The IGF axis and hepatocarcinogenesis

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Abstract

Deregulation of the insulin-like growth factor (IGF) axis, including the autocrine production of IGFs, IGF binding proteins (IGFBPs), IGFBP proteases, and the expression of the IGF receptors, has been identified in the development of hepatocellular carcinoma (HCC). Characteristic alterations detected in HCC and hepatoma cell lines comprise the increased expression of IGF-II and the IGF-I receptor (IGF-IR), which have emerged as crucial events in malignant transformation and the growth of tumours. Alterations of IGFBP production and the proteolytic degradation of IGFBPs resulting in an excess of bioactive IGFs, as well as the defective function of the IGF degrading IGF-II/mannose 6-phosphate receptor (IGF-II/M6PR), may further potentiate the mitogenic effects of IGFs in the development of HCC.

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Several different growth factors and their respective receptors have been identified and may act as positive or negative modulators of cell proliferation and differentiation of malignant cell systems. Among these, the insulin-like growth factors I and II (IGF-I and IGF-II), their receptors, and their binding proteins play an increasingly recognised role, being implicated in tumour formation, growth, and metastasis in vivo.[1 2] During this process, a crucial role has been attributed to the IGF-I receptor (IGF-IR), which has been shown to mediate mitogenic signals, to protect from a variety of apoptotic injuries, and to be necessary for the transformation of certain types of cells.[3 4] The IGF-II/mannose 6-phosphate receptor (IGF-II/M6PR), however, is involved in the transport of lysosomal enzymes, in binding, internalisation, and degradation of IGF-II, as well as in the activation of the mito-inhibitory transforming growth factor β (TGF-β),[5] and has therefore been considered to be encoded by a tumour suppressor gene.[6]

Within the circulation and tissue compartments, IGFs are bound with high affinity to a family of structurally related binding proteins (IGFBPs).[7–9] So far, six distinct IGFBPs have been characterised, which differ in molecular mass, binding properties for IGFs, and post-translational modifications such as phosphorylation and glycosylation.[10] Many functions have been proposed for the IGFBPs, including carrier proteins in the circulation, storage of IGFs in specific tissue compartments, inhibition of IGF action by preventing access to IGF receptors, or potentiation of the mitogenic response by providing a stable source of available growth factor.[9] IGFBP-3 is the most abundant IGFBP in the serum. It forms a ternary complex of 150 kDa with IGF-I and an acid labile subunit (ALS). The 150 kDa ternary complex is retained in the plasma and is a potential reservoir of IGFs in the circulation. The abundance of the IGFBPs can be regulated by gene expression and by limited proteolytic processing. Indeed, a role for IGFBP proteases in the regulation of IGF dependent physiological and pathophysiological processes has been reported.[11]

Here, we review the available data on the expression of the components of the IGF axis in normal liver. Based on this knowledge, alterations of the IGF axis during hepatocarcinogenesis are summarised and allow insights into the pathophysiological role of the IGF axis during this process.

The IGF axis in normal liver

IGF-I

Most of the circulating IGF-I and IGF-II is produced by the liver, although other tissues are capable of synthesising these peptides locally. Hepatic IGF-I production is principally regulated by growth hormone (GH), whereas the synthesis of IGF-II is relatively GH independent.[12] The GH/IGF-I axis is the major regulator of postnatal growth, whereas IGF-II appears to have an important role during fetal development. Recent studies showed that liver specific deletion of the IGF-I gene using the Cre/loxP recombination system reduced the serum IGF-I concentration by approximately 75% in mice.[13 14] However, the growth rates of these transgenic animals were not significantly different when compared with wild-type animals, suggesting a role of extrahepatic autocrine and/or paracrine IGF-I production in growth regulation. Within adult rat liver, IGF-I is mainly released from hepatocytes,[15–17] whereas the contribution of non-parenchymal cells to hepatic IGF-I production is less important.[18 19]

IGF-II

IGF-II expression is much higher during fetal development than in postnatal or adult life. A distinct activation pattern of four different promoters (P1–P4) correlates with IGF-II expression during development. Promoters P2, P3, and P4 are active in the fetal liver, whereas the

postnatal activities of P2–P4 decrease and P1 becomes dominant.[20] IGF-II mRNA expression was detected in hepatocytes as well as in non-parenchymal liver cells such as Kupffer cells (KCs), endothelial cells (ECs), and hepatic stellate cells (HSCs). A switch from the fetal to the adult IGF-II mRNA profile was observed in all the different types of liver cell between days 18 and 21 after birth.[21 22] In primary cultures of adult liver cells, IGF-II specific mRNA was only just detectable.[21 22]

