Original Research Article

Evaluation of stromal CD10 expression and its correlation with other clinico-pathological factors in invasive breast carcinoma

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Article Info

Abstract

Context: Breast cancer is the most common cause of cancer related deaths among women worldwide, thus forming a significant burden of disease. Various epithelial markers like ER, PR, and Her2/neu are well established factors predicting prognosis and treatment response in breast cancer. However, stroma has a definite role in tumor invasion and metastasis. Novel stromal markers are now emerging which aid in assessing the prognosis and treatment of invasive breast lesions.

Objectives: The present study aims to assess the frequency of stromal CD10 expression in malignant breast lesions and evaluate its prognostic significance and correlation with other clinico-pathological factors.

Design: Of the 172 cases of breast carcinoma, 158 were that of infiltrating ductal carcinoma (NOS) and were selected for CD10 staining forming the study group. Stromal CD10 was assessed immunohistochemically in these breast lesions and statistically correlated with known prognostic markers of breast carcinoma.

Results: Stromal CD10 significantly correlated with increasing tumor grade (P=0.0026), nodal positivity (P=0.0016), ER negativity (P=0.0007), Her2/neu positivity (P=0.0061), and worsening prognosis (P=0.04). No correlation was found between stromal CD10 expression with age, increasing tumor size and tumor stage. A higher CD10 positivity was seen in the triple negative group as compared to the non-triple negative group, but was statistically insignificant (P=0.0994).

Conclusion: Stromal CD10 expression showed a positive correlation with well-known poor prognostic factors in breast cancer [higher tumor grade, nodal positivity, ER negativity, Her2/neu positivity and worsening prognosis (NPI)], suggesting its definite role on tumor aggressiveness and its potential use as an independent prognostic marker.

Introduction

CD10, also known as common acute lymphoblastic leukemia antigen (CALLA), is a membrane bound zinc dependent metalloprotease that inactivates various kinds of biologically active peptides. Matrix metalloproteinases are metallopeptidases that cleave protein component of extracellular matrix and are one of the important factors that play a role in tumor progression. Upregulation of expression of extracellular matrix gene and enhanced matrix metalloprotein activity is associated with poor prognosis.[1]

CD10 is expressed on the surface of a variety of normal cells (bone marrow lymphoid stem cells, pro-B lymphoblasts, mature neutrophils, breast myoepithelial cells, bile canaliculi, and renal epithelial cells). Stromal CD10 expression is seen to be associated with aggressiveness in various epithelial malignancies including breast,[2,3] prostate,[4] gastric[5] and lung carcinomas.[6,7]

Breast cancer is the most frequent cancer and the leading cause of cancer related deaths among women worldwide, thus forming a significant burden of disease. Various epithelial markers like estrogen receptor (ER), progesterone receptor (PR), and Her2/neu are well established factors predicting prognosis and treatment response in breast cancer. However, studies have demonstrated an important role of stroma in tumor invasion and metastasis.[1] Stromal markers have not been extensively studied and are now emerging as new markers in predicting the prognosis and treatment in malignant breast lesions.

The present study was conducted to assess the frequency of expression of CD10 in the stroma of malignant breast lesions and to evaluate the prognostic significance of stromal CD10 expression and its correlation with other clinico-pathological factors like age, tumor size, grade, stage, lymph node status, hormone status and Her2/neu expression.

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Materials and Methods

The present study was carried out on 172 patients of malignant breast lesions treated by breast conservative surgery or modified radical mastectomy attending the department of Surgery and Pathology of the Jawaharlal Nehru Medical College and Hospital, after obtaining ethical approval from the institutional ethical committee. Of these 172 cases, 158 cases were diagnosed as Infiltrating Ductal Carcinoma, not otherwise specified (NOS) and these formed the study group. H&E stained histological sections were used for diagnosing and lesions were typed according to WHO classification of tumors of the breast (2012) and graded based on Scarff-Bloom-Richardson grading system. Nottingham's Prognostic index (NPI) was calculated, and patients were divided into six NPI groups (as advocated by Blamey et al, 2007).[8]

(EPG) Excellent Prognostic group - 2.08 to 2.4
(GPG) Good Prognostic group - >2.42 to ≤ 3.4
(MPG I) Moderate I Prognostic group - >3.42 to ≤4.4
(MPG II) Moderate II prognostic group - >4.42 to ≤5.4
(PPG) Poor prognostic group - >5.42 to ≤6.4
(VPG) Very poor prognostic group - ≥6.5

NPI was calculated as:

NPI = 0.2 × Tumor size (cm) + Lymph node stage (I/II/III) + Tumor grade (1/2/3)

4μ thick sections were cut for immunohistochemical assay of ER, PR, Her2/neu and CD10 immunomarkers. Table 1 shows specifications of primary antibodies used. Tumor epithelial cells of invasive ductal carcinoma of breast served as positive controls for ER, PR, and Her2/neu. Normal myoepithelial cells of breast tissue were used as positive built in control for CD10 (Fig1). Negative external controls, with omission of primary antibody were used for validating the reaction.

Evaluation of CD10 Immunostaining

Expression of CD10 by stromal cells in invasive breast lesions was considered negative if <10% of the stromal cells show membranous staining (Fig2), and positive if more than 10% of stromal cells exhibited membranous positivity (Fig. 3).[9] Scoring of slides was done by two pathologists separately, resolving the discrepancies by consensus.

Correlations between CD10 and various clinicopathological data as well as with ER, PR and Her2/neu status were studied.

