Distinct expression patterns in hepatitis B virus- and hepatitis C virus-infected hepatocellular carcinoma

Chun-Feng Lee, Zhi-Qiang Ling, Ting Zhao, Kuan-Rong Lee

AIM: To identify biomarkers indicating virus-specific hepatocarcinogenic process, differential mRNA expression in 32 patients with hepatitis B virus (HBV)-/hepatitis C virus (HCV)-associated hepatocellular carcinoma (HCC) were investigated by means of cDNA microarrays comprising of 886 genes.

METHODS: Thirty two HCC patients were divided into two groups based on viral markers: hepatitis B virus positive and HCV positive. The expression profiles of 32 pairs of specimens (tumorous and surrounding non-tumorous liver tissues), consisting of 886 genes were analyzed.

RESULTS: Seven up-regulated genes in HBV-infected group included 4 genes: VIM (cell structure), ACTB (cell structure), GAPD (glycolysis) and CD58 (cell adhesion). The expression patterns of the 11 genes, identified by cDNA microarray, were confirmed by quantitative RT-PCR in 32 specimens.

CONCLUSION: The patterns of all identified genes were classified based on the viral factor involved in HBV- and HCV-associated HCC. Our results strongly suggest that the pattern of gene expression in HCC is closely associated with the etiologic factor. The present study indicates that HBV and HCV cause hepatocarcinogenesis by different mechanisms, and provide novel tools for the diagnosis and treatment of HBV- and HCV-associated HCC.

Abstract

Aim: To identify biomarkers indicating virus-specific hepatocarcinogenic process, differential mRNA expression in 32 patients with hepatitis B virus (HBV)-/hepatitis C virus (HCV)-associated hepatocellular carcinoma (HCC) were investigated by means of cDNA microarrays comprising of 886 genes.

Methods: Thirty two HCC patients were divided into two groups based on viral markers: hepatitis B virus positive and HCV positive. The expression profiles of 32 pairs of specimens (tumorous and surrounding non-tumorous liver tissues), consisting of 886 genes were analyzed.

Results: Seven up-regulated genes in HBV-associated HCC comprised genes involved in protein synthesis (RPSS5), cytoskeletal organization (KRT8), apoptosis related genes (CFLAR), transport (ATP5F1), cell membrane receptor related genes (IGFBP2), signal transduction or transcription related genes (MAP3K5), and metastasis-related genes (MMP9). The up-regulated genes in HCV-infected group included 4 genes: VIM (cell structure), ACTB (cell structure), GAPD (glycolysis) and CD58 (cell adhesion). The expression patterns of the 11 genes, identified by cDNA microarray, were confirmed by quantitative RT-PCR in 32 specimens.

Conclusion: The patterns of all identified genes were classified based on the viral factor involved in HBV- and HCV-associated HCC. Our results strongly suggest that the pattern of gene expression in HCC is closely associated with the etiologic factor. The present study indicates that HBV and HCV cause hepatocarcinogenesis by different mechanisms, and provide novel tools for the diagnosis and treatment of HBV- and HCV-associated HCC.

© 2008 The WJG Press. All rights reserved.

Key words: Hepatocellular carcinoma; Hepatitis B virus; Hepatitis C virus-infected; cDNA microarray; Expression profiling

Peer reviewers: Peter Karayiannis, PhD, Associate Professor, Department of Medicine, Hepatology Section, St Mary’s Hospital Campus, South Wharf Road, London W2 1NY, United Kingdom; Jose JG Marin, Professor, Head of the Departamento Physiology and Pharmacology, University of Salamanca, CIBERehd, Campus Miguel de Unamuno, ED-S09, Salamanca 37007, Spain

Lee CF, Ling ZQ, Zhao T, Lee KR. Distinct expression patterns in hepatitis B virus- and hepatitis C virus-infected hepatocellular carcinoma. World J Gastroenterol 2008; 14(39): 6072-6077. Available from: URL: http://www.wjgnet.com/1007-9327/14/6072.asp DOI: http://dx.doi.org/10.3748/wjg.14.6072

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide[1]. The major risk factors for HCC are chronic hepatitis resulting from infection with HBV and HCV, and exposure to various exogenous carcinogens, including aflatoxin B1[2]. Several studies have shown that the incidence of HCC has increased substantially in East Asia, including China, Korea and Japan[3,4]. More than 350 million people worldwide are known to be chronic carriers of HBV[5]. Moreover, the incidence of HCC is increasing in many countries in...
parallel with the increase in chronic HCV infection\textsuperscript{[3-8]}. Therefore, clarification of the genetic portrait of hepatocarcinogenesis caused by HBV or HCV infection may provide clues to help reduce the incidence of HCC, and establish effective treatments for HCC. However, the molecular nature of this association is poorly understood.

