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Significant Correlation between Serum Level of Hepatocyte Growth Factor and Progression of Gastric Carcinoma

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Abstract. Hepatocyte growth factor (HGF) can promote proliferation of many types of tumor cells including gastric cancer cells. To study the role of HGF in the progression of gastric carcinoma, HGF levels were measured by an enzyme immunoassay (EIA) system in sera of gastric cancer patients and followed up the levels after the operation. The mean serum HGF level in 212 healthy control subjects, 140 patients with primary gastric cancer, and 13 patients with recurrent gastric cancer were 0.199 ± 0.073, 0.325 ± 0.209, and 0.578 ± 0.258 ng/ml, respectively. The increase of the levels was significantly correlated with the progression of tumor stage. The levels decreased to normal levels 1 month after curative resection of the tumors. However, the levels did not decrease significantly in nonresected cases. During the follow-up of the patients for several months, the level was significantly increased in recurrent gastric cancer patients, whereas there was no increase in nonrecurrent patients. In conclusion, the serum HGF levels significantly correlated with the aggressiveness of the tumors, suggesting an important role of HGF in the progression of gastric carcinoma.

Hepatocyte growth factor (HGF), a peptide growth factor well known as a potent stimulator of hepatocyte growth [1], can promote proliferation, motility, morphogenesis, and angiogenesis in many types of cells including various tumor cells [2–4]. The first step in the initiation of HGF action is its binding to a specific cell surface receptor, the HGF receptor, encoded by the proto-oncogene c-*met* [5]. The proto-oncogene c-*met* has been shown to be amplified in gastric cancers [6], which suggests involvement of HGF signaling in gastric cancer formation, progression, or both. It has recently been reported that serum or tumor HGF was an independent prognostic factor in breast cancer patients [7, 8], indicating that an elevated serum HGF level is involved in the systemic progression of breast cancer. Also, pleural effusion samples obtained from patients with lung cancer and various types of malignant disease contained high levels of HGF [9]. Therefore HGF, which plays a crucial role in tumor invasion, may have prognostic value for several malignancies. In our previous report [10], we showed the serum HGF levels in gastric cancer and hepatoma patients to be significantly higher than those of healthy controls. These observations led us to investigate the value of HGF in the progression of gastric carcinoma. We studied the HGF levels in sera of gastric cancer patients, compared them with those in 212 healthy controls, and evaluated the correlation of the levels of HGF with the tumor stages. Furthermore, we followed up the serum HGF level to determine if its changes are dependent on changes of the tumor burden after resection of the tumor.

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Patients and Methods

Patients and Healthy Controls

A series of 140 gastric adenocarcinoma patients, who were documented by endoscopic biopsy, were enrolled in this study. Serum samples were drawn from these patients 1 or 2 days before operations. All of the patients were treated at Ajou University Hospital during the period of December 1995 to April 1998. The average age of the patients was 54.0 ± 12.8 years (range 25–78 years), including 97 men and 43 women. No patient had received chemotherapy or radiation therapy before surgery. Patients consisted of 29 stage I patients, 14 stage II, 39 stage III, and 58 stage IV, according to the revised TNM classification by the International Union Against Cancer (UICC), fifth edition [11]. Altogether 117 patients underwent curative operations, and 23 underwent palliative operations. Curability of gastric resection was defined according to the General Rules for the Gastric Cancer Society, 12th edition, by the Japanese Research Society for Gastric Cancer [12]. Curative resection means that there is no residual tumor with high probability of cure. Control sera were obtained from 212 healthy persons who were proved healthy by routine checks, such as serologic examination, urinalysis, chest radiography, electrocardiography, and abdominal ultrasonography at Korea Health Care Center in Suwon, Korea. Serum HGF levels of 61 patients (54 curative resection, 7 nonresection due to unresectability) available 1 month postoperatively and 21 patients from the above 61 patients available at 6 months postoperatively were rechecked. When recurrence was confirmed by diagnostic imaging (computed tomography or ultrasonography), cytology, biopsy, or surgery, the levels were rechecked. The sites of recurrence were liver, peritoneal cavity, lymph node, and bone. Because the serum HGF levels were elevated in patients with liver disease [13, 14],

Table 1. Serum HGF levels and clinical stages of patients with primary gastric cancer.

