Serum Concentrations of Hepatocyte Growth Factor in Breast Cancer Patients

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ABSTRACT

Hepatocyte growth factor (HGF) was first identified as a potent stimulator of hepatocyte growth and DNA synthesis. Later, it was shown that HGF can promote cell motility and cell proliferation in various types of cells, including tumor cells and endothelial cells. We have examined serum concentrations of HGF in breast cancer patients using an ELISA. Of 134 primary breast cancer patients, 49 (36.6%) showed a significant increase in the circulating level of HGF as compared to healthy controls. The increase in the HGF level was significantly associated with axillary lymph node metastases and histological evidence of venous invasion. No significant correlation between serum HGF concentrations and intratumoral HGF concentrations was found; however, the removal of the primary tumor clearly decreased the serum HGF level, suggesting that the elevation of HGF in the serum was tumor related. Twenty-nine (82.9%) of 35 patients with recurrent breast cancer had an increase in the serum HGF level. The HGF level was significantly higher in patients with liver metastases compared to those with other sites of metastases. Postoperative sequential examinations confirmed that the increase in the serum HGF level was associated with the appearance of relapse. In conclusion, the serum HGF level was significantly increased in breast cancer patients. Circulating HGF might play important roles in tumor progression in this malignancy.

INTRODUCTION

HGF was first identified in the serum of heptatectomized rats as a potent growth factor for hepatocytes (1). Independently, scatter factor, which stimulates epithelial cell motility, was isolated from the media conditioned by cultured fibroblasts (2). Subsequent molecular analyses confirmed that two factors are identical (3). HGF/scatter factor is now known to be a pleiotropic growth factor not only for hepatocyte but also for various types of cells, including tumor cells and endothelial cells (4, 5). HGF can promote cell motility, invasiveness, and metastatic phenotype in a variety of tumor cells. In a recent clinical investigation, it was documented that the level of intratumoral HGF concentration was an independent prognostic indicator in primary breast cancer patients (6). In addition, an angiogenic activity of HGF was identified (7). These data indicate that HGF may play important roles in tumor growth and tumor angiogenesis.

It has also been observed that serum HGF concentrations are increased in patients with liver diseases like hepatitis and in heptatectomized patients (8–11). Biochemical studies showed that serum HGF in these patients was biologically active. Little is known, however, about serum HGF levels in cancer patients. In the present study, we measured immunoreactive HGF concentrations in sera of patients with primary and recurrent breast cancer. These data show significant differences in the circulating level of HGF in breast cancer patients.

PATIENTS AND METHODS

Patients. One hundred thirty-four patients with primary breast cancer and 35 with recurrent disease, treated at the Tokyo Metropolitan Komagome Hospital from 1990 to 1993, were enrolled in this study. The average age was 50.9 (range, 21–88) years. Primary breast cancer patients consisted of 23 stage I patients, 80 stage II, and 31 stage III according to the criteria of the Japanese Breast Cancer Society, which is based on the UICC criteria. The 35 patients with recurrent breast cancer included 12 with liver metastases and 23 without liver metastases, of whom 11 had soft tissue recurrences, 7 bone metastases, and 5 lung metastases. Liver, lung, and distant lymph node metastases were diagnosed using computed tomographic scan, and bone metastases were diagnosed using X-ray and bone scintigraphy. Patients with liver dysfunction due to hepatitis B and C virus infection were excluded from this study.

Samples. 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 used for determination of HGF. Primary tumors were resected to small cubes from fresh specimens, immediately frozen with liquid nitrogen, and stored at −80°C until analyzed.

ELISA. The level of HGF in sera was determined by a HGF-ELISA kit (Institute of Immunology, Tokyo, Japan). A specific sandwich method with a mouse mAb to recombinant human hepatocyte growth factor and mouse mAbs labeled by peroxidase was used in this ELISA system. Four-fold diluted sera was used for the measurement of HGF. The standard curve of HGF showed linearity from 0.075 to 1.6 ng/ml (data not shown). No healthy control had a level of over 0.4 ng/ml HGF in their serum.

The extract of primary tumor tissues was prepared according to the method of Yamashita et al. (12). Briefly, frozen samples (0.2 g) were homogenized and extracted with 50 mM Tris-HCl buffer (2 ml), pH 7.4, containing 0.25% Triton X-100. The tissue extracts were appropriately diluted according to protein levels and then HGF levels in the extracts were determined by HGF-ELISA.
Serum HGF in Breast Cancer

Statistical analysis demonstrated a significant difference in the circulating level of HGF between primary cases (n = 134) and recurrent cases (n = 35; P < 0.01), between primary cases and recurrent cases without liver metastases (n = 23; P < 0.01), and between recurrent cases with liver metastases (n = 12) and two other groups (P < 0.01). Bars, SD.

