Serum Concentrations of Human Hepatocyte Growth Factor Is a Useful Indicator for Predicting the Occurrence of Hepatocellular Carcinomas in C-Viral Chronic Liver Diseases

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BACKGROUND. Numerous reports have examined the relationship between hepatocyte growth factor (HGF) and either the facilitation or suppression of the occurrence of hepatocellular carcinoma (HCC).

METHODS. In this study, we measured serum HGF concentrations of blood samples and conducted prospective studies to examine the long-term outcome of C-viral chronic hepatitis (CH) and cirrhosis in patients. The subjects examined in this study include 99 patients with C-viral CH, cirrhosis, and HCC. The serum HGF level was measured in blood samples within 48 hours of collection using enzyme-linked immunosorbent assay kits.

RESULTS. The serum concentrations of HGF were significantly higher in patients with HCC than in patients with CH or cirrhosis. The detection rate of HGF and its mean serum level were significantly higher in patients with a low platelet count than in patients with a high platelet count. All of the patients with serum HGF concentrations of more than 0.6 ng/mL had HCC, irrespective of the levels of α-fetoprotein, vitamin K absence, or antagonist-II in the blood. Serum HGF concentrations increased concomitantly with increases in areas occupied by HCC. The cumulative incidence of occurrence of HCC was significantly higher in patients with high HGF concentrations than in patients with low HGF concentrations. Multivariate analysis revealed that the elevation in serum HGF level is the most important risk factor for the occurrence of HCC.

CONCLUSIONS. The serum level of HGF represents the degree of the carcinogenic state in the liver of patients with C-viral CH and cirrhosis. Therefore, the determination of serum HGF concentrations may be useful as a third tumor marker of HCC in detection as well as follow-up therapy. Cancer 2002;95:824–34.

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Human hepatocyte growth factor (HGF) is produced in various organs of the body and is characterized as a multifunctional factor with various biologic activities. Numerous reports have examined the relationship between HGF and liver diseases and several have sought a factor that would assist in predicting the prognosis of fulminant hepatitis, the development of chronic hepatitis (CH), and either the facilitation or suppression of the occurrence of hepatocellular carcinoma (HCC). The serum concentration of HGF in patients with C-viral liver diseases is elevated in patients with cirrhosis...
and HCC. However, HGF is activated by serine pro-
teases after the prolonged storage of blood samples in
a frozen state or after repeated freezing and thawing of
samples. In these blood samples, the concentrations of
HGF may be unduly high. Therefore, the HGF
concentration that is evaluated retrospectively in se-
rum samples kept frozen for long periods does not
represent accurately the HGF concentrations in the
patient, and, accordingly, may not be of clinical sig-
nificance.

HGF suppresses the expression of tumor growth
factor β1 mRNA, thereby relieving fibrosis of the liv-
er. In light of these results, HGF is expected to be
applied clinically for the treatment of CH and cirrhosis
in the future. However, in regards to HGF treatment
on the occurrence of HCC, contradictory effects have
been reported. Several groups have reported the sup-
pression of HCC by HGF treatment whereas other
groups have reported facilitatory effects. Unless
the effect of HGF on the occurrence of HCC is defined,
we cannot begin using HGF treatment to suppress
hepatic fibrosis in patients with chronic liver diseases.
In this study, we explore the relationship between
HGF and the occurrence of HCC. We collected blood
samples from patients with C-viral CH, cirrhosis, or
HCC and evaluated the serum concentrations of HGF
within 48 hours. These concentrations were compared
between patients with and without HCC. In addition,
in patients without HCC, we conducted a prospective
study on the occurrence of HCC immediately after a
first evaluation of the HGF level. We explored the
localization of HGF in liver tissues obtained by surgic-
al resection from patients with HCC. On the basis of
findings obtained in these examinations, we discuss
whether the evaluation of the serum HGF level is
useful in predicting the occurrence of HCC, in deter-
mining the efficacy of treatments for HCC, and as a
clinical follow-up of patients.

