Characteristics of Hepatic IGF-II Expression and Monitored Levels of Circulating IGF-II mRNA in Metastasis of Hepatocellular Carcinoma

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Key Words: Hepatocellular carcinoma; Insulin-like growth factor II (IGF-II); IGF-II mRNA; Gene amplification; Metastasis; Peripheral blood mononuclear cells

Abstract

The prognosis of hepatocellular carcinoma (HCC) remains dismal. Insulin-like growth factor II (IGF-II), a fetal growth factor, is highly expressed during HCC development. We examined serum IGF-II levels and circulating IGF-II messenger RNA (mRNA) expression and analyzed the clinicopathologic characteristics in patients with liver diseases. The higher IGF-II level in the serum of patients with HCC could be correlated with hepatitis B virus infection but not with patient sex, age, tumor size, and α-fetoprotein (AFP) level. Total RNAs were extracted from liver tissues or peripheral blood mononuclear cells, and IGF-II complementary DNA (cDNA) and AFP cDNA were synthesized through random primers and reverse transcriptase. Gene fragments were amplified by nested polymerase chain reaction and confirmed by sequencing. The incidence of the hepatic IGF-II gene was 100% in HCC, 54.3% in paracancerous tissues, and none in noncancerous tissues. The incidence rates for circulating IGF-II and AFP genes were 34.3% and 52.7%, respectively, and for both, 61.6% in patients with HCC. They were 100% in cases with extrahepatic metastasis. The IGF-II abnormality associates with HCC, and circulating IGF-II and IGF-II mRNA are useful molecular markers for HCC differential diagnosis and hematogenous metastasis.
liver diseases and its RNA in tissues and peripheral blood of patients with HCC were analyzed by reverse transcriptase–polymerase chain reaction (PCR) or enzyme-linked immunosorbent assay (ELISA) to estimate the clinical values of IGF-II and IGF-II mRNA as tumor markers in HCC differential diagnosis and hematogenous metastasis.

Materials and Methods

Patients

We evaluated 146 patients (108 men and 38 women) with HCC who were treated at the Affiliated Hospital of Nantong University, Nantong, China. The patients’ ages ranged from 25 to 81 years (median, 48.3 years). The incidence of hepatitis virus in the patients was 75.3% for the hepatitis B surface antigen (HBsAg) and 10.3% for the antibody to the hepatitis C virus. Other cases studied included 40 of chronic hepatitis, 35 of acute hepatitis, 38 of cirrhosis, and 25 of nonliver tumors (lung cancer, 6; gastric cancer, 6; esophageal cancer, 3; breast cancer, 3; colon cancer, 3; cervical cancer, 2; and pancreatic cancer, 2) and samples from 55 healthy people with hepatitis viral markers (hepatitis B virus [HBV] DNA, HBsAg, and anti–hepatitis C virus) and a normal alanine aminotransferase level obtained from the Nantong Central Blood Bank as control samples.

All cases were diagnosed by blood biochemical tests, viral histology, and B-ultrasonic examination. All peripheral blood samples, 5 mL of blood with heparin, were collected in the morning, and peripheral blood mononuclear cells (PBMCs) were separated at once. The serum AFP concentrations ranged from 35 to 4,650 ng/mL (35–4,650 μg/L; median, 568 ng/mL [568 μg/L]), and samples that exceeded 50 ng/L (50 μg/L) were taken as a positive result. The detection of circulating AFP mRNA and the separation of PBMCs in this study were performed as described elsewhere. The diagnosis of HCC and viral hepatitis was based on the criteria proposed by Chinese National Collaborative Cancer Research Group and at the Chinese National Viral Hepatitis Meeting, respectively.

Preparation of PBMCs

Ficoll (2.5 mL) was added to each sample. After centrifugation at 2,000 rpm for 20 minutes, the PBMCs were collected from the Ficoll/plasma interface. Then they were washed 3 times in normal saline and pelleted by using low-speed centrifugation. The cells were collected at 2 × 10⁵ per tube and then stored at –85°C for total RNA isolation.

