Prognostic Implications of the Expression of CXCL16 in Breast Carcinoma

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Background: Of the many prognostic factors for breast cancer, the relationship between an infiltration of inflammatory cells and the prognosis is debatable. Of the chemokines affecting cancer's inflammatory reactions, chemokine (C-X-C motif) ligand 16 (CXCL16) has attracted attention for its prognostic value in many cancers, including colorectal cancer and renal cell carcinoma. But the situation for breast carcinoma is unknown. The aim of this study was to examine the relationship between the prognostic factors and the CXCL16 expression in patients with breast carcinoma. **Methods:** The patients (n = 106) diagnosed with invasive ductal cancer of the breast were enrolled. We reviewed the clinicopathological factors of these patients, hematoxylin and eosin stains were prepared and estrogen receptor (ER), human epidermal growth factor receptor 2 (HER2/neu) and CXCL16 immunostaining was performed. **Results:** The ER expression was significantly correlated with age and inflammation. A CXCL16 expression was noted in 81.1% of the cases. No association was evident between a CXCL16 expression and any other parameter, including the survival rate. Multivariate analysis did not implicate ER, HER2/neu or CXCL16 as an independent prognostic factor, but the tumor size was independent predictive factor for the patient outcome. **Conclusions:** An inflammatory reaction mediated by CXCL16 is not associated with the prognosis of breast cancer or any clinicopathological factors.

Key Words: Breast neoplasms; CXCL16 protein, human; Prognosis; Survival

Breast carcinoma is the second most common malignant tumor of females in Korea.[1] There are many well-known prognostic factors for breast cancer, including the tumor size, nodal metastasis (the TNM stage), the hormone receptor status and human epidermal growth factor receptor 2 (HER2/neu) overexpression. The value of various factors concerned with the prognostic effect of intra-and peri-tumoral inflammation is debatable.[2-4]

Although many studies have revealed a relationship between inflammatory infiltrates and the prognosis of cancer, CXC chemokines have received particular attention with the discovery of chemokine (C-X-C motif) ligand 16 (CXCL16).[5] CXCL16 has been shown to be a prognostic factor for colorectal cancer[6] and renal cell carcinoma,[7] and it is broadly expressed in various inflammation associated cancers.[8] Yet there has been no published study about CXCL16 in breast cancer. Moreover, the relationship between inflammatory infiltrates and the prognosis of breast cancer is ambiguous at best.[3]

These gaps in knowledge prompted the present study. We investigated the relationship between a CXCL16 expression, an inflammatory reaction and the prognosis of breast carcinoma. We analyzed the relationship between the immunohistochemical results for estrogen receptor (ER), HER2/neu and CXCL16 and the clinicopathological factors for breast carcinoma.

MATERIALS AND METHODS

Patients

One hundred and six patients who were diagnosed with primary invasive ductal carcinoma of the breast at our hospital from 1993 to 2002 were considered for analysis. The patients had undergone either a mastectomy or segmental resection, along with axillary lymph node dissection. By reviewing the medical records and pathologic reports of the selected patients, we confirmed the patient age, tumor size and lymph node status and metastasis according to the TNM classification of the American Joint Committee on Cancer. Histological grading was performed according to the Nottingham modification. The survival rates of the patients were examined through the National Statistical Office of Korea.

Assessment of inflammation

The peritumoral and intratumoral inflammatory reactions were evaluated (yes or no) by reviewing the hematoxylin and eosin stained slides.

Immunohistochemistry and assessment

Three µm-thick sections were prepared from the representative formalin-fixed, paraffin-embedded tissue blocks for the immunohistochemical studies. Immunohistochemical stains were performed using an autostainer (Ventana Medical Systems, Tucson, AZ, USA). The primary antibodies were CXCL16 (1:25, rabbit anti-human CXCL16, 500-P200, Peprotech, London, UK), ER (1:50, 1D5, DakoCytomation, Glostrup, Denmark) and HER2/neu (1:200, CB11, Novocastra, Newcastle, UK). 3,3´-diaminobenzidine tetrahydrochloride was used to visualize the antibody/enzyme complexes; the samples were counterstained with Mayer's hematoxylin.

For ER, a receptor positive result was defined as nuclear staining in ≥10% of the tumor cells as scored by the Dako scoring system. HER2/neu overexpression was considered as moderate to strong membranous staining in >10% of the tumor cells, which was equivalent to a score of 2+ or 3+ by the HercepTest protocol.[9] We classified the HER2/neu scores of 0 and 1+ as negative, 2+ as undetermined and 3+ as positive. The CXCL16 positive results were defined as cytoplasmic staining in ≥10% of the tumor cells.

