background factors between the two groups showed that there were fewer female patients and that the serum albumin level was higher in the IFN group. On the other hand, the AFP level was higher and the Japanese TNM stage was advanced in many of the non-IFN group patients (Table 1). The median follow-up period was 37 and 31 months in the IFN and non-IFN groups, respectively.

Matching was performed using the propensity score to adjust for the background factors. The ten factors, as described above, were adopted for the covariates. Twenty patients were selected from each of the IFN and non-IFN groups through this matching. The *C*-statistic was 0.756. There were no significant differences between the matched patient groups in any of the host, tumor, or viral side background factors (Table 2). The 5-year cumulative survival rates were 100 and 76.9% in the IFN and non-IFN groups, respectively. This difference was not significant ($P = 0.075$) although the rate was higher in the IFN group (Fig. 1a).

HCC recurred after RFA in eight patients in the IFN group and in 12 patients in the non-IFN group. The 1- and 3-year cumulative recurrence rates were 5.0 and 38.0%, respectively, in the IFN group, and 22.2 and 64.2%, respectively, in the non-IFN group. The lower rates in the IFN group were statistically significant ($P = 0.019$) (Fig. 1b).

The patients were classified as "IFN responders" or as "Others". IFN responders consisted of 3 SVR patients and 11 patients in whom the serum ALT level had normalized at 30 IU/mL or lower on IFN therapy (14 patients in total). The "Others" group consisted of 26 patients. The cumulative recurrence rate was analyzed in the groups. The 1- and 3-year cumulative recurrence rates were 0 and 29.3%, respectively, in the IFN responders group and 20.7 and 63.7%, respectively, in the "Others" group. The lower rates in the IFN responders were statistically significant ($P = 0.001$; Fig. 2). The hazards ratio for recurrence in the IFN responders, based on the Cox proportional hazards model, was 0.158 (95% confidence interval = 0.045-0.561, $P = 0.004$). Local tumor progression was noted in three patients from the "Others" group, and in one patient of the IFN responders ($P = 0.45$; Fig. 3a). The cumulative ectopic recurrence rate was significantly lower in the IFN responders than in the "Others" group ($P = 0.008$; Fig. 3b).

Changes in the serum albumin level were also investigated. The difference between the level immediately before RFA and that measured for data analysis were compared between the two groups. The median duration of the measurement period was 21 and 17 months in the IFN responders and "Others" group, respectively. This difference was not statistically significant ($P = 0.08$). The serum albumin level was retained in the IFN responders, but decreased in the "Others" group ($P = 0.001$; Fig. 4).

