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Serum Heparin-binding Epidermal Growth Factor-like Growth Factor (HB-EGF) as a Biomarker for Primary Ovarian Cancer

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Abstract. *Ovarian cancer is the most lethal malignancy among gynaecological cancers. Although many anticancer agents have been developed for the treatment of ovarian cancer, it continues to have an extremely poor prognosis. Heparin-binding epidermal growth factor-like grown factor (HB-EGF) has been reported to be a rational therapeutic target for ovarian cancer. Here, we evaluated the clinical significance of serum HB-EGF by examining the association between prognosis and serum HB-EGF levels in patients with primary ovarian cancer. We found that high serum HB-EGF concentrations were significantly associated with poor prognosis in a combined cohort of patients with all stages of ovarian cancer, as well as in a subset of patients with advanced disease. In addition, serum HB-EGF levels increased as the cancer advanced. These data suggest that serum HB-EGF may be a target for the design of novel therapies for ovarian cancer.*

Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is one family of ligands that bind to the epidermal growth factor (EGF) receptor (1). HB-EGF is initially synthesized as a transmembrane protein and is cleaved at the cell surface by a protease, a process known as ectodomain shedding, yielding the mitogenic-soluble form of HB-EGF (2). HB-EGF is thought to play a pivotal role in cell proliferation and differentiation. In particular, soluble HB-EGF is a potent promoter of cell adhesion, cell motility and angiogenesis, which can lead to cell implantation, carcinogenesis, and atherosclerosis, among other disorders (3). Accumulating evidence suggests that HB-EGF is a promising target for breast, gastric, and ovarian cancer therapy (4). HB-EGF expression is significantly enhanced in ovarian cancer tissue compared with ovarian cysts and normal ovaries (5-7). In patients with ovarian cancer, the level of HB-EGF in serum correlates with the expression level in tumour tissue and peritoneal fluid (8), suggesting that the serum HB-EGF level faithfully reflects the expression in cancer tissue. It is plausible, therefore, that serum HB-EGF concentrations may correlate with clinical outcome in patients with ovarian cancer.

Ovarian cancer is the most lethal gynaecological malignancy and has an extremely poor prognosis, owing in large part to the wide extension of tumour cells into the peritoneal cavity (9). Metastasis from epithelial ovarian cancer can occur rapidly *via* transcoelomic, haematogenous, or lymphatic routes. More than 60% of patients with ovarian cancer are diagnosed at an advanced stage of disease. Cytoreductive surgery and platinum plus taxane-based chemotherapies introduced in the past decade have improved the survival of patients with epithelial ovarian cancer (10, 11). More recently, combination

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chemotherapy including platinum, taxane, and bevacizumab, an antibody to vascular endothelial growth factor (VEGF), has been recognized as the standard chemotherapy for ovarian cancer (12). However, identifying patients who would benefit from bevacizumab therapy has been challenging, particularly because there are no companion diagnostics for monitoring or predicingt response to treatment. To date, pharmacotherapy for ovarian cancer has had variable success (13). As a result, there is an urgent need to develop novel targeted agents as well as companion diagnostics for cancer therapy.

CRM197, the active ingredient of the investigational drug BK-UM, was originally isolated as a non-toxic mutant form of diphtheria toxin and has been shown to inhibit cancer cell proliferation by three modes of action (14-16). Addition of CRM197 reduced production of HB-EGF by a variety of cancer cells in culture, and strongly inhibited the growth of several types of cancer in nude mice (17-19). In clinical trials, CRM197 injected subcutaneously in the abdominal wall or administered intraperitoneally to patients with recurrent ovarian cancer was safe and relatively well tolerated (20, 21). Because administration of CRM197 is expected to inhibit the expression of HB-EGF in cancer cells, it is important to identify the clinical features of patients with ovarian cancer who have high serum HB-EGF levels and thus might benefit most from CRM197 therapy.

Here, we investigated the clinical significance of serum HB-EGF levels in patients with primary ovarian cancer by examining the relationship between serum concentrations and clinical outcome. We also assessed the clinical characteristics of patients with high serum HB-EGF concentrations.

Materials and Methods

Patients. This study analysed serum samples from 162 patients with primary ovarian cancer who had been treated at Fukuoka University Hospital (17 patients), Kyushu University Hospital (88 patients), and Tohoku University Hospital (57 patients) from January 2001 to January 2011. All patients underwent cytoreductive surgery followed by platinum plus taxane-based chemotherapy. None of the patients received preoperative radiotherapy or chemotherapy. The clinical and pathological characteristics of the patients, including age at diagnosis, histological subtype, preoperative serum cancer antigen (CA)-125 level, cytoreductive surgery status, and tumour stage are described in Table I. Tumour stage was determined according to the International Federation of Gynaecology and Obstetrics (FIGO) staging system (22). Cytoreductive surgery status was defined as no residual tumour (RT:0), residual tumour <1 cm (RT:1), <2 cm (RT:2), or >2 cm (RT:3). The patients provided their written informed consent for use of their data, and the study was approved by the Ethics Committees of Fukuoka University Hospital, Kyushu University Hospital, and Tohoku University Hospital (approval no. :14-9-04).

