified as Ct values; this value signifies relative gene expression (the ratio of target/control). qPCR results were analyzed by the comparative Ct method (17). Table I presents the primers used in the study. All the results are expressed as mean  $\pm$  standard deviation. Statistical analysis was performed with analysis of variance followed by Student's t-test using SPSS 11.0 (SPSS, Inc., Chicago, IL, USA).  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Screening of the gene expression profile of ED 14 rat liver by microarray analysis.** To screen the genes associated with ED 14 rat liver, a genome-wide microarray analysis was performed using the Illumina RatRef-12 Expression BeadChip (Illumina, Inc.), which contains 21,910 distinct rat oligonucleotide probes. This microarray chip encompasses the largest number of rat genes that were available at the time of the analysis. Total RNA was extracted from ED 14 and adult rat liver cells. The extracted RNA was of high quality and was homogeneous. Data analyses by average normalization of the hybridization signals showed that the expressions of 787 genes were upregulated in the ED 14 rat liver (DiffScore values,  $>20$ ). A number of these highly-expressed genes could be grouped into distinct, differentially-expressed functional groups based on the GO classification system. These upregulated genes included those that are associated with

Table I. Oligonucleotide sequences of the primers used for quantitative polymerase chain reaction.

| Gene symbol  | Forward primer (5-3') | Reverse primer (5-3') |
|--------------|-----------------------|-----------------------|
| <i>Hk2</i>   | CTGGTGCCCGACTGTGAT    | CCATTTCACCTTCATTCTT   |
| <i>Eno2</i>  | GGGACAAACAGCGTTACTT   | CAATGTGGCGATAGAGGG    |
| <i>HMGA1</i> | AGCCTTCGGTGAGTCCTGG   | GCTGTGCCCTTGTCTTGC    |
| <i>Pde9a</i> | CCACCATCTCCCTTCTGA    | CTCCACCACTTTGAGTCCTT  |
| <i>KIF4</i>  | TTCCACCTAAGCCCAAAC    | CTCCTCCTCAGCCACAGA    |
| <i>Plag1</i> | CCTTGCCCTCCAGCGAACT   | CGCCACCTTGTAATCCATCAG |
| <i>Orc1</i>  | GGATGATGCCGTCCAGTT    | CACCACGCTGATGGGAAA    |
| <i>Cspg2</i> | GTAATGTGACGGATAGAACG  | GTAAATGGCTGGGAAGAG    |
| <i>Foxm1</i> | ATCGCTACTTGACATTGGA   | CTCAGGATTGGGTCGTTT    |
| <i>Ccnf</i>  | ACACCCACCGCAGAACTA    | TCTCCTGGTCTCCCTCAT    |
| <i>SPAG5</i> | TGTAAAGGCCAAATAGAAC   | ACAATGGGAATGCTGACT    |
| <i>GAPDH</i> | AGACAGCCGCATCTTCTTGT  | CTTGCCGTGGGTAGAGTCAT  |

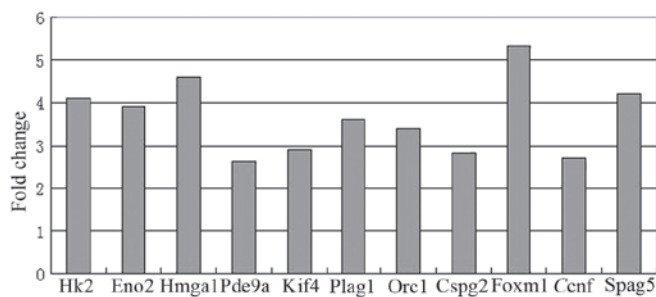


Figure 1. Fold changes for the expression of genes that are differentially expressed in rat liver at embryonic day 14 compared to adult rat liver. Fold changes were measured by quantitative polymerase chain reaction analysis of the 11 candidate genes initially selected by microarray analysis.

the metabolism pathway, the cell cycle, transcription, signal transduction, purine metabolism, cell structure, transportation and apoptosis (Table II).

