Increased hepatic expression of insulin-like growth factor-I receptor in chronic hepatitis C

José Tadeu Stefano, Maria Lúcia Corrêa-Giannella, Cristiane Maria Freitas Ribeiro, Venâncio Avancini Ferreira Alves, Paulo Celso Bosco Massarollo, Marcel Cerqueira Cesar Machado, Daniel Giannella-Neto

Abstract

AIM: Although increased insulin-like growth factor-I receptor (IGF-IR) gene expression has been reported in hepatocellular carcinoma, studies assessing IGF-IR in chronic hepatitis C (CHC) and cirrhosis are scarce. We therefore aimed to evaluate IGF-IR and IGF-I mRNA expression in liver from patient with CHC.

METHODS: IGF-IR and IGF-I mRNA content were determined by semi-quantitative RT-PCR and IGF-IR protein expression was determined by immunohistochemistry in hepatic tissue obtained from patients with CHC before (34 patients) and after (10 patients) therapy with interferon-α and ribavirin.

RESULTS: An increase of IGF-IR mRNA content was observed in hepatic tissue obtained from all CHC patients as well as from 6 cadaveric liver donors following orthotopic transplantation (an attempt to evaluate normal livers) in comparison to normal liver, while no relevant modifications were detected in IGF-I mRNA content. The immunohistochemical results showed that the raise in IGF-IR mRNA content was related both to ductular reaction and to increased IGF-IR expression in hepatocytes. A decrease in IGF-IR mRNA content was observed in patients who achieved sustained virological response after therapy, suggesting an improvement in hepatic damage.

CONCLUSION: The up-regulation of IGF-IR expression in hepatocytes of patients with CHC could constitute an attempt to stimulate hepatocyte regeneration. Considering that liver is the organ with the highest levels of IGF-I, our finding of increased IGF-IR expression after both acute and chronic hepatic damage highlights the need for additional studies to elucidate the role of IGF-I in liver regeneration.

© 2006 The WJG Press. All rights reserved.

Key words: Chronic hepatitis C; Insulin-like growth factor I; Insulin-like growth factor I receptor; Ductular reaction; Hepatocyte regeneration; Ischemia-reperfusion

Stefano JT, Corrêa-Giannella ML, Ribeiro CMF, Alves VAF, Massarollo PCB, Machado MCC, Giannella-Neto D. Increased hepatic expression of insulin-like growth factor-I receptor in chronic hepatitis C. World J Gastroenterol 2006; 12(24): 3821-3828

http://www.wjgnet.com/1007-9327/12/3821.asp

INTRODUCTION

There is a close relationship between the endocrine system and the liver, in which processes such as hormonal inactivation, synthesis of hormonal binding proteins, and growth factors take place. The insulin-like growth factor (IGF) system is an attractive target of study, since it may be modified by liver diseases and, reciprocally, it might play a relevant role in the progression of some hepatic diseases. This system is comprised of two ligands, IGF-I and IGF-II, their specific receptors, IGF-IR and IGF-IIR, and the IGF-binding proteins (IGFBPs). IGFs are peptides involved in proliferation, differentiation and inhibition of apoptosis of several cell types. IGF-IR has been shown to be capable of mediating both IGF-I and IGF-II signalling, whereas IGF-IIR (mannose 6-phosphate) is only able to decrease the bioavailability of IGF-II.

Circulating IGF-I is synthesized primarily by the liver in response to growth hormone, but most tissues express
IGF-I, which exerts autocrine and paracrine functions[7]. Within the normal adult liver, IGFs are believed to play relatively little effect, since IGF-II expression is down-regulated and IGF-1, although highly expressed, does not exert its actions due to low IGF-IR expression on hepatocytes[8]. Therefore, liver is the organ with the highest levels of IGF-I expression, while it exhibits almost undetectable levels of IGF-IR mRNA. This pattern of expression may be partially explained by down-regulation of IGF-IR promoted by locally produced IGF-I[9]. In contrast to hepatocytes, it is believed that the presence of IGF-IR in non-parenchymal rat liver cells (Kupffer cells, myofibroblasts and hepatic stellate cells [HSC])[10] and in human HCC[11] makes these cell types susceptible to IGFs mitogenic effects.