Subject	No. of cases	Mean ± SD (ng/ml)	Range (ng/ml)	Median (ng/ml)	*p*
Healthy control	212	0.199 ± 0.073	0.077–0.461	0.179	
Patients	140	0.325 ± 0.209	0.001–1.050	0.287	*
Stage					
I	29	0.190 ± 0.131	0.001–0.471	0.180	*
II	14	0.205 ± 0.120	0.001–0.383	0.224	**
III	39	0.280 ± 0.149	0.052–0.613	0.264	*
IV	58	0.451 ± 0.223	0.109–1.050	0.403	*

There was a significant difference in levels of serum HGF between healthy controls and patients with primary gastric cancer by independent Student's *t*-test. The levels of serum HGF in stage III were significantly higher than those of HGF in stage I; and the levels in stage IV were significantly higher than the levels in stages I, II, and III. The levels of serum HGF in stages III and IV were significantly higher than the levels of healthy controls, $^{*}p < 0.001$; $^{**}p < 0.05$.

patients with hepatitis or liver cirrhosis who were confirmed by serologic test or liver biopsy were excluded from this study.

Methods

Venous blood samples were drawn into tubes preoperatively, coagulated blood was centrifuged at 400 × *g* for 10 minutes, and the supernatants were stored at −70°C. The concentration of HGF was determined by an enzyme immunoassay (EIA) system using an Immunis HGF EIA kit (Institute of Immunology, Japan). A specific sandwich method with a mouse monoclonal antibody labeled with peroxidase was used in this system. The HGF concentration in samples was determined by the calibration curve prepared using the HGF standard solution.

Statistical Analysis

Student's *t*-test was used for analyses of unpaired samples, and the paired *t*-test was used when samples were paired. Values of $p < 0.05$ were considered statistically significant.

Results

Preoperative Serum HGF Levels

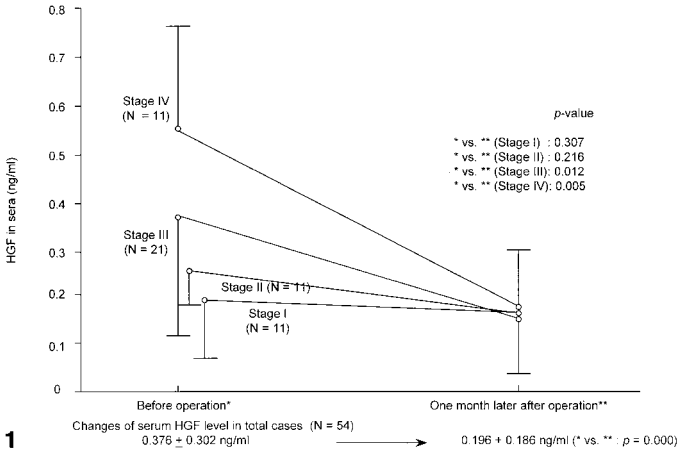
As shown in Table 1, the serum HGF concentration of 140 patients with gastric cancer was 0.325 ± 0.209 ng/ml (mean ± SD) (range 0.001–1.050 ng/ml), which was significantly higher than that of 212 healthy controls (0.199 ± 0.073 ng/ml, range 0.077–0.461 ng/ml) by Student's *t*-test ($p = 0.000$). The average HGF level for male patients was 0.299 ± 0.194 ng/ml and for female patients 0.297 ± 0.171 ng/ml (male vs. female, $p > 0.05$). Altogether 11 (5.2%) of 212 healthy controls and 52 (37.1%) of 140 patients had serum HGF levels higher than the cutoff value of 0.350 ng/ml. Above the cut-off value was defined as mean plus two standard deviations in healthy controls. The serum HGF levels in stage III and IV patients were significantly higher than those in healthy controls ($p = 0.000$). The serum HGF levels in stage III were significantly higher than those in stage I ($p < 0.05$), and the levels in stage IV were significantly higher than those in stage I, II, and III ($p = 0.000$).