PATIENTS AND METHODS

Patients

This study began with 62 patients with CH and 22
patients with cirrhosis. They were examined by M.M.
at Nihon University Itabashi Hospital from November
1994 through August 1995. All gave informed consent
for their participation in this study. Of these, 10 pa-
tients with CH (16.1%) stopped their visits to the hos-
pital within 3 years and 3 patients with cirrhosis
(13.6%) died of hepatic failure. The remaining 52 pa-
tients with CH (mean age, 45.6 years; proportion of
males, 50%; mean alanine aminotransferase [ALT]
value, 58.2; mean platelet count, 217,000/mm3; mean
observation period, 4.43 years) and 19 patients with
LC (mean age, 55.3 years; proportion of males, 52.6%;
mean ALT value, 55.0; mean platelet count, 99,000/
mm3; mean observation period, 4.05 years) were in-
cluded in this study. All of the patients were positive
for serum hepatitis C virus (HCV) RNA and were ob-
erved for more than 3 years. In addition, 28 more
patients (mean age, 62.1 years; proportion of males,
42.9%; mean ALT value, 51.7; mean platelet count,
106,000/mm3) were included in the study. They had
space-occupying lesions that were detected by ab-
dominal ultrasonography and computed tomography
(CT) scan and were diagnosed as having HCC by ab-
dominal angiography from November 1994 through
August 1995. All patients were negative for serum hep-
atitis B surface antigen (enzyme-linked immunosor-
bent assay [ELISA], Dinabot, Tokyo, Japan), LE cells,
anti-smooth muscle antibody (fluorescence antibody
[FA] method), and antimitochondria antibody (FA).
No heavy drinkers (more than 30 g ethanol intake per
day) were included in the study. Patients whose blood
ALT levels remained persistently abnormal for more
than 6 months, whose indocyanine green retention
rates (ICGRs) at 15 minutes were less than 10%, and
whose platelet counts were more than 130,000/mm3
were diagnosed as having CH. The criteria for diag-
nosing cirrhosis were persistent abnormal blood ALT
levels for more than 6 months, ICGRs of more than
10% at 15 minutes, platelet counts of less than
130,000/mm3, the presence of esophageal varices,
and the presence of cirrhosis pattern and splenomeg-
aly on abdominal diagnostic imagings. Splenomegaly
was defined as the product of the splenic hilus-apex
diameter and the perpendicular diameter measured on
the spleen images generated using ultrasonogra-
phy. The normal range of the spleen is less than 20
cm2. Blood samples were obtained only from patients
who gave informed consent. About 130 patients were
diagnosed as having CH or cirrhosis. Therefore, ap-
proximately 60% of these patients were subjects of this
study. Patients with CH or cirrhosis underwent ab-
dominal ultrasonography and CT scans once every
3–6 months to detect the occurrence of HCC. HCC
was diagnosed definitively by abdominal angiography
and liver biopsy after HCC was suspected by abdom-
inal ultrasonography and CT scan. Blood samples
were collected at this time. Patients with HCC also
underwent the following examinations. First, the rela-
tionship between the spread of pathologic changes
and the serum level of HGF was explored. Second,
changes in the serum HGF level before and after
transaortic embolization (TAE) were examined in
three patients who were observed regularly. Third, the
localization of HGF in the liver was examined by light
and electron microscopy using the indirect immuno-
peroxidase staining method.
Measurement of Serum HGF Level
The serum HGF concentrations were quantitated using ELISA kits (Otsuka Assay Institute, Tokushima, Japan). Titers of antigens were determined by reference to a standard curve, which had been constructed using the standard serum. HGF concentrations were measured at least three times in each serum sample and the highest value was used for later analysis. To measure the serum HGF concentrations, the serum was isolated from blood samples and kept frozen at −80 °C temporarily. HGF concentrations were obtained within 48 hours. From each of 20 patients, who gave informed consent to these procedures on explanation, 10 mL of whole blood was obtained after examination in the outpatient department. Five milliliters of the blood sample was placed immediately in a test tube treated with a serine protease inhibitor. Thereafter, the serum was separated from the blood and kept at −80 °C in an inhibitor-treated test tube. The serum isolated from the remaining 5 mL of the blood sample was placed in an untreated test tube and kept at −80 °C. HGF concentrations in the two test tubes were evaluated separately within 48 hours. As normal controls, 20 subjects with normal serum sedimentation rates, C-reactive protein, and liver function tests were also examined.