The role of IGF-I system in hepatic diseases and in liver regeneration remains uncertain. Although an elevation of IGF-IR expression during progression of preneoplastic hepatic foci to hepatocellular carcinoma (HCC) has been reported[12], studies using cultured HCC cells demonstrated a failure of IGF-I to stimulate mitogenesis[10,13]. On the other hand, hepatic over-expression of the IGF-II gene has been observed in animal models of hepatocarcinogenesis[13,14] as well as in human HCV-induced cirrhosis and HCC[17-19]. Increased IGF-IR gene expression was observed in HCC and in human hepatoma cell lines[8,12,20], however, there are few studies evaluating different chronic hepatitis C (CHC) staging and cirrhosis concerning IGF-IR expression[11,18]. Therefore, the present study was designed to semi-quantify the hepatic expression of IGF-IR and IGF-I in patients with CHC, searching for possible correlations with histopathological lesions in each compartment of hepatic acini.

MATERIALS AND METHODS

Patients and tissue samples
A series of 34 patients with CHC was evaluated at the Clinical Hospital of the University of Sao Paulo Medical School. The diagnosis of CHC was based on a positive serological test for HCV by at least a second-generation enzyme-linked immunoabsorbant assay, positive HCV-RNA by polymerase chain reaction (PCR) assay, and compatible liver biopsy. All patients received combined treatment with interferon-α (3 million U three times a week) and ribavirin (1000-1200 mg/d, accordingly to body weight) for 48 wk, since genotyping was not available. Hepatic tissue fragments obtained before treatment were divided in two samples: one was formalin-fixed paraffin-embedded for histopathological and for immunohistochemical studies; the other was collected in sterile containers and frozen in liquid nitrogen for RNA extraction. Additionally, in 10 patients, liver biopsy was performed within one month after the end of treatment. Among the 34 treated patients, 14 (41.1%) patients reached sustained virological response, 16 (47.05%) patients were non-responders and 4 (11.76%) patients relapsed in the follow-up period. Among the 10 patients histologically evaluated in the post-treatment period, 5 (50%) patients were sustained virological responders and 5 (50%) patients were non-responders.

In order to obtain normal hepatic tissue, hepatic fragments were collected from six cadaveric liver donors (8-12 h of cold ischemia) following orthopic transplantation. The organs obtained from the six cadaveric donors were non-diseased liver transplanted in patients presenting HCV-related end-stage liver disease. The transplants were performed at Experimental Surgery Division, Clinical Hospital, University of Sao Paulo Medical School.

This study was approved by the Ethical Committee of the University of Sao Paulo Medical School and informed consent was obtained from all patients.

Histological and immunohistochemical analyses
Formalin-fixed paraffin-embedded samples were sectioned at a thickness of 3 μm, stained by hematoxylin and eosin (H&E), Masson’s trichrome staining and reticulin impregnation, and evaluated by a single pathologist. Histopathological classification was based on criteria defined by the Brazilian Consensus on Chronic Hepatitis[21], which assesses individual histologic variables from zero (normal) to four (maximal degree of lesion): staging of architectural disturbances/fibrosis, necro-inflammatory lesions in portal tract, interface hepatitis and parenchymal lesions. Steatosis was also graded as follows: 0 = no fat deposition in hepatocytes; 1 = up to 25% of hepatocytes; 2 = 25%-50%; 3 = 51%-75%; and 4 = more than 75% hepatocytes presenting fat vacuoles.

Immunohistochemistry for IGF-IR was performed on sections deparaffinized with xylene, dehydrated with graded ethanol, incubated in 3% H2O2 to block endogenous peroxidase activity. To improve the staining pattern, antigen retrieval was obtained by heating in 0.5 mol/L EDTA (pH 8.0). The sections were incubated with Protein Block System and Biotin Blocking System (X0909, DAKOCytomation, USA). The primary antibody, mouse monoclonal IGF-IR (α-subunit) Ab-1 (Clone 24-31) (LAB VISION Corporation, Westinghouse, USA) at a dilution of 1:50 was incubated overnight. Signal amplification was achieved by kit LSAB-HRP (Labelled Streptavidin-Biotin) (DAKOCytomation, USA). Chromogen solution included 3, 3-diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemical Co., USA). A sample from liver with cholangiocarcinoma was considered as a positive control and a normal liver sample as negative control. The staining was graded according to the estimative of fraction of positive cells of the interlobular bile ducts, lymphocytes from portal tracts and lobular areas, Kupffer cells, hepatocytes, endothelial cells and ducts cells from periportal areas (canals of Hering). Scores were predefined as follows: 0 = without staining; 1 = small amount of immuno-positive liver cells; and 2 = large amount of immuno-positive liver cells.