**Histopathological Diagnosis.** Thin sections of about 5-μm thickness were cut from the surgical large sections with a microtome. These were mounted on large glass slides and stained with hematoxylin-eosin and elastica van Gieson. Venous invasion was defined as tumor cells filling the vein and appearing to adhere to the vein wall. Tumor cells in a lumen surrounded by endothelial cells without elastic fibers or smooth muscle were classified as lymphatic invasion.

**Microvessel Density and Hormone Receptors.** Intratumoral microvessels were identified by immunostaining using antihuman factor VIII-related antigen mAb (DAKO Japan, Tokyo, Japan), and microvessel density was evaluated as the count of endothelial deposits per mm² in the areas that were considered to be most active for neovascularization (13). The count was performed in three fields, and the average was calculated. These evaluations were done blinded.

**Hormone Receptors.** Estrogen receptor and progesterone receptor were measured according to the dextran-coated charcoal method using [1H]17β-estradiol. Tumors with more than 5 fmol/mg protein were defined as positive.

**Statistical Analysis.** The Student's t test was used for analyses of unpaired samples, and the paired t test when samples were paired.

**RESULTS**

The average level of HGF in the sera from patients with primary breast cancer was 0.41 ± 0.29 ng/ml (average ± SD). The highest HGF level was 2.31 ng/ml. Forty-nine (36.6% of 134) patients had more than a 0.4 ng/ml HGF level in the serum (Fig. 1). Background factor analyses demonstrated that the serum HGF level was significantly correlated with axillary lymph node metastases and intratumoral venous invasion (P < 0.05 and P < 0.05 by t test, respectively, Table 1). Twenty-two (44.7%) of 49 patients with 4 or more involved lymph nodes, and 14 (51.9%) of 27 patients with grade ++ venous invasion showed >0.4 ng/ml HGF. There was no significant correlation between serum HGF levels and menopausal status, tumor size, hormone receptors, and microvessel density. In serum samples collected 4–6 weeks after removal of the primary tumor by either mastectomy or partial mastectomy, the HGF levels were 0.41 ± 0.29 ng/ml (average ± SD). No significant correlation between serum HGF concentrations and tissue HGF concentrations was found (Fig. 3).

Of 35 patients with recurrent breast cancer, 29 (82.9%) exhibited HGF levels >0.4 ng/ml in the serum. The mean value

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**Table 1** Serum HGF levels and patients’ characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases</th>
<th>Average ± SD (ng/ml)</th>
<th>Positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menopause</td>
<td></td>
<td></td>
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<tr>
<td>Pre-</td>
<td>72</td>
<td>0.36 ± 0.17</td>
<td>33.3</td>
</tr>
<tr>
<td>Post-</td>
<td>62</td>
<td>0.47 ± 0.38</td>
<td>40.3</td>
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<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>23</td>
<td>0.41 ± 0.46</td>
<td>34.8</td>
</tr>
<tr>
<td>II</td>
<td>80</td>
<td>0.38 ± 0.24</td>
<td>30.0</td>
</tr>
<tr>
<td>IIIa</td>
<td>15</td>
<td>0.42 ± 0.20</td>
<td>53.3</td>
</tr>
<tr>
<td>IIIb</td>
<td>16</td>
<td>0.53 ± 0.29</td>
<td>56.3</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;2 cm</td>
<td>30</td>
<td>0.42 ± 0.41</td>
<td>33.3</td>
</tr>
<tr>
<td>2–5 cm</td>
<td>76</td>
<td>0.40 ± 0.26</td>
<td>34.2</td>
</tr>
<tr>
<td>5 cm&lt;</td>
<td>28</td>
<td>0.44 ± 0.25</td>
<td>46.4</td>
</tr>
<tr>
<td>No. of nodal metastases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>55</td>
<td>0.37 ± 0.19</td>
<td>34.5</td>
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<tr>
<td>1–3</td>
<td>30</td>
<td>0.40 ± 0.33</td>
<td>28.6</td>
</tr>
<tr>
<td>&lt;4</td>
<td>49</td>
<td>0.47 ± 0.36</td>
<td>44.7</td>
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<td>Estrogen receptor</td>
<td></td>
<td></td>
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<tr>
<td>–</td>
<td>27</td>
<td>0.46 ± 0.43</td>
<td>45.8</td>
</tr>
<tr>
<td>+</td>
<td>63</td>
<td>0.36 ± 0.16</td>
<td>26.7</td>
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<tr>
<td>++</td>
<td>44</td>
<td>0.46 ± 0.34</td>
<td>46.4</td>
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<tr>
<td>Progesterone receptor</td>
<td></td>
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<td></td>
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<tr>
<td>–</td>
<td>63</td>
<td>0.38 ± 0.28</td>
<td>30.2</td>
</tr>
<tr>
<td>+</td>
<td>54</td>
<td>0.39 ± 0.18</td>
<td>38.9</td>
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<tr>
<td>Vessel density</td>
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<tr>
<td>&lt;50</td>
<td>11</td>
<td>0.37 ± 0.15</td>
<td>45.5</td>
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<tr>
<td>51–100</td>
<td>21</td>
<td>0.42 ± 0.23</td>
<td>28.6</td>
</tr>
<tr>
<td>101–150</td>
<td>29</td>
<td>0.45 ± 0.25</td>
<td>41.4</td>
</tr>
<tr>
<td>&gt;150</td>
<td>12</td>
<td>0.53 ± 0.57</td>
<td>41.7</td>
</tr>
</tbody>
</table>