Hematologic and Biochemical Examinations
Serum levels of aspartate aminotransferase (AST), ALT, total bilirubin, alanine phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), and platelet counts were determined. In addition, serum levels of α-fetoprotein (AFP) as a tumor marker were determined by enzyme immunoassay. Protein induced by vitamin K absence or antagonist-II (PIVKA-II) was measured using an electrochemiluminescence immunoassay. The correlations between serum HGF levels and values of the above variables were examined. Miyazawa et al. reported that the F stage correlates well with platelet counts in patients with C-viral CH or cirrhosis. Because the evaluation of the F stage is influenced greatly by the subjective judgment of examiners, differences in the size of specimens, and the locus from which specimens were taken, we conclude that the platelet count may express the degree of fibrosis in the liver more objectively than the F score. Therefore, in this study, we used the platelet count as an indicator of intrahepatic fibrosis. Patients were divided into four groups according to their platelet counts: patients with platelet counts less than 100,000/mm³, patients with platelet counts ranging from 100,000 to less than 200,000/mm³, patients with platelet counts ranging from 200,000 to less than 300,000/mm³, and patients with platelet counts of more than 300,000/mm³. The correlation between the serum HGF concentrations and platelet counts was examined among these groups.

Detection of HGF in the Liver
The livers of five HCC patients, which were resected surgically, were immersed in OCT compound (Tissue-Tek, Sakura Finetecchnical Co., Ltd., Tokyo, Japan), rapidly frozen using dry ice and acetone, and stored at −80 °C until use. Frozen tissue was sliced into 4 μm thick sections and each section was fixed with acetone and subjected to two sessions of microwave treatment every 30 seconds. After standing for 20 minutes at room temperature, HGF localization was assessed by the indirect immunoperoxidase staining method using a polyclonal anti-HGF antibody (Institute Immunology, Tokyo, Japan, 1:300 dilution) as the primary antibody and peroxidase-labeled anti-rabbit IgG (MBL, Tokyo, Japan, 1:500 dilution) as the secondary antibody. HGF localization was determined in five patients with HCC. The patients were selected from those who had HCV genotype 1b, serum HCV RNA levels over 10⁵ copies per milliliter, and a well or moderately differentiated tumor. For immunoelectron microscopy (IEM), after treatment the DAB reaction (Sigma, St. Louis), the tissues were incubated with 2.5% glutaraldehyde (Electron Microscopy Sciences, Fort Washington, PA) and 2% osmic acid (Nissin Em) for 60 minutes at room temperature, and embedded in Epon 812 (Nissin Em) for 48 hours at 60 °C. Ultrathin sections were obtained using an Ultratome III® (LKB, Bromma, Sweden) and were observed on a JEM-100C electron microscope (JEOL, Tokyo, Japan).

Statistical Analysis
The serum concentrations of HGF were compared using the chi-square test for independence. Cumulative incidence curves were determined with the Kaplan–Meier method and the differences between groups were assessed using the log rank test. The remaining parameters were compared using analysis of variance and the Fisher protected least significant difference post hoc test with Statview 4.5 software (Abacus Concepts, Berkeley, CA). A P value of less than 0.05 was considered significant.

Multivariate Regression Analysis
The factors among the 71 cases (CH and cirrhosis) with serum HGF concentrations at the initial time or diagnosis of HCC were investigated. The independent factors of gender, age, history of blood transfusion, ALT level, platelet count, HCV RNA level, HGF levels, AFP levels, PIVKA-II levels, and HCV genotype were identified by Cox logistic regression using a step-wise
method analysis of factors for the risk for developing HCC using SPSS 6.0 software (SPSS, Chicago, IL).

RESULTS

Evaluation of Serum HGF Concentrations

The HGF concentrations in blood samples placed in test tubes treated with a serine protease inhibitor correlated well with the HGF concentrations of the samples placed in inhibitor-untreated test tubes ($r = 0.919, P = 0.0001$). This result suggests that the activation of HGF by serine proteases has no appreciable effect if the evaluation is made within 48 hours of collecting the blood samples. Accordingly, serum samples were separated from the blood collected in the outpatient department in inhibitor-untreated test tubes and kept frozen at $-80^\circ$C. The frozen serum samples were thawed and HGF concentrations were determined within 48 hours of blood collection.

In patients with C-viral chronic liver diseases, serum HGF concentrations were undetectable ($<0.3$ ng/mL) in 92.3% of cases with CH and in 36.8% of cases with cirrhosis. Conversely, HGF concentrations were detected in all cases with HCC. The detection rate of HGF in the patient group with platelet counts less than 100,000/mm$^3$ was significantly higher than that in the other groups. However, no significant correlation was observed between the mean HGF concentrations and the platelet counts in patients with serum HGF concentrations above 0.31 ng/mL ($r = 0.070, P = 0.6358$, Fig. 2). HGF concentrations were greater than 0.31 ng/mL in patients with HCC, irrespective of platelet counts. In patients with HCC without cirrhosis whose platelet count was more than 150,000/mm$^3$, the HGF concentrations were greater than 0.31 ng/mL.