Semi-quantitative RT-PCR
After tissue pulverization with a dismembrator (B. Braun Biotech International, Melsungen, Germany) at liquid nitrogen temperature, total RNA was extracted using TRIzol® reagent (Invitrogen Life Technologies, Carlsbad, USA) according to the manufacturer’s instructions. After phenol treatment and drying, RNA was dissolved in RNase-free water and RNA concentration was spectrophotometrically determined. RNA quality was checked on agarose gel electrophoresis. The expressions of IGF-
IR and IGF-I in the tissue series were performed by using semi-quantitative RT-PCR. Total RNA (3 µg) was reverse-transcribed into complementary DNA (cDNA) using the SuperScript™ II Reverse Transcriptase and random primers (Invitrogen Life Technologies) according to manufacturer’s recommendations and diluted with ddH2O to a final volume of 20 µL. The optimal number of PCR cycles was determined for each primer set so that the amplification process was conducted during the exponential phase of amplification. Concomitant amplification of the break-point control region (BCR) gene transcript was used as the internal control to stringently control for any variability in RNA degradation and RT efficiency. Reactions were carried out in a 45 µL final reaction volume containing 3 µL of cDNA template, 20 mmol/L Tris-HCl (pH 9.0), 50 mmol/L KCl, 1.5 mmol/L MgCl2, 0.22 mmol/L deoxynucleotide triphosphates (dNTPs), 2.0 U Taq DNA polymerase (GE Healthcare-Amersham plc, Buckinghamshire, UK) and specific primers for the target genes and for BCR gene. In order to achieve exponential co-amplification for both IGF-IR and IGF-I mRNA expressions in relation to the endogenous internal control (BCR) at the same number of cycles, sense and antisense primer concentrations were appropriately adjusted between 0.22 and 0.44 µmol/L. PCR amplifications for both genes were performed under the following conditions: initial denaturation at 94°C for 2 min 30 s, denaturation at 94°C for 30 s, annealing at specific temperature 57°C for 1 min and extension at 72°C for 1 min 30 s, followed by a final extension at 72°C for 1 min 30 s. The number of cycles to achieve the exponential phase was 40. As a positive control for IGF-IR and IGF-I, human placenta was used. For comparison purpose, normal human liver cDNA was commercially acquired (Human Liver QUICK-Clone cDNA [Clontech Laboratories, Inc., Palo Alto, CA, USA]) and, according to the manufacturer’s instructions, cDNA was generated from Poly A+ RNA from samples obtained from two normal human livers. The PCR products were submitted to electrophoresis through a 20 g/L agarose gel containing ethidium bromide and visualized under ultraviolet light. The band intensities were analysed by Molecular Analyst Software (Bio-Rad Laboratories, Hercules, USA). The expression levels of IGF-IR and IGF-I were normalized by the BCR housekeeping gene expression and the ratios of gene expression (IGF-IR or IGF-I/BCR) were calculated in all samples as arbitrary units of optical density (AU). The primers were designed with the primer3 www.cgi v 0.2 program[23] as follows: BCR (377-bp product): 5'-GAG AAG AGG GCG AAC AAG-3' (sense) and 5'-CTC TGC TTA AAT CCA GTG GC-3' (antisense); IGF-IR (230-bp product): 5'-ACC CGG AGT ACT TCA GCG GT-3' (sense) and 5'-CAC AGA AGC TTC GTT GAG AA-3' (antisense); IGF-I (303-bp product): 5'-TCT TGA AGG TGA AGA TGC ACA CCA-3' (sense) and 5'-AGC GAG CTG ACT TGG CAG GCT TGA-3' (antisense).

Statistical analysis
Data were tested for statistical significance using the JMP version 5.1 statistical computer program (SAS Institute Inc. Cary, NC, USA). Since assumptions for a parametric test were not valid (Kolmogorov-Sminov, P < 0.05), all data were evaluated by Kruskall-Wallis analysis of variance and the Mann-Whitney U test as a multiple comparison method. The Spearman test was used to assess the statistical significance of correlations among the different variables tested. Chi-square and Fisher’s exact tests were used to establish statistical relationship between groups regarding histopathological and immunohistochemical findings. P values less than 0.05 were considered statistically significant.