The phenotypic diversity of cancer is accompanied with a corresponding diversity in the gene expression patterns\textsuperscript{[6-10]}. Honda \textit{et al}\textsuperscript{[11]} showed the presence of different gene expression profiles in the liver lesions of chronic hepatitis caused by HBV and HCV, and suggested that the molecular mechanisms responsible for the pathogenesis of HCC differ between HBV and HCV infections. In the present study, we investigated the gene expression patterns of 32 HCC samples, using cDNA microarrays containing 886 clones in order to gain additional insight into hepatocarcinogenesis or cancer progression related to HBV and HCV infections. The aim of the present study was to characterize the gene expression associated with HCC, with a view to better understand the molecular pathophysiology, which may lead to better methods of detection, diagnosis, and classification of HCC.

**MATERIALS AND METHODS**

**Patient material**

The Institutional Review Board on Medical Ethics, Zhejiang Provincial People Hospital (China), approved the method of tissue collection. The present study was conducted in the department of surgery, Zhejiang Provincial People Hospital, on 32 patients who underwent hepatectomy for sporadic HCC without preoperative radio- or chemotherapy. All of tissue samples were immediately frozen in liquid nitrogen, and stored at -80°C until use. A total of 32 HCC samples from 15 lymph node negative and 17 lymph node positive cases were used (Table 1).

**Laser microdissection**

Eight μm-thick sections of the frozen tissue were cut at -20°C and stained with HE. Under microscopic observation, parts of cancer cells nests in the invasive and intraductal components were microdissected, using the LM100 laser capture microdissection system (Arcturus Engineering, Mountain View, CA, USA). A 15 μm-diameter beam was used to capture the tumor cells and the corresponding non-cancerous liver tissues. The cell nests were transferred to a LCM transfer film (CapSure TF-100S transfer film carrier, 5 mm-diameter optical-grade transparent plastic; Arcturus Engineering).

**RNA preparation and T7-based RNA amplification**

Total RNA was isolated from the dissected specimens using Trizol reagent (Gibco BRL) and a modified acidic guanidinium phenol-chloroform method, following the manufacturer’s recommendations. Total RNA was treated with DNase I for removal of genomic DNA, and the mRNA was purified using a poly(A) purification kit (Oligotex, Qiagen), according to the manufacturer’s instructions. The quality of mRNA was assessed by $A_{260}/A_{280}$ ratios and the contamination of genomic DNA was checked using the PCR method. cDNA was synthesized with T7-oligo (dT) primer (Ambion) and SuperScript II enzyme (Gibco BRL), as described in the instruction manual. cDNA was purified by cDNA clean-up column (DNA clear kit, Ambion). cRNA was generated by T7 MEGAScript kit (MEGAscript in vitro Transcription Kit, Ambion, AUSTIN, Tex), per the manufacturer’s recommendations. Column purification of cRNA was performed with RNaseasy kit (Qiagen), according to the manufacturer’s protocol. The concentration and quality of cRNA were analyzed by GeneQuant pro RNA/DNA Calculator (Amersharmacia biotech).

**Microarray hybridization and scanning**

Human Cancer Chip version 4.0 (IntelliGene, TaKaRa) was used for these studies. This array was spotted on a glass slide with 886 cDNA fragments of human genes, which are composed of 588 human identified genes related to cancer, and 298 cDNA fragments prescreened by differential display method between cancer tissue and normal tissue. Three μg of cRNA from the tumor and

