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Role of Hepatoma-derived growth factor (HDGF) in Hepatocellular carcinoma

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Abstract

HDGF (Hepatoma-derived growth factor) is a novel growth factor that belongs to a new gene family. HDGF was originally identified as a growth stimulating factor, and HDGF plays a significant role in the proliferation of benign and malignant hepatic cells. The endogenous overexpression of HDGF significantly increases the proliferation and DNA synthesis in hepatoma cells *in vitro*. In addition, HDGF-overexpressing HepG2 cells form larger tumors in nude mice in comparison to the control counterparts, thus indicating that HDGF promotes the proliferation of hepatoma cells *in vitro* and *in vivo*. Furthermore, HDGF is highly expressed in the HCC (hepatocellular carcinoma) tissues, and the expression level

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of HDGF is an independent prognostic factor for the disease-free and overall survival in patients of HCC.

On the other hand, HDGF has been known as an angiogenic factor. HDGF stimulates the proliferation of human umbilical vein endothelial cells, and recombinant HDGF induces vessel formation *in vitro*. The overexpression of HDGF in NIH3T3 fibroblasts induced the expression of VEGF (vascular endothelial growth factor), a potent angiogenic factor. The transplantation of HDGF-overexpressing cells suggested that the growth promoting effects of HDGF *in vivo* depends on its angiogenic activity in addition to its growth stimulating effects on hepatoma cell. Treatments that inhibit tumor angiogenesis improve the prognosis of patients with advanced HCC, thus HDGF could be a target molecule in the treatment of HCC.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world, and it is also one of the most aggressive tumors with a poor prognosis. The tumor biology of HCC with rapid growth and metastasis depends on two remarkable characteristics. One is the rapid proliferation of cancer cells, and the other is the hypervascularity of the tumors with neovascularization (1). Tumor angiogenesis is required for the progression and invasion of solid tumors (2); thus, clarifying the molecular mechanisms that regulate hepatoma cell proliferation and tumor neovascularization would provide important knowledge for the management of hepatocellular carcinoma.

Hepatoma-derived growth factor (HDGF) is a novel factor that was identified in the hepatoma-derived cell line Huh-7 (3, 4). HDGF is a growth factor for hepatoma cells (5). Furthermore, HDGF participates in liver development and regeneration by promoting the growth of hepatic cells (6-10). Although HDGF was originally identified as a growth factor, HDGF is also an angiogenic factor (11), thus suggesting that HDGF is involved in the progression of HCC through both hepatoma cell proliferation and the neovascularization. This article describes the dual activity of this novel growth factor and its possible roles in hepatocellular carcinoma.

HDGF acts as both a growth stimulating factor and an langiogenic factor

HDGF is a 26 kDa heparin-binding acidic glycoprotein that was purified from the conditioned media of the human hepatoma-derived cell line, Huh-7 (3, 4). In addition, several groups found 4 additional novel genes, HDGF- Related Proteins (HRPs: HRP1- HRP4) (12-14). The N-terminal

region of HDGF contains about 100 amino acids that are highly conserved among the HRPs. This N- terminal region is referred to as the "HATH (homologous to the amino terminus of HDGF) region". In addition, lens epithelium-derived growth factor (LEDGF), which was first reported to function as a survival factor for lens epithelium, contains a HATH region in its N-terminal region and is regarded as a member of the HDGF family (15).

Although HDGF was originally isolated from the conditioned media of cultured hepatoma-derived cells, several studies have shown that HDGF plays important roles in organ development in the fetus and tissue repair in adults, including the liver, kidney, lung, and gut (6, 7, 16-18). In addition, Everett et al. demonstrated that HDGF is highly expressed in proliferating fetal vascular smooth muscle cells (SMCs) and endothelial cells. They also demonstrated HDGF expression is induced in vascular SMCs proximal to abdominal aorta constriction and in neointimal cells after endothelial injury, suggesting some functional roles of HDGF in the development and tissue repair of the cardiovascular system (11). Therefore, any examination of the role of HDGF should consider the dual actions of HDGF, as both a growth factor and an angiogenic factor.

Role of HDGF as a hepatocyte growth stimulating factor

HDGF and normal hepatocyte proliferation

HDGF is highly expressed in immature fetal hepatocytes, especially in the mid-gestation stage, and its expression decreases remarkably near birth (6). An *in vitro* model which recapitulates hepatocyte maturation demonstrated that HDGF expression in hepatocytes decreases with cellular differentiation, suggesting that HDGF is closely related to the proliferative activity of hepatocytes. Furthermore, the exogenous administration of recombinant HDGF enhances the proliferation of fetal hepatocytes, whereas a reduction of HDGF severely suppresses the proliferation of these cells. These findings suggest that HDGF is an important growth factor for the proliferation of fetal hepatocytes during liver development (6).

Although mature hepatocytes rarely replicate in their normal state, their proliferative capacity is observed in the regenerating liver, such as after hepatectomy or drug-induced hepatic injury (19, 20). Many growth factors have been reported to participate in the various steps of liver regeneration (19, 20), so the induction of HDGF expression was examined in the proliferative hepatocytes of the regenerating

liver (8). Both CCl_4-treated and hepatectomized livers show an induced expression of HDGF in hepatocytes, and a single peak is observed prior to the peak DNA synthesis in the regenerating liver. This indicates that the HDGF expression increases in parenchymal hepatocytes before DNA synthesis in the regenerating liver. These findings suggest that HDGF plays a significant role in the proliferation of both adult hepatocytes as well as fetal hepatocytes.