Changes in Serum HGF Levels after Tumor Resection

We determined the serum HGF levels in 54 patients 1 month after curative resection of the tumors. Regardless of the original stages of the tumors, the mean level decreased significantly to as low as 0.196 ± 0.186 ng/ml after the curative resection, which was similar to the level of the healthy control (preoperative vs. postoperative level, $p = 0.000$ by paired *t*-test) (Fig. 1). Although no significant decrease was observed in stage I and II patients, the patients in stage III and IV had significantly decreased amounts of serum HGF after resection of the tumors ($p < 0.05$). In seven cases whose primary tumors could not be removed due to unresectability (five gastrojejunostomy only, two open and closure), six patients' levels increased. Hence we could find no significant decrease in the serum HGF levels in patients who did not undergo resection of the tumors ($p > 0.05$) (Fig. 2).

Serum HGF in Patients with Recurrence

We determined serum HGF levels in 13 patients with recurrent gastric cancer after curative resection (mean follow-up period 11.6 ± 5.7 months; range 4–24 months). These 13 patients included five with liver metastases, four with peritoneal metastases, three with bone metastases, and one with lymph node metastasis. All metastases were confirmed by computed tomographic (CT) scan or reexploration. Of the 13 patients, 10 (76.9%) exhibited HGF levels higher than the cutoff value. There were no differences in serum HGF levels according to site of recurrence. The mean serum HGF level of these patients was 0.578 ± 0.258 ng/ml, which was significantly different when compared with HGF levels of healthy controls and of patients with primary gastric cancer ($p = 0.000$, respectively) (Fig. 3). We also checked the serum HGF levels 6 months after the operations in 21 patients whose levels had been checked preoperatively and 1 month postoperatively. At 6 months after operation we confirmed no evidence of recurrence in these 21 patients by physical examination, diagnostic imaging (CT or ultrasonography), and serum levels of tumor markers. We also checked the serum HGF level at the time of recurrence (time from operation to recurrence 4–12 months) in another six patients, whose levels had been checked before and 1 month after operation. Although the levels did not show any significant change in nonrecurrent cases from 1 month to 6 months after operation ($p > 0.05$) (Fig. 4), the levels in six



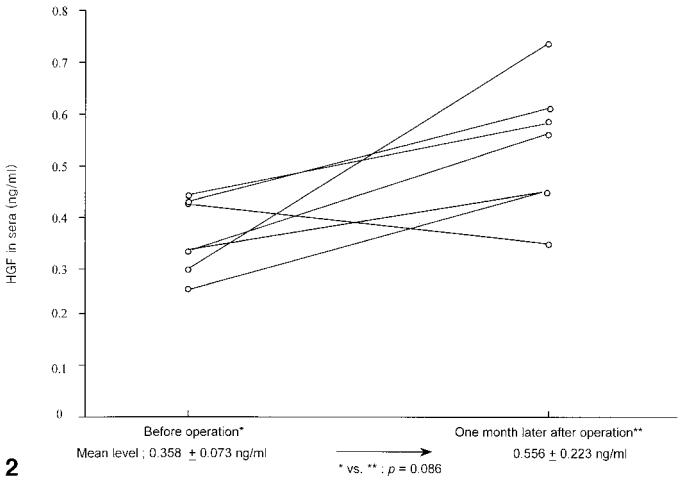
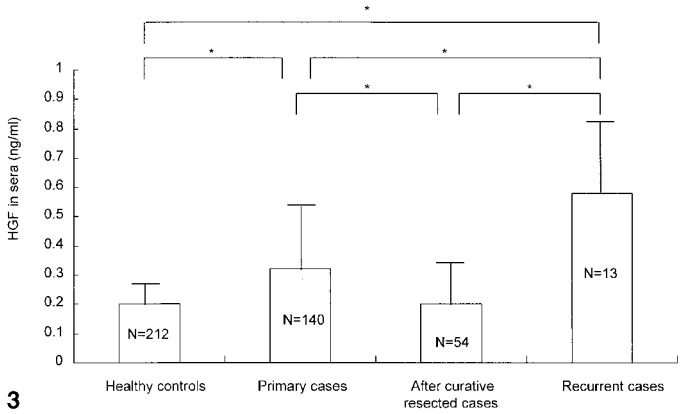
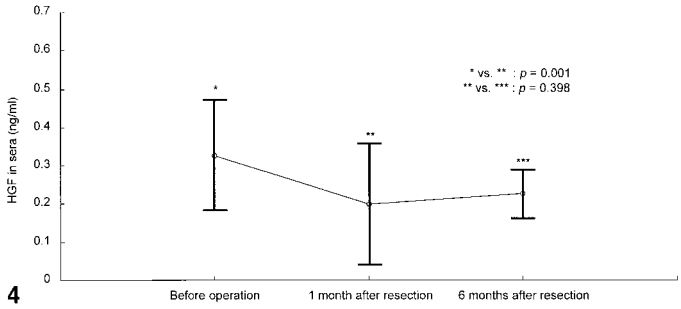


