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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ORIGINAL ARTICLE

Long-term interferon therapy after radiofrequency ablation is effective in treating patients with HCV-associated hepatocellular carcinoma

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Abstract
Purpose This study investigates the usefulness of long-term interferon (IFN) therapy following radiofrequency ablation (RFA) for HCV-associated hepatocellular carcinoma (HCC).
Methods This is a retrospective observational study. Patients underwent pegylated IFN-α/ribavirin combination therapy for 48 weeks and then were maintained on IFN-α administration on average for 68 weeks (mean total duration 116 weeks). Patients who underwent IFN monotherapy were maintained on IFN administration on average for 78 weeks.
Results There were biases in the background factors between the IFN and non-IFN groups. Therefore, a covariate adjustment was performed using the propensity score. An analysis of 20-matched patients from each group showed the 5-year cumulative survival rate was higher in the IFN group than in the non-IFN group (100 and 76%, respectively), and the 3-year cumulative recurrence rate was significantly lower in the IFN group than in the non-IFN group (38.0 and 64.2%, respectively). In 14 patients (i.e., IFN responders), the serum alanine aminotransferase (ALT) level remained normalized at 30 IU/mL or lower, regardless of disappearance of serum HCV RNA. In these patients, the cumulative recurrence rate was low, the hazard ratio was 0.158 (95% confidence interval = 0.045–0.561, $P = 0.004$), and the serum albumin level was retained.
Conclusion These results show the importance of maintaining the liver function and suggest that long-term IFN administration after RFA inhibits recurrence and contributes to an improved outcome in patients (in particular, IFN responders) who initially develop HCC.

Keywords Hepatitis C virus · Interferon · Hepatocellular carcinoma · Prevention · Radiofrequency ablation

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Introduction

More than 500 million people in the world are infected with hepatitis B and C viruses (HBV and HCV, respectively). Persistent infection with these hepatitis viruses is strongly associated with the development of hepatocellular carcinoma (HCC). The HCC patient is ranked fifth among cancer patients throughout the world and the number of deaths from HCC is ranked at third [1]. Its pathogenesis is being progressively elucidated; in most instances, HCC develops in patients who have a background of HBV- and HCV-induced chronic hepatitis and hepatic cirrhosis [2]. Advances in the early detection of and therapy for HCC have increasingly led to curative treatment. However, HCC is likely to recur and the incidences of intrahepatic metastasis and multicentric recurrences are high and are a

major problem in improving the treatment outcomes [3, 4]. There have been marked advancements recently in interferon (IFN) therapy for chronic hepatitis C. The incidence of HCC is, according to reports, significantly reduced in patients who achieved a sustained viral response (SVR) with IFN therapy and in patients for whom IFN therapy normalizes liver function. This indicates the importance of IFN therapy as a primary prevention against developing HCC [5–9]. IFN therapy after curative treatment of HCV-associated HCC is reportedly useful as secondary prevention against carcinogenesis [10–14]. There are reports that long-term IFN administration inhibits recurrence [15], but the evaluation of this has not been fully established [16]. In this study, after administering radical radiofrequency ablation (RFA), we performed long-term IFN therapy in patients with the initial development of HCV-associated HCC. We retrospectively investigated the recurrence-inhibitory effect of the treatment.

Patients and methods

Patients

Between April 2001 and March 2008, there were 226 patients who underwent RFA at the Division of Hepatobiliary and Pancreatic Diseases, Hyogo College of Medicine. Of these, 135 patients were negative for the HBs antigen and positive for HCV RNA, with tumor diameter $\leq$3 cm, with three or fewer tumors, and underwent RFA alone for the initial development of HCC. These 135 patients were selected for subjects. There were 71 male and 64 female patients who ranged from 39 to 85 years old (the median age was 68 years). The background liver was clinically and histologically evaluated. HCC was diagnosed using abdominal ultrasonography, dynamic computed tomography (CT), and magnetic resonance imaging (MRI). Markedly enhanced tumors were noted in the early phase of contrast imaging on CT or MRI in all patients and washout was observed in the portal phase or late phase 3 min after injection of the contrast medium. RFA was performed with ultrasonographic guiding using the Cool-tip Radiofrequency Ablation system (Tyco Healthcare Group LP, Burlington, MA). The lesions were evaluated by dynamic CT or MRI 1 and 8 weeks after RFA. The treatment was considered markedly effective when the intensely stained tumors disappeared and a treated area was sufficiently maintained.

