Serum Levels of Hepatocyte Growth Factor in Patients with Breast Cancer

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Abstract

Objective: **Hepatocyte growth factor (HGF) has been reported the cause of many biological events, including cell proliferation, movement, invasiveness, morphogenesis, and angiogenesis. Elevated hepatocyte growth factor content in tumor tissue was reported to predict a more aggressive biology in non–small cell lung cancer patients. However, there is still limited knowledge about the role of HGF in breast cancer. This study was designed with the aim to elucidate the possible relationship between the preoperative circulating soluble HGF and breast cancer.**

Materials and Methods: **One hundred twenty-four consecutive patients with invasive breast cancer undergoing surgery were prospectively included and evaluated. Venous blood samples were collected before the surgery. Sera were obtained by centrifugation and stored at −70°C until assayed. The control group consisted of 35 patients with benign breast tumor (20 with fibrocystic disease and 15 with fibroadenoma). Serum concentrations of soluble HGF were measured by the quantitative sandwich enzyme immunoassay technique. The data on primary tumor staging, age, estrogen receptor status, lymph node status, distant metastases status, histologic grading, and tumor-node-metastasis (TNM) staging were reviewed and recorded.**

Results: **The mean value of serum soluble HGF in patients with invasive breast cancer was 529.05 ± 123.33 pg/mL and that of control group was 343.00 ± 31.03 pg/mL and the difference was significant ($P < 0.001$). Furthermore, there were significantly higher serum levels of soluble HGF in patients with negative estrogen receptor ($P = 0.035$), in patients with poorer differentiated tumor ($P < 0.001$), in patients with more advanced primary tumor staging ($P < 0.001$), in patients with more advanced lymph node status ($P < 0.001$), in patients with distant metastases ($P < 0.001$), and in patients with more advanced TNM staging ($P < 0.001$). In multivariate analysis by the multiple linear regression method, TNM staging ($P < 0.001$) seemed an independent factor regarding the significant higher serum levels of soluble HGF.**

Conclusion: **Patients with more advanced TNM staging were shown to have higher serum soluble HGF. Thus, preoperative serum soluble HGF levels might reflect the severity of invasive breast cancer and deserve further evaluation.** (Cancer Epidemiol Biomarkers Prev 2005;14(3):715–7)

Introduction

Hepatocyte growth factor (HGF),which is known to be identical to scatter factor, has been reported the cause of many biological events, including cell proliferation (1), movement (2), invasiveness (3, 4), morphogenesis (5), and angiogenesis (6). Hepatocyte growth factor is found in many organs, including the mammary gland, lung, kidney, and liver (7, 8). Elevated hepatocyte growth factor content in tumor tissue was reported to predict a more aggressive biology in non–small cell lung cancer patients (9). However, there is still limited knowledge about the role of HGF in breast cancer. The evaluation of the possible outcome of the patient with breast cancer is important for planning optional treatment. Because no single prognostic factor can determine the whole status of a patient with breast cancer, the physician must consider all available prognostic data. This study was designed with the aim to investigate any correlation between the preoperative circulating hepatocyte growth factor and the clinicopathologic features and to evaluate the possible prognostic significance of the preoperative circulating hepatocyte growth factor in breast cancer.

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Materials and Methods

From November 1998 to October 2001, 124 consecutive patients with invasive breast cancer were included in this study. All the patients met the following criteria: (a) having been diagnosed as having primary invasive breast cancer, (b) having no clinical manifestation of infection, (c) having received no immunomodulatory agents during the previous 3 weeks, (d) having received no blood transfusion during the previous 3 weeks, (e) having no known liver dysfunction, (f) having no known lung or renal dysfunction, and (g) having no other known malignancy. Venous blood samples were collected before the surgery. Sera were obtained by centrifugation and stored at −70°C until assayed. All 124 patients were women ages 31 to 84 years (mean, 50.5 years). All patients underwent modified radical mastectomy except patients with stage IV and the diagnosis of breast cancer was confirmed by histologic examination. Invasive breast cancer was defined as carcinoma, regardless of origin (duct or lobule), with invasion to or beyond the basement membrane (10). The data of primary tumor staging, age, estrogen receptor status, lymph node and metastasis status, histologic grading, and tumor-node-metastasis (TNM) staging were collected. Thorough physical examination, chest radiography, level of serum alkaline phosphatase, and mammogram were the preoperative routine for all patients. Bone scan and abdominal ultrasonography were done for all patients with provisional clinical stage III or above to rule out the presence of distant metastases. Regardless of any provisional clinical stage, all patients with elevated serum alkaline phosphatase special complaints such as bone pain or any specific findings indicating the possibility of distant metastases such as

Table 1. Serum concentrations of HGF in relation to clinicopathologic variables

	n	Mean value of HGF (pg/mL)	*P*
Age (y)			
<50	65	535.5 ± 142.2	0.545
≧50	59	522.0 ± 99.2	
Estrogen receptor			
Negative	49	557.9 ± 135.7	0.035
Positive	75	510.2 ± 111.5	
Primary tumor staging			
T1	14	367.7 ± 56.9	<0.001
T2	70	491.0 ± 75.4	
T3	11	600.5 ± 119.7	
T4	29	672.0 ± 79.8	
Lymph node status			
No	58	454.0 ± 77.5	<0.001
N1	33	516.7 ± 97.7	
N2	33	673.2 ± 80.3	
Distant metastases			
Absent	114	505.2 ± 96.8	<0.001
Present	10	801.2 ± 25.5	
TNM staging			
I	11	343.4 ± 31.6	<0.001
II	66	463.9 ± 34.0	
III	37	627.0 ± 26.4	
IV	10	801.2 ± 25.5	
Histologic grading (differentiation)			
Well	45	438.9 ± 65.0	<0.001
Moderately	56	536.6 ± 100.2	
Poor	23	686.1 ± 92.8	

hepatomegaly also underwent bone scan and abdominal ultrasonography to detect if there was distant metastases. All of the cancer were graded according to the criteria described by Bloom and Richard (11). Estrogen receptor status was determined by immunohistochemical staining method (12-17). Thirty-five patients with benign breast tumor (20 patients with fibrocystic disease and 15 patients with fibroadenoma) were used as control group.