Comparison of HGF Concentrations among Patients with Different Platelet Counts

The proportion of patients with serum HGF concentrations greater than 0.31 ng/mL was 0% in the patient group with platelet counts of more than 300,000/mm$^3$, 15.8% in the patient group with platelet counts from 200,000 to less than 300,000/mm$^3$, 41.1% in the patient group with platelet counts from 100,000 to less than 200,000/mm$^3$, and 90.9% in the patient group with platelet counts less than 100,000/mm$^3$. The detection rate of HGF in the patient group with platelet counts less than 100,000/mm$^3$ was significantly higher than that in the other groups.
Comparison with Biochemical Variables

The serum ALT level was used as an indicator of intrahepatic activities. In patients with HGF concentrations above 0.31 ng/mL, no significant correlation was detected between the ALT and HGF concentrations, although there was the propensity for the HGF level to be elevated in patients with higher ALT values ($r = 0.152, P = 0.3053$). The serum HGF level was not significantly correlated with other indicators of liver functions, such as the AST level ($r = 0.234, P = 0.651$), the ALP level ($r = 0.311, P = 0.451$), and the $\gamma$-GTP level ($r = 0.334, P = 0.551$).

Cumulative Incidence of Occurrence of HCC in Patients with CH or Cirrhosis

Seventy-one patients with CH or cirrhosis were divided into two groups according to their serum HGF concentrations at the initial examination. Patients with HGF concentrations equal to or less than 0.3 ng/mL were assigned to a low HGF group and patients with concentrations equal to or greater than 0.31 ng/mL were assigned to a high HGF group. The cumulative incidence of occurrence of HCC was significantly higher in the high HGF group than in the low HGF group.
with HGF concentrations equal to or less than 0.3 ng/mL were classified into the low HGF group (51 patients; mean observation period, 4.47 years), whereas patients with serum HGF concentrations of more than 0.3 ng/mL were classified into the high HGF group (20 patients; mean observation period, 3.69 years). The cumulative incidence of occurrence of HCC, the cumulative of which was started prospectively after the first measurement of the HGF level, was compared between these two groups (Fig. 3). HCC occurred in only 1 patient (1.96%) in the low HGF group and in 11 patients (55%) in the high HGF group. The cumulative incidence of occurrence of HCC for 5 years was 3.0% in the low HGF group and 70% in the high HGF group. The difference between the two groups was statistically significant ($P < 0.0001$). In the one patient with HCC in the low HGF group, the HGF level rose with time to 0.32 ng/mL, and then to 0.40 ng/mL, and, thereafter, HCC was observed. The HGF level was 0.44 ng/mL when HCC was detected.

### Multivariate Analysis of the Development of HCC from CH and Cirrhosis

To identify risk factors for the development of HCC, multivariate analyses were performed using the Cox proportional hazards model. The analyses revealed that a serum HGF level greater than 0.31 ng/mL (the high HGF group) was the most cardinal risk factor, although the platelet count was also a significant risk factor (Table 1).

### Detection of HGF in the Liver

In the noncancerous regions of a liver with HCC, HGF was detected primarily in cells of mesenchymal origin, such as the sinusoidal endothelial cells, and in infiltrating cells in some necroinflammatory regions (Fig. 4a). HGF was also observed in hepatocytes with strong atypia in two patients (Fig. 4b). In cancerous regions, HGF was detected in infiltrating mesenchymal cells and in the cytoplasm and cell membranes of cancer cells in four (80%) of five patients (Fig. 5a–c). Carcinomas of these four patients were highly or moderately differentiated, single-nodular tumors of less than

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**TABLE 1**

Factors Contributing to the Occurrence of HCC, as Revealed by Multivariate Analysis using the Cox Proportional Hazards Model

| Factor                  | Hazard ratio | 95% CI            | $P$     |
|-------------------------|--------------|-------------------|---------|
| Gender (female)         | 0.425        | 0.034–4.226       | 0.4659  |
| HCV RNA level (< 100 kIU/mL) | 3.490        | 0.236–54.535      | 0.3728  |
| HCV genotype (1b)      | 1.279        | 0.095–17.191      | 0.8527  |
| Platelet count (< 150,000) | 0.819        | 0.691–9.970       | 0.0210  |
| AFP (< 20 ng/mL)       | 0.967        | 0.905–1.033       | 0.3184  |
| AST (< 80 U/mL)        | 0.984        | 0.916–1.057       | 0.6068  |
| ALT (< 80 U/mL)        | 1.014        | 0.964–1.068       | 0.5846  |
| HGF level (< 0.3 ng/mL) | 45.088       | 3.719–546.571     | 0.0028  |

HCC: hepatocellular carcinoma; CI: confidence interval; HCV: hepatitis C virus; AFP: $\alpha$-fetoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; HGF: hepatocyte growth factor.

