Activation of Fetal Promoters of Insulinlike Growth Factor II Gene in Hepatitis C Virus–Related Chronic Hepatitis, Cirrhosis, and Hepatocellular Carcinoma

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Increased prevalence of hepatitis C virus (HCV) infection has been found in patients with hepatocellular carcinoma (HCC). The expression of insulinlike growth factor II (IGF-II) has been linked to hepatocarcinogenesis in the experimental animal and in humans. Since reactivation of fetal IGF-II transcripts has been observed in human HCC, we have analyzed the levels of adult P1 and fetal P3 and P4 IGF-II promoter–derived transcripts in the liver of patients with HCV-related chronic active hepatitis (CAH), cirrhosis, and HCC by means of a semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR) assay. Transcripts derived from adult P1 promoter were increasingly expressed from normals to patients with CAH and cirrhosis, but were undetectable in the tumor area of 5 of 7 HCC patients and present at low levels in the nontumorous area of all HCC patients. Transcripts derived from fetal P3 promoter were not detectable in normal subjects, while they were expressed abundantly in most CAH and all cirrhotic patients. Transcripts from fetal P4 promoter were detected at high levels in 3 of 9 CAH patients and in the majority of cirrhotic patients. Increased expression of fetal promoter–derived transcripts was also found in the liver of HCC patients, although levels were lower in cirrho-
sis. Also, the activity of fetal P3 and P4 promoters was higher in the nontumorous than in the tumorous area of the liver of HCC patients. The expression of IGF-II transcripts was correlated with the rate of cell mitotic activity by measuring the expression of the proliferating cell nuclear antigen (PCNA) gene. PCNA messenger RNA (mRNA) levels progressively increased from normals to CAH and to cirrhotic patients, and persisted at a high level in the tumorous and in the nontumorous area of HCC subjects, thus showing that the increase of IGF-II transcripts in CAH and cirrhosis is accompanied by an activation of cell mitosis in these samples. These data suggest that the activation of IGF-II gene expression from adult and fetal promoters may play a role in premalignant proliferation observed in HCV-related chronic liver disease. (HEPATOL 1996;23:1304-1312.)

Hepatitis C virus (HCV) is a positive-stranded RNA virus that plays a major role in the development of chronic liver disease (CLD).1,2 Acute posttransfusion hepatitis due to HCV is followed by chronic hepatitis in more than 50% of cases,3,4 and 20% to 50% of these patients eventually progress to cirrhosis.3,5 Mounting evidence suggests that HCV infection may play a role in the development of hepatocellular carcinoma (HCC) in cirrhotic patients.5,7 Chronic injury of liver cells and the associated inflammatory and regenerative response that occurs in CLD are known to represent a preneoplastic process that may evolve toward malignancy.8-10 However, the mechanisms responsible for the development of HCC in HCV-related CLD are not well understood.

Insulinlike growth factor II (IGF-II) is a small peptide that is structurally related to proinsulin and displays multiform effects on cell growth and metabolism.15 IGF-II exerts its mitogenic activity through the insulinlike growth factor type I receptor (IGF-IR).15 In rodents, IGF-II is expressed at high levels in the fetus and at lower levels in the adult, and functions primarily as a prenatal growth regulator.12 In fact, IGF-II knockout mice show growth retardation,13 while IGF-IR knockout results in impaired formation and death at birth.14 In humans, IGF-II is expressed during the fetal life under the control of three different promoters (P2, P3, and P4) in most tissues, while IGF-II expression in the adult is driven by a liver-specific promoter (P1), and, to a lesser extent, by the three fetal promot
ers in the extrahepatic and hepatic tissues. Each promoter is followed by one or more alternative untranslated exons, which are all spliced to the last three exons, encoding the pre-prohormone molecule (Fig. 1).

Evidence demonstrates that the synthesis of IGF-II and the activation of its signaling pathway through the tyrosine kinase domain of the IGF-IR play important roles in tumorigenesis. The expression of IGF-II and IGF-IR genes is activated in several human and experimental tumors, and, in some of them, an autocrine/paracrine role of IGF-II has been demonstrated.

PATIENTS AND METHODS

Patients. The biochemical and histological characteristics of our patient population are described in Table 1. The study population consisted of 4 anti-HCV–negative, HCV-RNA–negative control subjects (age range, 44-66 years; 1 woman, 3 men) and 25 anti-HCV–positive patients (9 with CAH and hepatic tissues). Each promoter is followed by one or more alternative untranslated exons, which are all spliced to the last three exons, encoding the pre-prohormone molecule (Fig. 1).