Serum samples and heparin-binding epidermal growth factor-like growth factor (HB-EGF) ELISA. Blood samples were obtained from the 162 patients immediately before initiating treatment. Plasma was prepared by centrifugation (3,000 × *g* for 15 min) and stored at −80˚C until needed. Serum HB-EGF concentrations were determined using a commercially available sandwich ELISA (DuoSet Kit; R&D Systems, Minneapolis, MN, USA). The assay was performed using a modified protocol (8) in which the concentration of capture antibody (anti-human HB-EGF) was four-fold higher than in the manufacturer's protocol, and serum HB-EGF concentrations were calculated using a parallel line method (8).

Statistical analysis. The distributions of variables in this analysis are described by the mean±standard deviation or median [25 percentile, 75 percentile], and frequency.

The Mann–Whitney *U*-test and chi-squared test were used to compare the distributions or medians of continuous and ordered variables between two groups. Progression-free survival was calculated by the Kaplan–Meier test with 95% confidence intervals computed by Greenwood method. A *p*-value of less than 0.05 was considered statistically significant. Analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

Results

Association between serum HB-EGF concentrations and clinical prognosis. In order to assess the clinical significance of serum HB-EGF concentrations, we stratified 162 patients with all FIGO stages of ovarian cancer according to their serum HB-EGF level and then evaluated progression-free survival. Of the 162 patients, 92 and 70 patients had serum HB-EGF levels ≥150 pg/ml and <150 pg/ml, respectively. Poor prognosis was significantly associated with serum HB-EGF levels between 150 and 275 pg/ml (Table II). Of particular note, the difference in median progression-free survival of patients with serum HB-EGF concentrations <230 pg/ml compared with ≥230 pg/ml was highly significant, whereas no significant difference was detected when the threshold was 300 pg/ml (Table II). Of the 38 patients with serum HB-EGF levels ≥300 pg/ml, nine had FIGO stage I disease (five endometrioid adenocarcinoma, three clear cell carcinoma, and one mucinous adenocarcinoma) and experienced no recurrence during the observation period. Thus, the apparent lack of association between clinical progression and serum HB-EGF levels of ≥300 pg/ml could be because many patients with high levels of serum HB-EGF had early-stage disease. Serum HB-EGF levels were also strongly associated with clinical progression for the 79 patients with advanced ovarian cancer (FIGO stage III and IV). Of these patients, 36 with ≥230 pg/ml serum HB-EGF showed significantly poorer prognosis than the 43 patients with levels <230 pg/ml (Table III). However, there was no significant difference in the clinical prognosis of patients when the threshold serum HB-EGF value was ≥275 pg/ml. Seven patients had serum HB-EGF levels between 250 pg/ml and 270 pg/ml, five of whom

Table I. *Clinical characteristics of 162 patients with primary ovarian cancer.*

Variable	Value
Age (years)	56±11 (35-84)
FIGO stage	
I	65 (40%)
II	18 (11%)
III	56 (35%)
IV	23 (14%)
Histological subtype	
SAC	70 (43%)
EM	24 (15%)
MC	9 (6%)
CCC	39 (24%)
Others	20 (12%)
Residual disease	
RT:0	110 (68%)
RT:1	5 (3%)
RT:2	9 (6%)
RT:3	38 (23%)
CA125 (IU/ml)	1731±5,277 (8-56,140)
HB-EGF (pg/ml)	368±1,367 (9-16,812)

Data are the mean±standard deviation (range) or N (%). CA125: Cancer antigen 125, CCC: clear cell carcinoma, EM: endometrioid adenocarcinoma, HB-EGF: heparin-binding epidermal growth factor-like growth factor, MC: mucinous adenocarcinoma, RT: residual tumour, SAC: serous adenocarcinoma.

