The insulin-like growth factor (IGF) signaling axis and hepatitis C virus-associated carcinogenesis (Review)

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Abstract. Insulin-like growth factor (IGF) signaling plays an important autocrine, paracrine and endocrine role in growth promotion involving various tissues and organs. Synthesis of both IGFs (IGF-1 and IGF-2) in normal conditions takes place mainly in the liver even if the proteins can be produced in every cell of the human body. The alterations in the IGF signaling axis in human hepatocarcinogenesis are described, but mechanisms of the interactions between expression of oncogenic hepatitis C virus (HCV) proteins and components of the IGF system in progression of chronic hepatitis C to primary hepatocellular carcinoma (HCC) have been poorly recognised. In advanced stages of liver diseases, lowered serum levels of IGF-1 and IGF-2 have been documented. This was supposed to reflect significant damage to liver parenchyma, a decreased number of growth hormone receptors and a decreased genomic expression of IGF binding proteins (IGF BPs). In HCC, a decreased tissue expression of IGF-1, and an increased expression of IGF-1 receptor (IGF-1R) were noted, compared to the control. Potential mechanisms of augmented IGF-2 expression in HCC were also described and dysregulation of IGF signaling in HCC was concluded to occur predominantly at the level of IGF-2 bioavailability. The review aimed at presentation of involvement of IGF-1, IGF-1R and IGF BPs (mostly IGF BP-3 and IGF BP-6) in HCV-related hepatocarcinogenesis. Manifestation of various mRNA transcripts and IGF-1 proteins and their potential involvement in carcinogenesis are also discussed.

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1. Introduction

In HCV-related hepatocarcinogenesis, participation of viral proteins: core (C), non-structural 3 (NS3) and NS5 proteins themselves used to be accentuated (1-3). Several investigations point to a relationship between subcellular localisation, concentration, specific molecular form of the proteins and presence of specific domains and oncogenesis (4-7). HCV proteins were found to be involved in control of cell cycle, through their interaction with such cell cycle control proteins as p53, p21, cyclins, proliferating cell nuclear antigen (PCNA), transcription factors (mainly nuclear factor κB, NF-κB), proto-oncogenes (c-*fos*, c-*jun*), growth factors/cytokines, e.g. tumour necrosis factor (TNF)-α, transforming growth factor (TGF)-β and their receptors and proteins of apoptosis (8-13). A possible interactions between oncogenic HCV proteins and components of IGF axis in human HCC continue to be discussed (14).

2. Components of insulin-like growth factor (IGF) signaling axis

Two key regulatory proteins of this axis are known: IGF-1 and IGF-2, which manifest ~50% sequence identical to that of insulin (15). They are included in the insulin-related family together with relaxin while genes of the family members were located on distinct genomic fragments (chromosome 2 and 11p-q13) (16). To date, 6 types of the IGF BPs have been well characterised (IGF BP1-6), plus two subsequent ones, less well recognised (IGF BP-7 and 8) (17-19). The basic functions of IGF BPs include modulation of IGF-1 and IGF-2 bioactivity, mainly through interactions with receptors of the factors and with insulin receptor (IR). Moreover, IGF BPs extend half-life of IGFs in blood, store them in selected tissue compartments and inhibit activity of IGFs by lowering accessibility of their receptors. They may act independently of the receptors, inducing mitogenesis and cell migration (19). They participate in the interactions with other growth factors (e.g. TGF-β) (18). The most commonly manifested circulating form of IGF BP,
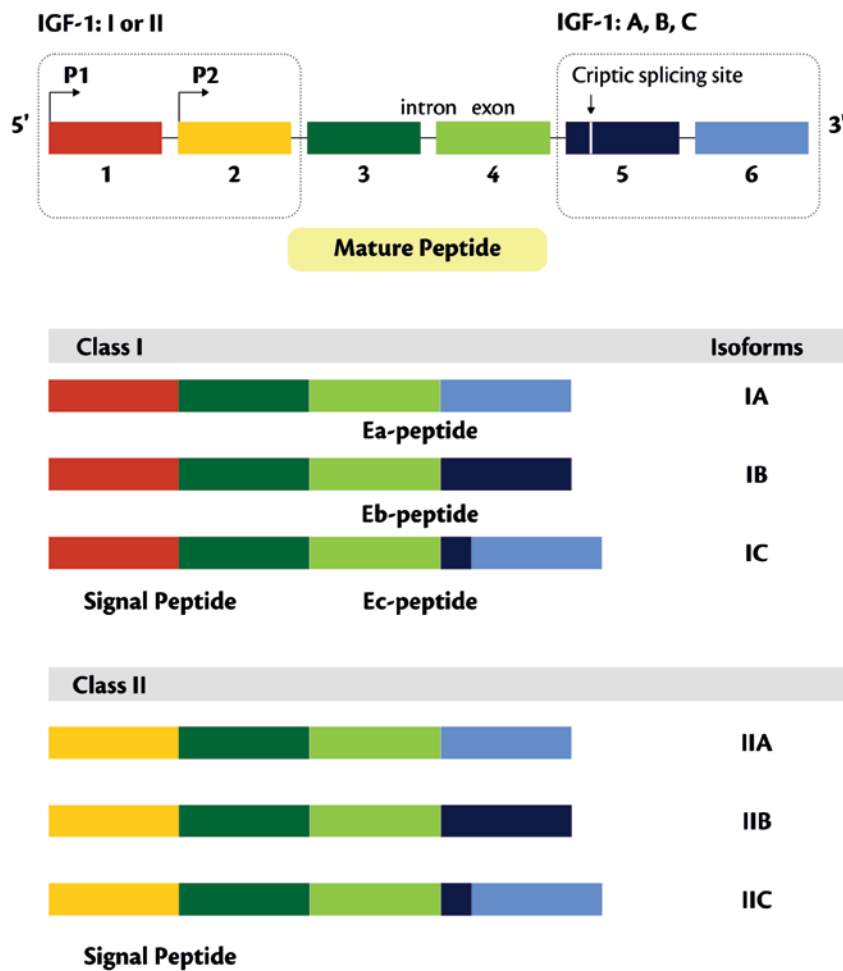
Figure 1. The structure of human *IGF-1* gene and different splicing of alternative isoforms and encoded precursor peptides. The human *IGF-1* gene contains 6 exons. Transcription starts from one of the two possible promoters (P1 and P2) located in exon 1 and 2, respectively. Exons 1 and 2 are alternatively utilized and comprise class I and II, respectively. Exons 3 and 4 are common to all splice variants. Exon 5 is normally absent (Class A), but when it is included, it forms Class B or Class C of mRNA isoforms. mRNAs containing exon 4 spliced directly to exon 6 are designed as Ea, exon 5 spliced to exon 4 are designed as Eb. The IGF-1Ec splice variant is an exon 4-5-6 variant. All peptide products, derived from pro-IGF-1 and referred to in the text, are shown. The figure is adapted from data of Temmerman *et al* (27) and Matheny *et al* (28).

is IGF BP-3, which binds over 95% of IGFs. The protein as a dimer forms a complex with the acid-labile protein (ALS) subunit (18). IGF BP-2, -3 and -5 contain a nuclear localisation signal and may influence on activity of transcription. The IGF BP-3 itself may act also as an inhibitor of cell growth (20,21). Free IGF-1 has a half-life of ~8 min in serum. This can be increased to ~30 min if bound to IGF BP-3 and to ~15 h in the ternary complex with IGF BP-3 and ALS (22).