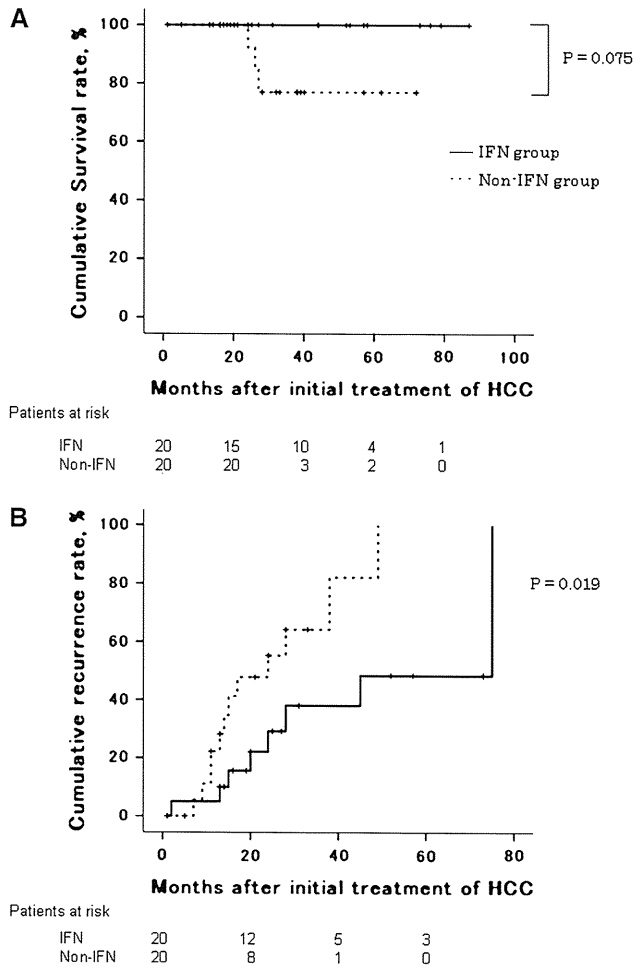

Fig. 1 **a** Cumulative survival rates after curative RFA treatment of matched patients with HCC. The cumulative rates were higher in the IFN group than in the non-IFN group ($P = 0.075$). **b** Cumulative recurrence rates after RFA treatment of matched patients with HCC. The recurrence rate of the IFN group was significantly lower than that of the non-IFN group ($P = 0.019$)

Discussion

The mechanism of HCV-associated carcinogenesis has been actively investigated, but it has not been fully elucidated [21]. Therapy for HCC has had marked advancements in recent years. However, this has not sufficiently increased the long-term survival rate. The annual recurrence rate of HCC is as high as 10–25%, even after curative treatment [22, 23]. In many patients, the background liver disease is hepatic cirrhosis, which gradually progresses to liver failure. Advances in treatment methods and determining how to inhibit recurrence are important in improving the prognosis of HCC. The usefulness of IFN therapy as a primary prevention of chronic hepatitis C and hepatic cirrhosis is well-recognized in Japan. IFN therapy apparently inhibits carcinogenesis in patients who achieve SVR [5–9]. We previously reported IFN's carcinogenesis-inhibitory effect

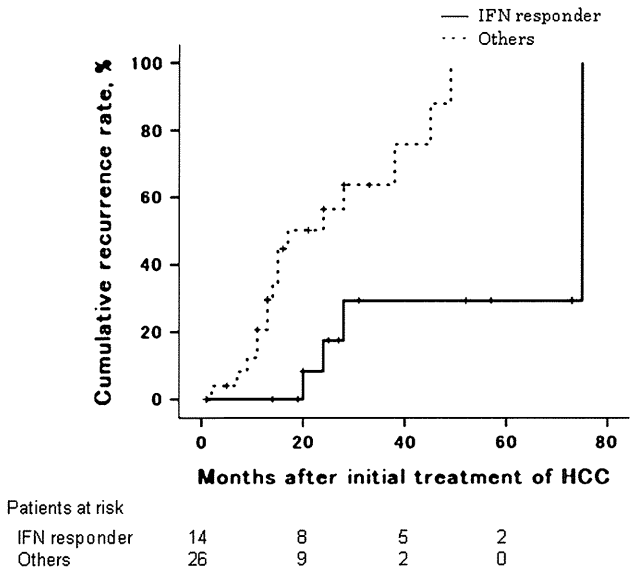

Fig. 2 Cumulative recurrence rates according to efficacy of IFN therapy after curative RFA treatment of matched patients with HCC. The rate of cumulative recurrence of HCC in the IFN responder was significantly lower than the "Others" ($P = 0.001$)

on HCV-induced hepatic cirrhosis [5, 6]. Many clinical studies, mainly in Japan, have confirmed this effect [7–9]. In this study, we performed matching using the propensity score since we noted biases in the background factors between the IFN and non-IFN groups. This method is used in many fields as a covariate adjustment method and modifies the dependent variables in observational studies in which randomized allocation is difficult [24, 25]. The *C*-statistic was 0.756, showing a favorable matching.