HDGF and hepatoma cell proliferation

HDGF was initially purified from the conditioned media of Huh-7 hepatoma cells and was observed to participate in the proliferation of both fetal and adult non-transformed hepatocytes (6, 8-10). However, the original purpose of this study was to find a novel growth factor which participates in the proliferation of hepatoma cells. Therefore, the role of HDGF in the proliferation of hepatic cancer cells was investigated (5, 21, 22).

The expression of HDGF was first examined in various hepatoma cell lines. As expected, HDGF is expressed in all hepatoma cell lines tested, including Huh-7, HepG2, Hep3B, PLC/PLF/5, SK-Hep1, and Mahlavu. In addition, the endogenous overexpression of HDGF significantly increases the proliferation and DNA synthesis in hepatoma cells (5), whereas antisense treatment targeting HDGF reduces the cellular proliferation (21). Furthermore, HDGF-overexpressing HepG2 hepatoma cells develop larger tumors in a xenograft model using nude mice in comparison to tumors derived from control cells (22). These *in vitro* and *in vivo* experimental studies strongly suggest that HDGF contributes to the progression of HCC by stimulating the growth of hepatoma cells.

The Fatty Liver Shionogi (FLS) mouse, which is an inbred mouse strain that spontaneously develops fatty change of the liver (23), was used to examine the role of HDGF in the development and progression of liver cancer. Ninety percent of FLS mice develop liver tumors at 72 weeks after birth, and these tumors are histologically diagnosed as hepatocellular adenoma and carcinoma. HDGF is more highly expressed in tumor tissue than in adjacent non-tumor tissue. Interestingly, the HDGF expression began to increase in the liver of FLS mice before the development of visible solid tumors, suggesting that HDGF functions as a growth stimulating factor at the early stage of hepatocarcinogenesis as well as at the progressive stage of HCC (24).

The expression of HDGF was further examined in human HCC tissue samples, to assess the relationship between the HDGF expression and clinicopathological features.

Patients with chronic liver disease show a higher HDGF expression in HCC tissue than in the adjacent tissue (24). Moreover, the expression level of HDGF is strongly associated with the prognosis of HCC after surgery and higher expression of HDGF led to poorer prognosis (25). Two other groups also reported that HCC patients with a higher HDGF expression showed an earlier recurrence and an unfavorable overall survival rate than those with lower expression levels of HDGF (26, 27). HDGF expression is an independent prognostic factor for the disease-free and overall survival in patients after curative resection of HCC. These findings suggest that HDGF plays a significant role in the progression of human HCC.

Transgenic mice that overexpressed HDGF in hepatocytes under the transcriptional control of the mouse albumin promoter/enhancer were generated to examine the effects on hepatocyte differentiation *in vivo* (28). The HDGF transgenic mice had no apparent morphological abnormalities in the liver. However, their gene expression patterns suggested that the maturational process of hepatocytes during the post-natal stage was partially inhibited. These observations suggest the HDGF expression to be important for sustaining the characteristics of immature cells, and it may also be involved in the increased proliferative activity of HCC cells.

Role of HDGF as an angiogenic factor

HDGF and angiogenesis

Although HDGF was originally identified as a growth stimulating factor, HDGF has also been shown to be involved in angiogenesis and vasculogenesis. Transplanted HDGF-overexpressing NIH3T3 cells develop large tumors in nude mice, and these tumors are macroscopically reddish and histologically abundant in vasculature (29). Everett et al. (11) demonstrated that that HDGF is highly expressed in the fetal cardiovascular system, and is induced in the regeneration of vascular vessels. HDGF stimulates the proliferation and migration of human pulmonary microvascular endothelial cells *in vitro*. In addition, recombinant HDGF promotes blood vessel formation in an experimental system using a chick chorioallantoic membrane. HDGF stimulates the proliferation of human umbilical vein endothelial cells and recombinant HDGF induces vessel formation *in vitro* (29). Interestingly, the overexpression of HDGF in NIH3T3 cells induces the expression of VEGF (vascular endothelial growth factor), a potent angiogenic factor. HDGF also stimulates the promoter activity of the VEGF gene, suggesting that HDGF promotes the transcription of the VEGF gene. Indeed, VEGF is highly induced in the tumors derived from HDGF-overexpressing NIH3T3 cells, and growth of the HDGF-

overexpressing tumors is partially suppressed by the administration of an anti-VEGF antibody (29). Therefore, apparently two factors seem to be associated with the angiogenic activity of HDGF, one is its direct effect on the proliferation of endothelial cells, while the other is the induction of VEGF.

HDGF-overexpressing HepG2 hepatoma cells develop larger tumors in comparison to control cells (22). However, the growth rate of tumors produced by the the transplantation of HDGF-overexpressing HepG2 cells in nude mice seems to be higher than that expected based on the proliferative activity of HDGF-overexpressing cells *in vitro*. In addition, HDGF-overexpressing NIH3T3 cells show only a slight transformation capacity in soft agar, whereas these cells develop large tumors in nude mice, thus indicating that HDGF-overexpressing cells had a more prominent growth stimulating activity *in vivo* than *in vitro* (29). DNA-chip analyses demonstrated an overexpression of HDGF to upregulate several genes involved in neovascularization, including PDGF-A and Tie-1(22). Therefore, the higher growth promoting effects of HDGF *in vivo* may depend on its angiogenic activity in addition to its growth stimulating effects on hepatoma cells, because HDGF-overexpressing tumors are rich in vasculature and plural angiogenic factors can be induced by HDGF.