| Case | Sex | Age | Hepatitis virus | Differentiated grade | TNM score |
|------|-----|-----|-----------------|---------------------|-----------|
| 1    | M   | 54  | HBV            | WD                  | T3N1M0    |
| 2    | M   | 60  | HCV            | WD                  | T3N1M0    |
| 3    | F   | 61  | HBV            | WD                  | T2N1M0    |
| 4    | M   | 62  | HBV            | WD                  | T2N1M0    |
| 5    | M   | 58  | HBV            | WD                  | T1N1M0    |
| 6    | F   | 56  | HCV            | MD                  | T3N1M0    |
| 7    | F   | 44  | HCV            | WD                  | T2N1M0    |
| 8    | M   | 49  | HCV            | WD                  | T1N1M0    |
| 9    | M   | 58  | HBV            | WD                  | T2N1M0    |
| 10   | M   | 67  | HCV            | PD                  | T3N1M0    |
| 11   | M   | 69  | HBV            | WD                  | T2N1M0    |
| 12   | F   | 63  | HCV            | WD                  | T1N1M0    |
| 13   | M   | 48  | HCV            | MD                  | T2N1M0    |
| 14   | F   | 63  | HBV            | WD                  | T1N1M0    |
| 15   | M   | 49  | HCV            | MD                  | T1N1M0    |
| 16   | F   | 51  | HBV            | PD                  | T3N1M0    |
| 17   | M   | 65  | HCV            | MD                  | T3N1M0    |
| 18   | F   | 58  | HBV            | PD                  | T4N1M1    |
| 19   | M   | 60  | HBV            | MD                  | T2N1M0    |
| 20   | F   | 56  | HCV            | PD                  | T3N1M1    |
| 21   | M   | 42  | HCV            | PD                  | T3N1M0    |
| 22   | M   | 55  | HBV            | PD                  | T4N1M1    |
| 23   | M   | 66  | HBV            | MD                  | T3N1M0    |
| 24   | F   | 70  | HCV            | WD                  | T2N1M0    |
| 25   | M   | 58  | HBV            | PD                  | T4N1M1    |
| 26   | M   | 53  | HCV            | PD                  | T3N1M0    |
| 27   | M   | 61  | HBV            | PD                  | T4N1M0    |
| 28   | F   | 65  | HBV            | MD                  | T3N1M0    |
| 29   | M   | 59  | HCV            | MD                  | T3N1M0    |
| 30   | M   | 50  | HBV            | PD                  | T3N1M0    |
| 31   | F   | 63  | HCV            | PD                  | T4N1M1    |
| 32   | M   | 66  | HCV            | PD                  | T3N1M0    |

M: Male; F: Female; HBV: Hepatitis B virus infection; HCV: Hepatitis C virus infection; WD: Well differentiated HCC; MD: Moderately differentiated HCC; PD: Poorly differentiated HCC.
the matched normal tissue were labeled with Cy3-dUTP and Cy5-dUTP respectively (Amersham Pharmacia Biotech, Buckinghamshire, England), using a labeling kit (RNA Fluorescence Labeling Core kit, TaKaRa), according to the manufacturer's instructions. The labeled probe was purified by centrifugation in a spin column (Centrisep, Priceton Separations, Adelphia, NJ). Two separate probes were combined, and 2 μL of 5 × competitor containing Cot I (Gibco BRL), poly dA (Amersham Pharmaca Bio), and tRNA (TaKaRa) were added. After addition of 50 μL of 100% ethanol and 2 μL of 3 mol/L sodium acetate (pH 5.2), the mixture was cooled at -80°C for 30 min, followed by centrifugation at 15 000 g for 10 min, and pelleted down. For final probe preparation, the pellet was washed in 500 μL of 70% ethanol twice, and eluted in 10 μL of 3 mol/L sodium acetate (pH 5.2). Supernatants were placed on the array and covered with a 22-mm × 22-mm glass coverslip. The coverslip was sealed with a glue, and the probes were incubated overnight at 65°C for 16 h in a custom-made slide chamber with humidity maintained by underlying moist papers. After hybridization, the slides were washed in 2 × SSC with 0.1% SDS, 1 × SSC, and 0.05 × SSC, sequentially for 1 min each, and then spin dried. Hybridized arrays were scanned using a confocal laser-scanning microscope (Affymetrix 428 array scanner, Santa Clara, CA). Image analysis and quantification were performed with ImaGene 4.2 software (BioDiscovery), according to the manufacturer's instructions.