IGFs affect a cell specifically binding three various surface receptors: IGF-1R, IGF-2R and IR. In most of activities of IGF-1 and IGF-2, IGF-1R plays the main role of a mediator. Apart from mediating in mitogenic and anti-apoptotic activities of IGFs, the receptor is engaged in cell transformation (23) and, therefore, this review accentuates first of all the role of IGF-1R.

IGF-1. Gene of *IGF-1* manifests a single copy in human genome located at the long arm of chromosome 12 (12q22-24.1), and contains 6 exons and 5 introns (24-26) (Fig. 1). The six exons are alternatively spliced into multiple transcripts, encoding specific circulating and tissue-specific isoforms of the IGF-1 peptide (27,28). At the 5' end of the gene, different promoters in combination with alternative transcription start sites and differential splicing generate the mutually exclusive Class I (1) and Class II (2) IGF-1 isoforms (29,30) (Fig. 1). At the 3' end of the gene, alternative splicing gives rise to at least three subsets of RNA transcripts, each encoding three distinct carboxy-terminal portions of the unique E-domain extension-peptide (E-peptide) as well as the 3'-untranslated region (3'UTR) (17,25,31-36). Exon 3 encodes parts of the signal peptide and the mature peptide common to all isoforms, while exon 4 encodes the rest of the mature peptide and the proximal part of the E domain. Even if according to some authors at least nine IGF-1 isoforms can be transcribed (35,37), the six main IGF-1 transcripts isoform in mice and men with apppropriate nomenclature are present (Fig. 1). Expression and composition of nucleotides in exons 5 and 6 determine formation of isoform variants: A (Ea), B (Eb) and C (Ec) within classes I and II (27). The predominant transcript IGF-1Ea has an exon 4 spliced directly to exon 6. Inclusion of exon 5 results in two transcript variants in humans: (1) IGF-1Ec (IGF-Ib in rodents) (mechano growth factor, MGF), and (2) IGF-1Eb (26,27,37-40). The variant designated as IGF-1Eb was described only in humans and it contains mRNA with exon 5 spliced to exon 4 (41).

IGF-1 mRNA isoforms yield, as a result, also various isoforms of IGF-1 protein (27,28). A post-translational modification may further influence the biological activity of IGF-1 (42). The mature molecule of IGF-1 is coded only by exons 3 and 4. In circulation, the prevailing IGF-1 peptide variant involves IGF-1Ea (35). IGF-1 transcript and protein isoforms play various functions, depending on tissue involved, e.g. in skeletal muscle many isoforms of IGF-1 play critical role in development, growth and repair of the tissue (28,43). IGF-1Eb mRNA correlates with markers of muscle satellite cells and myoblast proliferation, whereas upregulation of IGF-1Ea mRNA is correlated with differentiation to mature myofibers (28). The exon 1-encoded signal peptide seemed to have stronger secretory properties than the exon 2-encoded peptide (44).

In mammals, transcription of *IGF-1* gene remains under control of two promoters (P1 and P2) (26,45). The variety of IGF-1 isoforms stems from the use of alternative promoters combined with an elaborate pattern of alternative splicing of the primary transcript (35). In mammals, promoter P1 initiates over 90% transcripts (17). It is thought that P2 promoter encoding the endocrine IGF-1 form, remains under control of growth hormone (GH) (46). Both P1 and P2 promoters regulate transcription over scattered start sites, and it is suggested that heterogeneous transcription initiation compensates for the lack of typical proximal promoter elements, such as TATA- or CCAAT-boxes (47).

The nucleotide sequences of cDNAs predicting two different IGF-1 protein precursors and defining the size of these peptides (153 and 195 amino acids) were described for the first time by Rotwein (34). In liver cells IGF-1 isoform C (comprises 10% of IGF-1A isoform transcript) was also detected. Its presence was confirmed also in cultured HepG2 cells, in which its expression was increased following supplementation of GH. The transcript codes for the prepro-IGF-1 of 158 amino acids (aa), with an E-peptide sequence of 24 aa (33).

Within controlling sequences of both *IGF-I* gene promoters and their 5'UTR sequences several sites were noted for a potential specific binding of transcription factors, which may control its expression, including CCAAT/enhancer binding protein (C/EBP) α and β and hepatocyte nuclear factor-α (HNF-α) (48,49).

A distinct role is suggested for various *IGF-1* splicing profiles as prognostic biomarkers in human cancer development (50-52). Mature IGF-1 and IGF-2 peptides, similarly to proinsulin, consist of domains B, C, and A (amino-terminal end) and an additional domain D at the carboxy-terminal end, absent from proinsulin (15).

IGF-1 is a secretory protein of 7649 Da molecular mass, consisting of a single polypeptide chain with 70 aa, exerting a variable effect on cells and tissues (17). In postnatal period liver remains to be the main source of circulating IGF-1 and the protein is produced mainly under effect of GH. Secretion of GH is affected also by age, gender, diet and nutrition, insulin and sex hormones (17). Studies on RNA level demonstrated that in adipose tissue amounts of the IGF-1 transcript are equal to those in liver (17). IGF-1 produced in liver exerts mainly endocrine activity while IGF-1 synthetised by other tissues acts in a para- and/or autocrine way. Interestingly, even if normal liver represents the organ with the highest expression of IGF-1, it contains almost undetectable levels of IGF-1R mRNA. In contrast to hepatocytes, presence of IGF-1R was proven on Kupffer cells, myofibroblasts and hepatic stellate cells (20).