Fig. 1. Changes in serum HGF levels at various stages 1 month after curative resection ($n = 54$). There was a significant decrease in serum HGF level after resection of the tumor in stages III and IV by paired *t*-test.

Fig. 2. Changes in serum HGF levels in patients whose tumors were not resected owing to unresectability ($n = 7$). There was no significant change in serum HGF levels at 1 month after the operations by paired *t*-test.





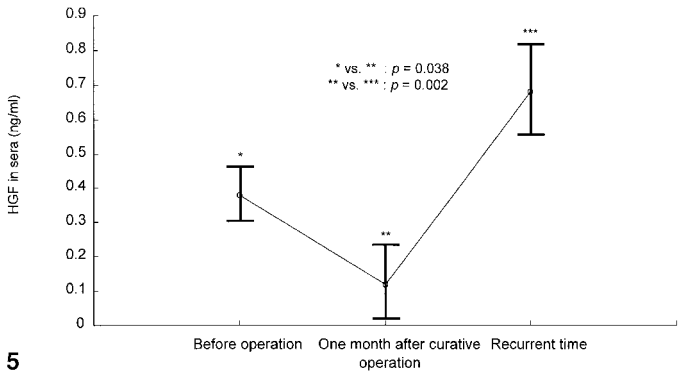


Fig. 3. Serum HGF level in healthy control subjects ($n = 212$), patients with primary gastric cancer ($n = 140$), patients with curative resection ($n = 54$), and patients with recurrent disease ($n = 13$). *$p = 0.000$ by independent Student's *t*-test.

Fig. 4. Serial follow-up of serum HGF levels after resection of tumors in patients who proved to have no recurrence ($n = 21$). There was a significant decrease in the serum HGF levels 1 month after tumor resection compared with the levels before operation by paired *t*-test. There was no significant change between the levels at 1 month and 6 months after resection.

Fig. 5. Serial follow-up of serum HGF levels after resection of tumors in patients who proved to have recurrences ($n = 6$, recurrence time 4–24 months). There was a significant decrease in the serum HGF levels 1 month after resection of the tumor by paired *t*-test. There was a significant increase in the levels at the time of recurrence compared with levels 1 month after resection.

patients with recurrence showed a significant increase at the time of recurrence comparing with their levels at 1 month after operation ($p = 0.002$) (Fig. 5).

Discussion

Hepatocyte growth factor was first identified as a potent mitogen for hepatocytes [1] and has recently been shown to be identical to the scatter factor [15]. It is mainly a paracrine factor produced by cells of mesenchymal origin and acts on epithelial cells [16], although autocrine activities have also been described [17]. As for the role in gastric cancer progression, Shibamoto et al. [18] showed that HGF significantly promoted the growth of gastric adenocarcinoma MKN-74 cells in a dose-dependent manner. Thus it is highly likely that HGF is involved in the progression of gastric cancer.

In this study, we evaluated the significance of the serum HGF level in gastric cancer patients. Although the mean serum HGF level was significantly higher than that of healthy controls ($p < 0.001$), we could not find a role for HGF as a useful tumor marker for the early diagnosis of gastric carcinoma because of low sensitivity (above the cutoff value = 37.1%). We found that the serum HGF levels correlated significantly with the progression of the stage of the tumors. Hence there was a direct correlation between the serum HGF level and the viable tumor burden. We also found

a significant decrease in the serum HGF level by 1 month after curative resection of the tumors and no increase during the follow-up 6 months later. Furthermore, the levels at 1 month after operation in those patients who underwent bypass or open and closure showed no significant changes compared with their levels preoperatively. The serum HGF levels showed significant increases at the time of recurrence compared with the levels at 1 month after resection of the tumor in the same patients. Furthermore, the average serum HGF level in patients with recurrent gastric cancer was significantly higher than that in healthy controls or in the patients with primary disease.