Liver Specimens

Fresh HCC specimens were used for this study. Cancerous, paracancerous (2 cm to cancer), and noncancerous (5 cm to cancer) tissues were collected from 35 patients who underwent operations for liver cancer at the Affiliated Hospital of Nantong University. The specimens were immediately frozen in liquid nitrogen and kept at –85°C until required. The patients included 28 men and 7 women, ranging in age from 22 to 70 years. Prior written informed consent was obtained from all patients according to the World Medical Association Declaration of Helsinki, and the study received ethics board approval from the Affiliated Hospital of Nantong University. The histologic types of all HCC specimens were graded in differentiation degrees as follows: well, 9; moderate, 12; and poor, 14. Of these specimens, 20 showed single tumor tubercles and the rest multiple; 14 were stage II, 13 were stage III, and 8 were stage IV. Each specimen was divided into 2 parts and analyzed by total RNA abstraction and pathologic examination.

Total RNA Isolation and Synthesis of Complementary DNA

Total RNAs were isolated from PBMCs and from liver tissues by the guanidine thiocyanate method with the RNAzole reagent (Promega, Madison, WI) and purified as described elsewhere. The RNAs were dissolved in tromethamine-hydrochloride buffer (10 mmol/L, pH 8.0) containing EDTA, 10 mmol/L. The concentration of total RNA was assessed by optical density measurements at 260 nm in a UV spectrophotometer and expressed as total RNA micrograms per milligram of wet tissue and then stored at –85°C. For synthesis of complementary DNA (cDNA), 2 μg of total RNA was denatured in the presence of random hexamers (200 pmol/L, Promega) at 95°C for 5 minutes and incubated with the Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL, Grand Island, NY) at 23°C for 10 minutes, 42°C for 60 minutes, and 95°C for 10 minutes; placed on ice for 5 minutes; and then stored at –20°C for subsequent PCR analysis.

Amplification of Nested PCR

The resulting cDNA was amplified by a nested PCR with 2 pairs of primers. The oligonucleotides were designed according to the IGF-II sequence and synthesized with a synthesizer (model 381 A, Applied Biosystems, Foster City, CA). The sequences of the 2 external primer pairs used for the initial PCR amplification were IGF-II-1 (sense), 5'-ATGGGAAATGCCAATGGGAAG-3' (nucleotide [nt] 251–271); and IGF-II-2 (antisense), 5'-CTTGCCCACGGGTTATCTGGG-3' (nt 566–586); the size of the amplified gene fragment was 336 base pairs (bp). The sequences of the 2 internal primer pairs used for the second PCR amplification were IGF-II-3 (sense), 5'-TGGCTGATTGCGTCTACGG-3' (nt 311–330); and IGF-II-4 (antisense), 5'-AGGTCACAGCTGGAGAAC-3' (nt 461–480). PCR amplification consisted of initial denaturation at 94°C for 5 minutes, followed by 94°C for 25 seconds, 55°C
for 30 seconds, and 72°C for 90 seconds for 30 cycles. The final product of the nested PCR was 170 bp. The human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genome was used as a control. The primer sequences for GAPDH were GAPDH-1 (sense), 5'-ACCACAGTCCATGCCATCAC-3' (nt 601–620), and GAPDH-2 (antisense), 5'-TCCACAC- CTTGTTCCTGA-3' (nt 1033–1052); the product of the PCR was 452 bp (GAPDH gene transcript, 40 pmol/L). The PCR products were electrophoresed on 2% agarose gels with ethidium bromide staining. The fragment sizes were evaluated using PCR markers (Promega) as molecular weight standards.

Sequencing of PCR Products
The 170-bp amplified product of the human IGF-II genome was purified with a Montage PCR centrifugal filter device (Millipore, Billerica, MA) according to the manufacturer’s instructions. One microgram of DNA was used for preparation of the sequencing reaction and directly sequenced using the MegaBACE DNA analysis system (MegaBACE DNA sequencer with the DYEnamic ET Dye Terminator Cycle Sequencing Kit, Amersham Biosciences, Piscataway, NJ), following the manufacturer’s protocol. The sequences were edited using the MegaBACE Sequence Analyzer, version 3.0 program (Amersham Biosciences) and aligned with the amplified sequences of the IGF-II genome, HCC tissue, and circulating IGF-II.

Quantitative Detection of Serum IGF-II Levels
The levels of serum IGF-II were detected by using a human IGF-II ELISA kit (Cusabio Biotech, Newark, DE) according to the manufacturer’s instructions. For the study, 50 μL of serum human IGF-II or standard were separately put into each well of a 96-well ELISA plate, and then 50 μL of HRP-conjugate was added to the wells, and incubated for 2 hours at 37°C. Next, 50 μL of substrate A and 50 μL of substrate B were added to each well for 15 minutes at 37°C. Then, 50 μL of stop solution was added to each well, and absorbance was read at 450 nm. During the procedure, washing the plate was according to the ELISA routine method.