Serum concentration of IGF-1 changes with patient's age and is dependent on gender. In childhood it grows systematically, most rapidly before and during pubescence, when it reaches the highest levels (53). The gender-related difference in IGF-1 concentration (of ~20 μg/l) appears already in the first three years of age and it is most pronounced (in girls higher by ~70 μg/l) at the age of ~11-13 years. Following 20th year of age a reverse change takes place and higher IGF-1 concentrations are noted in men and the mean inter-gender difference is 6-26 μg/l (54). Other investigators detected a gradually decreasing with age concentrations of IGF-1, independent of gender (55-57). Following a marked decrease in IGF-1 concentration following 25th year of life, a systematic slow reduction in the level to 80th year of age is noted (53). Free IGF-1 accounted for ~1% of the total IGF-1 and its variation with age was similar to total IGF-1 (58).

Independently of GH level, serum concentration of IGF-1 is affected by nutritional status. In childhood and during pubescence no correlation was noted between IGF-1 concentration and body mass index (BMI) (53). In persons with BMI <21 and BMI >29 decreased levels of IGF-1 are noted (59). In cases of obesity it achieves decreasingly lower values. Ther highest standard deviation score (SDS) values for IGF-1 were noted in women with BMI of 27.5 to 30, and in men with BMI ranging between 22.5 and 25 (60). In men of 40-75 years of age also race-dependent differences were noted in serum concentration of IGF-1. Caucasians had the highest median IGF-1 level (224 ng/ml), followed by Asian (208 ng/ml) and African Americans (205 ng/ml) (61).

In women an inverse correlation was documented between IGF-1 and insulin levels (62). Low concentrations of IGF-1 were proven to be strictly linked to growing risk of glucose intolerance and development of type 2 diabetes mellitus (DM) (63). In DM IGF-1 levels decreases (64) and manifests an inverse correlation with concentration of glycosylated haemoglobin (HbA$_{1c}$) (65). Insulin therapy of DM decreases portal concentrations of insulin. The decreased concentrations of insulin may lead to an insufficient production of IGF-1 in liver. In patients with DM concentration of IGF BP-1 increases also (65,66). In rats with induced diabetes, reductions in circulating IGF-1 levels and hepatic IGF-1 mRNA were noted as well as a significant increase (>400%) in liver IGF BP-1 mRNA (67). IGF-1 stimulates glucose uptake by peripheral tissues. This insulin-like effect may develop with mediation of IGF-1R or IR (68).

Concentrations of IGF-1 and IGF BP-3 are stable during a day even if the main factor which stimulates production and secretion of the two proteins is GH. In children and youth concentrations of IGF-1 and IGF BP-3 well correlate with 24 h output of GH and reflect spontaneous secretion of GH in healthy individuals. Levels of IGF-1 are more sensitive to GH control than levels of IGF BP-3 (69).

IGF-2. Human gene of *IGF-2* is located on chromosome 11 (11p15.5) and it consists of 9 exons. Control of the expression takes place from four promoters (P1-P4). Activity of IGF-2 gene is controlled by genomic imprinting (70). IGF-2 protein
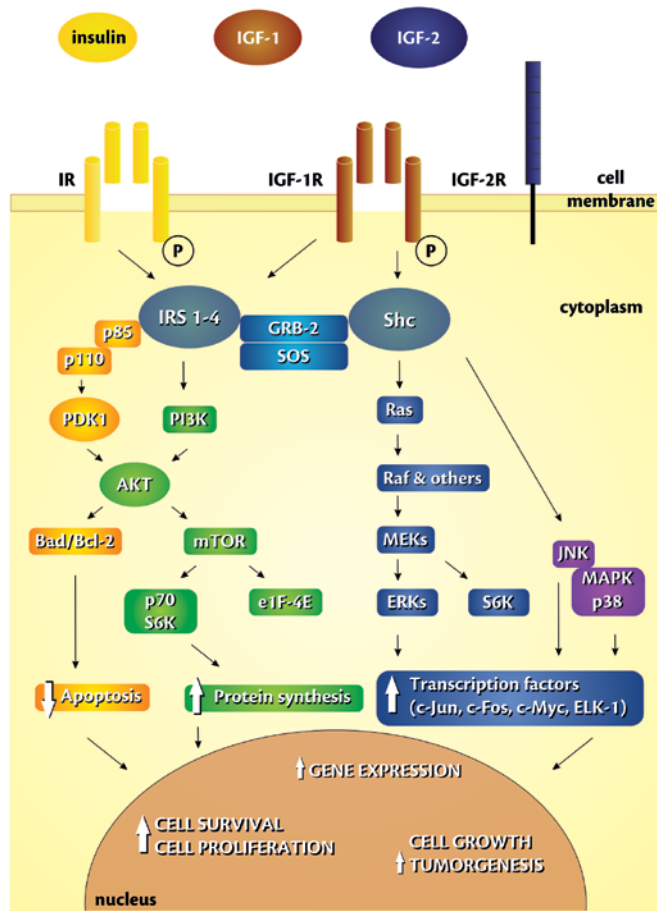
Figure 2. Signal transduction pathways of insulin, insulin-like growth factor 1 (IGF-1) and IGF-2 as potential factors in human hepatocarcinogenesis. IGF-1 binds mainly with IGF-1R to stimulate cellular proliferation or to inhibit apoptosis through different signal transduction pathways (76-79). IGF-1R is a tyrosine kinase which undergoes autophosphorylation and catalyzes phosphorylation of multiple cellular proteins, including members of IRS family and Shc molecule. Upon phosphorylation, IRSs interact with signaling proteins, including Akt, Ras/Raf, MEKs and ERKs. Activation of the PI3K and Akt pathway inhibits apoptosis. IGF-1R/IGF axis may positively control cell cycle progression in many phases, but the major direct effect is probably exerted at the G1-S interface, and this is mediated through the PI-3K/Akt and/or ERK pathways (79). In HCV-related HCC activation of IGF signaling was significantly associated with mTOR signaling and was clearly enriched in the Proliferation subclass of the molecular classification of HCC (84).

consists of 67 aa and manifests 65% of homology with IGF-1. Similarly to IGF-1 it exerts a mitogenic effect on various cells. A markedly higher expression of the protein is detected in embryonal life (71), in which it significantly modulates growth and differentiation of muscles (70). It exerts its effect on cells due to binding with, first of all, IGF-1R. It binds also to the cation-independent IGF-2 mannose-6-phosphate receptor (IGF-2R/MPR) (72,73). IGF-2 binds with high affinity also to isoform A of IR (IR-A), amplifying mainly the mitogenic effect. More numerous IR-A were demonstrated in fetal tissues and certain cancers which indirectly provides evidence for a significant involvement of IGF-2 in embryogenesis and carcinogenesis (73). Normal serum concentration of IGF-2 in adult individuals amounts to ~700 ng/ml. In contrast to IGF-1, concentrations of IGF-2 manifest no significant alterations during maturation. After birth they are low, increase in parallel

to age until pubescence and, then, remain unaltered till the end of life. Gender-related differences are small (2%) (58).