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**FIGURE 4.** Localization of hepatocyte growth factor (HGF) using the immunoperoxidase staining method, as detected with light microscope, in the noncancerous regions of a liver with hepatocellular carcinoma. HGF is observed primarily in mesenchymal cells, such as sinusoidal endothelial and infiltrating cells in necrotic inflammatory regions (a) and in dysplastic hepatocytes (b). Counterstained by hematoxylin. Original magnification: ×20 (a, b).
20 mm in diameter. The serum HGF concentrations of these four patients were 0.33, 0.34, 0.51, and 0.55 ng/mL, respectively. In the patient lacking detectable localized HGF, the serum HGF level was 0.31 ng/mL. In the cancerous regions of the four patients described above, HGF was not distributed diffusely, but was congregated in small regions. Using IEM, HGF was observed in the endoplasmic reticulum (ER) of the cytoplasm of cancer cells (Fig. 6).

**Comparison with Tumor Markers**
Because the localization of HGF was observed in cancer cells, we examined whether the serum level of HGF is useful as a tumor marker in patients with HCC. The relationship between the serum HGF level and the serum AFP level or the PIVKA-II level is depicted in Figure 7a and 7b, respectively, in patients with HCC, CH, or cirrhosis. No significant correlation was observed between the serum HGF level and the serum AFP level ($r = 0.162, P = 0.272$) or between the serum HGF level and the PIVKA-II level ($r = 0.323, P = 0.635$). In patients with serum HGF concentrations greater than 0.31 ng/mL, the serum HGF level correlated significantly with the AFP level ($r = 0.254, P = 0.0111$), although the serum HGF level did not correlate significantly with the PIVKA-II level ($r = 0.162, P = 0.2721$). As these data show, the serum HGF concentrations were elevated in patients with high levels of AFP and PIVKA-II. It is significant to note that all patients with serum HGF concentrations above 0.6 ng/mL had HCC, even if their AFP or PIVKA-II levels were low.

**Relationship between Areas Occupied by Tumors and the HGF Level**
The serum HGF concentrations were elevated with increases in the size of spaces occupied by tumors, as...
well as in the number of tumors in the liver. This was evident in patients having plural tumors larger than 20 mm in diameter (Fig. 8). In seven patients who underwent a course of TAE treatments for the first time from April through August 1998, we compared serum HGF concentrations before, during, and after the TAE treatments. Figure 9 shows the changes in the serum HGF level in these patients. The serum HGF level increased temporarily immediately after the treatments, but decreased significantly at 6 months after the treatments, in comparison with the serum HGF level before the treatments. The temporary increase in the serum HGF level may be a result of necrosis of the tumor cells. All of the patients, in whom the serum HGF level increased persistently after the completion of the treatments, died within 2 years.

**DISCUSSION**

The serum HGF level was markedly higher in patients with HCC than in subjects without cancer. Serum HGF concentrations greater than 0.31 ng/mL were observed in patients with HCC, whereas the concentrations were below the detection limit of our assay in many patients without HCC. Our finding that all of the
patients with HGF concentrations greater than 0.6 ng/mL had HCC suggests a clinical significance in the evaluation of the HGF level for the diagnosis of HCC. In addition, our results indicate that all of the patients with HGF concentrations above 0.6 ng/mL had HCC, even if their AFP or PIVKA-II levels remained within the normal range, suggesting that HGF may be a tumor marker of HCC occurrence. Our analysis of the cumulative incidence of occurrence of HCC suggests that CH and cirrhosis patients with HGF concentrations above 0.31 ng/mL at the initial examination are more likely to have HCC than those with HGF concentrations below the detection limit (0.3 ng/mL) at the initial examination. Therefore, patients with CH or cirrhosis may be in a highly carcinogenic state when their serum HGF concentrations are above 0.31 ng/mL. Multivariate analyses revealed that a serum HGF level above 0.31 ng/mL is a risk factor for the development of HCC. An evaluation of the HGF level may detect patients with a high carcinogenic state and a high risk for the occurrence of HCC. In fact, the HGF level of one of our patients was below the detection limit (0.3 ng/mL) at the initial examination. However, the concentrations increased over the time course of observation and exceeded 0.4 ng/mL just before HCC was detected (data not shown). Periodic measurements of the HGF level may be of clinical significance. Therefore, HGF may be a third tumor marker in HCC development, along with AFP and PIVKA-II levels. Platelet counts are also a significant risk factor.

