CD10 expression in stromal component of invasive breast carcinoma: A potential prognostic determinant

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BACKGROUND: CD10 is a cell surface metalloproteinase which inactivates various biologically active peptides. Earlier studies have suggested CD10 expression in the stroma of various carcinomas to be associated with more aggressive behavior of the tumor. The aim of this study was to assess CD10 expression in stromal component of invasive breast carcinoma and its relationship with well-known clinicopathologic parameters. METHODS: This study included 49 formalin-fixed and paraffin-embedded tissue specimens of invasive breast carcinoma from the pathology archive of a referral hospital. CD10 expression was detected by immunohistochemistry and scored based on the staining intensity and percentage of the stained cells. Kruskal-Wallis and Mann-Whitney tests, along with Spearman's correlation coefficients were used to determine the relationship between CD10 expression and clinicopathologic parameters. RESULTS: No association was found between stromal CD10 expression and age, carcinoma subtype, and HER2/neu status. A significant positive correlation was seen between stromal CD10 expression and tumor size (p = 0.01), axillary lymph node status (p = 0.02), and tumor grade (p = 0.004). Although negative correlations were detected between stromal CD10 expression and estrogen receptor and progesterone receptor status, these correlations were not statistically significant. CONCLUSIONS: Tumor grade is a major prognostic indicator of breast carcinoma. Tumor size and nodal status on the other hand, are important determinants of tumor stage. Therefore, our findings concerning the positive correlations between stromal CD10 expression and tumor grade, tumor size, and nodal status suggest a strong effect of stromal CD10 expression on aggressive behavior of breast carcinoma and introduce this marker as a potential prognostic determinant in breast cancer.

KEYWORDS: Invasive Breast Carcinoma, Stroma, CD10

BACKGROUND
CD10, also known as common acute lymphoblastic leukemia antigen (CALLA) and neprilysin, is a 90- to 110-kDa cell surface zinc dependent metalloprotease that inactivates various kinds of biologically active peptides. First thought to be specific to acute lymphoblastic leukemia, it was shown in subsequent studies to be expressed on the surface of a variety of normal and neoplastic cells.¹

CD10 expression is commonly seen in bone marrow lymphoid stem cells, pre-B lymphocytes, mature neutrophils, breast myoepithelial cells, bile canaliculi, and renal epithelial cells. Expression of CD10 by tumor cells is seen in some lymphoid malignancies including lymphoblastic, Burkitt's and follicular lymphomas, as well as some nonlymphoid malignancies such as endometrial stromal sarcoma. Moreover, CD10 positive cells have also been reported in the stroma of prostate, and breast, colorectal and lung carcinomas.²

Stromal CD10 expression is associated with more aggressive behavior in various epithelial malignancies.¹⁻³ In gastric carcinoma, CD10 positive stromal cells are correlated with vascular invasion and metastasis.⁴ In nasopharyngeal carcinoma, stromal CD10 expression correlates with tumor progression.⁵

To the best of our knowledge, until 2012, only 5 studies have addressed the clinical significance of stromal CD10 expression in invasive breast carcinoma. These studies have indicated an association between CD10 expression and poorer prognosis.¹⁻³⁶⁻⁷ The small number of the previous studies in this field and some discrepancies in their results led us to do the present study. In the present study, we examined the expression of CD10 in the stroma of invasive breast carcinoma and its relationship with some well-known clinicopathologic prognostic determinants of cancer.

METHODS
This study was performed on 49 women who had undergone modified radical mastectomy for...
invasive breast carcinoma in Alzahra Hospital (Isfahan, Iran) during 2010-11. None of the patients had received neoadjuvant therapy. Hematoxylin-and-eosin stained microscopic slides of the primary tumor were reviewed to confirm the diagnosis, define tumor subtype, and perform standardized grading of invasive ductal carcinomas according to the Nottingham modification of the Bloom and Richardson system. Tumor size in this study means the greatest tumor diameter in centimeter. Lymph node status was determined according to tumor node metastasis (TNM) staging system for breast carcinoma in which 0 to 3 correspond negative nodes, 1-3 positive nodes, 4-9 positive nodes, and 10 or more positive nodes, respectively. Estrogen receptor (ER) and progesterone receptor (PR) markers were considered as positive when at least 1% of tumor cell nuclei were immunoreactive for the marker.