Data processing
Each spot was defined by manual positioning of a grid of circles over the array image. For each fluorescent image, the average pixel intensity within each circle was determined, and a local background, outside of 3 pixel buffer range from the circle was computed for each spot. Net signal was determined by subtraction of the local background from the average intensity of each spot. Signal intensities between the two fluorescent images were normalized by the intensities of the house-keeping genes provided on the arrays. The fluorescence intensities of Cy5 (non-tumor) and Cy3 (tumor) for each target spot were adjusted so that the mean Cy3: Cy5 ratios of 32 housekeeping gene spots were equal to one. Because data derived from low signal intensities are less reliable, we first determined the cutoff values for signal intensities on each slide so that all of the filtered genes had greater S/N (signal to noise) ratios of Cy3 or Cy5 than three, and we excluded genes for further analysis when both Cy3 and Cy5 dyes gave signal intensities lower than the cutoff. To estimate the range of expression ratio within which the expression change could be considered as fluctuation in non-cancerous cells, we compared expression profiles of non-cancerous cells from 6 patients. Because 90% of expression ratios in non-cancerous cells fell within the range of 1.726 and 0.503, we categorized genes into three groups according to their expression ratios (Cy3: Cy5) up-regulated (ratio, 2.0); down-regulated (ratio 0.5); and unchanged expression (ratios, between 0.5 and 2.0); provided that signal counts of T (Cy3) and R (Cy5) were > 500. Genes with Cy3:Cy5 ratios > 2.0 or < 0.5 in more than 75% of the cases examined were defined as commonly up- or down-regulated genes, respectively.

Real-time reverse transcription PCR
LightCycler (Roche Diagnostics) technology was applied to confirm the data obtained by cDNA microarray. The primer sequences of 11 genes were obtained from the GDB Human Genome Database (http://www.gdb.org/gdb/)(Table 2). We used the same RNA from the disected cells for the microarray analysis. First-strand cDNA was obtained by reverse transcription using a commercially available kit (first strand synthesis kit, Amersham). For each PCR, 2 μL (20 ng) first strand cDNA template, 50 pmol of each primer, 2.4 μL (3 mmol) MgCl₂ and 2 μL 10 × SYBR Green I (Roche Laboratories) were mixed in 20 μL of PCR mixture. The running protocol was programmed as follows. In the first step, initial denaturation, reaction mixture was incubated for 10 min at 95°C. In the second step, DNA was amplified for 45 cycles at 95°C for 10 s, specific annealing temperature (the primer sequences dependent) for 0-10 s, and elongation at 72°C for some seconds [amplicon (bp)/25 s]. Finally, the temperature was raised gradually (0.2°C/s) from the annealing temperature to 95°C for the melting curve analysis. Twelve μL of PCR product were visualized by electrophoresis on 2% agarose gel stained with ethidium bromide.

### Table 2 Primers used to amplify cDNA at various genes in real time RT-PCR

| Target gene | Objective | Forward primer sequence (5’-3’) | Reverse primer sequence (3’-5’) | Genebank accession no./Amplicon size |
|-------------|-----------|---------------------------------|---------------------------------|----------------------------------|
| RPS2       | qRT-PCR   | GCCGTCGAGCCACGCTTCTTCGAGACAC   | CCGCTTTGAGGAGGCGGTAT             | NM_001009          152 bp        |
| KRT8       | qRT-PCR   | GCCGCTGATATCCAGCCTACGGACACAC   | GCGCCTCGGCTGCTGCTGCTGAAT         | NM_002273        637 bp         |
| CFLAR      | qRT-PCR   | GAGGACGTCTCTCTGAGACACACAC      | GCTTCCTGCATACAGCCTAATTAAT        | AF009618           116 bp        |
| ATP15F1    | qRT-PCR   | ACTGCGCTTATATGTGCTGCTAT       | GCAAGATCGTGCACAAAGGGA            | NM_001688         131 bp        |
| IGBP2      | qRT-PCR   | GCAAGACGTCTGCTGCTGCTGCT       | GCTGGAATAGCCTCTGCTGCTGAAT         | NM_000997         121 bp        |
| MAPK1K5    | qRT-PCR   | GAAAGAGATTTGATGCTCTGCTGCT      | GCTGGAATAGCCTCTGCTGCTGAAT         | NM_005923         233 bp        |
| MMP9       | qRT-PCR   | GACGAGACTCTTCTGACAGACACAC      | GCTTCCGCTACCCGACCTAGTA           | NM_004994         139 bp        |
| VIM        | qRT-PCR   | AGTTCACTCTCTTGCTGCTGCT        | GCTTCCGCTACCCGACCTAGTA           | NK007244         98 bp          |
| ACTB       | qRT-PCR   | GATGGACTTCCGCTGCACGACAC        | GCTTCCGCTACCCGACCTAGTA           | NM_003005         250 bp        |
| GAPD       | qRT-PCR   | CAACTCCTGCTGCTGCTGCTGCT       | GCTTCCGCTACCCGACCTAGTA           | AC003289         260 bp        |
| CD58       | qRT-PCR   | CTATCGGATAGTGGTCTGTGCTGCT     | GCTTCCGCTACCCGACCTAGTA           | NM_001779         154 bp        |

