Significance of Circulating Hepatocyte Growth Factor Level as a Prognostic Indicator in Primary Breast Cancer

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ABSTRACT

The circulating hepatocyte growth factor (HGF)/scatter factor level is frequently increased in advanced cancer patients. In this study, we have assessed the prognostic value of the circulating HGF level determined by enzymatic immunoassay in primary breast cancer patients. Of 200 primary breast cancer patients, 54 (27.0%) showed the increase of serum HGF level according to the age-matched cutoff values. The prognosis of the patients with the increased HGF level was statistically worse than that of the patients with normal HGF level (P = 0.0001, log-rank test). Multivariate analysis confirmed that the increase in HGF level was an independent prognostic indicator in primary breast cancer patients. In the background analysis, the increase in serum HGF level was significantly associated with tumor size, nodal status, and histological evidence of venous invasion. The data indicate that up-regulation of the circulating HGF level may predict systemic tumor spread and early relapse in primary breast cancer patients.

INTRODUCTION

Growth factors are known to function as autocrine and/or paracrine growth substances for solid tumor growth (1). However, recent investigations have indicated the importance of circulating growth factors in tumor progression. For instance, sialoadenectomy, removal of the submandibular gland which is a main source of epidermal growth factor in mice, markedly inhibited mouse tumor growth by reducing the circulating epidermal growth factor level (2). In addition, recently it was demonstrated that several types of intrinsic endothelial growth inhibitors appear in the systemic circulation and can inhibit tumor growth in mice xenograft models (3). In human cancer, the circulating levels of insulin-like growth factors, basic fibroblast growth factor, TGF-α, TGF-β, and HGF have been reported to be occasionally elevated, particularly in patients with advanced disease (4–8). These findings seem to suggest that the alternation of the circulating growth factor level, probably originating from cancer tissues, might play important roles in the regulation of solid tumor growth.

HGF was first identified as a potent stimulator of hepatocyte growth and DNA synthesis. Later, its pleiotropic functions for a variety of cell types, including epithelial tumor cells and endothelial cells, were characterized (9–11). Cell scatter activity of HGF was identified in several types of tumor cells possessing the HGF receptor c-met (12). In the endothelium, HGF was demonstrated to induce chemotactic activity and cell proliferation in vitro and in vivo (13, 14). These data suggest that up-regulation of the production or activation of HGF may promote not only tumor cell spread but also neovascularization.

In human breast cancer, HGF protein concentrations were significantly higher in tumor tissues than in adjacent normal tissues by biochemical EIA (15). On the other hand, immunocytochemical analysis showed that only stromal cells expressed HGF, but tumor cells and duct epithelial cells expressed c-met in breast cancer tissues (16). In addition, HGF expression was identified in few cultured breast cancer cell lines in vitro (17), which indicates that HGF plays a paracrine role in breast cancer tissues.

Recently, we have found that the serum HGF level is often elevated in breast cancer patients, particularly in patients with distant metastases (8). The aberrant increase of the serum HGF level was significantly associated with axillary lymph node metastasis and histological evidence of venous invasion. On the other hand, Yamashita et al. (15) reported the importance of the intratumoral HGF level as a prognostic indicator in primary breast cancer patients. In this study, we have investigated the prognostic value of the increase of the circulating HGF level in primary breast cancer patients. Because HGF can act on a variety of epithelial tumor cells, the results from this study will be informative for many types of other cancer.

PATIENTS AND METHODS

Serum HGF levels were determined in 200 primary breast cancer patients, 80 recurrent breast cancer patients, and 17 patients with benign breast diseases including 13 fibrocystic diseases, 3 fibroadenomas, and 1 phyllodes tumor who were treated in Tokyo Metropolitan Komagome Hospital from 1990 to 1994. Primary breast cancer patients were consecutively enrolled in this study. The average age of the primary breast cancer patients was 50.9 years of age (range, 21–78 years).
Table 1  Serum HGF levels in healthy controls*  
<table>
<thead>
<tr>
<th>Age group</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>Average + 2SD (ng/ml)</td>
</tr>
<tr>
<td>10s</td>
<td>18</td>
</tr>
<tr>
<td>20s</td>
<td>22</td>
</tr>
<tr>
<td>30s</td>
<td>21</td>
</tr>
<tr>
<td>40s</td>
<td>21</td>
</tr>
<tr>
<td>50s</td>
<td>18</td>
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*Correlation (t test). Female: 20s versus 50s (P = 0.0003), 30s versus 50s (P = 0.0003), 10s versus 50s (P = 0.0009), 40s versus 50s (P = 0.0092), 20s versus 40s (P = 0.0631). Male: 20s versus 50s (P = 0.0051), 20s versus 40s (P = 0.0102), 10s versus 50s (P = 0.0998), 10s versus 40s (P = 0.0870).