IGF-1 receptor, type I IGF receptor (IGF-1R). Gene of *IGF-1R* consists of 21 exons and is located on chromosome 15q25-26, synthetised as a single-chain preproprotein, composed of 1367 aa, from which a signal peptide of 30 aa is excised. The remaining part of the molecule undergoes glycosylation and fragments to two subunits, α and β, joined by disulphide bridges. The receptor manifests the most pronounced affinity to IGF-1 and a two-fold lower one to IGF-2 (17,68,74). Product of *IGF-1R* gene undergoes alternative splicing with formation of two different mRNAs, differing in only three nucleotides (CAG). Using the model of transfected hamsters the receptor devoid of the three nucleotides (CAG) was found to stimulate about two-fold more effectively IGF-1 in its mitogenic activity. Higher expression of either isoform of the transcript was documented in various tumours. Downregulation of the mature receptor resulted in massive apoptosis of neoplastic cells (75). Removal of the IGF-1R coding gene or inhibition of its expression eliminated cell transformation *in vitro* (23).

IGF-1 binds with IGF-1R to stimulate cellular proliferation or inhibit apoptosis through different pathways, increasing the risk of carcinogenesis (76-78) (Fig. 2). IGF-1R/IGF axis may positively control cell cycle progression in many phases, but the major direct effect is probably exerted at the G1-S interface, and this is mediated through the phosphatidylinositol 3-kinase/serine/threonine-specific protein kinase (PI-3K/Akt) and/or extracellular signal-regulated kinase (ERK) pathways (79). IGF-1 exerts a mitogenic effect influencing stimulation of cyclin D1 and of some proto-oncogenes (*c-FOS, c-JUN*) expression. Moreover, it acts anti-apoptotically and modulates body immune response by control of cytokine production (e.g. IL-3 and IL-14) (68). It was also demonstrated that short peptides of IGF-1 precursors may promote growth of normal and malignant cells in bronchial epithelium (17). IGF-1 controls expression of over 50 genes linked to mitogenesis and cell differentiation. However, it exerts mitogenic activity mainly through stimulation of DNA synthesis and of cyclin D1 expression (68).

IGF-2 receptor, type II IGF receptor (IGF-2R). The human IGF-2R-coding gene is situated on chromosome 6q25-q27 (80). Extracellular domain consists of 15 segments, each containing from 134 to 191 aa and a small region homologous to the collagen-binding domain of fibronectin (81). Mouse gene of 93 kb in length, includes 48 exons and codes for a protein with 2482 aa (72). The receptor itself is identical with cation-independent IGF-2/MPR (72,81). IGF-2R binds IGF-2 ~500 higher than IGF-1 (82). IGF-2R is a multifunctional protein and plays a principal role in sorting and transport of lysosomal enzymes from sites of their synthesis to appropriate cellular compartments and in control of proteins containing mannose-6-phosphate. The receptor is of potential significance for maturation and clearance of growth factors and it mediates IGF-2-activated signal transduction through a G-protein-coupled mechanism (72,81). In studies on mice IGF-2R was demonstrated to be paternally imprinted, and to be crucial for regulating normal fetal growth, circulating IGF-2, and heart development (82).
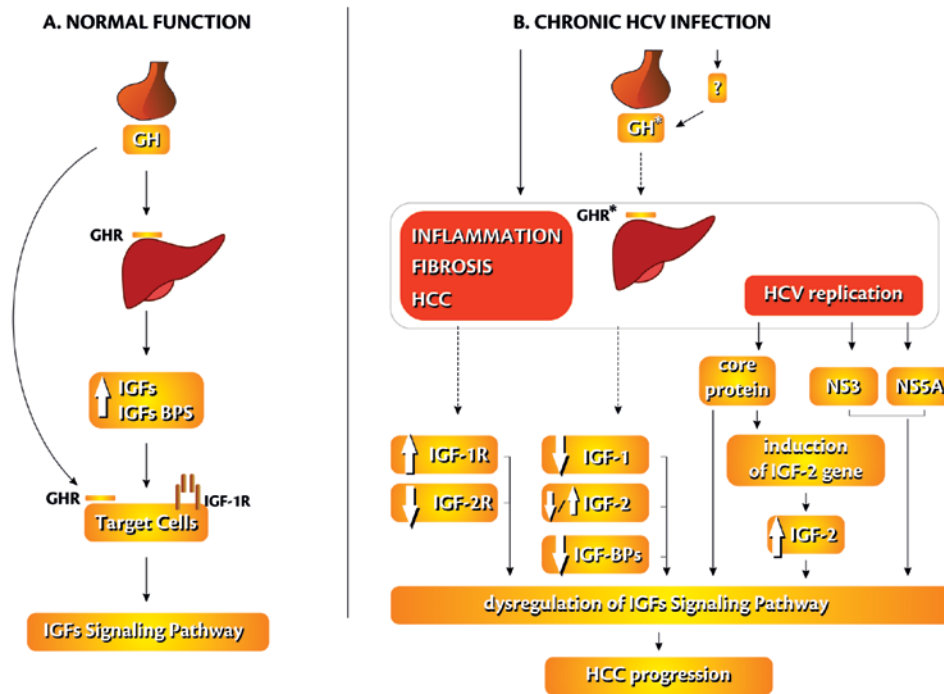
Figure 3. Schematic representation of the GH-IGF axis in normal physiology (A) and the proposed model for how it is affected by HCV-associated liver injury (B). (A) In postnatal period liver remains to be the main source of circulating IGF-1 and the protein is produced mainly under effect of growth hormone (GH) and its receptor (GHR) on liver cells (17). IGF-1 produced in liver exerts mainly endocrine activity while IGF-1 synthesised by other tissues acts in a para- and/or autocrine way (20). IGFs affect a cell specifically binding three various surface receptors: IGF-1R, IGF-2R and IR. In most of activities of IGF-1 and IGF-2, IGF-1R plays the main role of a mediator (23). (B) In HCV-infected patients, GH insufficiency (GH*) and persistent GH resistance (GHR*) of hepatocytes and low IGF-1 levels were documented (139). Other studies demonstrated an increased IGF-1R mRNA and a decreased IGF-1 protein synthesis in patients with chronic hepatitis C (144). HCV replication was associated with overexpression of IGF-2 in the cirrhotic livers (145). Studies on HCV-related HCCs documented a decreased *IGF-1* expression, an increased expression of *IGF-2* in 20-50% of HCCs (84,85,146), *IGF BP3* downregulation and allelic losses of IGF 2R (84). The increased synthesis of IGF-2 (mRNA and protein) was associated with an increased cellular proliferation in HCCs (146). Compared with a non-cirrhotic liver, all cirrhotic specimens showed reduced expression of IGF-2R/M6PR protein. It was suggested that downregulation of hepatocellular IGF-2R/M6PR and upregulation of IGF-2 might be early events in hepatocarcinogenesis (131). HCV core protein was shown to increase endogenous expression of IGF-2 in HepG2 cell line (149).