IGFBPS

In addition to IGF-I, the liver of adult rats has been recognised as a major source of circulating IGFBPs and ALS.[8] Several studies have indicated the production of these proteins in different hepatic cell populations. Rat hepatocytes in primary cultures have been shown to secrete IGFBP-1, IGFBP-2, IGFBP-4, and ALS,[23–25] whereas IGFBP-3 expression was found in KCs, ECs, and HSCs only.[18 19 26–31]

Primary cultures of rat liver cells have been used for investigations regarding the mechanism of regulation of IGFBP and ALS production. These studies demonstrated that the regulation of these factors was under the control of several hormones including insulin.[8] In primary cultures of hepatocytes, insulin has been shown to be a strong inhibitor of IGFBP-1 and IGFBP-2 expression,[30 32 33] whereas that of ALS and IGFBP-4[30] was stimulated by insulin treatment. Although released from different cellular sources within rat liver, biosynthesis of the individual components of the 150 kDa complex was apparently controlled by the same regulatory mechanism. Maintenance of cocultures of hepatocytes with KCs in the presence of cAMP decreased the biosynthesis of IGFBP-3 (released from KCs) and ALS (released from hepatocytes) synergistically,[19] whereas insulin treatment resulted in a synergistic stimulation of the biosynthesis of these components.[30] This synergistic regulation might be facilitated by a cellular interaction between hepatocytes and KCs because a soluble factor secreted by hepatocytes was necessary for the stimulated IGFBP-3 biosynthesis in KCs in response to insulin.[27 30] However, at present the nature of this soluble factor is still unknown.

IGFBP PROTEASES

Conditioned medium from primary cultures of rat liver cells lacked neutral IGFBP protease activity, whereas the acidification of the medium resulted in the activation of IGFBP proteases, which could be classified by their pH optimum and protease inhibitor profile as acidic precursor forms of aspartyl and cysteine proteases, probably belonging to the family of cathepsins.[18 19] Under physiological conditions, it is unlikely that the cell mediated acidification of the extracellular environment[34] is sufficient to activate secreted procathepsin. However, the acidic protease activities detected in conditioned medium of liver cells might reflect the ability of acidic proteases localised in the endosomal, cathepsin containing recycling compartment[35] or in lysosomes to participate in IGFBP proteolysis. In fact, when cocultures of hepatocytes with KCs were incubated at pH 7.4 and 37°C in the presence of iodinated IGFBP-3, a time dependent disappearance of intact IGFBP-3 and the generation of several IGFBP-3 proteolytic fragments of different sizes was observed.[19] This indicated that, after endocytosis, a cathepsin mediated proteolysis of IGFBP-3 in intracellular organelles occurs, accompanied by partial recycling and release of IGFBP-3 fragments into the extracellular medium. The process of endocytosis and subsequent proteolytic degradation of IGFBP-3, as demonstrated in the coculture model with hepatocytes and KCs, might reflect the capacity of the liver to clear IGFBP-3 from the circulation. Because KCs of both human and rat liver have been reported as a source of hepatic IGFBP-3,[18 19 27–30 36] the role of different liver cell populations in the degradation of IGFBP-3 and the mechanisms of its uptake remain to be determined.

IGF-IR

In the liver, IGF/insulin mediated signal transduction may occur through binding to either the insulin receptor (IR) and/or the IGF-IR. Hepatocytes have not been thought to be a major target tissue for the actions of IGF-I because only very few high affinity binding sites for IGF-I have been demonstrated on adult rat hepatocytes.[37 38] Furthermore, both the short term and long term metabolic actions of IGF-I on primary cultures of rat hepatocytes have been attributed to low affinity IGF-I binding to the IR.[39] In contrast, expression of the IGF-IR has been detected in non-parenchymal cells, namely KCs,[22 40] ECs,[22 31] and HSCs,[22 30 41] rendering these liver cells susceptible to the mitogenic effects of IGFs. In fact, a functionally active IGF-IR has been demonstrated on hepatic stellate cells at days two and three of primary culture because proliferation of these non-parenchymal cells was stimulated significantly by the addition of IGF-I.[18 42]