Other possible role of HDGF in HCC

HDGF and hepatic cancer stem/progenitor cells

Recent studies suggest that cancer-initiating/stem cells are closely associated with the development, progression and recurrence of malignant diseases. Lee et al (30) reported that patients with HCC that had a gene expression pattern similar to oval cells (hepato-cholangio progenitor cells) showed a poor prognosis, suggesting that this subtype of HCC can be derived from hepatic progenitor/stem cells. HDGF is expressed in rat oval cells as well as in fetal immature hepatocytes. In addition, HDGF can stimulate the proliferation of a rat oval cell line Oc15-5, which was established from the liver of Long-Evans-Cinnamon rats (31), thus suggesting that HDGF has a growth stimulating effect on hepato-cholangio progenitor cells (in preparation). Three groups have shown an increased expression of HDGF to be associated with a poor prognosis for HCC patients (25-27). Although such a poor prognosis could mainly depend on the growth stimulating effects and angiogenic activity of HDGF, HDGF may promote the proliferation of hepatic progenitor/stem-derived cells, thus leading to an unfavorable prognosis. The functional role of HDGF in hepatic stem/progenitor cells is interesting and should therefore be clarified in future studies.

Conclusion

HDGF is a novel growth factor belonging to a new gene family. HDGF is both a growth stimulating factor and an angiogenic factor. The functional role of HDGF in the stromal cells including the induction of neovascularization is important as well as its growth stimulating effects on hepatic cancer cells. A novel treatment that inhibits tumor angiogenesis (represented by the sorafenib) improves the prognosis of patients with advanced HCC, and HDGF is therefore considered to be a potential target molecule for the treatment of HCC.

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Review Article

Anticarcinogenic impact of interferon therapy on the progression of hepatocellular carcinoma in patients with chronic viral infection

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Hepatocellular carcinoma (HCC) is mainly caused by a persistent infection due to the hepatitis B or hepatitis C virus. The number of HCC cases is increasing in Asian and African countries, as well as in European and American countries. Interferon (IFN) therapy, used for type B chronic liver diseases, inhibits hepatic carcinogenesis in patients with compensated cirrhosis. However, there is insufficient evidence that IFN therapy inhibits hepatic carcinogenesis in patients with chronic hepatitis B. There are few cases of HCC due to chronic hepatitis B, and long-term follow-up periods verifying the inhibitory effect of IFN on hepatic carcinogenesis have not been obtained. To improve the prognosis of type B chronic liver diseases, it is important that hepatitis treatment follows guidelines in which a patient's age and the extent of hepatic fibrosis are taken into account. As for chronic hepatitis C, since a sustained virological response (SVR) in IFN therapy inhibits hepatic carcinogenesis and improves prognosis, treatment that aims for an SVR while taking into consideration host-sided and virus-sided factors is recommended for patients with type C chronic liver diseases. In areas with low incidence of HCC (e.g. USA), a large number of cases and a long-term follow-up period are needed before it can be accepted that IFN therapy inhibits hepatic carcinogenesis. After locally curative treatment of HCC, IFN therapy suppresses recurrence and improves survival rates.

Key words: chronic hepatitis, hepatitis B virus, hepatitis C virus, hepatocellular carcinoma, interferon, prevention

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) ranks fifth in the number of patients worldwide who are diagnosed with cancer; its death toll ranks third.[1] Approximately 600 000 to 700 000 patients worldwide die of HCC each year; the number of HCC cases is increasing in Asian and African countries, as well as in European and American countries.[2,3] HCC is mainly derived from a persistent infection due to the hepatitis B virus (HBV) or hepatitis C virus (HCV); thus, treating viral hepatitis inhibits hepatic carcinogenesis. In clinical and epidemiological studies of patients with chronic hepatitis B, active replication of HBV is linked to progression to cirrhosis and HCC.[4] Cessation of HBV replication reduces complications and improves prognosis. If, as a result of interferon (IFN) therapy, seroclearance of hepatitis B e antigen (HBeAg) can be achieved and the patient is negative for HBV DNA, then this might reduce the chances of HCC developing.[5] IFN therapy for chronic hepatitis C helps to reduce the risk of HCC developing in patients in whom a sustained virological response (SVR) has been achieved and that therapy also helps to reduce the risk of HCC developing in patients in whom viral clearance has proven difficult.[6,7] This paper reviews clinical research studies that have focused on the inhibitory effect of IFN therapy on hepatic carcinogenesis.