3. IGF system and hepatocellular carcinoma

Hepatocellluar carcinoma is the third leading cause of cancer-related death worldwide (14,84). The suggested roles of both IGFs and IGF-1R in development of HCC reflect, first of all, observations indicating strong mitogenic effects of the factors in *in vitro* conditions (21,85,86). Different types of cultured cells produce IGF-1 mRNA (87) and insulin receptor substrate 1 (IRS-1) (86). IRS-1 undergoes overexpression in human HCC (88,89). The dominating pathways activated by IGF-1 in hepatocytes and hepatoma cell lines involve the PI3K/Akt (90,91) and signal transducer and activator family protein (STAT) signaling pathways (92). IGF-1 has been implicated in NF-κB-mediated transcriptional regulation of inflammatory cytokines and endothelial cell adhesions receptors such as intracellular adhesion molecule-1 (ICAM-1) (93). Studies on HepG2 and HuH-7 cells demonstrated that the cells synthetised and secreted also IGF-2 and inhibition of the protein production resulted in a reduced cellular proliferation (87). Recent studies on HCV-related HCC demonstrated overexpression of *IGF-2* gene (resulting from reactivation of fetal promoters P3 and P4), IGF BP3 down-regulation, decreased IGF-1 expression and allelic losses of *IGF-2R*. Administration of IGF-1R-selective inhibitors (A12) reduced IGF-1-induced effects and was associated with a significant reduction of liver tumour growth (84) (Fig. 3).

IGF-1. A decreased serum levels of both IGFs were found to parallel progression of liver diseases, independently of their etiology (94,95). Besides, patients with HCC and hypogly-caemia were found to carry higher IGF-2 concentrations than patients without hypoglycaemia. The higher IGF-2 than those of IGF-1 levels were accompanied by more advanced histo-logical lesions in the liver (94). Kratzsch *et al* demonstrated a continuous decline in the concentrations of IGF-1, IGF BP-3 and serum GH-binding activity during progression of cirrhosis (95). Other investigations documented decreased levels of both IGFs in chronic liver diseases but the positive correlation with Child score was confirmed only for IGF-2 (96). It should be added that only in a small number of studied patients (17/55) viral etiology of chronic lesions in liver was confirmed (96). In chronic hepa-titis B and C were also demonstrated elevated levels of IGF-1 and lowered levels of IGF BP-3 (97). Studies on serum IGF-1 concentrations in HCC and in metastatic liver cancers demon-strated markedly lowered concentrations of IGF-1 in either type of hepatic tumour, as compared to the control (98). The decrease in IGF-1 was more pronounced in cases with viral-associated than in virus-negative HCCs (98). In liver cirrhosis lower IGF-1 concentrations were observed in comparison with control (99,100), as well as its increased concentration following anti-viral therapy (100). In HCV-infected children serum IGF-1 level was significantly lower compared with the control group (101).

In adult patients with chronic hepatitis C the reduction of serum IGF-1 level was found to precede by ~9 months development of HCC (102).

IGF-2. Similarly to IGF-1, concentration of IGF-2 also markedly decreases in patients with liver cirrhosis, as compared to healthy individuals. Advancement of liver cirrhosis, is paralleled by gradual decreases in IGF-2 levels. The lowest concentrations of the factor are encountered in patients with incurable ascites and extended activated partial tromboplastin time (APTT). During half-a-year observation of the patients, those with very low concentrations of IGF-2 (<200 ng/ml) died manifesting hepatic insufficiency (103). A successful liver transplantation was followed by a significant increase in IGF-2 concentration in almost all of the patients (104). In patients with HCC levels of IGF-2 decrease (but the difference was not statistically significant) and thus the protein cannot serve as a diagnostic marker of the tumour (105). The amount of IGF-2 mRNA was also lowered in patients with liver cirrhosis as compared to the control (106). The studies on rats with experimentally induced liver cancer, demonstrated a decreased expression of IGF-1 mRNA, and an increased expression of IGF-2 mRNA (107). Start of IGF-2 (fetal type of IGF expression) production in HCC is supposed to represent a late phenomenon in carcinogenesis of rats (107). Regarding human HCC activation of IGF axis (e.g. IGF-2 overexpression, IGF-1R activation) was observed in 21% of early liver tumours (83).

Recently, among 102 patients with HCC and 306 control patients, Weng *et al* detected a significant difference in manifestation of IGF-2 +3580 gene polymorphism (85). Moreover, they noted that the combination of IGF-2 +3580 AA and IGF-2R GG genotype may present a significantly lower risk for HCC. The authors concluded that the most significant IGF system-linked factors in development of HCC involved polymorphisms of IGF-2 and IGF-2R and their combinations (85). Hypomethylation of IGF-2 gene in exon 8-9 was present in 90% HCV-infected patients with liver cirrhosis, who would develop HCC within 10 years. The test could be used as a screening tool in diagnosis of HCC, since frequency of HCC in patients with hypomethylation of the gene is markedly higher than in patients with hypermethylation in the region (30.8% vs. 5.9%) (108).

IGF-1R. Role of IGFs receptors in liver carcinogenesis remains to be fully recognised. On one hand there exist papers pointing to an increased expression of IGF-1R in preneoplastic focal lesions in liver, leading to HCC, in HCC itself and in human hepatoma cells (20), on the other attempts to stimulate mitogenesis with IGF-1 in cultured rat cell lines of HCC proved to be unsuccessful (109). Hepatitis B virus (HBV) and HCV are known to be capable of activating promoters of *IGF-2* and *IGF-2R* genes, thereby increasing the production of this ligand and receptor by liver cancer cells (110-112). An increase in IGF-1R mRNA in liver cirrhosis has been demonstrated, but no change in the amount of IGF-2R mRNA was detected, as compared to the control (106). Activation of IGF-1R was significantly associated with activation of IGF-2 mRNA levels in ~20% of HCCs (84). Other authors observed decreased levels of IGF-1R in 39% patients with cirrhosis, as compared to the control (21).

IGF BPs. Studies on IGF BPs in hepatocarcinogenesis were conducted on animal models (113), in normal liver (114), in patients with chronic hepatitis, cirrhosis and HCC (21,115-118). In the studies, viral background of HCC could not be repeatedly demonstrated.