IFN therapy was indicated for patients meeting the following conditions: (1) platelet count $\geq$ 70,000 μL^{-1}, (2) white blood cell count $\geq$ 1,500 μL^{-1}, (3) hemoglobin $\geq$ 10 g/dL, (4) Child-Pugh classification stage A, and (5) age $\leq$ 80 years. IFN therapy was performed in 20 patients for whom RFA was markedly effective. Nineteen of 20 patients requested IFN therapy and met the criteria. One patient with Child-Pugh classification stage B underwent IFN therapy because of the patient's strong desire for this therapy. One hundred and fifteen patients did not undergo IFN therapy. Fifty-one of 115 patients did not meet the criteria. IFN therapy was not performed in 64 patients because of their unwillingness to undergo this therapy (despite meeting the indications for treatment) or because ursodeoxycholic acid or strong neominophagen C were being administered instead of IFN because the patients had complications such as hypertension and diabetes (Table 1).

Since this was a retrospective study, covariate adjustment using the propensity score was performed to adjust for between-group confounding factors [17, 18]. The following ten factors were adopted as matching covariates: age, gender, platelet count, serum alanine aminotransferase (ALT) level, α-fetoprotein (AFP) level, protein induced by vitamin K absence or antagonist-II (PIVKA-II) level, Child-Pugh classification, tumor diameter, number of tumors, and the Japanese tumor, node, and metastasis (TNM) stage [19]. Based on the results of the adjustments, 20 patients with IFN therapy and 20 patients without IFN therapy (i.e., IFN and non-IFN groups, respectively) were assessed. Recurrence of HCC was classified as a local tumor progression or as an ectopic recurrence. A local tumor progression was defined as recurrence in an area previously treated with RFA and an ectopic recurrence was defined as a recurrence outside this area.

IFN therapy

IFN therapy that was initiated 1 month after RFA was markedly effective in treating the initial HCC. In the IFN group, 19 patients had the genotype 1b and a high viral load. In Japan, pegylated IFN-α/ribavirin (PEG-IFN/RBV) combination therapy was approved at the end of 2004, and we have planned to give PEG-IFN/RBV combination therapy since 2005. IFN therapy was administered after requests from individual patients who gave informed consent. Twelve patients underwent PEG-IFN/RBV combination therapy. The therapy regimen consisted of PEG-IFN-α-2b (Peg-Intron, Schering Plough Corporation, Kenilworth, NJ) administered at a dose of 1.5 μg/kg/week; PEG-IFN-α-2a (Pegasys, Hoffman La Roche, Nutley, NJ) administered at a dose of 180 μg/week; and RBV administered for 48 weeks at a dose based on body weight (600 mg for $\leq$60 kg, 800 mg for 60–80 kg, and 1,000 mg for >80 kg). Two of the 12 patients achieved SVR, which was defined as the persistent negative conversion of serum HCV RNA after the 24th week of IFN therapy. After the combination therapy, nine patients underwent IFN-α (Sumiferon, Dainippon Sumitomo Pharma Co., Ltd.,

Table 1 Demographic and baseline characteristics of the patients

Variable	IFN group	Non-1 FN group	*P*
Patients (*n*)	20	115	
Age (range), years	65 (52–76)	67 (39–85)	0.06
Gender (*n*)			0.04
Male	15	56	
Female	5	59	
White blood count ($\times 10^2$ μL^{-1})	40 (38, 50)	37 (28, 46)	0.08
Hemoglobin (g/dL)	12.9 (11.7, 13.9)	12.8 (11.3, 13.5)	0.35
Platelet count ($\times 10^4$ μL^{-1})	9.7 (8.7, 11.9)	9.7 (8.0, 12.2)	0.36
Prothrombin activity (%)	80.9 (79.5, 88.9)	81.9 (77.5, 84.2)	0.80
Total bilirubin (mg/dL)	0.8 (0.6, 1.2)	0.9 (0.5, 1.2)	0.51
Albumin level (g/dL)	3.9 (3.5, 4.1)	3.7 (3.3, 3.9)	0.006
AST (IU/L)	60 (33, 82)	57 (45, 86)	0.42
ALT (IU/L)	50 (37, 83)	53 (39, 72)	0.71
AFP (ng/mL)	11 (6, 41)	27 (6, 79)	0.03
PIVKA-II (mAU/mL)	25 (17, 60)	37 (21, 137)	0.95
Child-Pugh classification (*n*)			0.41
A	19	96	
B	1	18	
C	0	1	
Tumor size (mm)	21 (17, 24)	20 (19, 29)	0.75
Number of tumors (*n*)			0.17
Solitary	18	88	
Multiple	2	27	
Japanese TNM stage (*n*)			0.044
I	6	43	
II	13	45	
III	1	27	
Follow-up period (month)	37 (19, 57)	31 (17, 69)	0.13

Except where indicated, data are expressed as the median (25th, 75th percentile)

Osaka, Japan) monotherapy (300 MU, 3 times/week) for a mean duration of 68 weeks (mean total duration 116 weeks).