In the matched groups, the cumulative survival rate was higher in the IFN group while the cumulative recurrence rate in this group was significantly lower. IFN exhibited an anti-viral effect, in addition to inhibiting liver cancer cell growth, in a basic study [26]. It also has a clinical anti-tumor effect on HCC [27, 28]. Ikeda et al. [10] and Kubo et al. [11] reported the efficacy of IFN-β and IFN-α, respectively, in inhibiting carcinogenesis after the curative treatment of HCC. Shiratori et al. [12] noted that IFN does not inhibit the initial recurrence of HCC, but it does inhibit subsequent recurrences. We performed long-term IFN monotherapy and prolonged IFN therapy by adding IFN monotherapy after PEG-IFN/RBV combination therapy. This suggests that IFN therapy is useful as a secondary prevention of carcinogenesis. However, the number of patients we used was small. Previous basic study has shown that continuous IFN administration induces anti-tumor effects [29]. Kudo et al. [15] also performed maintenance IFN therapy for HCC after RFA. In their study, the anti-tumor and the carcinogenesis-inhibitory effect of IFN therapy inhibited HCC recurrence and improved treatment outcomes.

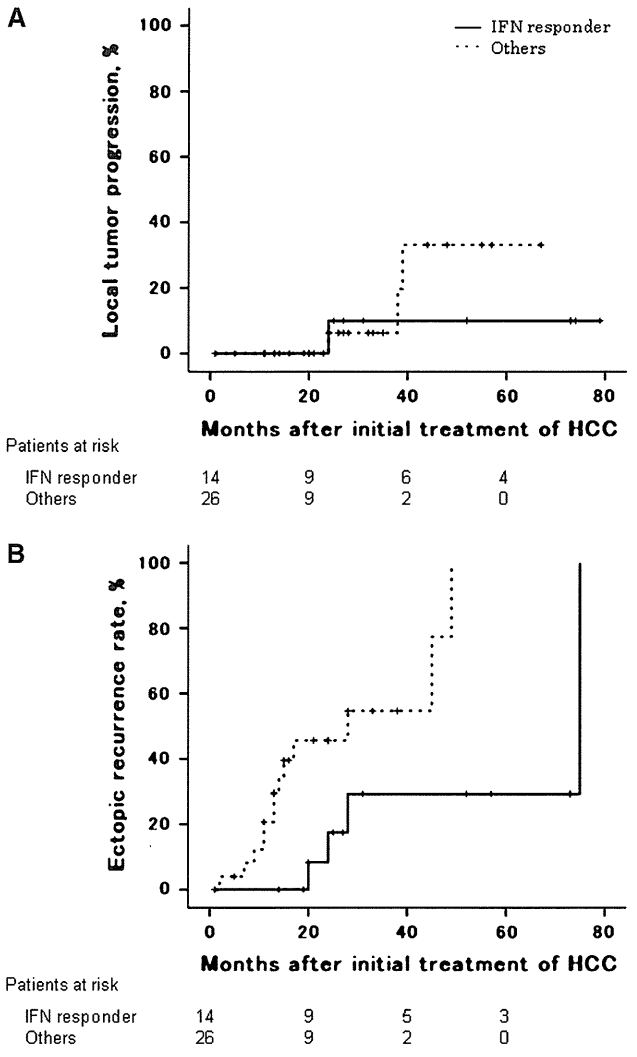

Fig. 3 Cumulative recurence rates after RFA treatment of matched patients with HCC. **a** Rates of local tumor progression was lower than that of the "Others", but the difference was not statistically significant ($P = 0.45$). **b** Rates of ectopic recurrence of the IFN responder was significantly lower than that of the "Others" ($P = 0.002$)

An analysis of the IFN responder patients and the "Others" groups shows that the IFN-induced reduction of the serum ALT level to 30 IU/mL or lower may be important for inhibiting intrahepatic recurrence. Yoshida et al. [7] performed IFN therapy in patients with chronic type C hepatitis and observed a carcinogenesis-inhibitory effect in biological responders. This was similar to the effect in the SVR patients. Wang et al. [30] reported that high-dose and long-term therapy with IFN-α inhibited intrahepatic tumor recurrence and lung metastasis in nude mice after curative resection. Uenishi et al. [31] performed IFN therapy after surgery for HCC and observed a recurrence-inhibitory effect in patients in whom the serum ALT level normalized, regardless of disappearance of serum HCV RNA. We also observed a low ectopic recurrence rate in the IFN responders. Recent studies of meta-analysis