RESULTS

Immunohistochemical analysis of IGF-IR
A section of cholangiocarcinoma tissue served as positive control for immunohistochemical analysis of IGF-IR. The positive pattern was observed in cytoplasmic membrane of the neoplastic duct epithelial cells (Figures 1A and 1B). Since positive staining for IGF-IR was detected in ductal cells, they served as internal control, both in normal liver where IGF-IR expression was not detected in hepatocytes (Figures 1C and 1D) and in chronic hepatitis liver samples. In CHC, an expressive increase in IGF-IR immunoreactivity was observed in membrane and in cytoplasm of proliferated bile ducts and in hepatocytes cytoplasm located in all acinar zones (Figures 2A-D). However, nuclear staining was not detected. Histological and immunohistochemical findings before treatment (samples from 34 patients) and post-treatment (samples from 10 patients) are shown in Figures 3 and 4. Immunohistochemical analyses demonstrated of IGF-IR positivity in hepatocytes in 85% (29/34) samples of CHC before treatment; twenty-three of these 29 samples showed positive immunostaining in hepatocytes located in all regions of the lobe (zones I, II and III of the Rappaport’s acinus), while in 6 of these 29 samples revealed positive immunostaining with grade 1 in periportal region, zone 1 (Figure 2E). On contrary, no IGF-IR positivity in

Figure 1 Immunohistochemical analysis of IGF-IR in liver tissue. A: Positive control cholangiocarcinoma tissue showing strongly positive staining for IGF-IR in neoplastic ducts (-100 ×); B: positive control cholangiocarcinoma tissue showing strongly positive staining for IGF-IR in neoplastic ducts (-200 ×); C: normal liver tissue showing IGF-IR immunoreactivity in ductal cells (<100 ×); D: normal liver tissue showing IGF-IR immunoactivity in ductal cells (<200 ×).
hepatocytes was observed in 15% (5/34) samples of CHC before treatment.

Among the patients with fibrosis of degree 0, 1 and 2, 13% (3/23) did not show positivity in hepatocytes, 70% (16/23) showed weak staining and 17% (4/23) showed intense staining. Within the group with fibrosis of degree 3 and 4, 9% (1/11) did not show any staining, 55% (6/11) showed weak staining and 36% (4/11) showed intense staining. In some cases, especially in cirrhotic livers, a granular pattern of positivity in hepatocytes cytoplasm from all lobular regions was visualized (Figure 2F).

All of the pre-treatment samples (34/34) showed IGF-IR positivity in bile ducts, with strong positivity (degree 2) in 91% (31/34) of the positive cases. Twenty-eight of 34 (82%) samples showed IGF-IR positivity in peribiliary duct cells (canals of Hering), with weak positivity (degree 1) in 25 of 28 samples and strong positivity (degree 2) in 3 of 28 samples.

Only 50% (17/34) and 15% (5/34) of the samples showed positivity in portal lymphocytes and Kupffer cells, respectively, with a weak (degree 1) positivity pattern. No staining in lobular lymphocytes and in endothelial cells was observed.

Histopathological and immunohistochemical variables

Figure 2 Immunohistochemical analysis of IGF-IR in different samples of liver. A: Strong positive staining in cytoplasm of hepatocytes in all acinar zones (-100 ×); B: strong positive staining in cytoplasmic membrane and cytoplasm of proliferate bile ducts and in cytoplasm of hepatocytes located in all acinar zones (-100 ×); C: strong positive staining in cytoplasmic membrane and cytoplasm of proliferate bile ducts and in cytoplasm of hepatocytes located in all lobular regions (-200 ×); D: strong positive staining in cytoplasmic membrane and cytoplasm of proliferate bile ducts and in cytoplasm of hepatocytes located in all acinar zones (-100 ×); E: strong positive staining in peribiliary duct cells (-100x); and F: granular pattern of positivity in cytoplasm of hepatocytes in all lobular regions (-400 ×).
did not show any statistically significant correlation. There were no statistically significant differences on IGF-IR immunohistochemical pattern in hepatic tissues from patients with and without sustained virological response between pre- and post-treatment periods (Figure 4).