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THE ANTITUMOR ACTION OF IFN

INTERFERON IS A cytokine with varied forms of bioactivity including antiviral action as well as action to inhibit cell growth, angiogenetic activity, action to regulate the immune response, and action to inhibit

telomerase activity. IFNs are generally grouped into type I IFNs, which include IFN-α, -β, and -ω, and type II IFN, which consists solely of IFN–γ.[8–10] IFN-α and -β are widely used clinically to treat viral diseases such as chronic hepatitis B and C and neoplastic diseases such as renal cell carcinoma and glioblastoma. Evidence of IFN's direct antitumor action has been reported, i.e. IFN-α and -β have been found to inhibit growth of a hepatoma cell line in a concentration-dependent manner.[11] In addition, IFN has been found to exhibit antitumor action by inducing apoptosis of tumor cells via p53 and by stopping the progression of the cell cycle.[12] Similarly, an *in vivo* study noted that an IFN dose similar to that used clinically suppressed the growth of hepatic carcinoma cells.[13] Moreover, alpha fetoprotein (AFP) levels decreased after administration of IFN to patients with chronic hepatitis C and consistently elevated AFP levels; the mechanism for this phenomenon may be antitumor action.[14] In addition, IFN is also assumed to have indirect antitumor action by immunopotentiation via natural killer cells.[15] Nevertheless, the current reality is that the mechanism of IFN's antitumor action has yet to be fully elucidated.

HBV-RELATED HCC

THERE ARE AN estimated 300 million or more HBV carriers in the world; many of them are concentrated in Asian and African areas.[1] About 15% of HCC cases in Japan are HBV-related.[16] The annual incidence of HCC in patients with type B chronic hepatitis is 0.1% to 1.0% and in patients with type B cirrhosis, 2.2% to 4.3%; the incidence of HCC is higher in Asia than elsewhere in the world.[17] A study of the natural history of HCC has reported that factors for a high risk of developing the condition are cirrhosis, being an elderly male, having genotype C or F1, having a double substitution (A1762T and G1764A) in the core promoter region, and high HBV DNA levels.[4] Since it is difficult to completely eliminate HBV, the primary goals of treatment are to eliminate or reduce HBV DNA in the blood and to normalize the levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT).[18]

We searched the medical published reports and found that the inhibitory effect of IFN therapy on hepatic carcinogenesis in patients with type B chronic hepatitis and cirrhosis was first reported in 1996; we also found four randomized controlled trials (RCT) – three from Europe and one from Asia (Table 1).[19–22] The paper from Asia indicated that IFN therapy inhibits hepatic carcinogenesis, but the papers from Europe did not have this

Table 1 Baseline characteristics of randomized control trials assessing effect of interferon (IFN) on hepatocellular carcinoma (HCC) development in hepatitis B virus (HBV)-infected patients

Study [reference]	Year	Sample size (*n*)	Male (%)	Mean age (years)	HBeAg positive (%)	Pre-existing cirrhosis (%)	IFN regimen	Mean follow-up (years)	VR (%)	Incidence of HCC (%)
Lampertico P *et al.*[21]	1997	T21	T80	T44	T0	T19	α-2b: 6 MU 3 times a week for 24 months	3.8	T28	T5
		C21	C90	C47	C0	C14			C0	C0
Krogsgaard K *et al.*[19]	1998	T210	81	36	T100	19	α-2a: 2.5–18 MU 3 times a week for 12–24 weeks	4.7	T24	T1
		C98			C100				C20	C1
Mazzella G *et al.*[20]	1999	T33	T76	T36	T100	T0	α: 5 MU/m^2 3 times a week for 24 weeks	7.1	T36	T3
		C31	C80	C40	C100	C0		6.6	C0	C6
Lin SM *et al.*[22]	1999	T67	T100	T32	T100	T10	α: 4–6 MU/m^2 3 times a week for 12 weeks	7.4	T42	T1.5
		C34	C100	C32	C100	C14		6.5	C24	C11.8

C, control group (no treatment); HBeAg, hepatitis B e antigen; MU, million units; T, IFN treated group; VR, virological response.

finding. In a study involving 308 patients with HBe antigen (HBeAg)-positive chronic hepatitis and cirrhosis, Krogsgaard *et al.*[19] administered a 2.5 to 10 million unit (MU)/m^2 dose of IFN-α2a three times weekly for 12 to 24 weeks to 210 patients (i.e. the treatment group). During a mean follow-up period of 4.7 years, HCC occurred in two patients in the treatment group and in one patient in the control group. In a study involving 64 patients with HBeAg-positive chronic hepatitis, Mazzella *et al.*[20] administered a 5 MU/m^2 dose of IFN-α three times weekly for 24 weeks to 33 patients (i.e. the treatment group). During a mean follow-up period of 7.1 years, HCC occurred in three patients whose chronic hepatitis had progressed to cirrhosis (one patient in the treatment group and two patients in the control group). In a study involving 42 patients with HBeAg-negative chronic hepatitis, Lampertico *et al.*[21] administered a 6 MU dose of IFN-α2b three times weekly for 96 weeks to 21 patients (i.e. the treatment group). During the mean follow-up period of 3.8 years, HCC occurred in one patient in the treatment group. In a study involving 101 patients with HBeAg-positive chronic hepatitis, Lin *et al.*[22] administered a 4–6 MU dose of IFN-α three times weekly for 12 weeks to 67 patients (i.e. the treatment group). During a mean follow-up period of 7 years, HCC occurred in one patient in the treatment group and in four patients in the control group ($P = 0.043$). They believed that IFN therapy had an inhibitory effect on hepatic carcinogenesis.