Statistical Analysis
The patients were divided by diagnosis into HCC, acute hepatitis, chronic hepatitis, liver cirrhosis, and nonliver tumor, and healthy people served as the control group. Results are expressed as mean ± standard deviation (SD). Differences between different groups were assessed by using a Student t test or a χ² test. A P value of .05 or less was considered significant. Sensitivity and specificity were calculated according to the following formulas: Sensitivity = a/(a + c); and Specificity = d/(b + d), where a = true-positive cases, b = false-positive cases, c = false-negative cases, and d = true-negative cases. Receiver operating characteristic (ROC) curves were constructed by calculating the sensitivities and specificities at several cutoff points.

Results
Abnormal Expression of Circulating IGF-II in HCC
The levels of IGF-II expression in the serum of patients with liver diseases are shown in Table 1. Of the 224 cases with chronic liver disease, the level of circulating IGF-II protein was significantly higher (P < .001) in patients with HCC than in patients with liver cirrhosis or chronic hepatitis. Also, the level of serum IGF-II in patients with HCC was significantly higher (P < .001) than in patients with nonliver tumors or acute hepatitis. The evaluation of serum IGF-II and AFP levels for HCC diagnosis using the ROC curves is shown in Figure 1. The analysis of 2 markers for the whole range of

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Cases</th>
<th>IGF-II, ng/mL</th>
<th>q</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocellular carcinoma</td>
<td>146</td>
<td>3.74 ± 0.67</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>38</td>
<td>3.10 ± 0.63</td>
<td>4.969</td>
<td>.000</td>
</tr>
<tr>
<td>Chronic hepatitis</td>
<td>40</td>
<td>1.93 ± 0.17</td>
<td>28.792</td>
<td>.000</td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>35</td>
<td>1.16 ± 0.12</td>
<td>41.424</td>
<td>.000</td>
</tr>
<tr>
<td>Nonliver tumor</td>
<td>25</td>
<td>3.10 ± 0.60</td>
<td>4.801</td>
<td>.000</td>
</tr>
<tr>
<td>Control subjects</td>
<td>55</td>
<td>1.14 ± 0.14</td>
<td>42.800</td>
<td>.000</td>
</tr>
</tbody>
</table>

IGF-II, insulin-like growth factor II.
* Data are expressed as mean ± SD. IGF-II values are given in conventional units; to convert to Système International units (nmol/L), multiply by 0.131.
† Compared with the hepatocellular carcinoma group.

**Figure 1** Receiver operating characteristic (ROC) curves for the serum insulin-like growth factor II (IGF-II) investigated marker for hepatocellular carcinoma. Sensitivity, true-positive rate; specificity, false-positive rate. The area under the ROC curves was 0.823 for α-fetoprotein (AFP) and 0.771 for IGF-II.
sensitivities and specificities using the area (0.823 for AFP and 0.771 for IGF-II) under ROC curves indicated that the abnormality of serum IGF-II level could be a useful molecular marker for HCC diagnosis.

Clinicopathologic Characteristics of IGF-II Expression

The pathologic characteristics of circulating IGF-II expression are shown in Table 2. The higher expression of hepatic IGF-II in patients with HCC was associated with HBV infection (\( P = .000 \)). However, no significant difference was found between IGF-II expression and patient sex, age, tumor size, extrahepatic metastasis, or AFP level (\( P > .05 \)).

Amplification of IGF-II mRNA in Different Liver Tissues

The amplification patterns of IGF-II mRNA from liver tissues or PBMCs and the alignment of the amplified sequences are shown in Figure 2. The fragments of the IGF-II genome were amplified by a nested PCR assay from HCC tissues and circulating blood of patients with HCC (Figures 2A-2C) and confirmed by sequencing (Figure 2D). The incidence of IGF-II mRNA in the cancerous tissues (35/35 [100%]) was significantly higher than in the paracancerous tissues (19/35