IGF-II/M6PR

The cellular 240 kDa IGF-II/M6PR is membrane bound, but is also released in a soluble form into the culture medium[43] or serum[44 45] by truncation of its 20 kDa C-terminal cytoplasmic tail. All the different types of liver cell in primary culture have been shown to express the membrane bound IGF-II/M6PR,[22 30 41 46] and the liver of the adult rat was shown to be the tissue with the highest release of soluble IGF-II/M6PR.[47]

The IGF axis in hepatocarcinogenesis

IGF-I

In seven human hepatoma tissues, IGF-I mRNA expression was lower compared with the adjacent non-tumorous tissue.[48] This low IGF-I gene expression might be related to the diseased tissue itself; alternatively, it might be the result of reduced GH stimulation because GH receptor expression in the hepatoma tissue was also low.[48] A recent study on 53 patients with hepatocellular carcinoma (HCC) revealed significantly lower serum IGF-I concentrations

in both virus negative and virus positive HCC patients compared with patients with metastatic liver cancer and normal controls.[49] After controlling for the degree of liver damage (as assessed by prothrombin time and serum albumin values), the reduction of serum IGF-I concentrations in patients with HCC appeared to be largely independent of liver damage.

IGF-II

Evidence that the IGF axis is involved in hepatocarcinogenesis was deduced from early observations showing overexpression of the IGF-II gene in liver tumours and preneoplastic hepatic foci in different animal models of hepatocarcinogenesis,[50–56] as well as in human HCC.[57 58] The amount of IGF-II expression in the HCCs was highly variable. Overexpression of IGF-II associated with re-expression of the fetal pattern of IGF-II transcripts in HCC occurred through activation of the fetal promoters P2–P4[59] and loss of activity of the adult promoter P1.[60] Allelic expression of the IGF-II gene in the liver is unique in that its expression is monoallelic (maternally imprinted) during the fetal period, but it becomes biallelic thereafter. Increased expression of IGF-II associated with allelic imbalance was demonstrated in preneoplastic hepatic foci,[61] as well as in HCC.[62] In this latter study, an extreme allelic expression imbalance, leading to restoration of monoallelic IGF-II expression, was observed in 15 of 15 informative HCCs.

In patients with HCC, increased prevalence of hepatitis B and C virus (HBV, HCV) infection has been found. In both HBV and HCV induced HCC, a link with increased IGF-II expression was demonstrated.[21 63–66] In HCV positive liver cirrhosis, HCV replication was significantly associated with the overexpression of IGF-II.[67] The HBV X protein (HBx), which is thought to be the causative agent in the formation of HCC in HBV positive patients, was shown to increase endogeneous IGF-II expression from the fetal promoters P3 and P4 of the IGF-II gene. Further studies indicated that HBx positively regulated IGF-II transcription through the Sp1 binding sites of P4.[68] Aflatoxin B1 induced mutation of the p53 gene at codon 249 (p53mt249) is crucial during the formation of HCC after HBV infection. P53mt249 greatly increased IGF-II transcription, largely from P4, through formation of transcriptional complexes via enhanced DNA–protein (Sp1 or TATA box binding protein (TBP)) and protein–protein (Sp1 and TBP) interactions.[69]

A pathophysiological link between IGF-II overexpression and cell proliferation was demonstrated by Lin *et al*,[70] who found high concentrations of IGF-II in the human hepatoma cell lines HuH-7 and HepG2. Antisense oligonucleotides complementary to IGF-II mRNA reduced both IGF-II mRNA and protein, and this was associated with decreased cell proliferative activity in these cell lines.