A semiquantitative assessment of CXCL16, ER and HER2/neu staining on the whole-tissue sections was performed independently by two observers, who were blinded to all the clinicopathologic variables.

Statistical analysis

The SPSS ver. 12.0K (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Cancer-specific survival was determined from the date of diagnosis to death by disease or the last follow-up date. The survival rates were calculated by the Kaplan-Meier method. Multivariate analysis was performed using the log-rank test and the Cox proportional hazards model. A p-value < 0.05 was considered statistically significant. The correlations between the ER, HER2/neu and CXCL16 expressions were evaluated by Spearman's correlation coefficient and the Kappa value.

RESULTS

Clinicopathological characteristics

All the 106 breast invasive ductal carcinoma patients were female. The patients were followed up for at least 7 years, and they showed a 68.5% survival rate. Of the patients who died, the average survival time was 61.5 months (range, 6 to 152 months).

Correlation between the clinicopathological factors and the ER expression

When the ER score was compared with the clinicopathological factors, the ER expression showed a significant relationship with age (p = 0.02) and inflammation (p = 0.01) (Table 1).

Correlation between the clinicopathological factors and the HER2/neu expression

When the relationship between HER2/neu and the clinicopathological factors was analyzed, a HER2/neu expression had no significant relationship with any variables (Table 1).

Correlation between the clinicopathological factors and the CXCL16 expression

CXCL16 protein was stained in the cytoplasm of the tumor cells, the normal epithelial cells and the inflammatory infiltrates (Fig. 1), and it was stained in 86 (81.1%) out of the 106 cases. An analysis of the relationships between CXCL16 and the clinicopathological factors revealed an increased CXCL16 expression related to an inflammatory reaction, but no association with the other variables (Table 1).

Correlation between the clinicopathological factors and survival

The mean age at diagnosis was 47.8 years (range, 20 to 78 years). This parameter was not statistically related to the survival time (p = 0.48). The histological grade was also not statistically associated with the survival time (p = 0.55). Tumor size was statistically related with the survival time (p = 0.00). The probability of death of the T3 and T4 patients was 4.081 times higher, respectively, than that of T1 and T2 patients. Lymph node metastasis was statistically related with the survival time (p = 0.00). The probability of death of the patients who had me-

Table 1. Correlation between the clinicopathological factors and the estrogen receptor (ER), HER2/neu and CXCL16 expressions in the patients with invasive breast cancer

	ER score			HER2/neu score				CXCL16		
	N	P	p-value	N	U	P	p-value	N	P	p-value
No. of cases	50	56		36	15	55		20	86	
Age (yr)			0.02				0.26			0.21
≤49	32	29		22	11	28		14	47	
≥50	18	27		14	4	27		6	39	
Grade			0.11				0.91			0.88
I	17	30		16	7	24		8	39	
II and III	33	26		20	8	31		12	47	
Tumor size (cm)			0.97				0.53			0.54
T1 (≤2.0)	27	30		22	7	28		12	45	
≥T2 (>2.0)	23	26		14	8	27		8	41	
Lymph node metastasis			0.29				0.37			0.51
N	31	29		21	6	33		10	50	
P	19	27		15	9	22		10	36	
TNM stage			0.81				0.77			0.76
I	20	22		15	5	22		7	35	
II	20	20		15	5	20		9	31	
III	10	14		6	5	13		4	20	
Inflammation			0.01				0.91			0.000
P	36	51		29	12	46		1	86	
N	14	5		7	3	9		19	0	

HER2/neu, human epidermal growth factor receptor 2; CXCL16, chemokine (C-X-C motif) ligand 16; N, negative; P, positive; U, undetermined; TNM, tumor, node and metastasis.
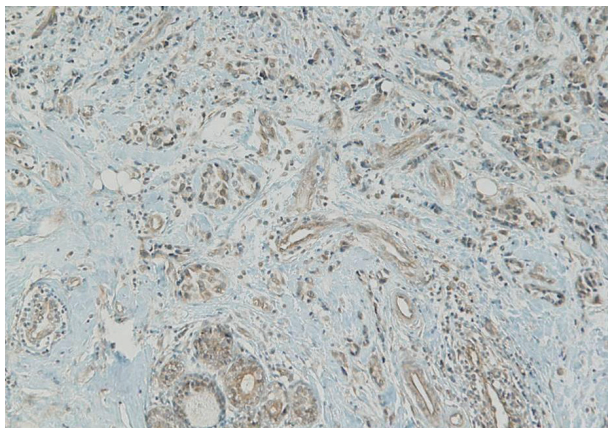
Fig. 1. Immunohistochemical staining for chemokine (C-X-C motif) ligand 16 (CXCL16). The staining shows diffuse cytoplasmic reactivity in the tumor cells, the normal acinar cells and the inflammatory infiltrates.