On the other hand, reports from a study involving patients with HBeAg-positive cirrhosis[23] and several studies involving patients with HBeAg-positive chronic hepatitis and HBeAg-negative chronic hepatitis[19,20,24,25] deny that IFN therapy inhibits hepatic carcinogenesis. Based on their meta-analysis of seven non-randomized controlled trial (NRCT) studies involving patients with cirrhosis,[20,23,26–30] Cammà *et al.*[31] believe that IFN therapy inhibits hepatic carcinogenesis (risk difference [RD], −6.4%; confidence interval [CI], −2.8 to −10; $P < 0.001$). However, their analyses of subgroups with small variations showed no significant differences and they found that IFN therapy did not inhibit hepatic carcinogenesis. Sung *et al.*[32] in a meta-analysis of 12 papers (which included an RCT study)[5,19,20,23–26,28–30,33,34] concluded that, compared with patients in the control group, patients in the IFN therapy group had a 34% reduced risk of developing HCC. This effect was especially beneficial for patients with cirrhosis. However, in a recent meta-analysis based on two RCT studies,[20,22] Zhang *et al.*[35] concluded that IFN therapy does not necessarily reduce the development of HCC.

At the current point in time, previous reports offer conflicting results with regard to whether or not IFN therapy suppresses hepatocarcinogenesis when used to treat hepatitis B virus-related chronic liver disease. Reasons for this conflict are presumably related to discrepancies in IFN's suppression of carcinogenesis brought about by differences in the clinical characteristics of the patients studied. In other words, numerous factors, such as: (i) patient age; (ii) sex; (iii) liver function tests; (iv) differences in the mode of infection (vertical or horizontal infection); (v) stage of liver fibrosis and grade of necroinflammatory activity; (vi) positivity or negativity for HBeAg; (vii) HBV genotype; (viii) levels of HBV DNA; (ix) treatment protocol; (x) therapeutic efficacy; and (xi) follow-up period, may affect study results. In a NRCT involving 313 patients with cirrhosis due to hepatitis B, Ikeda *et al.*[26] administered 6 MU IFN-α three times a week for 40 weeks to 94 patients in a treatment group (including 61 patients who were positive for HBeAg). A follow-up lasting an average of 7 years revealed 10 patients in the treatment group and 51 of 219 patients in an untreated group developed HCC; this finding indicated that use of IFN decreased the rate of carcinogenesis. In addition, Lin *et al.*[5] reported a case-control study matching for age, sex, HBeAg, ALT, and levels of HBV DNA. Their results revealed that five patients in a group receiving IFN therapy and 16 in an untreated group developed HCC ($P = 0.025$) in a mean follow-up of 6.8 years. A follow-up of 15 years indicated that the cumulative rate of hepatocarcinogenesis was significantly lower for patients who had cirrhosis and were receiving IFN therapy in comparison to the control group, but differences between the control group and patients who did not have cirrhosis and were receiving IFN therapy were not noted. Multivariate analysis indicated that independent risk factors for the progression of HCC were age, not having undergone IFN therapy, pre-existing cirrhosis, carrying HBeAg, and having the HBV genotype C (in comparison to genotype B). Based on previous studies, IFN therapy for patients with compensated cirrhosis B should be able to suppress hepatocarcinogenesis.[5,22,23,26,31,32,36] However, the inhibitory effect of IFN therapy on hepatic carcinogenesis for patients with type B chronic hepatitis has not yet gained a sufficient consensus. One reason is that there are few cases of hepatic carcinogenesis that develop from type B chronic hepatitis; thus, researchers cannot obtain either a sufficient number of cases or long-term follow-up periods to verify that IFN therapy inhibits hepatic carcinogenesis. An HBV carrier who has a high level of HBV DNA

rapidly progresses to cirrhosis, which is associated with a high rate of HCC.[37] In patients with HBeAg seroconversion and reduced levels of HBV DNA due to IFN therapy, the progression of cirrhosis slows and development of HCC is inhibited.[5] When serum transaminase returns to normal and HBV DNA falls below detection limits due to IFN therapy given to HBeAg-negative European patients, an improved prognosis is noted but IFN therapy has not been found to suppress hepatocarcinogenesis.[38] Miyake *et al.* reported that IFN therapy has been found to suppress hepatocarcinogenesis in Asians; they also reported that IFN has an effect in populations with a 10% or greater incidence of HCC that have not undergone IFN therapy and study populations with 70% or more subjects that are positive for HBeAg.[39]

Compared with the standard IFN, pegylated-IFN (PEG-IFN) has been reported as more effective in the elimination of HBeAg, reducing HBV DNA, and normalizing the serum ALT level.[36] However, there is no report on whether PEG-IFN (in comparison with the standard IFN and nucleos(t)ide analogs such as lamivudine) more greatly reduces the risk of developing HCC. Future research is needed.

In addition, IFN for type B cirrhosis is not price-listed in Japan, and the IFN administration period for type B chronic hepatitis is 6 months. Price-listing of IFN for type B cirrhosis, the extension of the administration period, and the approval of using PEG-IFN are pending.

HCV-RELATED HCC

THE HCV WOULD not be naturally eliminated when an infection is passed to humans. About 70% of persistently infected people become carriers and necroinflammatory reactions continue; as a result, hepatic fibrosis progresses to cirrhosis.[40] However, hepatic fibrosis progression rates in persistently HCV-infected people differ significantly among individuals and are influenced by the person's age when infected, the amount of alcohol intake, gender, and the extent of liver function abnormality. It has been demonstrated that, in people who have insulin resistance and fatty livers, the hepatic fibrosis progression rate is rapid and the sustained virological response (SVR) ratio in IFN therapy is reduced.[41,42] HCC incidence rates increase in relation to the progression of hepatic fibrosis.[43] The annual incidence of HCC from type C compensated cirrhosis is reportedly 7.1% in Japan and 3.7% in both Europe and America; and the annual incidence of HCC from chronic hepatitis is 1.8% in Japan and 0% in both Europe and America.[17] When such natural courses are taken into account, the treatment goals for type C chronic hepatitis are to prevent the progression to cirrhosis and to inhibit hepatic carcinogenesis.