Expression of human epidermal growth factor receptor 2 (HER2/neu) was scored as 0 to 3. Scores of 0 (negative), 1+ (negative), 2+ (weakly positive), and 3+ (strongly positive) represented no staining or membrane staining in less than 10% of the tumor cells, a faint/barely perceptible partial staining in the membrane of more than 10% of the tumor cells, a weak to moderate complete membrane staining in more than 10% of the tumor cells, a strong complete membrane staining in more than 30% of the tumor cells.

Formalin-fixed and paraffin-embedded tissue specimens of invasive breast carcinoma were used for immunohistochemical study of ER, PR, HER2/neu, and CD10 expression. Mouse monoclonal antihuman ER (Dako), mouse monoclonal antihuman PR (Dako), And rabbit antihuman c-erbB-2 oncoprotein (Dako) antibodies were used for ER, PR, and HER2/neu staining, respectively. The antibody employed for CD10 immunohistochemical staining was a mouse antihuman monoclonal antibody against CD10/CALLA, immunoglobulin G1 (IgG1) isotype, and clone 56C6 (Signet Company, England). The staining was performed according to the manufacturer’s instructions.

Afterwards, 4-micron sections, prepared from the paraffin-embedded tissue samples, were deparaffi-nized in xylene, rehydrated in a series of graded alcohols, and placed in a Tris buffer saline (TBS) (pH: 7.6). Endogenous peroxidase activity was quenched using 3% hydrogen peroxide. Slides were rinsed with deionized water and placed in TBS. The sections were then incubated with CD10 monoclonal antibody for 20 minutes at room temperature. The next steps were rinsing the slides for 5 minutes in TBS, applying the polymer (the secondary antibody), incubation for 30 minutes at room temperature, further rinsing for 5 minutes in TBS, and exposure to chromogen [diaminobenzidine (DAB)] for 5 minutes at room temperature. The slides were then rinsed with running tap water for 5 minutes. Finally, the slides were counterstained with hematoxylin and dehydrated, cleared, and mounted. CD10 expression in myoepithelial cells of normal breast tissue was used as the positive control. CD10 expression in the tumor stroma (in both stromal cells and extracellular matrix) was scored semiquantitatively as negative (no staining), weak (either diffuse weak staining or weak or strong focal staining in less than 30% of the stromal cells or extracellular matrix), and strong (strong staining in 30% or more of the stromal cells or extracellular matrix).

The collected data was analyzed using SPSS18 (SPSS Inc., Chicago, USA). The correlations between CD10 expression and subtypes of invasive breast carcinoma were determined using Kruskal-Wallis correlation coefficient. Mann-Whitney correlation coefficient was used to determine the correlations between CD10 expression and ER and PR status. The relationships between CD10 expression and other variables were evaluated by Spearman’s correlation coefficient. P-values of less than 0.05 were considered as significant.

RESULTS

The mean age of the patients was 49.8 ± 11.7 years (range: 22-78 years) (Table 1). Invasive ductal carcinoma, not otherwise specified (NOS) comprised the majority of our study population (40 cases, 81.6%), followed by 5 cases (10.2%) of medullary carcinoma, and 4 cases (8.2%) of invasive lobular carcinoma (Table 1). All cases of invasive ductal carcinoma (NOS) were graded based on the Bloom and Richardson grading system. Most cases (25 cases, 62.5%) were grade II, followed by 12 (30%) grade III, and 3 (7.5%) grade I cases (Table 1).

The mean tumor size was 3.49 ± 1.83 cm
CD10 immunostaining was performed on all 49 cases. No stromal expression was detected in the normal breast tissue. The myoepithelial cells lining the normal acinar and ductal structures in normal breast parenchyma adjacent to the tumor were considered as the positive control for CD10 expression (Figure 1). There was no expression of CD10 in normal ductal cells, fibroblasts, and adipose cells. The staining was scored as negative (Figure 2), weak, and strong as described previously in the methods section. CD10 was found to be positive in 81.6% (40 cases), out of which 63.2% (31 cases) showed weak immunoreactivity (Figure 3) and 18.4% (9 cases) showed strong immunoreactivity (Figure 4) (Table 1).