Evidence demonstrates that the synthesis of IGF-II and the activation of its signaling pathway through the tyrosine kinase domain of the IGF-IR play important roles in tumorigenesis. The expression of IGF-II and IGF-IR genes is activated in several human and experimental tumors, and, in some of them, an autocrine/paracrine role of IGF-II has been demonstrated.

TABLE 1. Biochemical and Histological Characteristics of Patient Population

| Case | Sex | Age (yr) | ALT* | AFP | HAI (ng/mL) | Diagnosis² |
|------|-----|----------|------|-----|-------------|------------|
| 1    | M   | 56       | 0.8  | 1.0 |             | Galletones  |
| 2    | M   | 66       | 0.5  | 2.0 |             | Galletones  |
| 3    | F   | 50       | 0.7  | 0.7 |             | Peptic ulcer|
| 4    | M   | 44       | 0.8  | 1.4 |             | Galletones  |
| 5    | M   | 54       | 2.0  | 1.8 |             | CAH         |
| 6    | M   | 28       | 2.5  | 5.4 |             | CAH         |
| 7    | M   | 65       | 1.0  | 4.8 |             | CAH         |
| 8    | M   | 58       | 3.5  | 1.1 |             | CAH         |
| 9    | M   | 47       | 4.5  | 1.4 |             | CAH         |
| 10   | M   | 45       | 4.0  | 2.0 |             | CAH         |
| 11   | M   | 65       | 5.0  | 8.0 |             | CAH         |
| 12   | M   | 56       | 5.0  | 5.4 |             | CAH         |
| 13   | M   | 61       | 2.5  | 5.0 |             | CAH         |
| 14   | F   | 31       | 1.0  | 8.3 |             | Cirrhosis   |
| 15   | M   | 53       | 3.0  | 2.9 |             | Cirrhosis   |
| 16   | M   | 60       | 10.0 | 2.8 |             | Cirrhosis   |
| 17   | F   | 60       | 1.0  | 5.4 |             | Cirrhosis   |
| 18   | M   | 49       | 3.0  | 7.0 |             | Cirrhosis   |
| 19   | M   | 68       | 1.5  | 3.0 |             | Cirrhosis   |
| 20   | F   | 51       | 4.5  | 5.4 |             | Cirrhosis   |
| 21   | M   | 47       | 1.5  | 3.3 |             | Cirrhosis   |
| 22   | F   | 63       | 2.0  | 7.5 |             | Cirrhosis   |
| 23   | F   | 71       | 2.5  | 150 |             | HCC         |
| 24   | M   | 46       | 3.0  | 16.3|             | HCC         |
| 25   | M   | 61       | 3.5  | 38.7|             | HCC         |
| 26   | M   | 75       | 4.5  | 54.4|             | HCC         |
| 27   | M   | 68       | 3.0  | 12  |             | HCC         |
| 28   | M   | 54       | 4.5  | 120 |             | HCC         |
| 29   | M   | 58       | 1.0  | 23.5|             | HCC         |

Abbreviations: ALT, alanine transaminase; AFP, a-fetoprotein; HAI, histological activity index.

* Times the upper limit of the normal range (≤37 U/L).

† All cirrhotic and HCC patients were in Child A class. All HCCs were well differentiated (Edmonson’s class I–II) except case 25, which was poorly differentiated (Edmonson’s class III–IV).
range, 28-65 years; 9 men], 9 with cirrhosis [age range, 31-68 years; 4 women, 5 men], and 7 with HCC [age range, 46-75 years; 1 woman, 6 men]). Antibodies to HCV were detected in serum samples by second-generation recombinant immunoblot assay (Chiron Corp., Emeryville, CA). All subjects were negative for hepatitis B surface antigen—negative and none were alcoholics. Liver tissue was obtained through ultrasound-guided fine-needle biopsy (Surecut 21 G, T.S.K. Laboratory, Tokyo, Japan) during diagnostic procedure in patients with CLD and from surgical specimens in control subjects. In patients with HCC, liver biopsy was performed in the tumorous and nontumorous areas at distance from the tumor. In each case, a portion of the liver sample was fixed in 10% buffered formalin for immunohistochemistry and routine histological examination. The remaining sample was immediately washed with 0.3% NaCl, snap-frozen in liquid nitrogen, and stored at −80°C until assayed. Informed consent was obtained from the patients.