6074 ISSN 1007-9327 CN 14-1219/R World J Gastroenterol October 21, 2008 Volume 14 Number 39
The amount of gene expression was normalized to the amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using Human GAPDH kit (GmbH Heidelberg, Germany). The qRT-PCR analysis was carried out in triplicate for each cDNA sample, and the median values were used for the three experiments. Up- and down-regulation were defined as the median value > 2.0 and < 0.5, respectively.

Statistical analysis
Statistical analysis among mean values was performed on the association of lymph node metastasis with expression levels by applying non-parametric Kruskal-Wallis and Mann-Whitney U tests. Statistical significance was defined as a P-value of < 0.05. Differential expression between the groups of HBV-infected and HCV-infected HCC was considered significant, with P < 0.05.

RESULTS
Quality analysis of total RNA after LCM and cRNA after T7-based amplification
About 20 slides were prepared from each sample, and the target cells were captured with at least 1000 cells per slide. Consequently, we captured a total of approximately 25000-30000 tumor cells and normal cells for RNA extraction. The quality of total RNA extracted after LCM was assessed by A_{260}/A_{280} and electrophoresis. To be considered for microarray analysis, the RNA samples were required to pass quality control criteria, with integrity of 28S and 18S, and A_{260}/A_{280} greater than 2.0. Products of cDNA synthesis and cRNA were also checked by A_{260}/A_{280} and electrophoresis. The results showed that A_{260}/A_{280} of all the RNA samples met the quality control criteria for sample preparation. Clear image appearance of 28S and 18S of ribosomal RNA was seen under the electrophorogram for each total RNA sample, which had to be intact and without degradation. RNA was subjected to two rounds of T7-based RNA amplification after removal of DNA contamination by RNase-free Dnase I treatment as described in the methods section. All RNA samples were successfully amplified by an estimated 250-fold, using T7 RNA polymerase. cDNA synthesis and cRNA showed satisfactory quality control criteria, with 1.5 kb < cDNA < 5.0 kb; 1.0 kb < cRNA < 4.5 kb; and A_{260}/A_{280} ratio of cDNA and cRNA greater than 2, respectively.

Identification of genes related to HBV-positive or HCV-positive status
After reverse transcription, each cDNA probe was labeled with Cy3- or Cy5-conjugated dyes and hybridized to microarray cDNAs with 886 genes. We compared the expression profiles of cancer cells and the corresponding normal cells in each case. A representative scatter plot of microarray analysis of carcinoma cells and non-cancerous tissue in case 20 (HCV-infected HCC) is shown in Figure 1. Up-regulated, down-regulated and unchanged genes are indicated by red, green and blue spots, respectively. We first arranged the relative expression of each gene (Cy3/Cy5 intensity ratio) into one of four categories: up-regulated (ratio > 2.0), down-regulated (ratio < 0.5), unchanged (ratio between 0.5 and 2.0), and not expressed (or slight expression but under the cutoff level for detection).

To identify the genes related to HBV-positive and HCV-positive status, 32 patients were divided into two groups: HBV-associated HCC group in which HBV was positive in 17 patients, and HCV-associated HCC group in which HCV was positive in 15 patients (Table 1). When comparing gene expression profiles in the two groups, there were 7 genes that were commonly up-regulated, and expressed more than 2.09-fold in the HCV-infected group compared with in the HCV-infected group. On the other hand, 4 down-regulated genes in HBV-infected group correlated significantly with the HCV-infected group. Table 3 shows the list of differentially expressed genes and their respective category based on the GO (Gene Ontology) system and TreeView. The up-regulated genes in HBV-infected group were involved in protein synthesis (RPS5), cytoskeletal organization (KRT8), apoptosis related genes (CFLAR), transport (ATP5F1), cell membrane receptor related genes (IGFBP2), signal transduction or transcription related genes (MAP3K5), and metastasis-related genes (MMP9). The up-regulated genes in HCV-infected group included genes such as VIM (cell structure), ACTB (cell structure), GAPD (glycolysis) and CD58 (cell adhesion).