Expression of stromal CD10 in breast cancer and its correlation with other clinicopathologic data.

Most patients in this study were ER positive (33 cases, 67.3%) and PR positive (31 cases, 63.3%). Mann-Whitney test showed a negative correlation between stromal CD10 expression and ER. However, the correlation was not statistically significant (p = 0.07) probably due to the rather small number of the cases in our study. The same finding was observed about PR (p = 0.08) (Table 2). On the other hand, a strong correlation was found between ER and PR status in chi-square test (p < 0.001).

The majority of our subjects (28 cases, 57.1%) were Her2/neu negative (0 or 1+), while 21 cases (42.8%) were HER2/neu positive including 11 cases (22.4%) with 2+ staining and 10 cases (20.4%) with 3+ staining. Spearman's correlation coefficient showed no significance relationship between stromal CD10 expression and HER2/neu status (r = 0.1; p = 0.24) (Table 2). Spearman's correlation coefficient showed a direct relationship between HER2/neu status and tumor grade (r = 0.31; p = 0.02).

Although Spearman's correlation coefficient failed to show any significant relationship between stromal CD10 expression and age (r = 0.005; p = 0.49), a direct correlation was observed between CD10 expression and tumor size (r = 0.32; p = 0.01), lymph nodes status (r = 0.29; p = 0.02), and tumor grade (r = 0.41; p = 0.004) (Table 2). In multivariate analysis of covariance, CD10 was found to have relationships with tumor grade, lymph node involvement, and tumor size in decreasing order. In fact, the P-value for the relationship between CD10 expression and tumor size increased from 0.01 to 0.04. The direct relationship between tumor grade and tumor size shown by Spearman's correlation coefficient (r = 0.309; p = 0.03) would well explain this increase.

Kruskal-Wallis test showed no significance relationship between stromal CD10 expression and subtype of the invasive breast carcinoma (p = 0.55).
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Table 1. Clinicopathologic data

| Age | Mean ± 2SD: 49.8 ± 11.7 years (range: 22-78 years) |
| Tumor size | Mean ± 2SD: 3.49 ± 1.83 cm (range: 1-10 cm) |
| Stromal CD10 Staining | Negative: 9 (18.4%) |
| | Positive (either weak or strong): 40 (81.6%) |
| Tumor Subtype | Invasive ductal carcinoma (NOS): 40 (81.6%) |
| | Invasive lobular carcinoma: 4 (8.2%) |
| | Medullary carcinoma: 5 (10.2%) |
| Tumor grade (invasive ductal carcinoma, NOS) | Grade I: 3 (7.5%) |
| | Grade II: 25 (62.5%) |
| | Grade III: 12 (30%) |
| Lymph node involvement | Zero: 23(46.9%) |
| | 1-3: 11(22.4%) |
| | 4-9: 10 (20.4%) |
| | ≥ 10: 5 (10.2%) |

The results are expressed as mean ± 2SD for age and tumor size and as number (%) for other parameters.

Table 2. The relationship between stromal CD10 expression and estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2/neu), involved lymph nodes, and grade status in invasive breast carcinomas