**Histology.** Diagnosis of CAH or cirrhosis was reached according to internationally accepted criteria.32,33 The histological activity index was assessed according to Knodell.34 All HCCs were graded histologically according to the criteria of Edmondson and Steiner.35

**RNA Extraction.** Total RNA was extracted using the guanidinium thiocyanate method, and the high quality of the product was assured by analysis on 1% agarose/formaldehyde electrophoresis stained with 1% ethyldium bromide.36,37

**Reverse Transcription.** First-strand complementary DNA was prepared using 200 units of reverse transcriptase (Superscript RT, Gibco BRL, Gaithersburg, MD), 1 μg of total RNA as template, and 10 pmol/L of random hexamers in the presence of 0.1 mmol/L dithiothreitol, 0.5 mmol/L dNTP (Pharmacia, Milan, Italy), and 20 units of RNase inhibitor (Promega, Madison, WI), as previously described.38 The reaction profile was 37°C × 10 minutes, followed by 42°C × 60 minutes. To control for contamination by genomic DNA, all RNA samples were run in duplicate with or without addition of reverse transcriptase.

**PCR Analysis.** Hybridization sites of primers used for polymerase chain reaction (PCR) analysis of IGF-II transcripts are shown in Fig. 1. P1-, P3-, and P4-specific IGF-II transcripts15-17 were analyzed using primer A: 5'-AGAACCTGGAGGTGGCAGCCA-3' (P1); primer B: 5'-CTGTTCCGGTTTGGCAGCA-3' (P3); primer C: 5'-GACCTTCGTTCTGAGCTGTA-3' (P4) as 5' primers; and primer D: 5'-GATGAGCAATGTC-TCAG-3' (exon 7) as 3' primer. The GAPDH-specific primers18 were 5'-CACCCTCTCCAGGAGCAG-3' (fore); 5' TCACGC- CAGTTTCTCCCGGA-3' (reverse). The PCNA-specific primers19 were 5'-CAAGAGGTTGTTGAGGAGCCA-3' (fore); 5'-TACTGCGCCAAGGTATCCG-3' (reverse). All primers were furnished by PRIMM Srl (Milano, Italy) and were used at the final concentration of 2 mmol/L.

P1, P3, and P4 IGF-II-specific transcripts and PCNA messenger RNA (mRNA) were coamplified with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using one twenty-fifth of first-strand complementary DNA in the presence of 0.2 mmol/L dNTP (Boehringer Mannheim, Mannheim, Germany) and 0.3 μM Ampli Taq DNA polymerase (Perkin Elmer, Branchburg, NJ). MgCl2 was added at the final concentration of 1.5 mmol/L, except for P1-GAPDH reaction, where the concentration was 1.75 mmol/L. After an initial denaturation step, 97°C × 10 minutes, the PCR amplification was performed in the presence of 1.5 μCi α-32P dGTP using the following profiles: P1-GAPDH: 94°C × 1 minute, 44°C × 1 min, and 72°C × 1 minute for three cycles, followed by 25 cycles at 94°C × 1 minute, 56°C × 1 minute, and 72°C × 1 minute. GAPDH primers were added after six cycles. P3-GAPDH: 94°C × 1 minute, 56°C × 1 minute, and 72°C × 1 minute for 28 cycles. P4-GAPDH: 94°C × 1 min, 56°C × 1 minute, and 72°C × 1 minute for 28 cycles. GAPDH primers were added after three cycles. PCNA-GAPDH: 94°C × 1 minute, 56°C × 1 minute, and 72°C × 1 minute for 28 cycles. In all cases, the final extension was at 72°C × 10 minute. RNA samples from all patients were coamplified simultaneously with IGF-II or PCNA- and GAPDH-specific primers to avoid differences due to the Taq DNA polymerase activity.

PCR products were phenol-chlorophorm—extracted, ethanol-precipitated, and subjected to 5% polyacrylamide gel electrophoresis and autoradiography. Sizes of the amplified fragments were estimated from migration of the 1-kb ladder molecular-weight marker (Gibco-BRL), and identity was assessed by restriction-enzyme digestion. PCR products were quantified by densitometric scanning of the autoradiograms using a Howteck Scanmaster 3 densitometer with RFL PrintTM Software (Pharmacia).