Verification of the genes related to HBV- and HCV-associated HCC using quantitative RT-PCR
To more quantitatively examine our data on hepatitis virus infection in HCC, we selected 7 up-regulated genes from the HBV-infected group, and 4 up-regulated genes from the HCV-infected group. The expression level of the selected genes was confirmed by quantitative RTPCR analysis in 32 patients. We used cDNA synthesized from 32 pair samples without amplification as template for real-time semiquantitative reverse transcription PCR. The results demonstrated that the samples obtained by means of T7-based amplification appropriately reflected the status of the original RNA in a proportional manner. The results of the DNA microarray were reproduced by reverse transcriptase PCR.

DISCUSSION
Genome-wide gene expression analysis of human cancer
may provide important clues in understanding HCC oncogenesis and may lead to improvement in predicting its clinical behavior\textsuperscript{[12]}. Using cDNA microarray, we examined the difference in gene expression profiles between normal liver tissues and HCC cells, as well as between HBV-positive and HCV-associated HCC. The data from cDNA microarray are consistent with RT-PCR data from HCC tissues and the corresponding non-tumor tissues. These expression profiles may be useful in elucidating the molecular carcinogenesis of HCC, especially HBV- and HCV-associated HCC.

In the present study, we attempted to establish a link between gene expression and the viral status of HCC. Comparative analysis of HBV- and HCV-associated HCC revealed that 11 genes, for which the expression levels differed between HBV- and HCV-associated HCC. Ribosomal-related genes such as RPS5 (RPL family genes) were up-regulated in HBV-associated HCC compared to HCV-associated HCC, suggesting the activation of protein translation in HBV-infected HCC. This observation is consistent with a previous report that major classes of genes encoding ribosomal proteins were up-regulated by the HBX protein\textsuperscript{[13]}. Cytoskeletal organization, such as KRT8 was shown to be up-regulated in HBV-associated HCC, as well as genes such as ACTB in HCV-associated HCC. Our results support the hypothesis that the deregulation of genes encoding proteins associated with cytoskeleton play a role in liver carcinogenesis\textsuperscript{[14]}. These findings also indicate that the pathway for liver carcinogenesis in the cytoskeleton may be different in HBV- and HCV-associated HCC. Cell adhesion genes such as CD58 were found to be up-regulated in HCV-associated HCC, but have not been reported to be related with human HBV-associated HCC. Xu et al\textsuperscript{[15]} showed that several signal transduction-related genes, including MAPK family genes were up-regulated in HBV-associated HCC. Up-regulation of MAPK has also been suggested as a common pathway for hepatocarcinogenesis caused by HBV and HCV infections\textsuperscript{[16]}. In the present study, MAP3K5 was up-regulated in HBV-associated HCC compared with the non-tumor liver tissue. However, MAP3K5 was down-regulated in HCV-associated HCC compared with the non-tumorous liver. Thus, additional studies are necessary to clarify the contribution of the MAPK pathway to each type of HCC. MMP9, which may promote metastasis, was up-regulated in HBV-associated HCC compared with HCV-associated HCC. Other genes such as IGFBP2, ATP5F1, VIM and GAPD, which are expressed differently in HBV- and HCV-associated HCC, were newly identified, although the findings of up-regulation of genes such as IGFBP2 and ATP5F1 in the HBV-associated HCC, and the up-regulation of genes such as VIM and GAPD in the HCV-associated HCC, were in agreement with previous observations\textsuperscript{[17]}. It has been suggested that liver carcinogenesis induced by HBV and HCV, in addition to common genetic and epigenetic alterations, may involve distinct pathways\textsuperscript{[18]}. Our expression profiles suggest that hepatitis viruses affect the expression of dozens of genes in HCC in a type-specific manner, thus invoking slightly different mechanisms of carcinogenesis. We believe that the results obtained in the present study will help our understanding of the molecular mechanisms underlying the pathogenesis of HBV- and HCV-associated HCC. The identification of genes defining virus type-specific expression profiles may contribute to our ability to develop virus type-dependent treatment regimens.