<table>
<thead>
<tr>
<th>Stromal CD10 expression</th>
<th>Negative</th>
<th>Weak</th>
<th>Strong</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>Negative</td>
<td>2 (12.5%)</td>
<td>9 (56.3%)</td>
<td>5 (31.3%)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>7 (21.2%)</td>
<td>22 (66.7%)</td>
<td>4 (12.1%)</td>
</tr>
<tr>
<td>PR</td>
<td>Negative</td>
<td>2 (11.1%)</td>
<td>11 (61.1%)</td>
<td>5 (27.8%)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>7 (22.6%)</td>
<td>20 (64.5%)</td>
<td>4 (12.9%)</td>
</tr>
<tr>
<td>HER2/neu</td>
<td>0 or 1+</td>
<td>6 (21.4%)</td>
<td>18 (64.3%)</td>
<td>4 (14.3%)</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>1 (9.1%)</td>
<td>7 (63.6%)</td>
<td>3 (27.3%)</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>2 (20%)</td>
<td>6 (60%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Number of involved lymph nodes</td>
<td>0</td>
<td>7 (30.4%)</td>
<td>14 (60.9%)</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td></td>
<td>1 - 3</td>
<td>1 (9.1%)</td>
<td>6 (54.5%)</td>
<td>4 (36.4%)</td>
</tr>
<tr>
<td></td>
<td>4 - 9</td>
<td>0 (0%)</td>
<td>9 (90%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td></td>
<td>≥ 10</td>
<td>1 (20%)</td>
<td>2 (40%)</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>Grade</td>
<td>I</td>
<td>2 (66.7%)</td>
<td>1 (33.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>5 (20%)</td>
<td>17 (88%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>1 (8.3%)</td>
<td>6 (50%)</td>
<td>5 (41.7%)</td>
</tr>
</tbody>
</table>

The values are expressed as number (%).

DISCUSSION

Proliferation of stromal cells is a common feature in cancer invasion and metastasis. Interactions of various types of cancer cells with stromal cells involve stimulatory and inhibitory factors which regulate cell adhesion and migration and affect the invasiveness and metastatic potential of cancer cells.[2] The modulating effect of matrix molecules on tumor invasion and metastasis gives the matrix an important role in cancers.[3] Increased knowledge concerning the role of stroma in breast cancer development and progression will help us to identify new prognostic markers and potential therapeutic targets in this frequent type of cancer among women.

Matrix metalloproteinases play a critical role in tissue remodeling by cleavage of protein components of the extracellular matrix. In normal breast tissue, CD10 is expressed by the myoepithelial cells lining the outer layer of the epithelial structures. In the majority of normal breast tissue, there are no CD10 positive stromal cells. However, a minor population of stromal cells has been shown to express CD10 in a small percentage of normal breast tissue.[2,11] The expression of CD10 by the stromal cells of nasopharyngeal carcinoma, gastric
carcinoma, and prostate cancer has been shown to be associated with tumor progression and metastasis.[4,5,12]

In the present study, we used immunohistochemical staining to investigate the expression of CD10 in the stroma of invasive breast carcinoma. The scoring system used in this study was based on the study of Makretsov et al. which divided the cases into three groups named as negative, weakly positive, and strongly positive as described previously.[1] Normal myoepithelial cells lining the acinar and ductal structures in normal parenchyma adjacent to the tumor were used as positive internal control. According to this scoring system, stromal CD10 expression was found in 81.6% of the cases (including 63.2% weakly positive and 18.4% strongly positive specimens) which is a significant frequency. Makretsov et al. found stromal CD10 expression in 79% of invasive breast carcinomas[1] that is close to the frequency observed in our study. Masaki et al. judged the expression of CD10 to be positive when more than 10% of the stromal cells around the neoplastic epithelial cells were positive. Based on this criterion, they detected stromal CD10 expression in 19% of invasive ductal carcinomas.[3] Iwaya et al. used the same criterion as Masaki et al. to define stromal CD10 expression and found its expression in 18% of invasive ductal carcinomas.[2] In a recent study by Puri et al., stromal CD10 expression was considered as negative when less than 10% of the stromal cells were immunoreactive with the marker. Cases with 10-30% positive stromal cells were considered as weakly positive and the presence of more than 30% positive stromal cells was defined as strongly positive. According to this definition, stromal CD10 expression was detected in 80% of the cases out of which 40% were weakly positive and 60% were strongly positive.[7] It seems that the different criteria used for scoring CD10 expression in the stromal component of breast carcinoma and the heterogeneity in the study populations regarding the frequency of various clinicopathologic parameters explain much of the difference observed in the frequency of the marker expression in various studies. While most cases of strong CD10 expression in our study were grade III tumors, the majority of our cases fitted into grade II tumors. Makretsov et al. reported similar findings.[1]