IGF-1, IGF BP-3 and ALS showed a similar pattern of change associated with age (58). Normal levels of the molecules were low upon birth and increased with age through puberty, with a slow age-dependent decrease thereafter. Slightly higher levels of the three compounds were present in women. Levels of IGF BP-6 increased with age and reached higher levels in men as compared to women (58). Healthy liver produces mainly IGF BP-1 and BP-3. IGF BP-1 (mRNA and protein) were demonstrated in hepatocytes while IGF BP-3 was detected mainly in Kupffer cells (114). IGF BP-3 mRNA was demonstrated in portal venous and sinusoidal endothelium (113).

Decreased levels of IGF BP-3 were shown in patients with cirrhotic liver, as compared to the control (115). The lowered serum concentration of IGF BP-3 was accompanied by absence of protease activity in cirrhotic patients (115,119). It should be noted that concentration of IGF BP-3 is also dependent on age (120,121). Serum concentrations increase with progressing age to reach maximum levels at pubescence. Comparison of IGF BP-3 and IGF-1 concentrations revealed that they did not exibit the same developmental pattern at puberty. IGF-1 levels increased to relatively higher levels than IGF BP-3 (120). Median IGF BP-3 concentration was similar between Caucasian and Asian middle-aged men but was more than 13% lower in African Americans. Median molar IGF-1:IGF BP-3 ratio was greatest in Caucasian and lowest in Asians (61). IGF BP-3 levels manifested no differences before and after menopause (122). Also, concentration of IGF BP-3 failed to correlate with BMI (117). A relationship was detected between lower levels of serum IGF-1, decreased IGF-1/IGF BP-3 ratio, higher serum concentrations of IGF BP-3 on one hand and liver steatosis on the other (116).

IGF BP-6 level significantly increases with age beginning at birth up to pubescence. In most age groups adult men manifested higher concentrations of IGF BP-6 than those noted in women (58).

In liver cirrhosis significantly lower serum level of IGF BP-3 is noted, as compared to healthy individuals (103). Also, it becomes reduced with an advancement in cirrhosis. Therefore, concentration of this protein provides a real potential index of progression manifested by hepatic lesions (103,117). A positive correlation was detected between concentration of IGF BP-3 and of albumin as well as a negative correlation with bilirubin concentration, size of spleen and activity of aspartate aminotransferase (AST) (117,121). Concentration of IGF BP-3 return to normal level following a successful liver transplantation (104). In patients with HCC concentrations of IGF BP-3 are decreased in cases with a disturbed nutrition, markedly deteriorated liver function and reduced secretion of GH (105). An increase in IGF-1/IGF BP-3 ratio in patients with HCC was also demonstrated as compared to patients with cirrhotic liver and a similar extent of liver insufficiency (118). In >70% HCC patients, IGF BP-3 expression was lowered as compared to normal liver. A less pronounced tissue expression of the protein was observed in tumour cells as compared to control (21). Decreased IGF BP-3 mRNA levels correlated with smaller

tumour size, less vascular invasion, and a lower incidence of early recurrence (84).

Analysis of gene expression using cDNA microarrays performed in 20 primary liver tumours demonstrated 170 genes with a decreased regulation in HCC, including IGF BP-3 and ALS (123). In turn, application of 75 antibodies to evaluate markers for early detection of viral-associated HCC, identified 7 proteins which significantly differentiated patients with HCC and those with chronic hepatitis, including IGF BP-6 (83).

A lowered serum concentration of IGF BP-3 was noted also in patients with variably advanced chronic hepatitis (including 12 HCV-positive patients). No correlation could be disclosed between grading/staging on one hand and activity of transaminases on the other (97). Other investigators failed to detect significant differences between concentration of IGF BP-3 in patients with liver cirrhosis which would be related to its aetiology (alcohol vs. HBV vs. HCV (117). Other studies demonstrated a significantly decreased concentration of IGF BP-3 in patients with chronic hepatitis C as compared to healthy individuals (121).

4. Tissue expression of IGF axis components in HCC

In all studied tissues of HCC (n=28) a decreased expression of IGF-1 and IGF BP-3 and in 32% of them an increased expression of IGF-2 was detected (21). Decreased amounts of IGF-1 mRNA (84) or both IGF's mRNA (124-126) in HCCs were demonstrated as compared to the control. In case of IGF-1 transcript, the decrease was more pronounced than that in IGF-2 (124-126). In hepatocarcinogenesis in rats appearance of IGF-2 mRNA was demonstrated upon a decrease in IGF-1 mRNA (107). An increased expression of IGF-2 (protein and mRNA) was demonstrated in HBV-related HCC (127). IGF-1, IGF-2 and IR mRNAs were detected at various stages of HCC development and in cultured cells. The studies showed that isolated hepatocytes synthesise IGF-2 mRNA with a switch between fetal and adult mRNA profiles occurring 21 days after birth (128).

Other studies demonstrated presence of IGF-2 in 60% HCC arising in HBV-associated cirrhosis, as compared to only 26% tumours in HBV-negative patients (129). Studies employing DNA microarrays showed that imbalances in levels of IGF-2 and H19 transcripts were correlated with advanced tumour stage and poor outcome in HCC patients (130). High focal levels of IGF-2 mRNA were found in some hepatocytes of all HBV and HCV-related cirrhotic livers (131). In subgroups of HCV-related HCC an increased tissue expression of IGF-2 (protein, mRNA) was noted (20,83,111). Compared with noncirrhotic liver, all cirrhotic specimens showed reduced expression of IGF-2R/M6PR protein. It was suggested that downregulation of hepatocellular IGF-2R/M6PR and upregulation of IGF-2 might be early events in hepatocarcinogenesis (131). Tovar et al observed losses in the IGF2R locus in ~25% of HCCs (84).

The increased expression of IGF-1R was detected in cirrhosis, in HCC and in human hepatoma cells, as compared to normal liver (84,86,87,132,133). More pronounced membraneous location of IGF-1R was documented in HepG2 cells, as compared to Chang liver cell lines (134). Overexpression of IGF-1R in the cell lines was supposed to indicate a malignant phenotype of the cells. Administration of anti-IGF-1R monoclonal antibody (αIR3) inhibited cell proliferation and induced apoptosis in HepG2 cells (134). It has been proven that mutations resulting in upregulation of IGF-1R gene in certain HCC include p53mt249 (135). The same mutation enhances transcription from fetal IGF-2 promoter P4 (136). Recent studies have drawn attention to the role of polymorphism in IGF-1 gene promoter in carcinogenesis. One of IGF-1 gene polymorphisms recognised in greatest detail seems to involve the sequence consisting of several CA repeats, present in 5'UTR of IGF-1 gene (41). The mutations of IGF-2R/M6PR gene give rise to truncated receptor protein and significant aa substitutions, and provide evidence that this gene functions as a tumour suppressor in human liver carcinogenesis (137).