### Table 3  The 11 genes for which expression levels differed between HBV- and HCV-associated HCC

| Gene name                                  | Symbol       | Accession       | Fold change | HBV:HCV  |
|--------------------------------------------|--------------|-----------------|-------------|----------|
| 7 genes up-regulated in HBV-associated HCC |              |                 |             |          |
| Ribosomal protein S5                       | RPS5         | NM_001009       | 6.35        | 2.38     |
| Keratin 8                                  | KRT8         | NM_002273       | 5.68        | 3.19     |
| ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1 | CFAR     | Y14039         | 2.86        | 2.09     |
| Insulin-like growth factor binding protein 2 (36 kDa) | IGF2B2     | NM_000597       | 3.37        | 2.49     |
| Mitogen-activated protein kinase kinase 5  | MAP3K5       | NM_005923       | 3.76        | 2.33     |
| Matrix metalloproteinase 9 (gelatinase B, 92 kDa gelatinase, 92 kDa type IV collagenase) | MMP9      | NM_004994       | 7.43        | 3.74     |
| 4 genes up-regulated in HCV-associated HCC |              |                 |             |          |
| Vimentin                                   | VIM          | NM_003380       | 8.61        | 0.28     |
| Actin-β                                    | ACTB         | X00351          | 4.13        | 0.37     |
| Glyceraldehyde-3-phosphate_dehydrogenase   | GAPD         | NM_002046       | 5.27        | 0.29     |
| CD58 antigen, (lymphocyte function-associated antigen 3) | CD58      | NM_001779       | 4.68        | 0.31     |

\textsuperscript{1}Symbol in LocusLink database; \textsuperscript{2}GeneBank accession number; \textsuperscript{3}Fold change, ratio of mean expression values in HBV- or HCV-associated cases (cancer cells vs non-cancerous cells); \textsuperscript{4}HBV:HCV ratio of mean expression values (HBV positive cases to HCV positive cases).

### Background

Hepatocellular carcinoma (HCC) is one of the most common fatal cancers worldwide. The major risk factors for HCC are chronic hepatitis resulting from infection with hepatitis B virus (HBV) and hepatocellular carcinoma (HCC), and exposure to various exogenous carcinogens, including aflatoxin B1. It has been reported that the incidence of HCC is increasing in several countries in parallel with the increase in chronic HBV and HCV infections. Therefore, clarification of the genetic portraits of hepatocarcinogenesis caused by HBV and HCV infection may provide clues to reducing the incidence of HCC, and establishing effective treatments for each type of HCC. However, the molecular nature of this association is poorly understood.

### Research frontier

The aim of the present study was to identify any useful biomarkers indicating virus-specific hepatocarcinogenic process. The differential mRNA expression in 32 patients with HBV-HCV-associated HCC was investigated by means of RT-PCR data from HCC tissues and the corresponding non-cancerous cells; 4 genes up-regulated in HCV-associated HCC; 7 genes up-regulated in HBV-associated HCC.
cDNA microarrays comprising of 886 genes.

**Innovations and breakthroughs**

It has been suggested that liver carcinogenesis induced by HBV and HCV, in addition to common genetic and epigenetic alterations, may involve distinct pathways. The results of the present study suggest that hepatitis viruses affect the expression of dozens of genes in HCC in a type-specific manner, thus invoking slightly different mechanisms of carcinogenesis. Genome-wide gene expression analysis of human cancer may provide important clues to understanding HCC oncogenesis and lead to improvements in predicting its clinical behavior.

**Applications**

We believe that the results obtained in this study will provide greater understanding of the molecular mechanisms underlying the pathogenesis of HBV- and HCV-associated HCC. The identification of genes defining virus type-specific expression profiles may contribute to our ability to develop virus type-dependent treatment regimens.

**Terminology**

DNA microarray is a meticulous technology used in molecular biology and in the field of biomedicine. This technique involves an arrayed series of thousands of microscopic spots of DNA oligonucleotides. It may involve a short section of a gene or other DNA elements that are used as probes to hybridize cDNA or cRNA samples (called target) under high-stringency conditions. Probe-target hybridization is usually detected and quantified by fluorescence-based detection of fluorophore-labeled targets to determine relative abundance of nucleic acid sequences in the target.

**Peer review**

This is a nice study on the changes in the expression patterns in cancer liver tissue associated with two different hepatitis viruses involved in hepatocarcinogenesis. The paper is well written and contains valuable data. The authors, using microarray technology, have compared gene expression between cancerous and non-cancerous liver tissue in both HBV and HCV infected patients. Seven genes were up-regulated in HBV and 4 genes, which were different, were up-regulated in HCV infected patients.