IGFBPS

In a study on surgical specimens from patients with HCC, the expression of IGFBP-1, IGFBP-3, and IGFBP-4 was significantly downregulated in HCC tissue.[71] In a study of 11 human hepatoblastomas, the expression of IGFBP-1 was decreased in tumours compared with the corresponding normal liver from the same individual, with the exception of two cases.[72] In this study, IGFBP-2 was constantly reduced in hepatoblastoma tissue, although the size of the decrease varied. The authors suggested that because of reduced IGFBP expression in tumours an excess of biologically active IGFs might be available to potentiate the proliferative effects; however, only a few studies have investigated the role of IGFBPs in the modulation of the effects of IGF during hepatocarcinogenesis. In the human hepatoma cell line PLC, the mitogenic activity of exogenously added IGFs was reduced by the presence of IGFBPs 1–4.[73] This inhibitory effect was attributed to IGFBP-3, which was the only IGFBP in the conditioned medium of PLC cells to be stimulated by exogenous IGFs. Similarly, treatment of the human Hep 3B cell line with all *trans* retinoic acid (RA) revealed a decrease of IGFBP-3, and treatment with RA over six days resulted in a time dependent stimulation of the growth of Hep 3B cells.[74] Addition of recombinant human IGFBP-3 also inhibited the growth of the human hepatoma cell lines PLC/PRF/5 and HepG2.[74] In LMH chicken hepatoma cells, the effects of IGF-I on proliferation were inhibited by a soluble and membrane bound 28 kDa IGFBP.[75] A study by Kondoh *et al* suggested a role for IGFBP-1 in cell proliferation in the human hepatoma cell lines HuH-7 and HepG2.[76] Among other genes, expression of the IGFBP-1 gene was undetectable in rapidly proliferating hepatoma cells, whereas its expression was high in dense, overcrowded cultures.

IGFBP PROTEASES

Limited proteolysis of IGFBPs is believed to be the major mechanism for the release of IGFs from IGFBP–IGF complexes, generating fragments with reduced affinity for IGFs.[11] Therefore, enhanced IGFBP proteolytic activity is thought to contribute to carcinogenesis through increased IGF-IR stimulation as a result of the increase in bioavailable IGF. Although several different proteases have been detected in tissue from HCCs, the precise role of these proteases with respect to IGFBP proteolysis and hepatocarcinogenesis is still obscure. In conditioned medium from the human hepatoma cell line PLC, cathepsin D was identified as an acid activated IGFBP-3 protease by its pH optimum, protease inhibitor profile, and by immunodepletion with specific antisera.[73] Similar to rat liver cells in primary culture, cell associated proteolytic degradation of IGFBP-3 was seen in PLC cells at neutral pH, which was mediated by cathepsin D localised intracellularly in endosomal recycling compartments or in lysosomes. These data point to a role for cathepsin D in the regulation of IGFBP bioavailability via endocytosis in acidic prelysosomal compartments. Of interest, increased plasma cathepsin D concentrations have been

detected in patients with hepatocellular carcinoma.[77 78]

More evidence of a role for IGFBP proteolysis in hepatic tumour development was provided by a study of Martin *et al*,[79] which used a double transgenic murine hepatic tumour model overexpressing the SV40 T antigen (TAg) and tissue inhibitor of metalloproteinase 1 (TIMP-1). This study demonstrated that TIMP-1 expression blocked liver hyperplasia during tumour development, despite TAg mediated reactivation of IGF-II. IGFBP-3 degradation was lower in TIMP-1 overexpressing livers and, as a consequence of reduced IGFBP-3 proteolysis and raised IGFBP-3 protein concentrations, IGF-II values were significantly lower in the transgenic animals. This decrease in bioavailable IGF-II resulted in diminished IGF-IR signalling in vivo, as demonstrated by diminished receptor kinase activity and decreased tyrosine phosphorylation of the IGF-IR.

IGF-IR

IGF-IR specific mRNA was detectable in 10 of 10 human hepatoma cell lines[80] and the chicken hepatoma cell line LMH.[75] In the human hepatoma cell lines PLC[73] and HepG2,[81] as well as the rat hepatoma cell line H4IIE,[82] IGF-IR protein and/or mRNA were detected. In the PLC hepatoma cell model, both IGF-I and IGF-II stimulated [^{3}H]-thymidine incorporation in a dose dependent manner.[73]

Several lines of evidence have suggested that the HBx protein plays a role in the process of HBV associated liver carcinogenesis. Kim and colleagues[83] found significantly higher expression of the IGF-IR in the human hepatoma cell line SNU 368, which produces the HBx protein, than in SNU 387 cells, which lack the HBx protein. This study indicated that the HBx protein might play a role in the development of HCC through activation of IGF-IR gene expression.