Patients with liver dysfunction due to any hepatitis B and C virus infection were excluded from this study. Clinical tumor stage and histological status were assessed according to the criteria of the Japanese Breast Cancer Society, which is based on the Union International Contre Cancer criteria. All primary breast cancer patients had either mastectomy or partial mastectomy with axillary node dissection. One-hundred seventy patients received adjuvant treatments including 56 patients on poly-chemotherapy. Chemotherapy included 40 patients on doxorubicin-containing combination, 20 on cyclophosphamide + 5FU/5FU derivatives, and 64 on single-agent chemotherapy. Chemotherapy was given to node-positive patients for 6 months, single chemotherapy was given for 6–24 months, and TAMB was given for more than 2 years. The indication and the protocol of adjuvant therapy were decided independently from any information on the serum HGF data. Postoperative physical examinations were performed every 2 months for all of the patients. Radiographic studies, including chest X-ray, liver ultrasonography, and bone X-ray or bone scintigraphy, were performed every 12 months or earlier, if clinically indicated. Hematological tests and the monitoring of tumor markers, including carcinoembryonic antigen and CA15-3, were performed every 3 months.

For the assessment of the normal HGF level in sera, the HGF level was examined in 205 age-adjusted healthy volunteers (15–59 years of age) including 105 men and 100 women who had no abnormality in their histories or in their laboratory tests, which included: blood counts, hemoglobin, liver function (transaminases, γGTP, lactate dehydrogenase, and alkaline phosphatase), kidney function (blood urea nitrogen and creatinine), cholesterol levels (low-density lipoprotein and high-density lipoprotein cholesterol), immunoglobulin levels, and the tests for hepatitis B and C. To determine the cutoff value in breast cancer patients, the average ± 2 SD of each generation in the healthy women’s data were used. For women >60 years, the cutoff value of the 50s age group was used.

Eighty recurrent breast cancer patients included 33 soft-tissue recurrences, 31 visceral recurrences, including 22 liver metastases and 9 others, and 16 bone recurrences. Liver, lung, and distant lymph node metastases were diagnosed by computed tomography scan, and bone metastases were diagnosed by X-ray and bone scintigraphy.

Venous blood samples were drawn into a tube and centrifuged at 3000 rpm for 10 min, and the serum samples were stored at −20°C until the determination of HGF level. Pieces of primary tumors were immediately frozen and stored at −80°C until various examinations were made.

The HGF level in the sera was determined by the HGF-EIA kit (Institute of Immunology, Tokyo, Japan) as described previously (8, 18). A specific sandwich method using a mouse monoclonal antibody to recombinant human HGF and mouse monoclonal antibodies labeled by peroxidase was used in the EIA system. Diluted sera (4×) was used for the measurement of HGF. The standard curve of HGF was linear in a plot at the concentration from 0.075 to 1.6 ng/ml (data not shown). When the sample was >1.6 ng/ml, 2× diluted sample was used for the determination.

Histological assessments were performed by two pathologists who had no information on the HGF levels. Histological lymphatic spread and venous spread were graded as −, +, and ++, respectively (19). ER and progesterone receptor were measured by the dextran-coated charcoal method using [3H]17β-estradiol or by the EIA method, and tumor with >5 fmol/mg protein was determined as positive.

The Kruskal-Wallis ANOVA, Student’s t test, and χ² test were used for assessing the relationship between the serum HGF levels and the various clinicopathological factors. Relapse-free survival curves were drawn by means of the Kaplan-Meier method, and the difference among the curves was analyzed by log-rank test (20). The relative risk for each prognostic indicator was evaluated by Cox’s proportional hazards regression model (21). The included factors for the prognostic analyses were menopausal status, age, nodal status, tumor size, serum HGF level, ER, progesterone receptor, and adjuvant therapy. Multivariate analysis was performed for the factors that provided P > 0.2 in the relative risk by the initial univariate analysis. The relationship between the prognostic indicator and the relapse-free survival rate was assessed.

RESULTS

In the healthy control, the serum HGF level decreased in proportion to aging with a peak in the 20s age group, as shown in Table 1. Particularly, the HGF level markedly dropped in the 50s, both in women and men. The age-adjusted upper limits (average ± 2 SD in each generation, as listed in Table 1) were used for the evaluation of the serum HGF level.

The serum HGF level in 200 primary breast cancer patients ranged from 0.075 to 2.312 ng/ml (average ± SD, 0.380 ± 0.31 ng/ml; Fig. 1). Of 200 patients, 54 (27.0%) showed an increase in the serum HGF level according to the age-matched cutoff values. Of 17 benign breast disease patients, only one case, who had a 4-cm-sized fibroadenoma, showed an increase in the serum HGF level. All of the 10 cases who had no evidence of recurrence after surgery showed a normal HGF level (Fig. 2). Background factor analysis demonstrated that the increase in the HGF levels in the sera was significantly associated with menopausal status, tumor size, number of axillary nodal metastases,
and histological evidence of venous invasion (Table 2). However, no significant correlation was detected with hormone receptor status.