**Statistical Analysis.** Correlation was evaluated by regression analysis. Significance of differences was evaluated by ANOVA, followed by Wilcoxon’s rank sum test.

**RESULTS**

**Semiquantitative RT-PCR Analysis of IGF-II Promoters.** We have studied the expression of the IGF-II gene from the adult (P1) and the two fetal (P3 and P4) promoters, which are more abundantly expressed in normal and transformed hepatocytes,18,29 in patients with HCV-related CLD by using a semiquantitative RT-PCR assay. Oligonucleotides were designed from the sequences of exon 3, exon 5, and exon 6, and used as 5' primers to specifically amplify by RT-PCR transcripts originating from adult P1 or fetal P3 and P4 promoters, respectively (Fig. 1). An oligonucleotide complementary to exon 7 sequence was used in all cases as 3' primer (Fig. 1). To compare the relative activities of P1, P3, and P4 IGF-II promoters in different RNA samples, each sample was simultaneously amplified with IGF-II promoter– and GAPDH-specific primers, and the intensities of IGF-II signals were normalized to those of GAPDH. To analyze the linearity of RT-PCR coamplification of IGF-II and GAPDH transcripts, aliquots withdrawn from the reaction tube after different numbers of cycles were electrophoresed and autoradiographed (Fig. 2A). Specific IGF-II and GAPDH transcripts were amplified with increasing efficiency up to 30 cycles, as shown by densitometric analysis of PCR products (Fig. 2B). No specific signal was evident in the control samples obtained by PCR amplification of RNA without the addition of reverse transcriptase (Fig. 2A).

**IGF-II Expression in Normal Liver Tissue, CAH, and Cirrhosis.** The expression of human IGF-II from fetal and adult promoters was tested in the liver of normal subjects and of patients with HCV-related CLD (Fig. 3 and Table 2). IGF-II transcripts derived from adult P1 promoter were increasingly expressed from normal patients to patients with CAH and cirrhosis. The increase in P1 transcripts was evident in 6 of 9 CAH patients (Fig. 3, cases 6, 7, 8, and 13, and, to a lesser extent, cases 5 and 12) and in all cirrhotic patients. The median densitometric scan values were 0.14 arbitrary units (range, 0.10-0.16) in normal patients, 0.30 (range, 0.12-3.01) in patients with CAH (P < .01 vs. normal
patients), and 0.72 (range, 0.44-2.66) in patients with cirrhosis ($P < .01$ vs. normal patients), as shown in Fig. 4A.

IGF-II transcripts derived from fetal P3 promoter were not detectable in normal subjects, while they were detected with progressively increasing expression in most of the patients with CAH (with the exception of case 11) and in all patients with cirrhosis (Fig. 3 and Table 2). The median densitometric scan values for IGF-II transcripts derived from fetal P3 promoter were 0.0 in normal patients, 0.45 (range, 0.0-0.88) in CAH patients ($P < .01$ vs. normal patients), and 7.11 (range, 2.14-13.51) in cirrhotic patients ($P < .01$ vs. normal patients and $P < .001$ vs. CAH) (Fig. 4). Five cirrhotic patients (cases 15, 18, 19, 20, and 22) had P3 mRNA levels 10-fold higher than the median densitometric scan values of CAH patients (Fig. 4B and Table 2).

IGF-II transcripts from fetal P4 promoter were detected in all subjects except case 21 (Fig. 3 and Table 2). Increased expression of P4 transcripts was found in
3 CAH patients (cases 7, 8, and 12) and in all cirrhotic patients, with the exception of case 21 (Fig. 3). The median densitometric scan values were 0.15 (range, 0.12-0.18) in normal patients, 0.24 (range, 0.15-1.63) in CAH patients ($P < .05$ vs. normal patients), and 2.29 (range, 0.0-9.19) in cirrhotic patients ($P < .05$ vs. normal patients and vs. CAH) (Fig. 4C).

Because of a certain degree of variability in the expression of IGF-II transcripts within each group of patients (Table 2), we evaluated whether this might be due to a different inflammatory activity. We did not find any significant correlation between histological activity index (Table 1) and IGF-II–RNA levels.