IGF-II/M6PR

Because of its essential functions for the degradation of mitogenic IGF-II, the activation of the growth inhibitor TGF-β, and the transport of lysosomal proteases, the gene encoding IGF-II/M6PR has been considered to be a tumour suppressor gene.[5] Thus, in a variety of tumour cell lines, as well as in rat and human HCCs, the expression of the IGF-II/M6PR gene has been reported to be significantly reduced.[84 85] Furthermore, in approximately 70% of patients with HCC in the USA, loss of heterozygosity at the IGF-II/M6PR locus, with point mutations in the remaining allele, has been detected.[86–89] Several of these mutations have been shown to disrupt the ligand binding functions of the intact IGF-II/M6PR,[90 91] further supporting the hypothesis that IGF-II/M6PR is a tumour suppressor gene. These data are in contrast to a recently published study on human HCC in Japan,[92] which failed to detect genetic alterations of the IGF-II/M6PR gene, rendering the role of IGF-II/M6PR in hepatocarcinogenesis controversial. In line with these results, the IGF-II/M6PR gene was not found to be mutated in an albumin SV40 large T antigen transgenic model and a chemically induced model of hepatocarcinogenesis in the rat.[93]

MODELS OF HEPATOCARCINOGENESIS

In different species, and in various animal models of hepatocarcinogenesis, a sequence of characteristic preneoplastic hepatic foci leading to hepatocellular adenomas (HCA) and HCC have been reported.[94] So far, a comprehensive study on IGF, IGFBP, and IGF receptor expression in various stages of hepatocarcinogenesis has only been performed in two animal models, namely: (1) the woodchuck hepatitis virus (WHV) carrier woodchuck liver and (2) low number pancreatic islet transplantation into the livers of streptozotocin induced diabetic rats. Therefore, alterations of the IGF axis occurring in these two animal models will be described in more detail.

In the model of pancreatic islet transplantation into the livers of diabetic rats, a sequence of clear glycogen and fat storing foci over mixed cell foci and basophilic cell foci leading to the development of HCA and HCC has been observed.[95] Because these lesions were anatomically close to the transplanted islets of Langerhans, it is likely that the local hyperinsulinaemia (probably together with other peptides) was responsible for the demonstrated changes.[96 97] By a few days after islet transplantation, pronounced alterations of the IGF axis were detectable in glycogen storing foci; namely, increased expression of IGF-I and IGFBP-4 associated with decreased expression of IGFBP-1 (fig 1).[98] At this stage of hepatocarcinogenesis, IGF-II was barely detectable in both preneoplastic foci and non-altered acini. IGF-II expression in HCC was heterogeneous, with some tumours showing a strong IGF-II overexpression and some tumours being negative for IGF-II. Although IGF-IR expression was low in preneoplastic foci, strong IGF-IR expression was seen in HCCs, rendering tumour cells susceptible to the mitogenic effects of autocrine and paracrine IGFs (fig 1).[98] Because of the low IGF-IR expression in preneoplastic foci, it is likely that the enhanced cell proliferation seen in preneoplastic hepatic foci[96] is mediated by the IR, rather than the IGF-IR. Indeed, increased IR expression associated with an activated signal transduction cascade such as IRS-1, raf-1, and MEK-1 (mitogen activated protein kinase kinase) has been demonstrated in preneoplastic hepatic foci in this animal model (F Dombrowski *et al*, 1998, unpublished). However, despite the high proliferative activity, the growth rate of these preneoplastic hepatic foci was limited by the high apoptotic activity also seen in these foci.[96] During the development from preneoplastic hepatic foci to HCA and HCC the increase in mitotic activity was greater than the increase in the rate of apoptosis.[95] This shift towards a net increase in proliferation might be related to a decrease of IR and IRS-1, raf-1, and MEK-1 expression, and/or an increase of IGF-IR