5. Role of IGF axis in HCV-related hepatocellular carcinoma

Serum concentrations of IGF-1 were significantly lower in HCV-associated HCC than in healthy subjects. Moreover, the lower levels of IGF-1 were detected in all patients below 55th year of age and with homeostasis model assessment of insulin resistance (HOMA-IR) <2.53 as compared to the control (138). Moreover in >80% of HCV-infected patients, severe GH insufficiency was documented and about half of these patients had low IGF-1 level. Basal and stimulated GH concentration increased significantly during therapy, but IGF-1 remained low (139).

IGF-1 is known to regulate, coordinatively with insulin, glucose homeostasis (106). Within nondiabetic HCV patients, IGF-1 serum levels correlated negatively with β-cell function (HOMA-β), and positively with insulin-sensitivity (HOMA-S) (140). In parallel, a negative correlation has been detected between IGF-1 serum levels and both alanine aminotransferase (ALT) and AST (140,141). In HCV patients with DM a positive correlation was observed between fasting insulin levels and HOMA-β (140). Such results suggest (but with caution) that low IGF-1 level might play a role in the development of insulin resistance in HCV viremic patients (140). This would be consistent with observations that IGF-1 may be an early marker of functional reserve or hepatocellular capacity (117,142). Hung et al detected DM more frequently in patients with HCV infection than those with HBV infection. Also in HCV-related HCC, blood glucose, insulin level and HOMA-IR were higher than in patients with chronic hepatitis and advanced fibrosis. The authors concluded that insulin resistance (regardless of the presence of diabetes) is significantly associated with HCC development in patients with chronic HCV infection (143).

Only few investigations pertained hepatic expression of IGFs and their receptors at different stages of chronic hepatitis C in humans (144). The studies demonstrated an increased IGF-1R mRNA, but not IGF-1 protein in patients with CHC as compared to the control (144). HCV replication was associated with the overexpression of IGF-2 in the cirrhotic livers (145). Studies on HCV-related HCCs documented increased expression of IGF-2 in 50% of HCCs. The increased synthesis of IGF-2 (mRNA and protein) was associated with an increased cellular proliferation in HCCs (146). The results observed in tissue samples suggest that IGF-2 might be responsible for IGF-1R activation (83).

Data from examination of serum and tissue levels involving IGF axis components in the patients with advanced liver

Table I. Expression levels of IGF axis components in human hepatocellular carcinoma (including HCV-related HCC).

Type of tumour	Etiology/ patients (n)	Method of detection	Summary of the findings	References
HCC	38	Northern blot	IGF-2 mRNA was increased 40-100 fold in 9 of 40 liver cancer surgical specimens	Cariani *et al*, 1988 (126)
HCC	25	RIA	Lower levels of IGF-1 and more advanced liver cancer in HCC patients; relatively higher IGF-2 levels in comparison with those who did not have hypoglycaemia	Wu *et al*, 1988 (94)
Hepatoma		Northern blot	Low IGF-1 mRNA expression in 7/7 specimens, low IGF-2 expression in 4/7 specimens	Su *et al*, 1989 (124)
HCC	HBV+	ICC and ISH	IGF-2 mRNA was increased in HCC specimens	Lamas *et al*, 1991 (127)
HCC	HBV+, HBV-, 54	ICC	Detection of IGF-2 expression in 9/15 HBV-positive and in 10/39 HBV-negative patients	D'Erico *et al*, 1994 (129)
HCC	HCV+, 7	RT-PCR	Undetectable expression of transcripts derived from adult IGF-2 P1 promoter; increased expression of fetal promoter-derived transcripts, but lower than in cirrhosis	Nardone *et al*, 1996 (111)
HCC	HCV+, 35	ICC	Positive immunoreaction of IGF-2 in 69% of HCC; IGF-2 was positive in 80% of HCC with fatty change but only in 60% of those without steatosis	Sohda *et al*, 1997 (112)
HCC	HCV+, 7; HBV+, 3	ICC, ISH	Increased expression of IGF-2 mRNA and protein in 5/10 HCCs	Sohda *et al*, 1997 (146)
HCC	HBV+, 25; HBV-, 5	Northern blot	Normal adult promoter of IGF-2 was repressed in all but two of HCC; re-expression of two fetal transcripts in 12 tumours; significant association of IGF-2 with direct tumour invasion	Ng *et al*, 1998 (125)
HCC	HCV+, 10; HBV+, 10	cDNA microarrays (23 040 genes)	Upregulation of mitosis-promoting genes (e.g. cyclin G1, MAPK1, MAPK3), tumor-associated genes (CD34, glypican 3, recoverin, matrix metalloproteinase 11), increased expression of genes encoding for 4 enzymes (CYP2E, AKR1C4, EPHX1 and FMo3) exclusively in HCV-positive HCC	Okabe *et al*, 2001 (123)
HCC	28	ICC, Western blot	Decreased expression of IGF-1 in 100% HCCs; low levels of IGF BP3 and type I receptor (IGF-1R) in 39% of HCCs; overexpression of IGF-2 in 32%	Huynh *et al*, 2002 (21)
HCC		DNA microarray	Increased IGF-2 expression was correlated with advanced tumor stage and poor prognosis	Iizuka *et al*, 2004 (130)
HCC	HCV+, 10; HBV+, 26; B/C +, 1; None/ unknown, 11	Antibody array platform and immunoassay	Identification of 7 proteins that significantly differentiate HCC patients from hepatitis patients (e.g. IGF BP-6); among the 75 unique probes, three: AFP, β-catenin, CSF1 were upregulated in HCC; L-selectin, IGF BP-6, IL-6R and VCAM-1 were very slightly downregulated in HCC vs. hepatitis	Sun *et al*, 2008 (83)
HCC	HCV+, 104	RT PCR; SNP array, oligonucleotide microarray; cell viability, proliferation and death assays and miRNA transfection; Western blot; ICC	Overexpression of IGF-2 (due to reactivation of fetal promoters P3 and P4) in 21% of early HCCs, IGFBP3 downregulation and allelic losses of IGF2R (25% of cases); IGF-1 mRNA expression levels were significantly decreased among HCC samples	Tovar *et al*, 2010 (84)
HCC	65	RIA	Decreased serum IGF-1 level in HCCs (especially HCV-infected patients) as compared with healthy subjects	Su *et al*, 2010 (138)
HCC	63	ICC	Decreased IGF-1 tissue expression in comparison with control livers; no correlations between hepatic expression of IGF-1 in HCC and histologic malignancy	Kasprzak *et al*, 2011 (150)

RIA, radioimmunoassay; ICC, immunocytochemistry; ISH, *in situ* hybridization; RT-PCR, reverse transcription polymerase chain reaction; MAPK, p38 mitogen-activated protein kinase; AFP, α-fetoprotein; CSF, colony stimulating factor; IL, interleukin; VCAM, vascular cell adhesion molecule.