In 1995, we examined (using an RCT) the inhibitory effect of IFN therapy on hepatic carcinogenesis for type C cirrhosis.[44,45] Ninety patients with type C cirrhosis were divided into two groups: the IFN treatment group and the untreated group. We examined the long-term clinical effects of IFN therapy. In the IFN treatment group, an SVR occurred in seven patients and a biological response (BR) occurred in six patients. In the untreated group, the spontaneous disappearance of the HCV and sustained normalization of ALT level did not occur. During the mean follow-up period of 8.2 years, the cumulative incidence rate of HCC was significantly lower in the IFN treatment group than in the untreated group (27% vs. 73%, respectively) ($P = 0.001$). The relative risk (RR) was 0.256. A multicenter Japanese study – the Inhibition of Hepatocarcinogenesis by Interferon Therapy (IHIT) study – showed that, compared with the untreated group, the risk of hepatic carcinogenesis was inhibited by 0.51-fold in the IFN treatment group; the RR of hepatic carcinogenesis was 0.197 in patients who achieved SVR with IFN therapy.[46] To our knowledge, seven RCT papers have been published since 1995 that investigated the inhibitory effect of IFN therapy on hepatic carcinogenesis (Table 2).[44,47–52] In a study involving 99 patients with compensated cirrhosis, Valla *et al.*[47] administered a 3 MU dose of IFN-α2b three times weekly for 48 weeks to 52 patients (i.e. the treatment group). A mean follow-up period of 3.3 years showed that HCC occurred in five patients in the treatment group and in nine patients in the control group; however, there was no statistically significant difference between the groups. On the other hand, the results of a meta-analysis by Cammà *et al.*[31] confirmed that IFN therapy inhibits hepatic carcinogenesis in patients with type C cirrhosis. Their investigation of 3109 patients in three RCT studies[44,47,53] and 11 NRCT studies[28,30,46,54–61] showed that the risk of developing HCC in the IFN treatment group was reduced by 12.8% (95% CI, −8.3% to −17.2%), compared with the risk in the untreated group. They reported that, especially in patients who obtained a SVR, there was a marked inhibition of hepatic carcinogenesis (as indicated by an RD of −19.1%). Even in people who did not have a SVR, the RD was significantly reduced (at −11.8%). Miyake *et al.*[62] reported that hepatic carcinogenesis was inhibited in the IFN-treated group, compared with the untreated group (RR, 0.45; 95% CI, 0.31–0.65), based on their meta-analysis of three RCT studies[47–49] and six

Table 2 Baseline characteristics of randomized control trials assessing effect of interferon (IFN) on hepatocellular carcinoma (HCC) development in hepatitis C virus (HCV)-infected patients

Study [reference]	Year	Sample size (*n*)	Male (%)	Mean age (years)	Pre-existing cirrhosis (%)	IFN regimen	Mean follow-up (years)	SVR (%)	Incidence of HCC (%)
Nishiguchi S *et al.*[44]	1995	T45	T62	T55	T100	α: 6 MU 3 times a week for 24 weeks	T4.4	T16	T4
		C45	C51	C57	C100		C5.5	C0	C38
Valla DC *et al.*[47]	1999	T45	T73	T57	T100	α-2b: 3 MU 3 times a week for 48 weeks	3.3	NA	T11
		C49	C65	C56	C100				C18
Bernardinello E *et al.*[48]	1999	T38	T50	T56	T100	β: 6 MU 3 times a week for 24 weeks followed by 3 MIU for another 24 weeks	5	T3	T5
		C23	C61	C58	C100			C0	C4
Francesco A *et al.*[50]	2004	T30	T57	T55	T100	α-2b: 6 MU daily for 1 month followed by 3 MIU daily for 11 months plus ribavirin 1 g daily for 12 months	5	T43	T0
		C30	C60	C57	C100			C0	C30
Soga K *et al.*[49]	2005	T103	T49	T52	T0	α, α-2a or α-2b: 3–10 MU daily for 2–4 weeks and 3 times a week for total of 14–28 weeks or β; 3–6 MU daily for 6–8 weeks	7.8	T32	T5
		C30	C43	C54	C0			C0	C23
Fartoux L *et al.*[51]	2007	T51	T45	T60.5	T100	α-2a: 3 MU 3 times a week for 2 years	2	T0	T12
		C51	C45	C60.5	C100			C0	C12
Lok AS *et al.*[52]	2009	T495	T71	T50	T40	PEG-IFN α-2a: 90 μg weekly for 3.5 years	T4.6	T0	T4.6
		C510	T79	C53	C41		C4.9	C0	T4.9

C, control group (no treatment); MU, million units; NA, not available; PEG-IFN, pegylated interferon; SVR, sustained virological response; T, IFN treated group.

NRCT studies[50,55,58,63–65] published between 1989 and 2009.