All of the above findings suggest that HGF may have a role as an ideal tumor marker for the follow-up of gastric cancer patients after operation. According to our data, the serum HGF levels in gastric cancer patients with stage I or II disease were not significantly different from those of healthy controls and did not decrease significantly after resection of the tumor. In contrast, the levels in patients with stage III and IV disease were significantly higher than those of healthy controls and decreased significantly after resection of the tumor. That is, the amount of HGF produced from tumors at an early stage was too small to be detected in serum; and the serum HGF level was increased in advanced-stage disease, which might need HGF to progress.

While our study was in progress there appeared a report [19] on the HGF level in gastric cancer patients whose results were similar to our findings. The authors demonstrated a significant increase in the serum HGF level in 104 gastric cancer patients compared to 89 healthy controls. In contrast, we checked the levels of serum HGF after removal of the tumors as well as the preoperative levels and followed up the levels until recurrences appeared. Therefore, we believe that our study provides more in-depth information about the significance of HGF as a tumor marker for follow-up of the gastric cancer patients. Further prospective studies on a large number of patients with a longer follow-up are necessary to establish its clinical usefulness.

Résumé

Le facteur de croissance des hépatocytes (HGF) peut promouvoir la prolifération de plusieurs types de cellules tumorales dont les cellules gastriques. Afin d'étudier le rôle du facteur HGF dans la progression de cancer gastrique, on a mesuré les taux de HGF par dosage enzymatique immunologique (EIA) dans le sérum de patients atteints de cancer gastrique et suivis en postopératoire. Les taux sériques moyens de HGF ont été, respectivement, de 0,199 +/− 0,073 ng/ml, de 0 +/− 0,209 ng/ml, et de 0.578 +/− 0,258 ng/ml chez 212 sujet sains, 140 patients ayant un cancer gastrique primitif, et 13 patients ayant une récidive de cancer gastrique. L'augmentation de ces taux corrélait de façon significative avec le stade tumoral. Les niveaux se sont abaissés un mois après la résection de ces tumeurs à visée curative. Cependant, les taux n'ont pas diminué de façon significative lorsque la tumeur n'a pu être réséquée. Pendant le suivi de plusieurs mois, les taux ont augmenté de façon significative en cas de récidive du cancer gastrique, alors que ces taux sont restés stables en l'absence de récidive. En conclusion, les taux sériques de HGF sont corrélés de façon significative avec l'agressivité de ces tumeurs, suggérant un rôle important de HGF dans l'évolution du cancer gastrique.

Resumen

El factor de crecimiento hepatocítico (HGF) puede estimular la proliferación de diversos tipos de células cancerosas, incluidas las gástricas. Con objeto de estudiar el papel del HGF en el desarrollo del cáncer gástrico, determinamos los niveles séricos de HGF [utilizando un inmuno-ensayo enzimático (EIA)] en el pre y postoperatorio de pacientes con cáncer gástrico. Se determinó el nivel sérico de HGF en 212 sujetos sanos (grupo control), en 140 pacientes con cáncer gástrico primario y en 13 enfermos con cáncer gástrico recidivado, obteniéndose los siguientes resultados: 0,199 ± 0,073 ng/ml, 0 ± 0,209 ng/ml y 0,578 ± 0,258 ng/ml. El incremento de los niveles séricos se correlacionó significativamente con la progresión del estadio tumoral. Los niveles de HGF retornaron a límites normales al mes de haberse realizado una resección curativa del cáncer gástrico; por el contrario, dichas tasas no disminuyeron significativamente en los casos en que el tumor gástrico no fue resecado. En el seguimiento, durante varios meses, de estos enfermos, observamos que los niveles séricos de HGF aumentaban significativamente en los casos de recidiva del cáncer gástrico; estos incrementos no se produjeron en pacientes sin recidiva. Conclusión: Los niveles séricos de HGF guardan una significativa correlación con la agresividad del tumor, hecho que indica el importante papel que desempeña el HGF en la progresión del carcinoma gástrico.

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