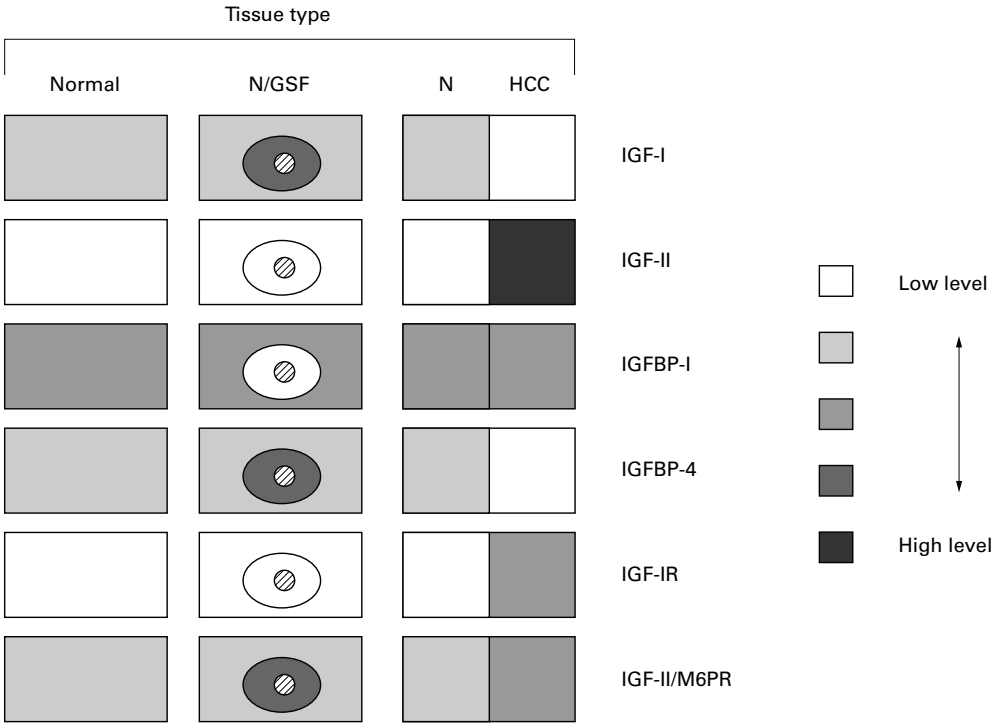

Figure 1 Expression of insulin-like growth factor I (IGF-I), IGF-II, IGF binding protein 1 (IGFBP-1), IGFBP-4, the IGF-I receptor (IGF-IR), and IGF-II/M6PR (mannose 6-phosphate receptor) in rat liver after low number pancreatic islet transplantation into the livers of streptozotocin induced diabetic rats. Schematic illustration demonstrating representative results from immunohistochemical analysis and in situ hybridisation of antisense mRNA to normal liver (normal; N), to glycogen storing foci (GSF; circular area within the square), and hepatocellular carcinoma (HCC) developing after pancreatic islet transplantation. The intensity of gene expression is directly proportional to the darkness of the section. The circular area (hatched) within the circle is a schematic representation of a transplanted pancreatic islet within the GSF.

expression during the progression to HCC in this model.

Studies investigating the IGF axis in the WHV model revealed an overexpression of IGF-II in over 90% of preneoplastic hepatic foci in precancerous woodchuck liver as well as in HCC derived from this model.[53 54] Furthermore, in situ hybridisation studies showed that IGFBP-1 and IGFBP-2 were downregulated in preneoplastic hepatic foci, as well as in HCC tumour tissue, compared with the adjacent normal liver, whereas IGFBP-4 was upregulated.[99] Although in the HCC model of pancreatic islet transplantation IGF-II overexpression was only detectable in HCC, but not in preneoplastic foci, the altered IGFBP expression in preneoplastic foci was comparable with that reported for the WHV model.[99] Despite the similarities of IGFBP expression observed in these two models, the biological actions of IGFBPs—whether they enhance or attenuate the effects of IGFs—cannot be predicted in an in vivo animal model. To define the role of IGFBPs during the process of hepatocarcinogenesis, the above described model of islet transplantation needs to be transferred to mice carrying a targeted disruption for one of these IGFBPs or vice versa to IGFBP transgenic mice.

In summary, we demonstrated pronounced alterations in the expression of components of the IGF axis during hepatocarcinogenesis. Prominent features detected in hepatoma cell lines, in animal models of hepatocarcinogenesis, and in HCC comprise the re-expression of the fetal pattern of IGF-II mRNA transcripts as well as the presence of the IGF-IR; these events have been identified as crucial to the process of malignant transformation and the growth of tumours. Alterations of local IGFBP production and the presence of IGFBP proteolytic activities, resulting in an excess of bioactive IGFs, may further potentiate the mitogenic effects of IGFs in the development of hepatocellular carcinoma. A pathophysiological role for the IGF-II degrading IGF-II/M6PR in hepatocarcinogenesis, either through downregulation or mutation of the IGF-II binding site, has not been uniformly detected in all animal models and HCCs studied so far.

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