The inhibitory effect of IFN is furthermore demonstrated in non-responders (NRs) to IFN therapy (RR, 0.48; 95% CI, 0.26–0.66). Zhang *et al.*[35] recently performed a meta-analysis on the effect of non-maintenance IFN therapy on hepatic carcinogenesis. They used only four RCT papers (in three papers, the subjects were patients with type C cirrhosis).[45,47–49] The results indicated that IFN therapy inhibited hepatic carcinogenesis in the IFN treatment group, compared with the untreated group (RR, 0.39; 95% CI, 0.26–0.59). The results of IFN therapy, when focusing only on patients with cirrhosis, also showed the same inhibitory effect (RR, 0.44; 95% CI, 0.28–0.68). In one study, patients who were initially NR to IFN therapy were divided into two groups: a maintenance IFN treatment group and an untreated group.[51,52] An analysis of the results showed that IFN therapy has no inhibitory effect on hepatic carcinogenesis.

In the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) Trial,[52] 1005 patients had cirrhosis and had chronic hepatitis that progressed to fibrosis (i.e. bridging fibrosis). Of these, patients who were unresponsive to a combination therapy with PEG-IFN and ribavirin (RBV) were divided into two groups – the maintenance treatment group (PEG-IFN α-2a, 90 μg) and the untreated group. The incidence of HCC in each group was investigated. During a mean follow-up period of 4.6 years, there was no difference between the two groups in the incidence of HCC. In a continuation of the HALT-C report,[7] the mean follow-up period was extended to 6.1 years. The results showed that IFN therapy inhibited hepatic carcinogenesis in patients with cirrhosis in the maintenance IFN treatment group, compared with its inhibitory effect in the untreated group (HR, 0.45; 95% CI, 0.24–0.83). On the negative side, maintenance IFN therapy insufficiently inhibited hepatic carcinogenesis in patients with chronic hepatitis that had progressed to fibrosis. However, the incidence of HCC was reduced in these patients if their liver had a histological improvement with IFN therapy.

One report shows that maintenance IFN therapy reduces the incidence of HCC in elderly patients with chronic hepatitis, compared with patients in the untreated group.[66] Kumada *et al.* state that the administration of IFN therapy is important in normalizing serum ALT level or reducing the AFP level, even if HCV does not disappear.[6] For non-SVR patients receiving IFN therapy, a patient's age is an important risk factor for hepatic carcinogenesis, and the annual incidence of HCC in patients with chronic hepatitis and hepatic fibrosis is significantly higher in aged people than in young people.[67] It can be accordingly conjectured that the reason for a higher complication rate of HCC among HCV carriers in Japan than among HCV carriers in the USA is that Japanese carriers have a higher mean age and a higher extent of hepatic fibrosis.[68] These factors may be responsible for the difference in the inhibitory effect of IFN therapy on hepatic carcinogenesis noted between patients in Japan and patients in the USA.

Based on past studies investigating the inhibitory effect of IFN therapy on hepatic carcinogenesis in patients with type C chronic liver diseases, a consensus has been reached concerning the following three points:

- Point 1: Patients achieving SVR with IFN therapy have a reduced HCC incidence rate and an improved HCC prognosis.[46,69,70]
- Point 2: Because of the use of combination therapy (e.g. PEG-IFN and RBV) in recent years, the treatment outcome has improved and the SVR ratio is about 50% to 80%.[71] However, for patients with chronic hepatitis that has progressed to fibrosis and for patients with compensated cirrhosis, IFN therapy alone reduces the SVR ratio and reduces the incidence of complications associated with the liver (including hepatic carcinogenesis).[69]
- Point 3: IFN therapy reduces the incidence rate of HCC when a BR is achieved or when there is a histological improvement.[66,72]

Further examinations are needed to determine whether maintenance IFN therapy inhibits hepatic carcinogenesis and whether there is any difference between IFN therapy NR patients and IFN-untreated patients in the rate of hepatic carcinogenesis. Studies are needed that take into account the amount of IFN administered, the administration period, and the use of RBV and novel concurrent drugs. In addition, we expect that combination therapy with PEG-IFN and RBV for patients with compensated cirrhosis will be promptly price-listed in Japan.

RECURRENCE INHIBITION AFTER LOCALLY CURATIVE TREATMENT FOR HCC

EVEN IF LOCALLY curative treatment for HCC is performed, HCC relapses occur at an annual rate of 15% to 20%. This high rate is not caused by any other malignant neoplasms, and it results in a high mortality.[73] To improve the prognosis of patients with HCC, measures are needed that advance HCC treatment and inhibit recurrence.

Basic investigations reveal that IFN has anti-viral activity and it inhibits the growth of HCC.[74,75] In a retrospective examination, Someya *et al.* reported that singlevariate and multivariate analyses showed that IFN therapy inhibits recurrence in patients with HCC-complicated type B cirrhosis after locally curative treatment.[76] Lo *et al.*[77] performed an RCT, using as subjects 40 patients who had undergone a radical hepatic resection because of HBV-related HCC. On examining the IFN treatment group (in which patients were administered 10 MU/m^2 of IFN-α2b three times weekly for 12 weeks) and the untreated group, they found that the one-year and 5-year survival rates were 97% and 79%, respectively, in the IFN treatment group and 85% and 61%, respectively, in the untreated group. Therefore, the IFN treatment group had a better prognosis ($P = 0.137$). A multivariate analysis demonstrated that IFN therapy may reduce the risk of death (HR, 0.42; 95% CI, 0.17–1.05; $P = 0.063$). In the examination of subgroups, there was no difference between the IFN treatment group and the untreated group in the 5-year survival rate in patients at stage I/II; however, in patients at stage III/IV A, IFN therapy inhibited the early recurrence of HCC and improved the 5-year survival rate from 24% to 68% ($P = 0.038$). Sun *et al.*[78] in their RCT also reported that IFN therapy was useful after an operation for HCC and that the median overall survival time and median disease-free time were significantly longer in treated patients, compared with the untreated patients.