Table II. *Median progression-free survival of 162 patients with primary ovarian cancer (FIGO I–IV) stratified by serum heparin-binding epidermal growth factor-like growth factor (HB-EGF) concentration.*

Serum HB-EGF (pg/ml)	Progression-free survival (months)		*p*-Value
	Below cut-off	Above cut-off	
125	16.8 (1.1-86.1) (N=53)	8.7 (2.5-78.0) (N=109)	0.114
150	38.6 (1.1-86.1) (N=70)	14.0 (2.5-78.0) (N=92)	0.024
175	34.9 (1.1-86.1) (N=78)	14.0 (2.5-78.0) (N=78)	0.013
200	34.9 (1.1-86.1) (N=89)	13.8 (2.5-78.0) (N=73)	0.009
230	40.2 (1.1-86.1) (N=106)	10.5 (2.5-74.6) (N=56)	<0.001
250	34.9 (1.1-86.1) (N=108)	10.5 (2.5-74.6) (N=54)	<0.001
275	28.3 (1.1-86.1) (N=119)	11.7 (3-74.6) (N=43)	0.027
300	26.7 (1.1-86.1) (N=124)	13.4 (3-74.6) (N=38)	0.063

Data are the median (range) and the number of patients (N).

Table III. *Median progression-free survival of 79 patients with advanced primary ovarian cancer (FIGO III–IV) stratified by serum heparin-binding epidermal growth factor-like growth factor (HB-EGF) concentration.*

Serum HB-EGF (pg/ml)	Progression-free survival (months)		*p*-Value
	Below cut-off	Above cut-off	
125	16.8 (3.6-70.7) (N=53)	8.7 (1.3-74.6) (N=109)	0.348
150	12.2 (3.6-70.7) (N=28)	8.7 (1.3-74.6) (N=51)	0.238
175	12.2 (3.6-70.7) (N=33)	8.7 (1.3-74.6) (N=46)	0.121
200	12.2 (3.6-70.7) (N=40)	9.1 (1.3-74.6) (N=39)	0.093
230	12.2 (3.6-70.7) (N=43)	7.9 (1.3-74.6) (N=36)	0.028
250	12.2 (3.6-70.7) (N=44)	8.3 (1.3-74.6) (N=35)	0.043
275	9.5 (3.6-70.7) (N=51)	8.7 (1.3-74.6) (N=28)	0.227
300	8.0 (2.5, 70.7) (N=55)	10.5 (1.3-74.6) (N=24)	0.509

Data are the median (range) and the number of patients (N).

experienced disease recurrence within a few months of completion of chemotherapy. These patients had high residual tumour status and histological subtypes of clear cell carcinoma or endometrioid adenocarcinoma. Collectively, these data indicate that clinical prognosis is significantly associated with serum HB-EGF levels of ~230 pg/ml but not with levels ≥275 pg/ml. This suggests that patients with serum HB-EGF levels of ~230 pg/ml might benefit from novel HB-EGF–targeted therapy.

Clinical features of patients with serum HB-EGF concentrations ≥230 pg/ml. We next evaluated the clinical features of all patients (FIGO stages I-IV) and the subset of patients with advanced ovarian cancer (stages III and IV) according to a serum HB-EGF cut-off of 230 pg/ml. When the full patient cohort was analyzed, the number of patients with HB-EGF level ≥230 pg/ml increased as the disease advanced, whereas no specific associations were observed according to histological subtype (Table IV). Among the patients with advanced ovarian cancer, no postoperative residual disease (RT:0) was significantly associated with serum HB-EGF level <230 pg/ml compared with ≥230 pg/ml (Table V). However, there were no other differences in clinical characteristics between the patients with advanced ovarian cancer based on serum HB-EGF level. Taken together, these results suggest that serum HB-EGF may participate in the growth and extension of ovarian cancer.

Discussion

In the present study, we found that serum levels of HB-EGF correlated significantly with clinical prognosis in both the full cohort of 162 patients with ovarian cancer and the subset of 79 patients with advanced ovarian cancer. In addition, serum HB-EGF levels increased significantly with clinical stage of disease, suggesting that HB-EGF may be involved in tumour growth and extension.

HB-EGF-targeted agents have been tested in two phase I clinical trials, which investigated the safety, tolerability, and efficacy of the inhibitory monoclonal antibody to HB-

Table IV. *Clinical characteristics of 162 patients with primary ovarian cancer (FIGO I-IV) partitioned by serum heparin-binding epidermal growth factor-like growth factor (HB-EGF) concentration cut-off of 230 pg/ml.*

Variable	HB-EGF <230 pg/ml (N=106)	HB-EGF ≥230 pg/ml (N=56)	*p*-Value
Age (years)	58±11 (35-84)	57±11 (36-76)	0.576
FIGO stage			
I	51 (78%)	14 (22%)	
II	12 (67%)	6 (33%)	
III	34 (61%)	22 (39%)	
IV	9 (39%)	14 (61%)	0.006
Histological subtype			
SAC	43 (59%)	30 (41%)	
EM	13 (57%)	10 (43%)	
MC	8 (89%)	1 (11%)	
CCC	29 (74%)	10 (26%)	
Other	14 (74%)	5 (26%)	0.176
Residual disease			
RT:0	83 (75%)	27 (25%)	
RT:1	2 (40%)	3 (60%)	
RT:2	6 (67%)	3 (33%)	
RT:3	15 (39%)	23 (61%)	<0.001
CA125 (IU/ml)	1135±2,801 (8-20.291)	2840±8,035 (12-56,140)	0.052
HB-EGF (pg/ml)	130±57 (9-229)	818±2,269 (230-16,812)	0.027