Eight patients underwent IFN monotherapy. One patient had the genotype 2a, one patient had the hemoglobin of 11.1 g/dL and six patients had low levels of the platelet count (<100,000 μL^{-1}). In accordance with Japan package insert of PEG-IFN-α-2b, we planned to undertake IFN monotherapy in eight patients on enough informed consent. Treatment was initiated with PEG-IFN-α-2a monotherapy (90 μg/week or 90 μg/2 weeks) in five patients, and IFN-α monotherapy (300–600 MU, 3 times/week) was initiated in three patients. The therapies were continued in these patients, except for one patient who ultimately achieved SVR. The mean duration of PEG-IFN-α-2a or IFN-α monotherapy was 78 weeks.

Follow-up procedure

After RFA for HCC, liver function testing and tumor marker measurement (AFP, PIVKA-II) were performed every month in all patients. Abdominal ultrasonography was performed every 3 months and dynamic CT or MRI was performed every 6 months. Histological examination was performed on suspected hypovascular HCC using fine-needle aspiration biopsy. When a recurrence occurred, the patient was admitted for appropriate therapy.

Statistical analysis

For between-group comparisons, the Mann–Whitney *U* test was used for continuous variables and the Chi-square and Fisher exact tests were used for categorical variables. The survival time and HCC recurrence were evaluated using the Kaplan–Meier method and between-group comparisons were performed using the log-rank test. The influence of IFN administration on recurrence after RFA was investigated using the Cox proportional hazards model. Covariate adjustment was performed using the propensity score, as previously reported [17, 18]. The *C*-statistic (ROCKIT software, Kurt Rossmann Laboratories, University of Chicago, Chicago, IL) [20] was used for the goodness-of-fit

index of patients matched using the propensity score. Analysis was performed using statistical software SPSS ver. 16.0 (SPSS, Inc., Chicago, IL) and SAS ver. 9.13 (SAS Institute, Inc., Cary, NC). A two-sided *P* value of less than 5% was regarded as significant.

Results

The virological and adverse effects of IFN therapy

Two of the 19 patients with the genotype 1b who had a high viral load and one patient with the genotype 2a achieved SVR. HCV RNA did not become negative in 14 patients. HCV RNA disappeared in the remaining three patients, but assessing SVR was difficult since the patients were undergoing IFN therapy. Normalization of the serum ALT level to 30 IU/mL or lower occurred in 11 of the 17 non-SVR patients. Since there were no severe adverse events, IFN therapy was continued in 12 patients who underwent PEG-IFN/RBV combination therapy and in eight patients who underwent IFN monotherapy. IFN therapy was suspended due to recurrence of HCC in two patients who underwent IFN monotherapy, but the treatment was resumed after a second RFA treatment in the patients. In the non-IFN group, normalization of the serum ALT level to 30 IU/mL or lower did not occur in any patient.

Recurrence of HCC

After radical RFA in 135 patients, the 1- and 3-year cumulative recurrence rates were 5.0 and 38.0%, respectively, in the IFN group, and 25.0 and 68.6%, respectively, in the non-IFN group. The lower rate in the IFN group was statistically significant ($P = 0.007$). A comparison of the