### IGF-II Expression in HCC.

The expression of fetal and adult human IGF-II promoters was tested in tumorous and nontumorous area of the liver of patients with HCV-related CLD (Fig. 5 and Table 2). IGF-II transcripts derived from adult P1 promoter were not detected in the tumorous area of HCC patients with the exception of two patients (cases 23 and 25) and were expressed at low levels in the nontumorous area of all HCC patients (Fig. 5 and Table 2). The median densitometric scan values for adult P1 promoter in the tumorous and nontumorous areas of HCC patients were 0.0 (range, 0.0-0.19) and 0.13 (range, 0.05-0.19), respectively (Fig. 4). These values were in the range of those found in the liver of normal controls, but were lower than those found in CAH and cirrhotic patients ($P < .05$ vs. CAH and $P < .001$ vs. cirrhosis) (Fig. 4A and Table 2).

Transcripts from fetal P3 promoter were detected in all HCC patients but in tumorous area of case 27 (Fig. 5 and Table 2). Higher P3-derived RNA levels were found in the nontumorous area than in the tumorous area of 5 of 7 HCC patients. The median densitometric scan value was 1.9 in the nontumorous area (range, 0.31-2.06) and 0.48 in the tumorous (range, 0.0-2.66) area (Fig. 4B). This difference was not statistically significant. The expression of P3 transcripts in the tumorous and nontumorous areas was higher than in normal controls ($P < .01$), but lower than in cirrhotic patients ($P < .001$) (Fig. 4B and Table 2).

IGF-II transcripts derived from fetal P4 promoter were detected in all HCC patients except in the tumorous area of case 25 (Fig. 5 and Table 2). P4-derived mRNA levels were higher in the nontumorous area than in the tumorous area ($P < .01$). The median densitometric scan value of P4-RNA levels in the nontumorous area (0.45; range, 0.10-2.61) was slightly higher than in normal patients ($P < .05$) and not significantly different from CAH and cirrhotic patients. P4 transcripts detected in the tumorous area (median densitometric scan value, 0.125; range, 0.0-0.17) were not significantly different than in normal patients and significantly lower than in CAH and cirrhotic patients ($P < .01$) (Fig. 4C and Table 2).

### PCNA Expression in Normal Controls and HCV-Related CLD.

To correlate the expression of transcripts derived from P1, P3, and P4 promoters with the rate of cell mitotic activity, we assessed the expression of PCNA, which is a well-established marker of cellular proliferative activity.

Autoradiogram expression and densitometric scan values are shown in Fig. 6 and Table 2. PCNA-mRNA levels progressively increased from normal patients to CAH and cirrhotic patients (Fig. 6). The median densitometric scan values were 0.09 (range, 0.0-0.11) in normal patients, 0.15 (range, 0.07-0.30) in CAH patients ($P < .05$ vs. normal patients), and 0.60 (range, 0.28-0.86) in cirrhotic patients ($P < .01$ vs. normal patients and $P < .001$ vs. CAH) (Fig. 4D).

The level of expression of PCNA in HCC subjects was comparable in the tumorous and nontumorous areas (median densitometric scan values, 0.59 and 0.51;
range, 0.36-1.25 and 0.30-1.07, respectively). These values were higher than in normal patients ($P < .01$) and CAH patients ($P < .001$), but comparable with those found in cirrhotic patients (Figs. 4D and 6).

The increase in the expression of P1, P3, and P4 IGF-II promoters from normal patients to CAH and cirrhotic patients correlated positively with PCNA transcript levels in the same subjects ($r = .46, P = .030; r = .57, P = .0054; r = .49, P = .018$, for P1, P3, and P4, respectively).

**DISCUSSION**

HCV is a major causative agent of cirrhosis, which is a known risk factor for the development of HCC. As well, a strong association between HCV infection and HCC has been described. However, the mechanisms by which HCV contributes to development of HCC is unknown. Recently, two different transgenic mouse models of HCC (i.e., hepatitis B virus large envelope polypeptide and Z2 $\alpha_1$-antitrypsin transgenic mice) indicated that, independently of the causative agent, liver cell injury and chronic inflammation may stimulate mediators of hepatocellular proliferation, which in turn leads to the development of precursor lesions of HCC, thus suggesting the existence of a common endogenous pathway for liver carcinogenesis.

Evidence supports the hypothesis that IGF-II plays a role during liver carcinogenesis in rodents and humans. In addition, a number of studies have shown increased expression of IGF-II RNA and/or protein levels in HBV-related CLD and HCC. The role of IGF-II in HCV-related CLD and HCC has not yet been studied.