We found six RCT studies that examined the inhibitory effect of IFN therapy on recurrence after locally curative treatment for HCV-related HCC.[79–84] Ikeda *et al.*[79] and Kubo *et al.*[80] showed that IFN therapy significantly inhibits the recurrence of HCC. Shiratori *et al.*[81] reported no difference between the IFN-treated group and the control group with the first relapse of HCC, but noted that IFN therapy inhibits a second or later recurrence of HCC. Only Mazzaferro *et al.*[83] reported that IFN therapy shows no significant difference between the IFN treatment group and the control group; however, at the first relapse of HCC, IFN therapy inhibits recurrence in patients having a single tumor that is free from vascular invasion and has a diameter of less than 3 cm. An examination of NRCT, which were performed in Japan, also showed that IFN therapy significantly inhibits the relapse of HCC (especially in patients who receive IFN treatment aimed at eliminating HCV), achieves an SVR,[85–87] and improves survival rates.[88] Maintenance IFN therapy after the locally curative treatment of HCC reportedly inhibits recurrence.[85,89,90] Kudo *et al.*[89] reported that IFN therapy inhibits the first relapse (as well as a second or third relapse) and improves the prognosis. We also demonstrated that long-term maintenance IFN therapy, given after the combination therapy with PEG-IFN and RBV, effectively inhibits HCC recurrence and improves prognosis.[91] Singal *et al.*[92] performed a meta-analysis of five RCT papers[79,81–83,93] and five NRCT papers.[87,89,94–96] They reported that IFN therapy inhibits HCC recurrence (odds ratio [OR], 0.31; 95% CI, 0.26; $P < 0.0001$) and significantly extends the overall survival time. Furthermore, Zhang *et al.*[97] conducted a meta-analysis of six RCT papers (Two papers focused on HBV-related HCC and four papers focused on HCV-related HCC).[77,78,80–83,93] Their meta-analysis showed that IFN therapy inhibits early recurrence (OR, 0.62; 95% CI, 0.42–0.93; $P = 0.02$) and improves the one-year survival rate (OR = 3.14; 95% CI = 1.79–5.52; P (00.0001). Shen *et al.*[98] similarly performed a meta-analysis of 13 papers on HBV-related and HCV-related HCC (nine papers involved RCT[77–84,93], and four papers involved NRCT[87,89,94,99]). From this, they concluded that IFN therapy improved the one-year, 2-year, and 3-year recurrence-free survival rates in the IFN treatment group, compared with the control group.

Based on past studies investigating the inhibitory effect of IFN therapy on HCC recurrence after locally curative treatment, HCC recurrence is reduced through HCV clearance. Thus, IFN therapy for viral eradication is recommended for patients with hepatitis C if possible. Meanwhile, in patients with hepatitis B, IFN therapy after locally curative treatment may improve their prognosis. Further examinations are needed to determine whether IFN therapy after locally curative treatment reduces HCC recurrence in patients with hepatitis B.

FINAL COMMENTS

FOR PATIENTS WITH chronic hepatitis B, IFN therapy reduces the risk of hepatic events (including the inhibitory effect for developing HCC) particularly among responders to treatment in Asian, but not in European patients. The progression to cirrhosis and a high level of HBV DNA (greater than 10^5 copies/mL) are strong risk factors for hepatic carcinogenesis from type B chronic liver diseases.[37] Liaw *et al.*[100] reported that therapy with lamivudine, a nucleos(t)ide analog, significantly reduces the progression to non-compensated cirrhosis and inhibits the development of HCC. Matsumoto *et al.*[101] also had similar results in a multicenter study of Japanese patients with type B chronic hepatitis. As for inhibition of hepatic carcinogenesis from type B chronic liver diseases, measures for hepatitis

are important, after taking age, amount of HBV DNA, extent of background liver disorder, HBV genotype, and others into account, according to guidelines, use of IFN or nucleos(t)ide analogs needs to be determined.[18,37]

It is important that IFN-based therapy obtains SVR to inhibit the development of hepatic carcinogenesis from type C chronic liver diseases. Thus, IFN therapy is recommended for patients with chronic hepatitis C. Tanaka N *et al.*[102] reported single nucleotide polymorphisms (SNPs) in the IL28B locus. These polymorphisms are extremely effective for estimating the effects of IFN therapy; they provide a novel indicator to help determine a patient's therapy, and will be used clinically.[103] New anti-viral drugs are being developed for treating type C chronic hepatitis. Combination therapy using PEG-IFN, RBV, and a protease inhibitor reportedly improves the SVR rate.[68] In addition, the acyclic retinoid, studied and developed in Japan, is expected to show a strong inhibitory effect on hepatic carcinogenesis.[104]

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