**qPCR analysis.** Fig. 1 shows the results of the qPCR experiment for 11 genes. The expression of the 11 genes were all significantly increased in ED 14 rat liver ( $P < 0.05$ ), compared to their expression in adult rat liver, confirming the validity of the microarray results.

## Discussion

In the present study, the gene expression pattern of ED 14 rat liver compared to adult rat liver was investigated. The microarray analysis initially suggested that there were 787 candidate genes. Some of the identified genes are known to be involved in hepatogenesis. A previous study reported that high levels of Bmp4 expression within the septum transversum mesenchyme implied a significant role for Bmp4 signaling during hepatogenesis (5). A number of these highly-expressed genes can be grouped into distinct, differentially-expressed functional groups based on the GO classification system. These upregulated genes include genes that are associated with the metabolism pathway, cell cycle, transcription, signal transduction, purine metabolism, cell structure, transportation and apoptosis. qPCR confirmed that 11 genes were significantly and differentially expressed.

These differentially-expressed genes may provide certain information for the investigation of hepatogenesis mechanism.

In the adult rat liver, numerous genes belonging to the group of genes associated with the metabolism pathway were expressed at extremely low levels or were not expressed. However, the function of certain genes remains unknown. The *Hk2*, *Eno2*, *Eno1*, *Galnt7*, *Slc1a3*, *Galnt1* and *Galnt3* genes are involved in glucose metabolism. *Hk2* can catalyze glucose, mannose, glucosamine and fructose into their respective 6-phosphate form through an irreversible phosphorylation reaction. Enolase (also known as phosphoglycerate hydrolase) is a critical enzyme for adenosine triphosphate synthesis in glycolysis, and is responsible for catalyzing the conversion of 2-phosphoglycerate to phosphoenolpyruvate and in catalyzing the reverse reaction during gluconeogenesis (18). *Eno1* exists in the majority of tissues. However, recent studies have shown that *Eno1* has a transcriptional regulatory function as it can combine with the c-Myc promoter region, which can inhibit c-Myc transcriptional function. The present study found increased *Eno1* expression in the embryonic liver. This action may be associated with increased glycolysis in the embryonic liver tissue. *Eno2* (also known as NSE) is the homodimer of  $\gamma$  subunits and is primarily found in neurons and neuroendocrine tissues.

An example of a gene associated with the cell cycle is cyclin F, which belongs to the F-box protein family that is characterized by a ~40-amino acid motif. In contrast to the majority of cyclins, it does not bind or activate any cyclin-dependent kinases. However, cyclin F, similar to other cyclins, oscillates during the cell cycle with protein levels; it peaks in the G2 phase. Through its ability to restrain the expression of CP110, cyclin F plays a significant role in limiting centrosome duplication and in maintaining chromosome stability (19).

A spindle-binding protein, SPAG5 (also known as astrin, hMAP126), plays an important role in the regulation of mitosis. SPAG5 is located in the centromere during the medium term of mitosis. The downregulation of SPAG5 expression results in spindle deformity and may delay mitosis and therefore result in cell cycle arrest at the G2/M phase (20). Glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) binds with SPAG5 and subsequently phosphorylates and regulates the location of SPAG5 in the spindle

Table II. Genes with increased expression in the embryonic day 14 rat liver<sup>a</sup>.