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Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Cases</th>
<th>Mean ± SD IGF-II (ng/mL)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>108</td>
<td>3.73 ± 0.65</td>
<td>0.224</td>
<td>.823</td>
</tr>
<tr>
<td>Female</td>
<td>38</td>
<td>3.77 ± 0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥50</td>
<td>109</td>
<td>3.74 ± 0.64</td>
<td>0.117</td>
<td>.907</td>
</tr>
<tr>
<td>&lt;50</td>
<td>37</td>
<td>3.75 ± 0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5.0</td>
<td>66</td>
<td>3.77 ± 0.76</td>
<td>0.491</td>
<td>.624</td>
</tr>
<tr>
<td>&lt;5.0</td>
<td>80</td>
<td>3.71 ± 0.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extrahepatic metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With</td>
<td>38</td>
<td>3.88 ± 0.69</td>
<td>2.0128</td>
<td>.058</td>
</tr>
<tr>
<td>Without</td>
<td>108</td>
<td>3.62 ± 0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Fetoprotein (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥400.0</td>
<td>67</td>
<td>3.83 ± 0.67</td>
<td>1.564</td>
<td>.120</td>
</tr>
<tr>
<td>&lt;400.0</td>
<td>79</td>
<td>3.66 ± 0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B surface antigen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>110</td>
<td>3.93 ± 0.50</td>
<td></td>
<td>.000</td>
</tr>
<tr>
<td>Negative</td>
<td>36</td>
<td>3.16 ± 0.80</td>
<td>5.390</td>
<td>.000</td>
</tr>
</tbody>
</table>

*IGF-II values are given in conventional units; to convert to Système International (SI) units (nmol/L), multiply by 0.131. For AFP, multiply by 0.131. To convert the conventional units for α-fetoprotein to SI units (nmol/L), multiply by 0.131. To convert the conventional units for AFP to SI units (nmol/L), multiply by 0.131. To convert the conventional units for α-fetoprotein to SI units (nmol/L), multiply by 0.131.

HCC, hepatocellular carcinoma; IGF-II, insulin-like growth factor II.

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**Figure 2** The amplification of insulin-like growth factor II (IGF-II) messenger RNA (mRNA) from liver tissues or peripheral blood mononuclear cells (PBMCs) and the alignment of the amplified sequences. The IGF-II mRNA was synthesized to IGF-II complementary DNA and detected by nested polymerase chain reaction (PCR; 170 base pairs [bp]). A, The sensitive limitation for IGF-II mRNA analysis was 2 ng/L in the detection system using total RNA with \( 10^{-2} \) - to \( 10^{-8} \)-fold dilution and then amplified by nested PCR. B, The amplified fragments (452 bp) of the glyceraldehyde-3-phosphate dehydrogenase genome from liver or blood was used as a control. C, The amplification of IGF-II genomes in liver tissues and circulating blood. Lanes 1 and 2, positive IGF-II fragments from hepatocellular carcinoma (HCC) tissue; lane 3, positive IGF-II fragments from paracancerous tissue; lane 4, negative result from noncancerous tissue; lane 5, negative result from circulating PBMCs of patients with liver cirrhosis, and lane 6, positive fragments from PBMCs in patients with HCC. M, DNA molecular weight marker. D, The homology analysis of the amplified sequences: Origin, the cited sequence (170 bp, nt 311-480) of the human IGF-II genome; Hepatoma, the fragment of the IGF-II genome from HCC tissue; PB, the fragment of blood IGF-II gene from a patient with HCC.
or in the noncancerous tissues (0%; \( P < .01 \)). The fragments of the IGF-II genome were clearly detected in the PBMCs of patients with HCC (Figure 2C) but not in patients with acute hepatitis, chronic hepatitis, liver cirrhosis, or non-liver tumors.

**Significance of Circulating IGF-II mRNA Amplification**

The amplification of circulating IGF-II mRNA and comparison with circulating AFP mRNA in patients with different liver diseases is shown in **Table 3**. The sensitive limitation for AFP mRNA analysis was 2 ng/L in the detection system using total RNA with 10^-2- to 10^-8-fold dilution and then amplified by nested PCR. There were different positive frequencies of IGF-II mRNA and AFP mRNA in the PBMCs of patients with liver cirrhosis, chronic hepatitis, acute hepatitis, and HCC and in healthy control subjects: the respective incidence rates of IGF-II mRNA and AFP mRNA were 0% and 18.4% in liver cirrhosis, 0% and 7.5% in chronic hepatitis, and 34.3% and 52.7% in HCC; there was no positive fragment in acute hepatitis or in control subjects. Although the frequency of the presence of IGF-II mRNA in patients with HCC was 34.3% and its positive rate was lower than that of AFP, the sensitivities of IGF-II mRNA and AFP mRNA were both significant.