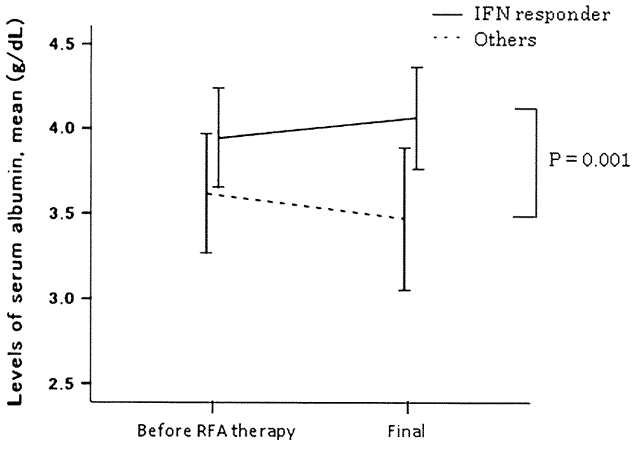

Fig. 4 Effect of IFN therapy after curative RFA treatment of matched patients with HCC on the levels of serum albumn. The *bars* indicate mean ± 1 SD. Serum albumin in the IFN responders was significantly better preserved than the "Others" ($P = 0.001$)

have shown that IFN-α treatment could significantly decrease early recurrence, so-called intrahepatic metastasis, and improved 1-year survival of patients with HCC after complete resection or ablation [32, 33]. This study suggests that IFN therapy is effective for the suppression of intrahepatic metastasis of HCC. This effect of IFN might be related with the direct suppression of tumor growth on an already-existing undetectable malignant lesion. However, mechanisms of IFN's effect on recurrence were very complex so that no single study could explain them fully.

Jeong et al. [14] reported maintenance of the Child-Pugh score in patients in whom IFN therapy achieved SVR after curative treatment of HCV-associated HCC. This study suggested that the improvement in and the maintenance of the serum albumin level (an important index of liver function) contributed to improved treatment outcomes. In other words, the recurrence is inhibited and liver function is improved in the IFN responders after HCC treatment. This indicates that curative treatment can be performed, even if recurrence occurs. The statistical method used to adjust for the background factors indicated that long-term IFN administration for HCC after RFA inhibited recurrence and improved the treatment outcome—particularly in the IFN responders.

There are several problems with IFN therapy. It is not applicable to patients with a poor liver function. Diverse adverse effects appear with the therapy such as thrombocytopenia. These problems need to be considered in reaching a conclusion concerning IFN administration to prevent recurrence after the curative treatment of HCC. A large-scale prospective study is needed that covers the type and dose of IFN, use of concomitant drugs (such as ribavirin), and the duration of IFN administration.

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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 hepatecmized 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.

Acknowledgements

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The role of hepatoma-derived growth factor (HDGF) in cancer development and progression

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Abstract

HDGF (hepatoma-derived growth factor) is a novel growth factor that belongs to a new gene family. Although HDGF was originally identified from the conditioned medium of a hepatoma cell line, HDGF is suggested to promote the proliferation of various kinds of cell via two different mechanisms: a receptor-mediated pathway followed by the activation of MAP kinase (MAPK) signaling, and direct action through its DNA binding after nuclear translocation. HDGF is a unique factor which has multiple functional characteristics, such as anti-apoptotic activity and angiogenic activity, as well its growth stimulating activities. Recent studies have shown that HDGF is considered

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to play significant roles in the development and progression of malignant diseases through these various activities. Furthermore, the expression level of HDGF is an independent prognostic factor for the disease-free and overall survival of various kinds of malignant disease, including hepatocellular carcinoma, pancreatic cancer, cholangiocarcinoma, esophageal cancer, gastric cancer, colorectal cancer, gastrointestinal stromal tumor, and some non-gastroenterological cancers. Therefore, HDGF is suggested to be involved in the development and progression of cancer in various organs.