| Gene symbol                   | Definition   | Accession no.  | Detection P-value | DiffScore |
|-------------------------------|--|----------------|-------------------|-----------|
| <b>Metabolism pathway</b>     |  |                |                   |           |
| <i>Hk2</i>                    | Hexokinase 2   | NM_012735.1    | 0                 | 360.045   |
| <i>Eno2</i>                   | Eenolase 2, $\gamma$   | NM_139325.1    | 0                 | 360.045   |
| <i>Galnt7</i>                 | UDP-N-acetyl- $\alpha$ -D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7  | NM_022926.1    | 0.00121           | 106.51    |
| <i>Gcnt1</i>                  | Glucosaminyl (N-acetyl) transferase 1, core 2  | XM_579453.1    | 0.00121           | 88.097    |
| <i>Hsd11b2</i>                | Hydroxysteroid 11- $\beta$ dehydrogenase 2   | NM_017081.1    | 0.00121           | 86.04     |
| <i>Sphk1</i>                  | Sphingosine kinase 1   | NM_133386.2    | 0.00242           | 79.566    |
| <i>Galnt11</i>                | UDP-N-acetyl- $\alpha$ -D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 1, transcript variant 2 (predicted) | XM_001053416.1 | 0                 | 74.865    |
| <i>Galnt3</i>                 | UDP-N-acetyl- $\alpha$ -D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3  | NM_001015032.2 | 0.00242           | 53.1      |
| <i>Eno1</i>                   | Enolase 1, $\alpha$  | NM_012554.1    | 0.00242           | 48.033    |
| <i>Gad1</i>                   | Glutamic acid decarboxylase 1  | NM_017007.1    | 0.00121           | 34.698    |
| <i>Gfpt1</i>                  | Glutamine fructose-6-phosphate transaminase 1  | NM_001005879.1 | 0.00242           | 31.043    |
| <i>Dbh</i>                    | Dopamine $\beta$ hydroxylase   | NM_013158.1    | 0.00364           | 24.062    |
| <b>Cell cycle</b>             |  |                |                   |           |
| <i>Cdc23</i>                  | Cell division cycle 23, yeast, homolog   | XM_214588.4    | 0.00242           | 67.5      |
| <i>Ccne1</i>                  | Cyclin E   | XM_574426.1    | 0.00242           | 58.898    |
| <i>Ccnf</i>                   | Cyclin F   | XM_001054372.1 | 0                 | 360.045   |
| <i>E2f1</i>                   | E2F transcription factor 1   | XM_230765.4    | 0.00121           | 130.672   |
| <i>Tgf<math>\beta</math>3</i> | Transforming growth factor, $\beta$ 3  | NM_013174.1    | 0.00242           | 22.983    |
| <i>Mapk8</i>                  | Mitogen-activated protein kinase 8   | XM_341399.2    | 0.00242           | 28.211    |
| <i>SPAG5</i>                  | Sperm-associated antigen 5   | XM_340848.3    | 0                 | 360.045   |
| <i>Nfkb1</i>                  | Nuclear factor of $\kappa$ light chain gene enhancer in B-cells 1, p105  | XM_001075876.1 | 0.00242           | 34.263    |
| <b>Transcription</b>          |  |                |                   |           |
| <i>E2f1</i>                   | E2F transcription factor 1   | XM_230765.4    | 0.00121           | 130.672   |
| <i>HMGA1</i>                  | High-mobility group AT-hook 1  | NM_139327.1    | 0                 | 360.045   |
| <i>Bmp4</i>                   | Bone morphogenetic protein 4   | NM_012827.1    | 0.00242           | 79.573    |
| <i>Nkx2-5</i>                 | NK2 transcription factor related, locus 5 ( <i>Drosophila</i> )  | NM_053651.1    | 0.00242           | 63.247    |
| <i>Rai14</i>                  | Retinoic acid induced 14   | NM_001011947.1 | 0.00121           | 108.339   |
| <i>Twist1</i>                 | Twist gene homolog 1 ( <i>Drosophila</i> )   | NM_053530.2    | 0                 | 143.869   |
| <i>Foxm1</i>                  | Forkhead box M1  | NM_031633.1    | 0                 | 360.045   |
| <i>Zfhlx1b</i>                | Zinc finger homeobox 1b  | NM_001033701.1 | 0.00242           | 35.255    |
| <i>Sst</i>                    | Somatostatin   | NM_012659.1    | 0.00121           | 65.352    |
| <i>Myog</i>                   | Myogenin   | NM_017115.2    | 0.00242           | 36.437    |
| <i>Hdac7a</i>                 | Histone deacetylase 7A   | XM_345868.3    | 0.00242           | 60.799    |
| <i>Lef1</i>                   | Lymphoid enhancer binding factor 1   | NM_130429.1    | 0                 | 57.738    |
| <i>Runx1</i>                  | Runt-related transcription factor 1  | NM_017325.1    | 0.00121           | 34.814    |
| <b>Signal transduction</b>    |  |                |                   |           |
| <i>Cpt1c</i>                  | Carnitine palmitoyltransferase 1c  | XM_001078512.1 | 0.00242           | 49.495    |
| <i>Fbxl12</i>                 | F-box and leucine-rich repeat protein 12   | NM_001025700.1 | 0.00242           | 27.332    |
| <i>Pde9a</i>                  | Phosphodiesterase 9A   | NM_138543.1    | 0                 | 360.045   |
| <i>Dnmt2</i>                  | DNA methyltransferase 2  | NM_001031643.1 | 0.00242           | 40.064    |
| <i>Bmp4</i>                   | Bone morphogenetic protein 4   | NM_012827.1    | 0.00242           | 79.573    |
| <i>Fbxl12</i>                 | F-box and leucine-rich repeat protein 12   | NM_001025700.1 | 0.00242           | 27.332    |
| <i>HMGA1</i>                  | High-mobility group AT-hook 1  | NM_139327.1    | 0                 | 360.045   |
| <i>Hnrpa1</i>                 | Heterogeneous nuclear ribonucleoprotein A1   | NM_017248.1    | 0.00242           | 81.233    |
| <i>Hrc</i>                    | Histidine-rich calcium-binding protein   | NM_181369.2    | 0.00242           | 30.671    |
| <i>Zfhlx1b</i>                | Zinc finger homeobox 1b  | NM_001033701.1 | 0.00242           | 35.255    |