We hypothesized that, in the course of HCV-related CLD, there might be an increase in the expression of IGF-II, which might contribute to the proliferative hit ultimately leading to development of HCC. Our data show a progressive increase in the expression of tran-
whereas it was activated in CAH and cirrhosis. Since P3-derived transcripts are expressed abundantly in human HCC, we postulate that the activation of IGF-II fetal promoter in CLD may represent a preneoplastic lesion. The increase in IGF-II expression significantly correlated with the expression of PCNA, which is a known marker of cell mitotic activity. Therefore, our study indicates that there is a significant relationship between liver cell proliferation and IGF-II expression in the course of HCV-related CLD. IGF-II expression during hepatitis B virus-related CLD and HCC has been localized mainly to the hepatocytes. Additionally, isolated and cultured rat hepatocytes and human hepatoma cell lines are known to express IGF-II and IGF-IR that mediates IGF-II proliferative effects. Therefore, the increase in IGF-II-mRNA levels during HCV-related CLD suggests that IGF-II might contribute through an autocrine mechanism to the enhanced proliferative activity of liver cells that may ultimately lead to the development of HCC. One additional possibility is that the increased IGF-II expression in the cirrhotic liver is contributed to by non-parenchymal cells. In this case, IGF-II would act in a paracrine mechanism to stimulate the growth of hepatocytes and promote hepatocarcinogenesis.

We also studied the expression of transcripts derived from IGF-II promoters in patients with HCV-related HCC. In nearly all HCC patients, transcripts from the adult P1 promoter were not detected; however, there was an increased expression of transcripts from fetal P3 (but not P4) promoter, though to a lesser degree when compared with cirrhosis. In the nontumorous area of the same HCC patients, transcripts from adult P1 promoter were expressed to the same extent as in normal subjects, while those from both P3 and P4 fetal promoters were expressed more abundantly. IGF-II expression in the nontumorous area was higher than in the tumorous area but still lower than in cirrhosis. This data is in agreement with a recent report by Su et al., demonstrating that IGF-II immunoreactivity was
stronger in the nontumorous, pericancerous area than in the tumor area of hepatitis B virus–related HCC. Interestingly, one of the two HCCs showing the highest RNA levels from P3 promoter (case 25) was the less-differentiated one (Edmonson’s class III-IV). This is in accord with data obtained in HCC of hepatitis B virus–chronically infected woodchucks, where the anaplastic, poorly differentiated tumors were highly positive for IGF-II expression, while the well-differentiated tumors expressed IGF-II at very low levels.25

The expression of the adult P1 and fetal P3 and P4 promoters of IGF-II was higher in cirrhotic patients than in patients with HCC, both in the tumor and in the cirrhotic, nontumorous areas. This raises the possibility that in the tumor and, to a lesser degree, in the nontumorous areas of HCC patients, there is a selection against IGF-II expression. Alternatively, a specific population of cells in the cirrhotic liver might be responsible for IGF-II expression, and that population of cells is not present in the clonal HCCs. The most likely candidates for this would be Ito cells or fibroblasts. Unlike IGF-II, the rate of liver cell proliferation as assessed by evaluation of PCNA expression was comparable in cirrhosis and HCC patients. Thus, one may hypothesize that once the neoplastic transformation has been triggered, the sustained hepatocyte proliferative activity in HCC patients is contributed to by events other than increased expression of IGF-II.

Carcinogenesis is a multistep process in which several mutations or epigenetic alterations of genes positively or negatively affecting cell growth accumulate in a clonal cell population.26 We postulate that in HCV-related CLD, as a result of liver cell death and inflammation, there is an increased synthesis of IGF-II that stimulates hepatocyte proliferative activity. This may subsequently cause alteration of growth regulatory genes, which, in turn, leads to development of HCC.47,48 However, one cannot exclude that growth factors other than IGF-II are activated as an early event in liver carcinogenesis. In particular, increased expression of transforming growth factor α has been reported during CLD and HCC in humans.49,50

In conclusion, our study suggests that IGF-II may play an important role in the preneoplastic proliferative response that follows prolonged cellular injury during HCV infection, and it as well lends support to the hypothesis that IGF-II may be involved in the development of HCC in HCV-related CLD.

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