**Table 3**

**Comparative Analysis of Circulating IGF-II mRNA and AFP mRNA From Peripheral Blood Mononuclear Cells of Patients With Liver Diseases**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Cases</th>
<th>IGF-II mRNA</th>
<th>AFP mRNA</th>
<th>IGF-II and AFP mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocellular carcinoma</td>
<td>146</td>
<td>50 (34.2)</td>
<td>77 (52.7)</td>
<td>90 (61.6)</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>38</td>
<td>0 (0) †</td>
<td>7 (18) †</td>
<td>7 (18) †</td>
</tr>
<tr>
<td>Chronic hepatitis</td>
<td>40</td>
<td>0 (0) †</td>
<td>3 (8) †</td>
<td>3 (8) †</td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>35</td>
<td>0 (0) †</td>
<td>0 (0) †</td>
<td>0 (0) †</td>
</tr>
<tr>
<td>Nonliver tumor</td>
<td>25</td>
<td>0 (0) †</td>
<td>0 (0) †</td>
<td>0 (0) †</td>
</tr>
<tr>
<td>Normal control</td>
<td>55</td>
<td>0 (0) †</td>
<td>0 (0) †</td>
<td>0 (0) †</td>
</tr>
</tbody>
</table>

AFP, \( \alpha \)-fetoprotein; IGF-II, insulin-like growth factor II; mRNA, messenger RNA.

* Data are given as number positive (percentage). Nonliver tumors included the following: lung cancer, 6; gastric cancer, 6; esophageal cancer, 3; breast cancer, 3; colon cancer, 3; cervical cancer, 2; and pancreatic cancer, 2.

† \( P = .000 \) vs the hepatocellular carcinoma group.
AFP mRNA (52.7%) in the same group, its positive fragments could not be amplified in patients with benign liver diseases and control subjects; thus, IGF-II mRNA was a more specific marker for an HCC diagnosis. Otherwise, of the 146 cases with HCC, 45 cases (30.8%) were positive for AFP mRNA and IGF-II mRNA, 32 cases (21.9%) were positive for only AFP mRNA, and 5 cases (3.4%) were positive for only IGF-II mRNA. The detection of circulating IGF-II mRNA in combination with AFP mRNA could increase the frequency of HCC diagnosis (61.6%).

Circulating IGF-II mRNA in HCC Metastasis

The relationship between circulating IGF-II mRNA and HCC with or without distal metastasis for the 146 patients with HCC is analyzed in Table 4. IGF-II mRNA fragments were detected in all 38 patients with HCC with extrahepatic metastasis (100%). The positive frequency of circulating IGF-II mRNA fragments was 35.3% in cases in which the AFP value was less than 50 ng/mL (50 μg/L) and 34.0% in cases in which the AFP value was 50 ng/mL (50 μg/L) or more, with no significant differences between the 2 groups.

Discussion

HCC is one of the most common cancers, with more than 1 million deaths occurring annually worldwide. Multiple genetic alterations, including the activation of oncogenes and inactivation of tumor suppressor genes, are required for malignancy in human cancers and are correlated with increased stages of carcinogenesis and further tumor progression with many characteristics, such as fast infiltrating growth, metastasis in early stage, high-grade malignancy, and poor therapeutic efficacy. HCC prognoses are poor, and early detection is of the utmost importance. Treatment options are severely limited. Recent studies have discovered changes in the IGF axis that affect the molecular pathogenesis of HCC, and IGF-II is a polypeptide hormone secreted by many organs of the fetus. In the present study, we analyzed serum IGF-II and its gene expression in patients with HCC in order to estimate their clinical values in HCC differential diagnosis and hematogenous metastasis.