Table II. Expression levels of IGF axis components in various stages of chronic liver diseases (including chronic hepatitis C).

Liver pathology	Etiology/patients (n)	Method of detection	Summary of the findings	References
Chronic hepatitis Cirrhosis	15 15	RIA	Both IGF-1 and IGF-2 levels decrease as liver disease progressed	Wu et al, 1988 (94)
Cirrhosis		Immunoassay	A continuous decline in the concentrations of IGF-1, IGF BP-3 and GH-binding activity	Kratzsch et al, 1995 (95)
Active chronic hepatitis Cirrhosis	HCV+, 9; HCV+, 9	RT-PCR	Increased expression of transcripts derived from adult IGF-2 P1 promoter; abundantly expressed transcripts from fetal P3 promoter in most CAH patients and all cirrhotic patients; transcripts from fetal P4 promoter detected at high levels in 3/9 CAH patients and in the majority of cirrhotic patients	Nardone et al, 1996 (111)
Cirrhosis	HCV+, 31	PCR	HCV replication was significantly associated with the overexpression of TGF-α and IGF-2	Tanaka et al, 1996 (145)
Cirrhosis	HCV+, 11; HBV+, 6; others, 21	RIA	IGF-1 and IGF-2 were lower in patients with cirrhosis than in control; only IGF-2 level was correlated with Child score	Nikolić et al, 2000 (96)
Chronic hepatitis	HCV+, 12 HBV+, 5	RIA	IGF-1 levels were significantly higher than that of the control group; IGF BP-3 levels in chronic hepatitis C were significantly lower than in control group	Okan et al, 2000 (97)
Cirrhosis	HCV+, 114	Immunoradiometric assay	Significant reduction in IGF-1 level preceded the diagnosis of HCC by 9.3±3.1 months independent of the grade of impairment of liver function	Mazziotti et al, 2002 (102)
Cirrhosis	HCV+, 3 HBV+, 6 B/D coinfection, 1	ICC, ISH	High focal levels of IGF-2 RNA were found in some hepatocytes of all, livers HBV- or HCV-induced cirrhosis; all cirrhotic specimens showed reduced hepatocellular expression of M6P/IGF-2R protein	Sedlaczek et al, 2003 (131)
Cirrhosis	of viral origin, 22	Immunoradiometric assay	Serum IGF-1 levels were found very significantly lower than in healthy individuals	Vyzantiadis et al, 2003 (99)
Cirrhosis	HBV+, 44	Immunoradiometric assay	Serum levels of IGF-1, IGF-2 and IGF BP-3 were significantly lower than in controls and were associated with the severity of liver dysfunction; IGF-1<30 ng/ml; IGF-2<200 ng/ml and IGF BP-3<6 ng/ml implied a negative prognosis for patients with liver cirrhosis	Wu et al, 2004 (103)
Chronic hepatitis Cirrhosis	14	ICC, ISH	Patients with chronic liver disease have a significant reduction in their hepatic production of IGF-1, whereas IGF-2 tends to be elevated (mRNA, protein)	Morali et al, 2005 (106)
Chronic hepatitis before and after therapy	HCV+, 34; after therapy, 10	RT-PCR, ICC	An increase of IGF-IR mRNA content in all CHC patients in comparison with normal liver; no relevant modification in IGF-1 mRNA content; a decrease IGF-IR mRNA in patients who achieved sustained virological response	Stefano et al, 2006 (144)
Chronic hepatitis before and after therapy	HCV+, 40	Immunoassay	Mean IGF-1 values were significantly lower in patients with advanced fibrosis than in the others; serum levels of IGF-1 increased during combined therapy	Lorenzo-Zúñiga et al, 2007 (100)
Chronic hepatitis	HCV+, 29; HBV+, 12; others, 9	ELISA	Significantly reduced serum levels of IGF-1 in all children with liver diseases	Mahdy et al, 2007 (101)
Chronic hepatitis	HBC+ or HBV+, 19	Immunoassay	Among top scoring 7 proteins that significantly differentiate HCC from chronic hepatitis was IGF BP-6 (higher levels in CHC than in HCC)	Sun et al, 2008 (83)
Chronic hepatitis	HCV+, 30	ICC	Significantly lower expression of IGF-1 tissue expression in patients with CHC compared with control livers, but with no differences in comparison with HCC; positive correlations between IGF-1 and C and NS3 HCV proteins; negative correlations between IGF-1 and ALT and AST activities	Kasprzak et al, 2011 (150)

RIA, radioimmunoassay; RT-PCR, reverse transcription polymerase chain reaction; CAH, chronic active hepatitis; CHC, chronic hepatitis C; ICC, immunocytochemistry; ISH, in situ hybridization.

diseases (including HCV-associated HCC) are summarized in Tables I and II.

6. IGF axis components and HCV genome

IGF-1 may play a role both in persistence of chronic hepatic inflammation through control of signaling pathways linked to proinflammatory cytokines and receptors for endothelial adhesion molecules (e.g. ICAM-1) (93), and in induction of acute inflammatory reaction, triggered by tumour cells during early stages of liver metastasis (79,147,148). In recent years increased attention is devoted also to complex interactions between HCV proteins and IGF axis. HCV core protein was shown to increase endogenous expression of IGF-2 in HepG2 cell line, regulating positively its transcription, and it may promote cell divisions (149).

Our own studies in chronic hepatitis C patients demonstrated positive correlation between tissue expression of IGF-1 and two HCV proteins: core and NS3 while studies on tissue material originating from HCC detected no significant correlations between tissue expression of IGF-1 and the histological malignancy of the tumour (150).

7. Conclusions

Several components of the IGF signaling axis, such as IGF-1, IGF-2 and IGF-1R, are dysregulated during HCV-related human HCC. Only few investigations pertained hepatic expression of IGFs and their receptors at different stages of chronic hepatitis C. The studies demonstrated an increased IGF-1R synthesis, the abberant IGF-2 expression (decreased/increased), and decreased synthesis of IGF-1 as an events in human hepatocarcinogenesis. Recognition of the role played by HCV in different splicing profiles of *IGF-1* gene in progression of chronic hepatitis C will require further studies. Better understanding of the HCV protein and IGF axis component interactions will facilitate development of novel approaches to prognose and to treat the virus-related HCC.

Acknowledgements

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