**mRNA expression of IGF-IR and IGF-I**

An increased IGF-IR mRNA content was detected in hepatic tissue of all 34 patients with CHC before treatment as compared with normal liver (Clontech Laboratories) which did not express IGF-IR mRNA (Figure 5). There were no relevant modifications in IGF-I mRNA content in hepatic tissue from patients with CHC as compared with normal liver, however, since there was only one sample of normal hepatic tissue, it was not possible to perform a statistical evaluation (data not shown).

IGF-IR and IGF-I mRNA contents were evaluated in hepatic tissue obtained within one month of the end of treatment in ten patients. There was a statistically significant decrease ($P = 0.05$) in mean IGF-IR mRNA content in hepatic tissues from patients who achieved sustained virological response in comparison to non-responders (Figure 6), while there was no statistically significant difference in IGF-I mRNA content in hepatic tissues from patients with and without sustained virological response between pre- and post-treatment periods (Figure 7). Furthermore, no statistically significant correlations were observed between IGF-IR and IGF-I mRNA contents and histopathological or immunohistochemical variables.

In an attempt to better characterize the expression pattern of these two genes in normal hepatic tissues, IGF-IR and IGF-I mRNA contents were evaluated in hepatic fragments from six cadaveric liver donors (8-12 h of cold ischemia) following orthopic transplantation. Interestingly, despite being considered as normal livers by histopathological examination, all samples presented an increased IGF-IR mRNA content (Figure 8), regardless the degree of ischemia-reperfusion injury, which ranged from 1 to 3, accordingly to Gayotto & Leitão classification[23]. In addition, there were no relevant modifications in IGF-I mRNA content in hepatic tissue from liver donors following orthopic transplantation in comparison to normal liver (data not shown).

**DISCUSSION**

Although almost all tissues and cells express IGF-IR during embryogenesis, in the normal adult liver, IGF-IR mRNA is practically undetectable[5] and very low IGF-I binding has been shown in cultured hepatocytes[8] possibly due to IGF-IR down-regulation by the locally produced IGF-I. However, hepatic non-parenchymal cells, such as Kupffer cells, endothelial cells and HSC, express IGF-IR and are susceptible to their mitogenic effects[11,24,25].

In the current investigation, we demonstrated an increase of IGF-IR mRNA content in hepatic tissue from patients with different CHC staging. The immunohistochemical findings suggested this augmented mRNA content might be secondary to ductular proliferation, since these epithelial cells expressed IGF-IR, and to increased IGF-IR expression in hepatocytes. Although IGF-IR im-
damage. The role of IGF-IR in liver regeneration following orthopic transplantation (lanes 1 and 2). The experiment was carried out in triplicates. Human placenta was used as a positive control (PC) and no DNA template was observed in this model. Additionally, Desbois-Mouthon et al., have recently reported that hepatocyte proliferation in chronic hepatitis, including CHC. Although in previous findings showing an increase in the number of cells that originate newly proliferated ductal cells, the hepatic progenitor cells (“oval cells”), as hepatitis C severity progresses, as well as the recent report of a strong correlation between portal fibrosis and periporal ductular reaction with progenitor cells expansion in CHC illustrate the need for further studies addressing the mechanisms underlying progenitor cells activation and liver remodelling in CHC.

The up-regulation of IGF-IR expression in hepatocytes of patients with CHC could constitute an attempt to stimulate hepatocyte regeneration in response to liver damage. The role of IGF-IR in liver regeneration following subtotal hepatectomy has been demonstrated in animal models. Caro et al. showed a significant increase in IGF-I binding in freshly isolated hepatocytes from regenerating adult rat liver. In addition, Santos et al. demonstrated both a transient increase in IGF-I binding to liver membranes and an increase on IGF-IR mRNA concentrations. Up-regulation of IGF-IR as well as IGF-I was also observed in hepatocytes of rats chronically treated with carbon tetrachloride (CCl4) and immunoreactivity increased proportionally to fibrosis development, which might be a compensatory reaction to the continuous loss of hepatocytes. However, the role of IGF-I system in the injured liver has not been completely understood. While experiments in cultured HSC support the participation of IGF-I in HSC transformation toward a fibrogenic phenotype, promoting HSC proliferation and collagen synthesis, targeted over-expression of IGF-I in activated HSCs attenuates fibrogenesis and accelerates liver regeneration after acute CCl4-induced injury. These effects appear to be mediated in part by up-regulation of HGF and down-regulation of TGF-β1, suggesting that IGF-I can modulate the cytokine response to liver injury, facilitating regeneration and reducing fibrosis. Based on our finding of increased IGF-IR expression even after an acute challenge such as ischemia-reperfusion injury, we question if a direct effect of HSC-derived IGF-I on hepatocytes expressing IGF-IR might contribute to the accelerated hepatic regeneration observed in this model. Additionally, Desbois-Mouthon et al. have recently reported that hepatocyte proliferation...
during liver regeneration is impaired in liver-specific IGF-IR knockout mice as evidence that IGF-IR contributes to liver regeneration.