Table 2 Demographic and baseline characteristics of the matched patients

Variable	IFN group	Non-IFN group	*P*
Patients (*n*)	20	20	
Age (range), years	66 (52–76)	67 (56–73)	0.30
Gender (*n*)			1.00
Male	15	15	
Female	5	5	
White blood count ($\times 10^2$ μL^{-1})	40 (38, 50)	39 (29, 46)	0.14
Hemoglobin (g/dL)	12.9 (11.7, 13.9)	13.3 (11.9, 14.2)	0.67
Platelet count ($\times 10^4$ μL^{-1})	9.7 (8.7, 11.9)	10.9 (7.8, 14.6)	0.49
Prothrombin activity (%)	80.9 (79.4, 88.9)	83.8 (74.3, 90.1)	0.89
Total bilirubin (mg/dL)	0.8 (0.6, 1.2)	1.0 (0.7,1.2)	0.30
Albumin level (g/dL)	3.9 (3.5, 4.1)	3.7 (3.3,4.0)	0.08
AST (IU/L)	60 (33, 82)	62 (41, 87)	0.19
ALT (IU/L)	50 (37, 83)	52 (35, 67)	0.69
AFP (ng/mL)	11 (6, 41)	18 (8, 69)	0.29
PIVKA-II (mAU/mL)	25 (17, 61)	33 (18, 47)	0.75
Child-Pugh classification (*n*)			1.00
A	19	20	
B	1	0	
Tumor size (mm)	21 (17, 24)	21 (18, 26)	0.67
Number of tumors (*n*)			1.00
Solitary	18	18	
Multiple	2	2	
Japanese TNM stage (*n*)			0.46
I	6	9	
II	13	9	
III	1	2	
HCV genotype			1.00
1 b	19	18	
2a	1	2	
Viral load (kIU/mL)	900 (370, 1,600)	850 (270, 1,250)	0.76
Follow-up period (month)	37 (19, 57)	28 (17, 39)	0.24

Except where indicated, data are expressed as the median (25th, 75th percentile)

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).

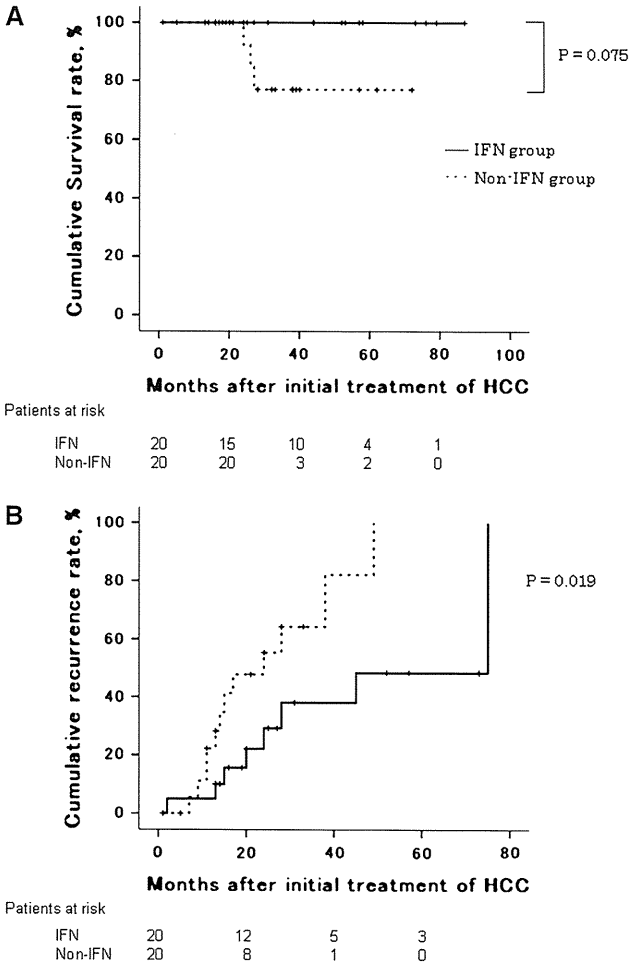

Fig. 1 **a** Cumulative survival rates after curative RFA treatment of matched patients with HCC. The cumulative rates were higher in the IFN group than in the non-IFN group ($P = 0.075$). **b** Cumulative recurrence rates after RFA treatment of matched patients with HCC. The recurrence rate of the IFN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

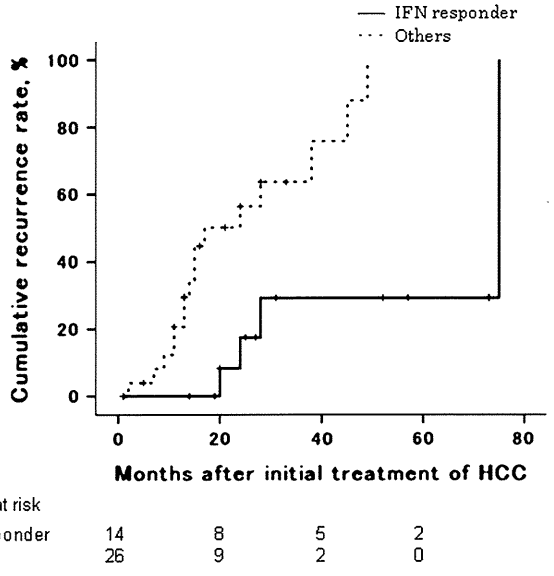

Fig. 2 Cumulative recurrence rates according to efficacy of IFN therapy after curative RFA treatment of matched patients with HCC. The rate of cumulative recurrence of HCC in the IFN responder was significantly lower than the "Others" ($P = 0.001$)

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.