Previous reports argue whether cancer cells express HGF. Hu et al. reported that high serum concentrations of HGF in patients with HCC do not result primarily from HGF synthesis by the tumor cells. However, Bilezikci et al. concluded that hepatoma cells express HGF. Although our study does not directly address this issue, our findings offer some evidence that cancer cells express HGF. In noncancerous tissues of livers with HCC, HGF was localized in cells of mesenchymal origin and in hepatocytes with strong atypia. In cancerous regions, HGF was observed in infiltrating mesenchymal and cancer cells. In the ultrastructural findings, HGF was localized in the ER of cancer cells. This observation, in conjunction with the finding that the serum HGF level increased with increases in the size of spaces occupied by tumors, suggests that HGF is produced by cancer cells. Elevated serum HGF concentrations in patients with HCC over that in patients without HCC are probably due to the production of HCC by infiltrating mesenchymal and cancer cells. The observation that HGF did not distribute diffusely in cancerous foci, but congregated in cancer cells in some restricted regions, is not inconsistent with the fact that the serum HGF level varied from 0.3 to 1.0 ng/mL among patients. We found that HGF concen-
Concentrations were higher in patients with diffuse carcinomas or with two or more cancerous foci than in patients with a single cancerous focus. In addition, our results indicate that the HGF level was reduced after TAE therapy for tumors, but was elevated thereafter to a concentration similar to that before treatment. Together, our findings suggest that the evaluation of the HGF level in CH or cirrhosis patients may have clinical significance. The serum HGF level represents the degree of high carcinogenic state in the liver and patients with HGF concentrations above 0.31 ng/mL have a high risk of developing HCC. The probability of the presence of HCC is high in patients with HGF concentrations greater than 0.6 ng/mL. Often, patients with HGF concentrations greater than 0.8 ng/mL have an ectopic recurrence of HCC in the liver or a diffuse infiltration of cancer cells. When the HGF concentrations were monitored throughout the course of treatment, the prognoses were unfavorable in patients whose high HGF concentrations persisted after treatment. Therefore, the evaluation of the serum HGF concentration predicts the prognoses of HCC patients after treatment.

There is an important technical issue concerning the evaluation of HGF. HGF is produced not only by the liver, but also by the spleen and the bone marrow. When patients with cirrhosis are in highly carcinogenic states, the functions of the spleen increase because of splenomegaly. Therefore, the serum HGF level may be elevated in these patients as a result of overproduction of HGF by the spleen. To examine this possibility, we calculated the volume of the spleen in 10 patients with cirrhosis from data obtained by imaging and compared the assessed sizes of the spleen with serum HGF concentrations. No correlations were observed between the volume of the spleen and the HGF level ($r = 0.321$, $P = 0.3421$). This result suggests that the serum HGF level represents the amount of HGF produced in the liver.

Some authors have reported contradictory effects of HGF, namely, inhibitory, $^8$–$^{11}$ as well as facilitatory effects, $^{12}$–$^{15}$ on the generation of cancers. Serum concentrations of HGF and its expression in the liver of rats with chemically induced cancers have been examined.$^{24,25}$ HGF was expressed in hepatocytes with strong atypia. By analyzing the base sequence of HGF genes, we detected the presence of a variant of HGF lacking an amino acid residue. On the basis of these findings, we presumed that the variant HGF, which coexists with HGF of the consensus sequence, facilitates the generation of hepatocytes with strong atypia, induces their irregular regeneration, and leads to the development of HCC. In the current study, we found that the cumulative incidence of occurrence of HCC is high in patients with CH or cirrhosis and whose HGF concentrations were elevated. This finding is consistent with our hypothesis that, in livers with a highly carcinogenic state and prevalent irregular regeneration, increases in the paracrine production and secretion of HGF from mesenchymal cells facilitate the irregular regeneration of hepatocytes and, thereby, promote the development of cancer.

In conclusion, HGF may be a critical marker for emerging HCC and may possess facilitatory autocrine capability. The clinical application of HGF in the treatment of liver diseases should be examined with caution, considering the possibility that HGF may increase carcinogenesis in the liver.

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