*The increase in the level of HGF was significantly associated with the status of metastatic nodes and with the grade of histological venous invasion.*

*NS, not significant; ly and v, histological evidence of lymphatic invasion and venous invasion in tumor, respectively.*
Fig. 2 Decrease in the circulating level of HGF before and after operation in patients with primary breast cancer. The mean value of the HGF level in sera of preoperative patients was 0.69 ± 0.23 ng/ml and that of postoperative patients was 0.33 ± 0.12 ng/ml. The difference was statistically significant (P < 0.05, paired t test).

Fig. 3 Correlations between HGF levels in sera and HGF levels in tumor tissue (pg/ml) in 43 primary breast cancer patients. No correlation was found.

Fig. 4 Changes in the circulating level of HGF between primary operation time and relapse time (nine patients). When the relapse appeared, HGF levels were significantly increased as compared to the primary operation time.

DISCUSSION

In this investigation, it was found that more than one third of primary breast cancer patients and more than 80% of those with recurrent disease had a significant increase in the circulating level of HGF. Since the serum HGF levels were <0.4 ng/ml in healthy controls, this increase in primary breast cancer patients seemed to be due to the presence of tumor. In fact, the removal of the primary tumor clearly decreased the serum HGF level in primary breast cancer patients, with the exception of one patient with distant metastasis at the time of primary surgery. Although the significance of circulating HGF elevated in cancer patients is uncertain, HGF from the sera of patients with fulminant hepatic failure stimulated DNA synthesis in cultured rat hepatocyte, although multiple forms of HGF with different molecular weights were present in the serum (14). HGF (0.4 ng/ml) can induce various effects on target cells, including tumor cells and endothelial cells. If the immunoreactive serum HGF in our patients is active, it might function as an endocrine growth factor for breast cancer. In a recent study, it was reported that the concentration of transforming growth factor α, which is a potent growth factor for breast cancer cells, was increased in the serum of breast cancer patients (15). Therefore, it is important to know the significance of endocrine functions of growth factors.

Basically, HGF is thought to be a stromally derived paracrine growth factor in breast cancer, because human cultured breast cancer cells express the HGF receptor, c-met, but not to produce HGF by themselves (16–18). In fact, human primary fibroblast cultures from the breast were demonstrated to express HGF mRNA (19). In the present study, large amounts of HGF were detected in breast cancer tissues. Several growth factors or cytokines including tumor necrosis factor α, interleukin 1, and transforming growth factor β are known to be responsible for the production of HGF in stromal cells (20). In addition, recent studies underlined the importance of release and activation mechanism of HGF from its precursor form or extracellular matrix-bound forms (21, 22). We also found a marked induction of serum HGF by heparin (23). Therefore, intratumoral matrix cells, including fibroblasts and endothelial cells, seem to produce or release the circulating HGF, although no significant correlation between serum HGF concentrations and tumor tissue HGF concentrations was found.
In the background analyses, the increase in the serum HGF level was significantly associated with markers representing the aggressiveness of the primary tumor. The HGF level was increased in patients with metastatic nodes and high-grade intratumoral venous invasion. Furthermore, the serum HGF level was frequently increased in breast cancer patients with recurrent disease. In particular, this increase was marked in patients with liver metastases. This also strongly suggests a close correlation between the appearance of HGF in sera and tumor progression. In a preliminary postoperative follow-up study, the serum HGF level was significantly elevated in association with relapse.

The monitoring of the serum HGF level might be useful as a tumor marker, particularly for the early detection of liver metastases. HGF levels seemed to be different in the spectrum for the detection of relapse from the level of carcinoembryonic antigen and CA 15–3. Evaluation of HGF in combination with these conventional tumor markers may be clinically useful.

Finally, it seems that the increase in the circulating level of HGF is significantly associated with tumor progression in breast cancer. The suppression and control of HGF action might have value as a treatment in breast cancer patients.

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Serum concentrations of hepatocyte growth factor in breast cancer patients.
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