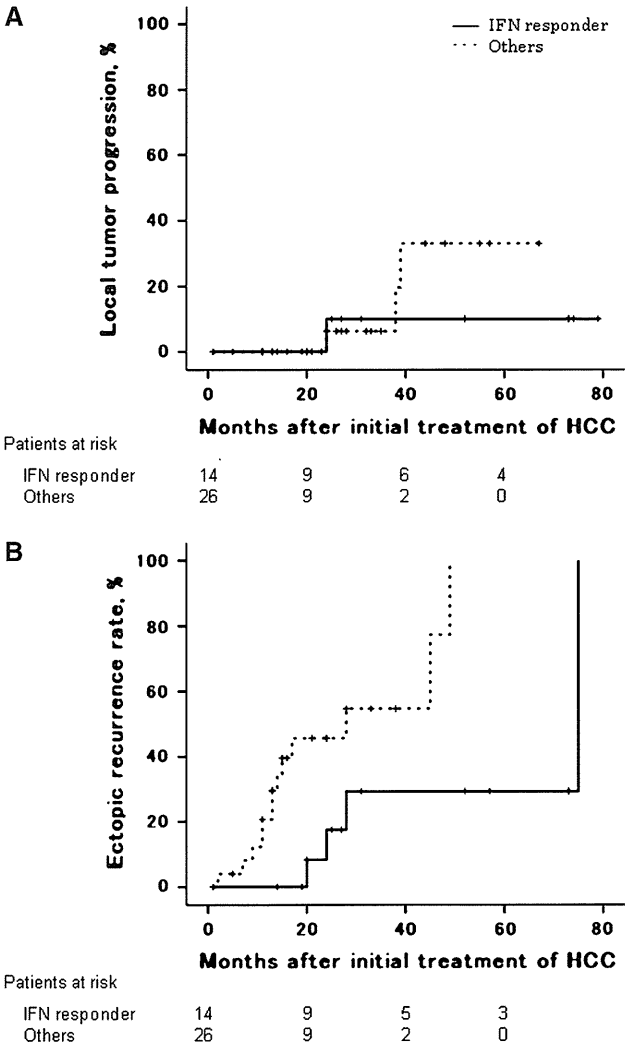

Fig. 3 Cumulative recurence rates after RFA treatment of matched patients with HCC. **a** Rates of local tumor progression was lower than that of the "Others", but the difference was not statistically significant ($P = 0.45$). **b** Rates of ectopic recurrence of the IFN responder was significantly lower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

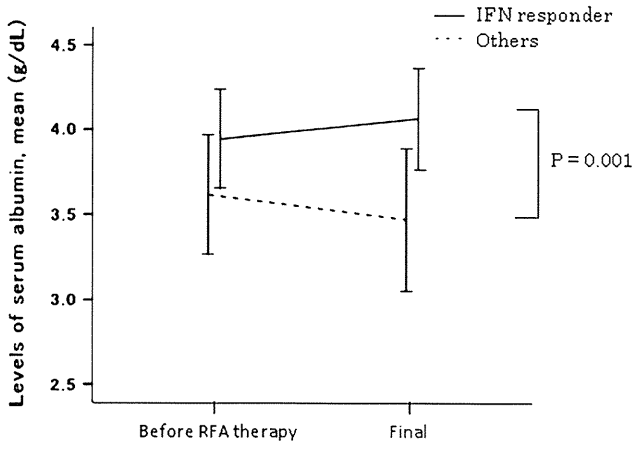

Fig. 4 Effect of IFN therapy after curative RFA treatment of matched patients with HCC on the levels of serum albumn. The *bars* indicate mean ± 1 SD. Serum albumin in the IFN responders was significantly better preserved than the "Others" ($P = 0.001$)

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 hepato-carcinogenesis 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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