Introduction

Cancer development and progression consist of many complicated processes (1, 2). One major event is an unlimited cellular proliferation associated with the escape of malignant cells from apoptotic cellular death. In addition, mutual reactions between cancer cells and surrounding stromal cells are also important, and tumor angiogenesis is considered to be essential for the progression and invasion of solid tumors. Therefore, clarifying the molecular systems involved in the anti-apoptotic/ unregulated cancer cell proliferation and tumor neovascularization processes would provide clinically important knowledge for the treatment of malignant diseases.

Hepatoma-derived growth factor (HDGF) is a novel factor that was purified from the hepatoma-derived cell line, Huh-7 (3, 4). Although HDGF was originally identified as a growth factor, HDGF has been also reported to be an anti-apoptotic factor and an angiogenic factor (5, 6), suggesting that it is involved in the development and progression of cancers through several potential mechanisms, including the stimulation of the cellular proliferation, the inhibition of the apoptosis of cancer cells and neovascularization. This article describes the characteristics of this novel factor and its possible roles in cancer development and progression, mainly focusing on hepatocelluar carcinoma (HCC).

Characteristics of HDGF

Molecular characteristics and possible signal transduction of HDGF

HDGF is a 26kDa heparin-binding acidic glycoprotein consisting of 240 amino acids. This growth factor was initially identified from the conditioned media of Huh-7 hepatoma cells. In addition, four novel genes have been identified and reported as HDGF- related proteins (HRPs) (7-9). These proteins share a highly homologous N-terminal region consisting of about 100 amino acids, which is called the HATH (homologous to the amino terminus of HDGF) region. Lens epithelium-derived growth factor (LEDGF),

which was originally reported as a survival factor for the lens epithelium, contains a HATH region, and is considered to be a member of the HDGF family (10).

Although the primary sequence of the HDGF protein does not contain the N-terminal hydrophobic sequence which is characteristic of signal peptides, it is detected in the conditioned media of various types of cells. HDGF is therefore thought to be secreted through a process which differs from the classical Golgi secretion system (5, 6). Recently, Thakar et al. (11) have reported that the N-terminal 10 amino acids of HDGF are required for the secretion of this growth factor. They also showed that phosphorylation of serine 165 in the C-terminal region of HDGF has a significant role in its secretion process.

Although the mechanism(s) in the secretion of HDGF remains unclear, the exogenous administration of HDGF significantly stimulates the proliferation of cells, including fibroblasts, endothelial cells, and fetal hepatocytes, as well as malignant cells (4, 6, 12-14). Furthermore, exogenously supplied HDGF stimulates the phosphorylation of MAP kinase (MAPK) in endothelial cells and gastric epithelial cells (15, 16). These findings strongly suggest the possibility of receptor-mediated signal transduction by HDGF. Recently, part of the HATH region (amino acids 81-100) was reported to be a possible receptor-binding site (17), and we have identified the putative receptor for HDGF (Liu et al, in submission). Based on these findings, the growth promoting effects of HDGF should occur via a receptor-mediated signal transduction pathway, and are followed by the intracellular activation of MAPK.

On the other hand, HDGF has two putative nuclear localization signals (NLSs); the first NLS (NLS1) resides in the HATH region and the second NLS (NLS2) is located in the gene-specific region. HDGF can be transported to the nucleus of cells, as demonstrated by its immunohistological detection, thus suggesting that HDGF has the characteristics of a nuclear factor (18, 19). We have shown that the nuclear translocation of HDGF is essential for the mitogenic activity of HDGF-overexpressing cells. We also found that the NLS2 is especially important for the growth stimulating effects of HDGF (18).