Characteristic alterations detected in HCC and hepatoma cell lines comprise the overexpression of IGF-II and the IGF-I receptor emerging as critical events in malignant transformation and growth of tumors. Very little information is available on the expression of IGF-II mRNA in HCC. Previous studies have demonstrated that IGF-II is a fetal growth factor and abnormally expressed in hepatocarcinogenesis. The sinusoidal cells in paracancerous cirrhotic nodule tissues and the malignant hepatocytes in HCC tissues expressed IGF-II. As we know that liver cirrhotic nodules are the precancerous condition of HCC, so it is clearly suggested that, in the precancerous condition, IGF-II-mediated hepatocyte proliferation is mainly via IGFIIR by a paracrine mechanism. IGF-II could be secreted by hepatoma cells themselves or by malignant hepatocytes and stimulate their proliferation via an autocrine mechanism. Hepatoma cells may regain some embryonic development characteristics like AFP secretion. The levels of serum IGF-II were significantly higher in the HCC group than in the liver cirrhosis and chronic hepatitis groups (Table 1), with higher diagnostic value for HCC (Figure 1). The data indicated that the overexpression of serum IGF-II in patients with HCC should be a useful marker for HCC diagnosis.

The higher expression of hepatic IGF-II in patients with HCC was associated with HBV infection. However, no significant difference was found between serum IGF-II expression and patient sex, age, tumor size, or AFP level (Table 2). The observations that HCC cells express less IGF-I than control liver cells, whereas IGF-II expression is higher in a high proportion of HCC cells, are consistent with previous reports that showed IGF-I mRNA levels were lower in HCC compared with nontumorous hepatic tissue from an adjacent area, whereas IGF-II mRNA was higher in tumor (Figure 2) compared with paracancerous or distant cancerous tissues; the molecular mechanisms responsible for reduction in IGF-I and reactivation of IGF-II in HCC remain to be determined. The marked increase of IGF-II is associated with occurrence and development of HCC and promoted metastasis of hepatoma cells.

Molecular markers (biomarkers) for HCC metastasis and recurrence could provide additional information to that gained from traditional histopathologic features. A large number of biomarkers have been shown to have potential predictive significance. Significant differences in the RNA level between HCC and cirrhotic tissues were quite informative. Although the serum free IGF-II level increased in patients with chronic hepatitis or liver cirrhosis, the circulating IGF-II mRNA was detected only in patients with HCC. Although the frequency of IGF-II mRNA was not so high in patients with HCC, it is more specific for an HCC diagnosis.
The hepatocyte-specific mRNAs in the circulation negative rate with AFP level alone has been found for small monitoring HCC development; however, the higher false-negative rate of 2Diagnostics, 3Oncology, 4General Surgery, and 5Pathology, mechanisms responsible for the reactivation of the IGF-II signaling pathway in HCC. The peripheral blood AFP mRNA and IGF-II mRNA were more specific and more sensitive tumor markers for monitoring and finding a few circulating HCC hepatocytes.

The prognosis of HCC is poor, and early detection is of the utmost importance. Treatment options are severely limited by the frequent presence of metastases. Although the mechanisms of hepatocarcinogenesis have not been elucidated, a long-lasting inflammation induced by hepatitis virus infection is a definite risk for neoplastic degeneration and accumulation of genetic alterations.1,3,6,37 Serum AFP is a useful marker for monitoring HCC development; however, the higher false-negative rate with AFP level alone has been found for small HCCs.7 The hepatocyte-specific mRNAs in the circulation can predict the likelihood of hematogenous metastasis (Table 4). The expression of IGF-II mRNA was detected in all HCC tissues, half in their paracancerous cirrhotic tissues and none in their noncancerous tissues. The relationship between circulating IGF-II mRNA and HCC with or without metastasis was analyzed. The fragments of circulating IGF-II mRNA could be detected in all patients with HCC with extrahepatic metastasis (Table 4); like circulating AFP mRNA, these results argue for growth factor–dependent HCC development and could provide novel markers of severity and prognosis for HCC.

The present data indicate that the expression levels of IGF-II and IGF-II mRNA were different in different parts of HCC tissues, and IGF-II mRNA could be detected only in the peripheral blood of patients with HCC. The frequency of circulating IGF-II mRNA and its diagnostic value increased with clinical stage of HCC and with distal metastases of HCC hepatocytes. The reactivation of the hepatic growth factor IGF-II is associated with occurrence, development, and metastasis of HCC.3,8,39 Circulating IGF-II mRNA could be a useful molecular marker for HCC diagnosis, especially in monitoring extrahepatic metastases of tumor cells. Further studies will permit us to quantitate IGF-II mRNA in liver tissues and peripheral blood and to explore the molecular mechanisms responsible for the reactivation of the IGF-II signaling pathway in HCC.

References
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