**REFERENCES**

1. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999; 340: 745-750
2. Kasai Y, Takeda S, Takagi H. Pathogenesis of hepatocellular carcinoma: a review from the viewpoint of molecular analysis. *Semin Surg Oncol* 1996; 12: 155-159
3. Parkin DM, Pisani P, Ferlay J. Global cancer statistics. *Cancer J Clin* 1999; 49: 33-64, 1
4. Okuda K, Fujimoto I, Hanai A, Urano Y. Changing incidence of hepatocellular carcinoma in Japan. *Cancer Res* 1987; 47: 4967-4972
5. Lee WM. Hepatitis B virus infection. *N Engl J Med* 1997; 337: 1733-1745
6. Perou CM, Jeffrey SS, van de Rijn M, Rees CA, Eisen MB, Ross DT, Pergamenschikov A, Williams CF, Zhu SX, Lee JC, Lashkari D, Shalon D, Botstein D. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proc Natl Acad Sci USA* 1999; 96: 9212-9217
7. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JF, Yang L, Marti GE, Moore T, Hudson J Jr, Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Levy R, Wilson W, Grever MR, Byrd JC, Botstein D, Brown PO, Staudt LM. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000; 403: 503-511
8. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. *Nature* 2000; 406: 747-752
9. Ross DT, Scherf U, Eisen MB, Perou CM, Rees C, Spellman P, Iyer V, Jeffrey SS, Van de Rijn M, Waltham M, Pergamenschikov A, Lee JC, Lashkari D, Shalon D, Myers TG, Weinstein JN, Botstein D, Brown PO. Systematic variation in gene expression patterns in human cancer cell lines. *Nat Genet* 2000; 24: 227-235
10. Welsh JB, Zarrinkar PP, Sapinoso LM, Kern SG, Behling CA, Monk BJ, Lockhart DJ, Burger RA, Hampton GM. Analysis of gene expression profiles in normal and neoplastic ovarian tissue samples identifies candidate molecular markers of epithelial ovarian cancer. *Proc Natl Acad Sci USA* 2001; 98: 1176-1181
11. Honda M, Kaneko S, Kawai H, Shiroya T, Kobayashi K. Differential gene expression between chronic hepatitis B and C hepatic lesion. *Gastroenterology* 2001; 120: 955-966
12. Iizuka N, Hamamoto Y, Tsunedomi R, Oka M. Translational microarray systems for outcome prediction of hepatocellular carcinoma. *Cancer Sci* 2008; 99: 659-665
13. Wu CG, Forgues M, Siddique S, Farnsworth J, Valerie K, Wang XW. SAGE transcript profiles of normal primary human hepatocytes expressing oncogenic hepatitis B virus X protein. *FASEB J* 2002; 16: 1665-1667
14. Le Bail B, Faouzi S, Bousserarie L, Balabaud C, Bioulac-Sage P, Rosenbaum J. Extracellular matrix composition and integrin expression in early hepatocarcinogenesis in human cirrhotic liver. *J Pathol* 1997; 181: 330-337
15. Xu XR, Huang J, Xu ZG, Qian BZ, Zhu ZD, Yan Q, Cai T, Zhang X, Xiao HS, Qu J, Liu F, Huang QH, Cheng ZH, Li NG, Du JJ, Hu W, Shen KT, Lu G, Fu G, Zhong M, Xu SH, Gu WY, Huang W, Zhao XT, Hu GX, Gu JR, Chen Z, Han ZG. Insight into hepatocellular carcinogenesis at transcriptional level by comparing gene expression profiles of hepatocellular carcinoma with those of corresponding noncancerous liver. *Proc Natl Acad Sci USA* 2001; 98: 15089-15094
16. Okabe H, Satoh S, Kato T, Kitahara O, Yanagawa R, Yamaoka Y, Tsunoda T, Furukawa Y, Nakamura Y. Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression. *Cancer Res* 2001; 61: 2129-2137
17. Yoon SY, Kim JM, Oh HJ, Jeon YJ, Lee DS, Kim JH, Choi JY, Ahn BM, Kim S, Yoo HS, Kim YS, Kim NS. Gene expression profiling of human HBV- and/or HCV-associated hepatocellular carcinoma cells using expressed sequence tags. *Int J Oncol* 2006; 29: 315-327
18. Delpuech O, Trabut JB, Carnot F, Feuilard J, Brechot C, Kremsdorf D. Identification, using cDNA macroarray analysis, of distinct gene expression profiles associated with pathological and virological features of hepatocellular carcinoma. *Onkogene* 2002; 21: 2926-2937

S- Editor Tian L  L- Editor Anand BS  E- Editor Ma WH