In the liver, it has been shown that ischemia-reperfusion injury occurs as biphasic pattern, with an initial acute phase characterized by hepatocellular damage at 3-6 h and a subacute phase characterized by massive neutrophil infiltration at 18-24 h[38]. The ischemia-reperfusion injury also induces the activation of intracellular pathways clearly involved in the hepatocellular regenerative response[10], such as expression of tumor necrosis factor-α (TNF-α)[40,41] and IL-6-associated signalling pathways[42], in an attempt to replace cells lost to ischemic and immunologic damage[43]. To our knowledge, augmented IGF-IR expression has never been reported in animal or human livers submitted to ischemia-reperfusion injury, while it was described in a murine model of renal ischemia-reperfusion injury.[44]. Considering that liver is the organ with the highest levels of IGF-I expression, our finding of increased IGF-IR expression in an acute model of hepatic damage such as ischemia-reperfusion injury as well as in the condition of chronic hepatitis C highlights the need for additional studies to elucidate the role of IGF-I in the complex events of liver regeneration.

Regarding IGF-IR expression in the post-treatment period, although few patients were available for this analysis, the decrease in IGF-IR mRNA content observed in the patients who achieved sustained virological response suggests an improvement in hepatic injury without returning to the expression pattern observed in normal liver, which exhibits undetectable levels of IGF-I mRNA. We hypothesized that the change on IGF-IR mRNA content in the absence of modifications on the immunostaining pattern, as well as the positivity of IGF-IR mRNA in all patients who achieved sustained virological response without return to the expression pattern observed in normal liver, it has been shown that ischemia-reperfusion injury occurs as biphasic pattern, with an initial acute phase characterized by hepatocellular damage at 3-6 h and a subacute phase characterized by massive neutrophil infiltration at 18-24 h[38]. The ischemia-reperfusion injury also induces the activation of intracellular pathways clearly involved in the hepatocellular regenerative response[10], such as expression of tumor necrosis factor-α (TNF-α)[40,41] and IL-6-associated signalling pathways[42], in an attempt to replace cells lost to ischemic and immunologic damage[43]. To our knowledge, augmented IGF-IR expression has never been reported in animal or human livers submitted to ischemia-reperfusion injury, while it was described in a murine model of renal ischemia-reperfusion injury.[44]. Considering that liver is the organ with the highest levels of IGF-I expression, our finding of increased IGF-IR expression in an acute model of hepatic damage such as ischemia-reperfusion injury as well as in the condition of chronic hepatitis C highlights the need for additional studies to elucidate the role of IGF-I in the complex events of liver regeneration.

Because of the importance role of the IGF system in mediating not only normal growth and tissue repair but also carcinogenesis, the expression and function of all components of this system must be strictly controlled. Although we speculate that up-regulation of IGF-IR might participate in hepatocyte regeneration, the suggestion that HCV replication might mediate expression of the IGF-IR ligand IGF-II in cirrhotic livers[43] rise concerns that these two simultaneous changes could act as possible factors for HCV-associated hepatocarcinogenesis in cases of advanced hepatic lesion, since an autocrine and/or paracrine activation of the IGF-II/IGF-IR axis has been implicated in the development of HCC[46]. This hypothesis is corroborated by the finding that hepatitis B virus (HBV) X protein (HBx), which participates in the process of HBV-associated liver carcinogenesis, activates the IGF-IR gene expression[47,48]. It remains to be elucidated if a direct activation of IGF-IR gene by HCV could contribute to the increased IGF-IR expression in hepatocytes in CHC.