Data are the mean±standard deviation (range) or N (%). CA125: Cancer antigen 125, CCC: clear cell carcinoma, EM: endometrioid adenocarcinoma, HB-EGF: heparin-binding epidermal growth factor-like growth factor, MC: mucinous adenocarcinoma, RT: residual tumour, SAC: serous adenocarcinoma.

Table V. *Clinical characteristics of 79 patients with advanced primary ovarian cancer (FIGO III-IV) partitioned by serum heparin-binding epidermal growth factor-like growth factor (HB-EGF) concentration cut-off of 230 pg/ml.*

Variable	HB-EGF <230 pg/ml (N=43)	HB-EGF ≥230 pg/ml (N=36)	*p*-Value
Age (years)	59±10 (35-79)	59±11 (40-76)	0.984
FIGO stage			
III	34 (61%)	22 (39%)	
IV	9 (39%)	14 (61%)	0.080
Histological subtype			
SAC	27 (52%)	25 (48%)	
EM	3 (43%)	4 (57%)	
MC	1 (100%)	0 (0%)	
CCC	2 (33%)	4 (67%)	
Other	10 (77%)	3 (27%)	0.279
Residual disease			
RT:0	22 (71%)	9 (29%)	
RT:1	2 (50%)	2 (50%)	
RT:2	6 (67%)	3 (33%)	
RT:3	13 (37%)	22 (63%)	0.041
CA125 (IU/ml)	638±3,928 (12-20.291)	876±9,811 (34-56,140)	0.204
HB-EGF (pg/ml)	127±52 (9-225)	398±233 (230-1,573)	<0.001

Data are the mean±standard deviation (range) or N (%). CA125: Cancer antigen 125, CCC: clear cell carcinoma, EM: endometrioid adenocarcinoma, HB-EGF: heparin-binding epidermal growth factor-like growth factor, MC: mucinous adenocarcinoma, RT: residual tumour, SAC: serous adenocarcinoma.

EGF KHK2866 in patients with advanced ovarian cancer and of BK-UM in patients with recurrent ovarian cancer (21, 23). Companion diagnostics for each agent were also developed (8, 24). The studies found that serum HB-EGF levels were reduced by intravenous injection of KHK2866 (23), and significantly reduced by intraperitoneal administration of BK-UM in patients with high levels of serum HB-EGF (>125 pg/ml by ELISA) (21). However,

the KHK2866 antibody caused reversible neuropsychiatric toxicity and its development has been discontinued (23). However, the trial of BK-UM showed not only that it was safe and tolerable, but also that high serum HB-EGF concentrations were associated with poor clinical prognosis (21). These data suggest that patients with high serum HB-EGF levels could be good candidates for HB-EGF-targeted therapy.

Many companion diagnostics developed for cancer therapeutics require tissue immunohistochemistry or specific gene analysis (13). Bevacizumab is a well-known anticancer agent that targets VEGF, a growth factor for colon, lung, and ovarian cancer (24). However, companion diagnostics based on analysis of VEGF expression in serum or tissue are not yet available for use in patients treated with bevacizumab.

VEGF expression is stimulated by numerous pathways and it can be produced by many cells and tissues, including cancer tissues; therefore, the origin of serum VEGF in patients with cancer (25, 26). Accordingly, we speculate that serum VEGF levels may not be specifically related to the properties of cancer cells. HB-EGF promotes VEGF signalling (27-29), and in breast cancer, HB-EGF plays a pivotal role in the acquisition of tumour aggressiveness by orchestrating a molecular hierarchy regulating VEGF and angiopoietin-like 4 (ANGPL4) (28). Therefore, HB-EGF might be involved in the clinical outcome of ovarian cancer through contributions to tumour growth and extension.

In this study, we found that high levels of HB-EGF in serum were linked to poor prognosis in patients with ovarian cancer. Administration of BK-UM has been reported to reduce serum HB-EGF concentrations (8). Therefore, a phase II study of BK-UM in patients with high serum HB-EGF levels would facilitate the development of both a HB-EGF-targeted therapy and its companion diagnostic tool.

Conflicts of Interest

The Authors declare that they have no conflicts of interest in regard to this study.

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