Like Iwaya et al.,[2] we observed no statistically significant relationship between stromal CD10 expression and age. Our results showed that stromal CD10 expression is positively correlated with tumor size, nodal metastasis, and tumor grade. Kim et al. have also found a significant correlation between stromal CD10 expression and tumor size,[8] while Makretsov et al.,[1] Iwaya et al.[2] Masaki et al.[3] and Puri et al.[7] failed to show a significant correlation between the two parameters. Iwaya et al.,[2] Masaki et al.,[3] and Kim et al.[6] suggested stromal CD10 expression to be significantly correlated with nodal involvement. However, Makretsov et al. found no correlation between stromal CD10 expression and lymph node status.[1] While Makretsov et al.[1] and Kim et al.[8] detected a statistically significant positive correlation between stromal CD10 expression and tumor grade, Iwaya et al.[2] and Puri et al.[7] reported the absence of a significant correlation between the two parameters.

We failed to establish a significant relationship between stromal CD10 expression and tumor subtype. Although the same has been observed by Makretsov et al.,[1] the small number of tumor subtypes other than invasive ductal carcinoma in our study decreases the validity of this result.

We found a negative correlation between stromal CD10 expression on one hand and ER and PR status on the other. However, the correlations were not statistically significant. Puri et al. obtained the same result.[7] Makretsov et al.[1] and Kim et al.[8] showed a statistically significant negative correlation between stromal CD10 expression and ER status. However, Makretsov et al. found no statistically significant correlation between stromal CD10 expression and PR status,[1]

In our study, stromal CD10 expression and HER2/neu status were not significantly correlated. While the same observation was reported by Makretsov et al.,[1] Puri et al. detected a statistically significant positive correlation between stromal CD10 expression and HER2/neu status.[7] The small number of studies evaluating this relationship necessitates further investigation on the subject.

The study by Puri et al. is the only one that has investigated the relationship between stromal CD10 expression and Ki67 index in breast carcinoma. According to the results of this study, there is a statistically significant positive correlation between stromal CD10 expression and Ki67 index.[7]

The effect of CD10 expression in the stromal component of breast carcinoma on survival has also been investigated in some previous studies. In general, stromal CD10 expression in breast cancer has been correlated with decreased overall and/or metastasis free survival.[1,3,6,7,13] As mentioned before, we did not have access to survival data of the patients.

As a whole, the discrepancies observed between the results of the studies focused on the relationship be-
Between CD10 expression of the stromal cells and clinical-pathologic prognostic factors in breast carcinoma may be attributable to several factors including different sample sizes and population heterogeneity regarding the studied parameters. Further studies with greater sample sizes and more homogenous populations are needed to validate the results.

Since tumor size and nodal status are important determinants of tumor stage in breast carcinoma, our data concerning the positive correlation between stromal CD10 expression and tumor size and nodal involvement suggests a strong effect of the stromal CD10 expression on aggressive behavior of breast carcinoma. Moreover, the positive correlation between stromal CD10 expression and tumor grade strengthens this conclusion. However, it is still unclear how CD10 positive stromal cells induce more aggressiveness of breast carcinoma. Since CD10 is a matrix metalloproteinase, it may support more invasiveness of the carcinoma cells by facilitating matrix degradation and remodeling.[2]

CD10 positive stromal cells in normal bone marrow also induce differentiation of pre-B cell lineage and accelerate their proliferation and motility. The interaction of CD10 positive stromal cells with breast carcinoma cells might also induce the carcinoma cells to proceed through the cell cycle and activate their movement.[12,14] The observation of Puri et al. concerning the positive correlation between stromal CD10 expression and Ki67 index in breast carcinoma supports the potential role of CD10 positive stromal cells in inducing carcinoma cells to proceed through the cell cycle.[7]

Finally, our results in parallel with those by other investigators open new horizons of therapeutic strategies in future. Treatments targeted to decrease the role of CD10 positive stromal component in aggressive behavior of breast carcinoma may be promising in this regard.

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REFERENCES