In the univariate analysis, the patients with the increase in serum HGF levels showed significantly poor prognosis compared with those with normal HGF levels ($P = 0.0001$, log-rank analysis; the median follow-up period was 42 months; Fig. 3). There was no significant difference in the types of drugs or duration of adjuvant therapy between the two groups. The serum HGF level was a significant prognostic indicator in node-positive patients ($P = 0.0096$, log-rank analysis; Fig. 4). In node-negative patients, patients with high-serum HGF levels also showed a worse prognosis than those with normal HGF levels ($P = 0.050$, log-rank analysis). Among the factors examined by univariate analysis (nodal status, serum HGF level, tumor size, ER, and presence or absence of adjuvant endocrine therapy), $P < 0.2$ was achieved (Table 3). Then, a multivariate analysis was performed (Table 4). In the multivariate analysis, HGF, nodal status, ER, and tumor size were significant ($P < 0.05$).

**DISCUSSION**

The increase of the circulating HGF level was significantly correlated with tumor size, nodal metastasis, and histological evidence of venous invasion, which are well-known conven-
The present study showed that an increase in the serum HGF level is a novel significant indicator of poor prognosis in primary breast cancer patients. In both node-positive and node-negative patients, the circulating HGF level was a significant prognostic indicator. Particularly, it was a potent factor in node-positive patients. Multivariate analysis confirmed that the increase in the serum HGF level was an independent prognostic indicator as potent as nodal status. In addition, in patients with clinically confirmed distant metastases, 60% of the cases showed an elevation of the serum HGF level. These findings strongly suggest that the up-regulation of the circulating HGF level is closely associated with systemic tumor spread and with early relapse in primary breast cancer patients.

HGF is noted to be involved in carcinogenesis. A recent study reported that cotransfection of HGF and c-met was capable of inducing morphological transformation in vitro and tumorigenicity in vivo in a nontumorigenic mouse cell line C127 (24). In the bladder cancer cell line NBT-II,
transfection of HGF increased the invasive phenotype and growth rate of these cells (25). Furthermore, the overexpression of c-met has been detected in various types of carcinomas including stomach, pancreas, and bladder cancer (26–28). On the other hand, recent investigations indicated an activity of HGF as a potent endothelial growth factor, suggesting that HGF can stimulate not only tumor cell invasion but also neovascularization. In breast cancer, HGF is known to function in a paracrine manner, because human cultured breast cancer cells are present to express the HGF receptor, c-met, but are unlikely to produce HGF by themselves (16, 17). HGF can induce a tube-like formation in cultured breast cancer cells T47D (29). Huguet et al. (30) reported that HGF is also involved in the down-regulation of Wnt5a, which has a function in the development of the normal mammary gland in cultured breast cancer cells. Furthermore, the intratumoral HGF level determined by EIA was an independent prognostic indicator in primary breast cancer (15). Therefore, our present data seem to be compatible with the accumulated fundamental evidence showing crucial roles for HGF in breast cancer progression.

However, still little is known about the mechanism of how the circulating HGF level is up-regulated in cancer patients. The removal of the main tumor can provide a marked drop in the elevated serum HGF level (8). The effect of anticancer treatment is also able to down-regulate the serum HGF level (data not shown). However, we failed to prove the direct correlation between increased serum HGF levels and intratumoral HGF protein concentrations. Because several types of proteases, such as plasminogen activators and heparitinase, can release and activate HGF from its precursor form or extracellular matrix-bound forms (31–33), the activities of these proteases might be important in the release of HGF into the systemic circulation. Venous hepatic injection is also possible to elevate serum HGF level transiently (34).

In fulminant hepatic failure patients, HGF from the serum was biologically active because it stimulated DNA synthesis in cultured rat hepatocytes (35). Pleural effusion fluids from cancer patients showed cell scatter activity (36). In fact, using MDCK cells, we also confirmed the induction of morphological changes and cell scatter activities of semipurified HGF from the pleural effusion of recurrent cancer patients (data not shown).

The increase in the serum HGF level was found not only in breast cancer patients but also in other types of neoplasms including gastric cancer, lymphomas, and leukemias (37). On the other hand, as for HGF, several growth factors including TGF-α, basic fibroblast growth factor, and vascular endothelial growth factor and TGF-β are known to be up- or down-regulated in the circulation of cancer patients (5–7, 38). The alternations of the circulating growth factor or cytokine levels might be common events in cancer progression. In conclusion, the determination and monitoring of the serum HGF level seem to be of value for predicting systemic tumor spread and prognosis.

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References


Significance of circulating hepatocyte growth factor level as a prognostic indicator in primary breast cancer.


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