Table II. Continued.

| Gene symbol       | Definition  | Accession no.  | Detection P-value | DiffScore |
|-------------------|---|----------------|-------------------|-----------|
| <i>Cutl1</i>      | Cut-like 1 ( <i>Drosophila</i> )  | XM_001070482.1 | 0.00242           | 84.871    |
| <i>Mapk8</i>      | Mitogen-activated protein kinase 8  | XM_341399.2    | 0.00242           | 28.211    |
| <i>Ptk2</i>       | PTK2 protein tyrosine kinase 2  | NM_013081.1    | 0.00242           | 47.335    |
| <i>Crk</i>        | v-Crk sarcoma virus CT10 oncogene homolog (avian)                           | NM_019302.1    | 0.00242           | 38.966    |
| <i>Pscd3</i>      | Pleckstrin homology, Sec7 and coiled-coil domains 3                         | NM_053912.2    | 0.00242           | 48.629    |
| <i>Fgf12</i>      | Fibroblast growth factor 12   | NM_130814.1    | 0.00242           | 21.89     |
| <i>Kit</i>        | v-Kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog               | NM_022264.1    | 0.00242           | 32.155    |
| Purine metabolism |   |                |                   |           |
| <i>Gmpr</i>       | Guanosine monophosphate reductase   | NM_057188.1    | 0                 | 63.736    |
| <i>Adcy8</i>      | Adenylate cyclase 8 (brain)   | NM_017142.1    | 0.00242           | 43.906    |
| <i>Adcy2</i>      | Adenylate cyclase 2 (brain)   | NM_031007.1    | 0                 | 36.184    |
| <i>Adcy3</i>      | Adenylate cyclase 3   | NM_130779.1    | 0.00242           | 25.177    |
| <i>Pde9a</i>      | Phosphodiesterase 9A  | NM_138543.1    | 0                 | 360.045   |
| Cell structure    |   |                |                   |           |
| <i>Actn1</i>      | Actinin $\alpha$ 1  | NM_031005.2    | 0.00242           | 30.32     |
| <i>Actn3</i>      | Actinin $\alpha$ 3  | NM_133424.1    | 0.00242           | 39.463    |
| <i>Cspg2</i>      | Chondroitin sulphate proteoglycan 2   | XM_215451.4    | 0                 | 360.045   |
| <i>Ptk2</i>       | Protein tyrosine kinase 2   | NM_013081.1    | 0.00242           | 47.335    |
| Transportation    |   |                |                   |           |
| <i>Slc18a2</i>    | Solute carrier family 18 (vesicular monoamine), member 2                    | NM_013031.1    | 0.00242           | 29.17     |
| <i>Slc29a2</i>    | Solute carrier family 29 (nucleoside transporters), member 2                | NM_031738.1    | 0.00242           | 31.183    |
| <i>Slc39a6</i>    | Solute carrier family 39 (metal ion transporter), member 6                  | NM_001024745.1 | 0.00121           | 111.965   |
| <i>Slc6a4</i>     | Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 | NM_013034.1    | 0.00242           | 50.804    |
| <i>Slc9a5</i>     | Solute carrier family 9 (sodium/hydrogen exchanger), member 5               | NM_138858.1    | 0.00121           | 133.543   |
| <i>KIF4</i>       | Kinesin family member 4   | XM_343797.3    | 0                 | 360.045   |
| Apoptosis         |   |                |                   |           |
| <i>Anp32a</i>     | Acidic (leucine-rich) nuclear phosphoprotein 32 family, member A            | NM_012903.1    | 0.00242           | 46.678    |
| <i>Orc1</i>       | Origin recognition complex, subunit 1                                       | NM_177931.2    | 0                 | 360.045   |
| <i>Plagl1</i>     | Pleiomorphic adenoma gene 1   | NM_001008316.1 | 0                 | 360.045   |
| <i>Mapk8</i>      | Mitogen-activated protein kinase 8  | XM_341399.2    | 0.00242           | 28.211    |
| <i>E2f1</i>       | E2F transcription factor 1  | XM_230765.4    | 0.00121           | 130.672   |
| <i>Smo</i>        | Smoothened homolog ( <i>Drosophila</i> )                                    | NM_012807.1    | 0.00242           | 71.987    |
| <i>Ccne1</i>      | Cyclin E  | XM_574426.1    | 0.00242           | 58.898    |
| <i>Actn3</i>      | Actinin $\alpha$ 3  | NM_133424.1    | 0.00242           | 39.463    |
| <i>Inha</i>       | Inhibin $\alpha$  | NM_012590.1    | 0.00242           | 55.708    |
| <i>Trim35</i>     | Tripartite motif protein 35   | NM_001025142.1 | 0.00121           | 124.313   |
| <i>Phlda1</i>     | Pleckstrin homology-like domain, family A, member 1                         | NM_017180.1    | 0.00242           | 43.949    |
| <i>Sst</i>        | Somatostatin  | NM_012659.1    | 0.00121           | 65.352    |