Although it has not been fully clarified how HDGF stimulates cellular growth after nuclear translocation, recent studies have suggested functional roles of the HATH region. The HATH regions of the HDGF family contain a PWWP motif (20, 21), which was originally reported in a candidate gene, WHSC1, for Wolf-Hirschhorn syndrome. An NMR study revealed that the PWWP domain of HDGF has a

characteristic hydrophobic cavity, thus indicating that HDGF likely binds to some component of chromatin through this cavity (22). In addition, HDGF has been suggested to bind to a conserved DNA sequence in the promoter region and suppress the transcription of its target genes, and the presumed DNA binding site is thought to reside in the PWWP domain (23). These findings suggest that the PWWP motif in the HATH region serves as a DNA binding domain for the HDGF protein.

In light of these findings, we hypothesized that HDGF would have two different mechanisms of stimulating cell growth: via a receptor-mediated pathway followed by the activation of MAPK signaling, and direct action through the DNA binding after nuclear translocation. Therefore, HDGF is a unique growth factor which has dual mechanisms for stimulating cellular proliferation.

HDGF functions as both a growth stimulating factor and an angiogenic factor

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, kidneys, lungs, and gut (5, 6, 16, 24, 25). In addition, Everett et al. (12) demonstrated that HDGF is highly expressed in proliferating fetal vascular smooth muscle cells (SMCs) and endothelial cells. They also demonstrated that HDGF expression is induced in vascular SMCs proximal to abdominal aorta constriction, and in neointimal cells after endothelial injury, suggesting functional roles for HDGF in the development and tissue repair of the cardiovascular system. Therefore, HDGF is suggested to function as an angiogenic factor, as well as a growth stimulating factor.

HDGF as a growth stimulating factor

HDGF and non-transformed hepatocyte proliferation

HDGF is highly expressed in immature fetal hepatocytes, especially in the mid-gestation stage of the liver, and its expression was dramatically decreased near birth (13). HDGF expression in hepatocytes decreases with cellular maturation, thereby suggesting that HDGF expression is related to the proliferative activity of hepatocytes. Furthermore, the exogenous administration of recombinant HDGF stimulates the proliferation of cultured fetal hepatocytes, whereas a reduction of HDGF expression severely suppresses the proliferation of these cells. These findings strongly suggest that HDGF plays a significant role in the proliferation of fetal hepatocytes during liver development (13, 14).

In the normal state, the liver is a quiescent tissue and most of mature hepatocytes are out of the replicating phase. However, the liver has the capacity to regenerate in response to cell loss, such as after hepatectomy or drug-induced hepatic injury (26, 27). In both hepatectomized and CCl_4-treated livers, the expression of HDGF was induced in hepatocytes prior to the peak of DNA synthesis. These findings indicate that the HDGF expression increases in parenchymal hepatocytes before DNA synthesis in the regenerating liver, thus suggesting that HDGF is involved in the proliferation of adult hepatocytes, as well as fetal hepatocytes (28).

Involvement of HDGF in the proliferation of various types of non-transformed cells

In the fetus, HDGF is abundantly expressed not only in the liver, but also in various other tissues, including the kidneys, heart, lungs and gut. Oliver et al. purified an endothelial growth factor from the conditioned media of a rat metanephrogenic mesenchymal cell line, and demonstrated that this purified growth factor was identical to HDGF. They have reported that HDGF should have an important role on glomerular capillary formation during nephron morphogenesis (24). HDGF was also expressed abundantly in fetal cardiovascular systems, including the heart and aorta. The HDGF protein is first detected in atrial myocytes, then its expression expands to the ventricular myocytes, endothelial and ventricular outflow cells. In addition, HDGF is strongly expressed in the proliferating vascular SMCs and endothelial cells in fetus (12). Furthermore, exogenous HDGF and endogenous overexpression of HDGF stimulated the growth of vascular SMCs. These findings suggest that HDGF can regulate vascular SMC proliferation during cardiovascular development and neointimal formation in response to vascular injury.