Measurement of Hepatocyte Growth Factor (Principle of the Assay)

This assay (R&D Systems, Inc. Minneapolis, MN) employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for HGF has been precoated onto a microplate. Standards and samples are pipetted into the wells and any HGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for HGF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of HGF bound in the initial step. The color development is stopped and the intensity of the color is measured. The minimum detectable dose of HGF is typically <40 pg/mL.

Statistical Analysis. The Student's *t* test was used to assess the significance of difference in the levels of HGF between the patient group and control group. The following clinicopathologic variables were first entered into the univariate analysis by Student's *t* test or ANOVA test: primary tumor staging, age, estrogen receptor status, lymph node status, distant metastases status, histologic grading, and TNM staging. These clinicopathologic variables were then assessed by the multiple linear regression method. $P < 0.05$ was accepted as significant. Results in pg/mL were expressed as the mean ± SD.

Results

The mean value of serum soluble HGF in patients with invasive breast cancer was 529.1 ± 123.3 pg/mL and that of control group was 343.0 ± 31.0 pg/mL and the difference was significant ($P < 0.001$). Furthermore (Table 1), there were significantly higher serum levels of soluble HGF in patients with negative estrogen receptor ($P = 0.035$), in patients with poorer differentiated tumor ($P < 0.001$), in patients with more advanced primary tumor staging ($P < 0.001$), in patients with more advanced lymph node status ($P < 0.001$), in patients with distant metastases ($P < 0.001$), and in patients with more advanced TNM staging ($P < 0.001$). In multivariate analysis by the multiple linear regression method, TNM staging ($P < 0.001$) seemed an independent factor regarding the significant higher serum levels of soluble HGF.

Discussion

The evaluation of the possible outcome of the patient with breast cancer is important for planning optional treatment. Because no single prognostic factor can determine the whole status of a patient with breast cancer, the physician must consider all available prognostic data. This study was conducted to evaluate the correlation of circulating soluble hepatocyte growth factor with clinicopathologic variables and its possible prognostic value with the hope to find additional meaningful information for making a treatment decision. In this study, serum was chosen for measurement of hepatocyte growth factor because serum is easily available and sufficient for an objective analysis. The data could be achieved preoperatively and could be useful to an optimal preoperative planning. This method for measurement of hepatocyte growth factor is feasible, reproducible, inexpensive, and widely available; the serum levels of hepatocyte growth factor have already been shown to be of prognostic value in other malignancy (9). Although semiquantitative analysis by immunohistochemical staining of tumor specimen has been claimed to be a useful method to detect a tumor mark (18), this method indeed has several drawbacks. First, although semiquantitative evaluation is sufficient to differentiate negative versus positive reaction, it is sometimes not accurate enough to evaluate the intermediate patterns of staining. Second, the possibility of heterogeneity within tumor specimens might cause different results. Finally, different types of antibodies and procedures were used in different series and could lead to different results. The choice of serum for a quantitative analysis in this study could possibly avoid the abovementioned disadvantages of a semiquantitative analysis by immunohistochemical staining. Only patients with invasive breast carcinoma was included, because noninvasive carcinoma usually carries a quite different clinical course. Patients with clinical infection were excluded because infection itself would theoretically cause an altered concentration of serum cytokine. In addition, immunomodulatory agents and blood transfusions were avoided to allow for an objective, accurate assay of cytokines. Patients with known liver, lung, or renal dysfunction were also excluded to avoid the possible interfering factors. Patients with known malignancy other than breast cancer were excluded since the increase in the serum HGF was found in patients with other neoplasms including gastric cancer, lymphomas, and leukemias (19).

HGF is found to be involved in carcinogenesis. Jeffers et al. (20) reported that cotransfection of HGF and c-met was able to induce morphologic transformation *in vitro* and tumorigenicity *in vivo* in a nontumorigenic mouse cell line C127. In the bladder cancer line NBT-II, transfection of HGF upgraded the invasive phenotype and growth rate of these cells (21).

The mammary stroma is reported to exert a paracrine influence on the neighboring epithelial cell population (22). It has been noted that an intimate interaction may exist in fibroblast HGF expression between epithelial and mesenchymal compartments in human breast cancer depending on the *in vivo* conditions (23). It was reported that HGF receptor is widely distributed in various epithelial cells including tumor cells but obviously not in mesenchymal cells (24). On the other hand, HGF production was found in the stromal component but not in the epithelial component of the breast (8, 23). Because it has been reported that HGF is a modulator of epithelial cell proliferation and motility for a broad spectrum of cell types (7, 25), it is tempting to speculate that HGF originating from breast stromal cells may play a crucial role in facilitating breast cancer cell invasion and metastasis.

In multivariate analysis by the multiple linear regression method, TNM staging ($P < 0.001$) seemed an independent factor regarding the significant higher serum levels of soluble HGF. Based on the results, the higher preoperative level of serum HGF is shown closely related to a more advanced TNM stage (Table 1). Thus, the preoperative level of serum HGF may reflect the severity of invasive breast cancer and may be useful to pick up higher risk patients for more aggressive treatment. It is worthwhile to have further investigation by larger group of patients with longer follow-up to achieve more substantial conclusion.

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