\*Only genes with an explicit function are listed.

and centrioles. Kif2b, CLASP1 and SPAG5 (astrin) form a molecular switch in the centromere to regulate centromere and microtubule dynamics, promote the process of mitosis and maintain mitosis loyalty (21).

For the genes associated with transcription, high-mobility group AT-hook 1 (HMGA1) is a non-histone protein involved in numerous cellular processes, including the regulation of

inducible gene transcription, integration of retroviruses into chromosomes and metastatic progression of cancer cells (22). The level of HMGA is high in embryonic tissues and insignificant in normal adult tissues. As early as ED 8.5, HMGA1 transcripts are highly detectable in all embryonic tissues. During mid-late gestation, its expression is gradually confined to specific body organs and tissues, including the central

nervous system (primarily confined to the germinal layer), primordial liver, kidney and the retina. The high expression of the *HMGA1* gene is associated with cell proliferation and to establishing the correct identity of particular cells types (23). As a transcription regulatory factor, HMGA1 could stimulate the binding of nuclear factor- $\kappa$ B to the interferon- $\beta$  promoter interacting with the p50 and p65 subunits. The HMGA1 proteins may be involved in organizing chromatin at the local level to provide the correct architecture for other transcription factors that are associated with playing a significant role in controlling growth.

The Foxm1 protein is a proliferation-specific member of the Fox family. Liver regeneration markedly induces Foxm1b expression at the G1/S transition and continues throughout hepatocyte proliferation.

Several signal transducers are also upregulated in rat embryonic liver. *Pde9a*, which maps to human chromosome 21q22.3 between *TFF1* and *D21S360*, includes >20 splice variants, potentially changing the N-terminal amino acid sequences of the encoded proteins. *Pde9a* is highly expressed in the brain, heart, placenta, adult and embryonic kidney, spleen, prostate and colon (24), has a high affinity for cyclic guanosine 3',5'-monophosphate (cGMP) and can catalyze the hydrolysis of cGMP to the corresponding nucleoside 5'-monophosphate.