tastasized lymph nodes was 2.82 times higher than that of the N0 patients. The TNM stage was closely related with the survival rate (p = 0.00). The survival curve according to the TNM stage showed that the probability of death of the stage II and III patients was 1.19 and 0.93 times higher, respectively, than that of the stage I patients. Inflammation and the CXCL16 expression were not statistically related to the survival time (p = 0.90 and p = 0.77). Cox multivariate analyses revealed tumor

size was of prognostic significance (Table 2).

Correlation between an ER expression and survival

ER positivity showed a higher relative risk than ER negativity. Yet an ER expression was statistically unrelated with the survival time (p = 0.19).

Correlation between HER2/neu overexpression and survival

The probability of death of a HER2/neu score 2 and 3 was 3.09 and 1.82 times higher, respectively, than a HER2/neu score 0 and 1. A HER2/neu expression was statistically related with the survival time (p = 0.02).

Correlation between a CXCL16 expression, an inflammatory reaction and survival

When the CXCL16 expression was negative, the survival rate and mean survival period were 65% and 56 months, respectively. When the CXCL16 expression was positive, the survival rate and mean survival period were 69.8% and 63 months, respectively. But the CXCL16 expression was of no prognostic significance (p = 0.77) (Fig. 2). Nineteen cases had no inflammatory

Table 2. Cox multivariate analysis of the estrogen receptor (ER), HER2/neu, CXCL16 expressions and the other clinicopathologic variables of the patients with invasive breast cancer

Variables	Hazard ratio	95% CI	p-value
CXCL16	0.19	0.15-2.32	0.19
Inflammation	4.02	0.34-47.78	0.27
ER	1.46	0.57-3.47	0.46
HER2/neu	1.82	0.75-4.44	0.19
Grade	1.11	0.27-4.49	0.88
TNM stage	0.93	0.10-8.88	0.95
Lymph node metastasis	2.87	0.84-9.40	0.09
Size (≤2.0 vs >2.0)	4.08	1.22-13.62	0.02

HER2/neu, human epidermal growth factor receptor 2; CXCL16, chemokine (C-X-C motif) ligand 16; CI, confidence interval; TNM, tumor, node and metastasis.

cells in the intratumoral or peritumoral stroma, and they did not show a CXCL16 expression. An inflammatory reaction was statistically related to a CXCL16 expression (p = 0.00), but it was not related to the survival rate (p = 0.90).

Correlation between the ER, HER2/neu and CXCL16 expressions

ER and CXCL16 were statistically related to inflammation. The Spearman correlation coefficient between these markers was 0.269 (p = 0.005) and the Kappa value was 0.218 (p = 0.006). There was no statistical relationship between HER2/neu and the other markers.

DISCUSSION

Antecedent studies have implicated many factors as prognostic factors for breast cancer.[10] Patient age, the tumor histological grade and size, the TNM stage and the ER, HER2/neu and CXCL16 expressions were selected for this study.

In this study tumor size, lymph node metastasis and the TNM stage were statistically associated with patient survival on the univariated analysis. However, only tumor size among these factors was statistically associated with patient survival on the Cox multivariate analysis, which convincingly implicated it as a predictor of patient survival.

Some authors have claimed that ER-positive tumors are associated with longer disease-free survival.[11] In contrast, minimal and statistically insignificant differences in long term prognosis have also been reported.[12] In our study, an ER expression was statistically related with age (p = 0.02), supporting the idea that an older patient may be more likely to have ER receptor posi-
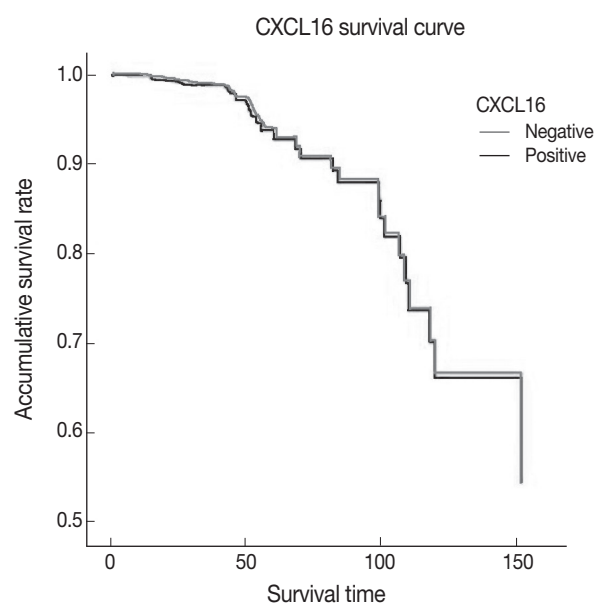
Fig. 2. Survival curve according to the chemokine (C-X-C motif) ligand 16 (CXCR16) expression in the patients with invasive breast carcinoma.