HDGF is highly expressed in the endothelial cells of developing blood vessels in the fetal lungs (16, 29). In a bleomycin-induced lung damage model, HDGF expression is dominantly induced in the bronchial and alveolar epithelial cells, including type II alveolar cells (30), thus suggesting that HDGF is related to the development and tissue repair of the respiratory system. With regard to the proliferation of the gut system, HDGF expression is suggested to have a suppressive role in the maturation of fetal intestinal cells and to be associated with the proliferation of these cells (25).

These research results suggest that HDGF functions as a growth stimulating factor for non-transformed cells and is involved in the development of various organs and in tissue repair processes.

HDGF as an angiogenic factor

HDGF and angiogenesis

Everett et al. (12) demonstrated that HDGF is highly expressed in the fetal cardiovascular system, and is induced during the regeneration of vascular vessels. Transplanted HDGF-overexpressing NIH3T3 cells develop large tumors in nude mice, and these tumors are histologically abundant in vasculature (31). HDGF also stimulates the proliferation and migration of human pulmonary microvascular endothelial cells *in vitro*. In addition, administration of recombinant HDGF significantly promoted blood vessel formation in the chick chorioallantoic membrane assay (16). HDGF has been demonstrated to stimulate the proliferation of human umbilical vein endothelial cells, and recombinant HDGF induces vessel formation *in vitro* (31). Interestingly, the introduction of HDGF in NIH3T3 cells induces the expression of VEGF (vascular endothelial growth factor), which is regarded as the most important angiogenic factor. The overexpression of HDGF significantly upregulates the promoter activity of VEGF, thus suggesting that HDGF promotes the transcription of the VEGF gene. Indeed, VEGF is highly expressed in the tumors developed from HDGF-overexpressing NIH3T3 cells, and growth of the HDGF-overexpressing tumors was partially suppressed by treatment with an anti-VEGF neutralizing antibody (31). Furthermore, HDGF was also reported to induce VEGF in a gastric cancer cell line (32). Therefore, the angiogenic activity of HDGF appears to occur via two mechanisms; one is its direct effect on the proliferation of endothelial cells, and the other is its induction of VEGF.

The introduction of HDGF cDNA into HepG2 hepatoma cells resulted in the formation of larger tumors in comparison to the tumors developed from the control cells (33). Tumors derived from HDGF-overexpressing HepG2 cells rapidly increased in size, although their proliferative activity *in vitro* only moderately increased. In addition, HDGF-overexpressing NIH3T3 cells show only a slight transformation capacity in soft agar, while these cells develop large tumors in nude mice, thus indicating that cells expressing high levels of HDGF had a more prominent growth activity *in vivo* than that expected from the *in vitro* studies (31). Several DNA-chip analyses demonstrated that the overexpression of HDGF upregulated several genes involved in neovascularization, including PDGF-A and Tie-1 (33). Since HDGF-overexpressing tumors are rich in vasculature and several different angiogenic growth factors can be induced by HDGF, the higher growth stimulating effects of HDGF *in vivo* may result from its angiogenic activity, in addition to its growth stimulating effects on cells.

HDGF in apoptosis

Malignant cells are sometimes able to suppress or avoid apoptotic signals, and their unregulated proliferative capacity and expression of many growth factors are suggested to contribute the tumor progression through their anti-apoptotic effects (34). However, the role of HDGF in the apoptotic pathway is still controversial.