In association with the cell structure, the protein Cspg2 belongs to the extracellular matrix chondroitin sulphate proteoglycan family. Cspg2 has four isoforms, labeled V0-V3. The isoforms V0 and V1 are highly expressed during embryonic development and their expression decreases following tissue maturation. Cspg2 is involved in extracellular matrix assembly and in controlling cell adhesion, proliferation, migration and apoptosis (25). In the mesenchymal cell condensation area, Cspg2 is highly expressed during the development of cartilage, heart, hair follicles and kidney. *In vitro* evidence shows that the Cspg2 V0 and V1 isoforms are involved in precartilaginous mesenchymal condensation and subsequent chondrogenesis (26). The requirement of Cspg2 in development is highlighted by the finding that a Cspg2 deficiency (in a transgenic mouse model) is embryonically lethal due to defects in cardiac formation, limb mesenchymal aggregation and chondrogenesis (27). However, the overexpression of Cspg2 in the embryonic liver has not been reported previously.

The group that consists of the genes associated with transportation includes a variety of genes involved in material transportation, such as solute carrier family 18, member 2 (*Slc18a2*), which transports vesicular monoamine; *Slc29a2* (which transports nucleosides); *Slc39a10*, which transports zinc ions; *Slc39a6*, which transports metal ions; *Slc6a4*, which is a neurotransmitter transporter; *Slc9a5*, which is a  $\text{Na}^+/\text{H}^+$  transporter protein; and *KIF4*, which transports cytoplasmic vesicles. The transport of these substances can regulate the activities of certain significant biological enzymes. Specific substances form cofactors and particular substances are involved in the signal transduction processes of growth factors or hormones.

Hepatogenesis requires the growth and proliferation of certain cells and the removal of others by apoptosis. However, only a limited number of genes associated with apoptosis were present on the chips in the present study. The pleomorphic adenoma gene 1 (*Plag1*) is a novel developmentally-regulated

$\text{C}_2\text{H}_2$  zinc finger gene. The deduced amino acid sequence of the *Plag1* protein shows seven canonical  $\text{C}_2\text{H}_2$  zinc finger domains and a serine-rich C terminus; the latter may have a regulatory function. According to Pendeville *et al* (28), the embryonic lung and liver, but not adult organs, primarily express *Plag1*. However, *Plag1* has been found in a variety of tumors. The oncogenic activity of *Plag1* results from its positive regulation of insulin growth factor 2 (IGF-II) expression. The peptide growth factor IGF-II plays an important role in embryonic development and carcinogenesis. The expression of IGF-II is primarily located in hepatocytes of the embryonic and neonatal liver (29). These findings indicate that the upregulation of *Plag1* could reduce the apoptosis rate of cancer cells.

Orc1 is the largest subunit of the origin recognition complex (ORC), which plays a critical role during the initiation of DNA replication in eukaryotes. The expression level of the Orc1 subunit oscillates throughout the cell cycle (which defines an Orc1 cycle); it accumulates during the G1 phase and is degraded during the S phase. By controlling the progression of the S phase, Orc1 regulates the growth of animal cells. Orc1 is closely correlated with cell proliferation and the cell cycle and strictly controls the progress of the cell cycle. Saha *et al* (30) found that low Orc1 levels could rapidly induce caspase-3 activity, which subsequently induces p53-independent apoptosis. Orc1 is expressed in the adult brain and muscle and in numerous embryonic tissues. However, no studies have been performed regarding its expression in the liver. The results of the present study show that Orc1 may play a multifunctional role in the embryonic liver, and it possibly functions as a mediator of p53-independent apoptosis.

In conclusion, 11 upregulated genes were found in the ED 14 rat liver compared to the adult rat liver, which may provide novel insights into the molecular mechanisms that control hepatogenesis. These overexpressed genes are potential markers for identifying hepatic progenitor cells.

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