tive tumor cells. Indeed, aged breasts have more ER receptors.[13] We hope to reveal the relationship between an ER expression and inflammation in further studies.

A HER2/neu expression is an excellent predictor of the response to Herceptin, but it is a weak predictor of a response to chemotherapy.[14] Although this factor can identify a subset of patients with a poor prognosis, and particularly when lymph node metastases are present,[15] it is closely related with the tumor grade[16] and it loses much of its independent prognostic significance on multivariate analysis.[17] In our study, a HER2/neu expression was statistically associated with cancer survival on the univariate analysis (p = 0.02). The patients with HER2/neu scores of 0 or 1 showed a longer survival period than that of the patients with scores 2 or 3.

Tumor cells can produce cytokines and chemokines, which can create a microenvironment that protects tumor cells. Of the numerous known cytokines, CXC chemokines are especially increased in a diverse set of malignancies.[18,19] CXC chemokines and their receptors have important roles in the angiogenesis, growth, invasiveness and metastasis of tumors.[20] CXCL16 was originally identified as CXCL16/SR-PSOX, and it has been shown to be a transmembrane protein and ligand of the human immunodeficiency virus coreceptor CXCR6/Bonzo.[5] CXCL16 has since been demonstrated to be induced by proinflammatory cytokines such as tumor necrosis factor-alpha and interferon-gamma, and to exist as a soluble form in addition to the transmem-

brane-bound form. The soluble form can be separated from the cell surface by the metalloproteinases ADAM10 and ADAM17.[21] The CXCL16 receptor functions as a cell adhesion molecule by combining to CXCR6 and it is expressed in activated T-cells, natural killer T-cells, macrophages, dendritic cells, fibroblasts, liver sinusoidal endothelial cells, bone marrow plasma cells, reactive astrocytes and glioma cells.[6,22,23]

A CXCL16 expression has also been identified in renal cell carcinoma and colorectal cancer,[6,7] and in 48% of all breast cancers.[8] In the present study, the CXCL16 expression rate was 81.1%, which is higher than previously reported. This discrepancy of the expression rate may be due to the higher proportion of inflammation associated cases in the present study. A CXCL16 expression was not only observed in tumor cells, but also in endothelial cells and inflammatory cells. We presumed this expression pattern was due to the CXCL16 distribution in various cell types. The expression of CXCL16 in organs such as pancreas and kidney is associated with a favorable prognosis.[6,7] In these reports, a CXCL16 expression was statistically associated with inflammation (p = 0.00), and a more intensive expression of CXCL16 was associated with a more extensive chronic lymphocytic infiltration. Many breast cancers have peritumoral and intratumoral chronic inflammatory infiltrates, and some studies have tried to find a relationship between these inflammatory reactions and the prognosis. A 10-year follow-up study of medullary carcinoma patients conducted in 1977 reported a better (but statistically insignificant) prognosis of the patients with tumors that had inflammatory infiltrates.[2] In other reports, an inflammatory infiltration of breast cancer implied a better prognosis[3] or it was either not associated with the prognosis or it foretold a worsened prognosis.[4] While it has been reported that breast cancer with an accompanying inflammatory reaction may have a poorer prognosis,[24] this concept seems unreasonable in light of the relatively good prognosis of medullary carcinoma.

In the present study, 19 cases showed no peritumoral or intratumoral inflammation and no CXCL16 expression. This result closely links a CXCL16 expression with an inflammatory reaction in breast cancer. But since a CXCL16 expression was not statistically related with patient survival, an inflammatory reaction seems to be unrelated with the prognosis. Further studies on this are certainly needed given the debate still surrounding this suggestion, and particularly those studies that will use molecular techniques.

In this study, the ER and CXCL16 expressions showed a statistical relationship with an inflammatory reaction and the correlation analyses demonstrated a very weak statistical relationship between the expressions of these two markers. However, there has been no previous study about this correlation, and so further studies on this are needed.

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