REFERENCES

1. Jones JI, Clemons DR. Insulin-like growth factor factors and their binding proteins: biological actions. Endocr Rev 1995; 16:3-34
2. Nakar J, Kido Y, Acilli D. Distinct and overlapping functions of insulin and IGF-I receptors. Endocr Rev 2001; 22:819-855
3. Baserga R, Peruzzi F, Reiss K. The IGF-1 receptor in cancer biology. Int J Cancer 2003; 107:873-877
4. Le Roith D, Bondy C, Yakar S, Liu JI, Butler A. The somatomedin hypothesis. 2001. Endocr Rev 2001; 22:53-74
5. Le Roith D, Werner H, Beitner-Johnson D, Roberts CT Jr. Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocr Rev 1995; 16:143-163
6. Ghosh P, Dahms NM, Kornfeld S, Mannose 6-phosphate receptors: new twist in the tale. Nat Rev Mol Cell Biol 2003; 4:202-212
7. Werner H, Le Roith D. New concepts in regulation and function of the insulin-like growth factors: implications for understanding normal growth and neoplasia. Cell Mol Life Sci 2000; 57:932-942
8. Caro JF, Poujol J, Ittoop O, Pories WJ, Flickinger EG, Sinha MK. Insulin-like growth factor I binding in hepatocytes from human liver, human hepatoma, and normal, regenerating, and fetal rat liver. J Clin Invest 1988; 81:976-981
9. Laron Z. Insulin-like growth factor 1 (IGF-1): a growth hormone. Mol Pathol 2001; 54:311-316
10. Zindy F, Lamas E, Schmidt S, Kern A, Brechot C. Expression of insulin-like growth factor II (IGF-II) and IGF-I, IGF-II and insulin receptors mRNAs in isolated non-parenchymal rat liver cells. J Hepatol 1992; 14:30-34
11. Svegliati-Baroni G, Ridolfi F, Di Sario A, Casini A, Marucci L, Gaggiotti G, Orlandoni P, Macarri G, Perego L, Benedetti A, Folli F. Insulin and insulin-like growth factor-I stimulate proliferation and type I collagen accumulation by human hepatic stellate cells: differential effects on signal transduction pathways. Hepatology 1999; 29:1743-1751
12. Scharf JG, Dombrowski E, Ramadori G. The IGF axis and hepatocarcinogenesis. Mol Pathol 2001; 54:138-144
13. Price JA, Kovach SJ, Johnson T, Koniaris LG, Cahilll PA, Sitzmann JV, McKillop IH. Insulin-like growth factor I is a comitogen for hepatocyte growth factor in a rat model of hepatocellular carcinoma. Hepatology 2002; 36:1089-1097
14. You L, Lukes H, McDoumail H, Werner J, Zahradka P. Insulin-like growth factor-I (IGF-I)-dependent activation of pp42/44 mitogen-activated protein kinase occurs independently of IGF-I receptor kinase activation and IRS-1 tyrosine phosphorylation. Eur J Biochem 1999; 266:1147-1157
15. Nosrati G, Levinovitz A, Moller C, Eriksson LC, Andersson G. Expression of insulin-like growth factor I (IGF-I) and IGF-II mRNA during hepatic development, proliferation and carcinogenesis in the rat. Carcinogenesis 1988; 9:209-213
16. Harris TM, Rogler LE, Rogler CE. Reactivation of the maternally imprinted IGF2 allele in TGalpha induced hepatocellular carcinomas in mice. Oncogene 1998; 16:203-209
17. Sohda T, Yun K, Iwata K, Soejima H, Okumura M. Increased expression of insulin-like growth factor 2 in hepatocellular carcinoma is primarily regulated at the transcriptional level. Lab Invest 1996; 75:307-311
18. Sedlacek N, Hasilik A, Neuhaus P, Schuppan D, Herbst H. Focal overexpression of insulin-like growth factor-2 by hepatocytes and cholangiocytes in viral liver cirrhosis. Br J Cancer 2003; 88:733-739
19. Carieni E, Lasserre C, Seurin D, Hamelin B, Kemeny F, Franco D, Czech MP, Ullrich A, Brechot C. Differential expression of insulin-like growth factor II mRNA in human primary liver cancers, benign liver tumors, and liver cirrhosis. Cancer Res 1988; 48:6844-6849
20. Tsai TF, Yauk YK, Chou CK, Ting LP, Chang C, Hu CP, Han SH, Tu TS. Evidence of autocrine regulation in human hepatoma cell lines. Biochem Biophys Res Commun 1988; 153:39-45
21. Gayotto LCC e Comitê SBP/ SBH. Visão histórica e consenso nacional sobre a classificação das hepatites crônicas - Projeto
The relation of mesenchymal cell products to hepatic epithelial systems. Prog Liver Dis 1990; 9: 27-38