HDGF expression has been reported to decrease in radio-resistant cells, and high HDGF expression is related to the sensitivity to irradiation in esophageal cancer cells (35). During the process of TNF/cycloheximide-induced apoptosis of endothelial cells, dephosphorylation of HDGF was shown to be an essential process for the initiation of caspase-dependent apoptosis (36), and knock-down of HDGF inhibits the apoptosis in TNF/cycloheximide-treated HeLa cells (37). In contrast, recent studies have shown that HDGF was involved in the resistance to apoptosis (REF). HDGF has also been reported to be a survival factor for CNS neurons, motor neurons and olfactory epithelium (38-40). The downregulation of HDGF induces the expression and dephosphorylation of the pro-apoptotic protein Bad, and suppresses the Erk-Akt signaling of MAPK, thus leading to the activation of an apoptotic pathway (41). In colorectal cancer cells, knockdown of HDGF induced apoptosis through the mitochondrial pathway, whereas overexpression of HDGF inhibited drug-induced apoptosis, suggesting that HDGF is associated with the resistance of these cells to chemotherapy (42, 43). Furthermore, the blockage of HDGF activated both the Fas-mediated extrinsic and Bad-mediated intrinsic apoptotic pathways in hepatoma cells (41, 44). Therefore, HDGF is also thought to function as a survival factor in hepatoma cells by exerting multiple anti-apoptotic effects.

Based on these recent reports, HDGF can be regarded as an anti-apoptotic survival factor in various cancer cells, although additional studies are required to elucidate the precise roles of HDGF in apoptosis.

HDGF in cancer

HDGF in hepatocellular carcinoma

HDGF is expressed in various hepatoma cell lines, including Huh-7, HepG2, Hep3B, PLC/PLF/5, SK-Hep1, and Mahlavu (5, 6, 18, 33). In addition, the endogenous overexpression of HDGF significantly increases the proliferation and DNA synthesis in hepatoma cells (18), whereas antisense treatment targeting HDGF reduces the cellular proliferation (45). Moreover, HDGF-overexpressing HepG2 hepatoma cells developed larger tumors in a xenograft model using nude mice in comparison to the control tumors (33).

These *in vitro* and *in vivo* experimental studies strongly suggest that HDGF acts as a growth factor for hepatoma cells.

We examined the HDGF expression in the livers of two rodent HCC models. Fisher F344 rats fed with a choline-deficient amino acid (CDAA) diet develop steatohepatitis with the progression to liver fibrosis, and HCC is observed beginning after 52 weeks of age. The Fatty Liver Shionogi (FLS) mouse is an inbred mouse strain that spontaneously develops fatty changes, and at 52 weeks, 90% of male FLS mice develop liver tumors that are histologically confirmed to be hepatocellular adenoma and carcinoma (46). HDGF was expressed more highly in HCC than in the adjacent cirrhotic liver in CDAA-fed rats, and it was also expressed more strongly in HCC than in the adjacent liver with steatohepatitis in the FLS mice. Of note, the HDGF expression increased in the liver of FLS mice before the development of visible solid tumors, suggesting a growth stimulating function of HDGF during the early stage of hepatocarcinogenesis as well as during the progression of HCC (47).

In the previous reports, the protein expression of HDGF in human HCC tissue samples was evaluated by immunostaining, and the levels of the HDGF protein were found to be higher in human HCC tissues than in the adjacent tissues (47). Moreover, the expression level of HDGF is strongly associated with the prognosis of HCC after surgery, and a higher expression of HDGF was found to be related to poorer prognosis (48). In fact, three independent groups (including our group) have demonstrated that HCC patients with a higher HDGF expression level showed an earlier recurrence and an unfavorable overall survival rate compared to those with lower expression levels of HDGF (48-50), and that the HDGF expression was found to be 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.

HDGF in pancreatic cancer

Pancreatic ductal carcinoma is one of the most fatal cancers, and shows a high proliferative and invasive activity, with a poor prognosis. HDGF is strongly expressed in pancreatic cancer cells, as well as hepatoma cells, including various pancreatic ductal carcinoma cell lines such as MIA PaCa-2, PANC-1, PL45 and KP-4 (51). We examined the HDGF expression by immunohistochemistry for 50 patients with primary ductal pancreatic carcinoma who received surgical treatment, and reported that the univariate and multivariate analyses showed nuclear HDGF expression to be an independent prognostic factor for pancreatic