Desmet V, Roskams T, Van Eyken P. Ductular reaction in the liver. Pathol Res Pract 1995; 191: 513-524

Harada K, Kono N, Tsuneyama K, Nakamura Y. Cell-kinetic study of proliferating bile ductules in various hepatobiliary diseases. Liver 1998; 18: 277-284

Libbrecht L, Desmet V, Van Damme B, Roskams T. Deep intralobular extension of human hepatic ‘progenitor cells’ correlates with parenchymal inflammation in chronic viral hepatitis: can ‘progenitor cells’ migrate? J Pathol 2000; 192: 373-378

Eleazar JA, Memeo L, Jhang JS, Mansukhani MM, Chen S, Memeo L, Jhang JS, Mansukhani MM, Chen S, Chen FL, Brenner DA, Fuller CR, Simmons JC, Pardo A, Martinez-Chantar ML, Fagin JA, Prieto J. Expression of insulin-like growth factor I by activated hepatic stellate cells reduces fibrogenesis and enhances regeneration after liver injury. Gut 2005; 54: 134-141

Desbois-Mouthon C, Wendum D, Cadoret A, Rey C, Leneuve P, Blaise A, Housset C, Tronche F, Le Bouc Y, Holzenberger M. Hepatocyte proliferation during liver regeneration is impaired in mice with liver-specific IGF-1R knockout. FASEB J 2006; 20: 773-775

Hernandez LA, Grisham MB, Twohig B, Arfors KE, Harlan JM, Granger DN. Role of neutrophils in ischemia-reperfusion-induced microvascular injury. Am J Physiol 1987; 253: H699-H703

Fausto N, Webber, EM. Liver Regeneration. In: Arias I, Boyer J, Fausto N, Jakoby W, Schachter D, Shafritz D, eds. The Liver: Biology and Pathobiology. New York: Raven Press, 1994: 1059-1084

Bradham CA, Stachlewitz RF, Gao W, Qian T, Jayadev S, Jenkins G, Hannun Y, Lemasters JJ, Thurman RG, Brenner DA. Reperfusion after liver transplantation in rats differentially activates the mitogen-activated protein kinases. Hepatology 1997; 25: 1128-1135

Bradham CA, Schemmer P, Stachlewitz RF, Thurman RG, Brenner DA. Activation of nuclear factor-kappaB during orthotopic liver transplantation in rats is protective and does not require Kupffer cells. Liver Transpl Surg 1999; 5: 282-293

Debonera F, Aldeguey X, Shen X, Gelman AE, Gao F, Que X, Greenbaum LE, Furth EE, Taub R, Othoff KM. Activation of interleukin-6/STAT3 and liver regeneration following transplantation. J Surg Res 2001; 96: 289-295

Othoff KM. Hepatic regeneration in living donor liver transplantation. Liver Transpl 2003; 9: S35-S41

Matejka GL. Expression of GH receptor, IGF-I receptor and IGF-I mRNA in the kidney and liver of rats recovering from unilateral renal ischemia. Growth Horm IGF Res 1998; 8: 77-82

Lee YI, Han YJ, Lee SY, Lee YI, Park SK, Park YJ, Moon HB, Shin JH, Lee JH. Activation of insulin-like growth factor II signaling by mutant type p53: physiological implications for potentiation of IGF-II signaling by p53 mutant 249. Mol Cell Endocrinol 2003; 203: 51-63

Tanaka S, Takenaka K, Matsumata T, Mori R, Sugimachi K. Hepatitis C virus replication is associated with expression of transforming growth factor-alpha and insulin-like growth factor-II in cirrhotic livers. Dig Dis Sci 1996; 41: 208-215

Kim SO, Park JC, Lee YI. Increased expression of the insulin-like growth factor I (IGF-I) receptor gene in hepatocellular carcinoma cell lines: implications of IGF-I receptor gene activation by hepatitis B virus X gene product. Cancer Res 1996; 56: 3831-3836

Tao X, Shen D, Ren H, Zhang X, Zhang D, Ye J, Gu B. Hepatitis B virus X protein activates expression of IGF-IR and VEGF in hepatocellular carcinoma cells. Zhonghua Gan Zang Bing Za Zhi 2000; 8: 161-163

S-Editor Wang J L-Editor Kumar M E-Editor Bai SH