Statistical Analysis

The statistical analysis was carried out using SPSS software (v.20.0) and p-value of <0.05 was considered to be statistically significant. IHC expression and its correlation with various histopathological parameters were carried out by independent t-tests like Chi square test and Fischer exact test.

Results

Of the 176 cases, 158 cases were diagnosed as infiltrating ductal carcinoma (NOS), forming the study group. In the present study, mean age at presentation was 46.46 ± 11.74 years with the highest incidence of breast cancer in the age group of 40-60 years. No stromal CD10 expression was detected in the normal breast tissue. Out of 158 cases, 109 (69.0%) cases turned out to be CD10 positive while 37 (31.0%) were CD10 negative. Correlations between CD10 and various clinico-pathological parameters were evaluated as depicted in Table 2.

A positive correlation was noted between CD10 expression and lymph node metastasis which was statistically significant (P=0.0016). A statistically significant trend was seen with increasing tumor grade (P=0.0026) and with worsening prognosis (P=0.0061). CD10 positivity was also significantly associated with ER negativity (P=0.0007) and Her2/neu positivity (P=0.0149).

A higher CD10 positivity was seen in the triple negative group as compared to the non-triple negative group. However, the difference was not found to be significant (P=0.0947).

Discussion

Stroma plays a very important role in tumor invasion and metastasis as well as response to therapy. Interaction of surrounding cell types with tumor cells in the tumor microenvironment is pivotal in cancer progression and response to therapy.[10]

Matrix metalloproteinases are the family of metallopeptidases that have an important role in tissue remodeling, by cleaving the protein component of extracellular matrix. CD-10 is a zinc dependent metalloproteinase present in the stromal cells of some breast carcinoma and is said to be up-regulated in the breast cancer cells.[11] CD10 has come up as a potential new marker for prognostication and possible therapeutic intervention in invasive breast cancer, as shown in various studies published earlier. Previous studies have demonstrated that expression of CD10 in breast stromal cells is associated with biological aggressiveness and poor prognosis.[2,3,9,12]

In the present study, we witnessed CD10 expression in the stromal cells of invasive ductal carcinoma, but not in the stromal cells of normal breast suggesting that tumor-stromal interaction exists between breast cancer cells and CD10-positive stromal cells.

We noted a positive correlation between CD10 expression and lymph node metastasis which was statistically significant, a finding similar to the studies by other authors.[12,13]

Our results showed a statistically significant correlation between stromal CD10 expression and tumor grade (P=0.0026), a finding concordant to Makretsov et al,[2] Puri et al,[3] Jana et al,[9] Taghizadeh et al,[13] and Kim et al.[14] However, there was no statistically significant correlation of CD10 expression with tumor stage. This indicates that both tumor grade and tumor stage are independent prognostic factors.

There was no statistically significant correlation of CD10 expression with tumor size in the present study similar to the results of Makretsov et al.[2] On the contrary, Kim et al.[14] and Mohammadizadeh et al.[15] have found a significant correlation between stromal CD10 expression and tumor size.

We also studied the association between stromal CD10 expression and Nottingham's prognostic index and noted a significant correlation. Same result was observed by Jana et al.[9] and Devi et al.[12] Only a small number of investigators studied this relationship and further studies with a larger sample size are required to firmly establish the correlation.

Similar to previous studies, our study also showed a statistically significant inverse correlation between CD10 and ER expression suggesting a higher CD10 expression in ER negative cases. However, PR did not demonstrate a statistically significant correlation with CD10.

We noted a statistically significant correlation between stromal CD10 expression and Her2/neu status similar to Puri et al.[3] and Jana et al.[9] However, Makretsov et al.[2] did not report a statistically significant correlation between stromal CD10 expression and Her2/neu status. In the present study, higher stromal CD10 expression was seen in the triple negative group (ER-/PR-/Her2-) compared to the non-triple negative group. However, the difference was not found to be significant. The same finding was observed by Jana et al.[9] Since only a few studies evaluated this

correlation further studies are required to validate this finding.

The effect of stromal CD10 expression in breast carcinoma on survival has also been investigated in some previous studies where it has been correlated with decreased overall and/or metastasis free survival[2,14] but we could not perform long term follow up in our patients.

As a whole, the discrepancies observed between the results of various studies investigating the correlation between stromal CD10 expression and clinico-pathologic factors in breast carcinoma may be attributed to several factors including different sample sizes and population heterogeneity regarding the studied parameters.

CD10 has been shown to have a role in drug designing. Pan C et al,[16] in a study demonstrate that CD10 is capable of cleaving CPI-0004Na (a prodrug of doxorubicin) and generating intracellular Dox, which has a higher potency. Cytotoxicity of CPI-0004Na is inhibited by phosphoramidon, a known inhibitor of CD10 enzymatic activity.

Thus routine CD10 staining may help in planning the treatment for breast cancer patients.

Table 1: Specifications of primary antibodies used

Antibody against	Source	Clone	Dilution	Antigen retrieval
ER	Thermo scientific	SP1	1:100	Microwave citrate buffer pH- 6.0, 95ºC, 10 min
PR	Thermo scientific	SP2	1:100	Microwave citrate buffer pH- 6.0, 95ºC, 10 min
Her2/neu	Thermo scientific	SP3	1:100	Microwave citrate buffer pH- 6.0, 95ºC, 10 min
CD 10	Thermo scientific	(Clone 56C6)	1:30	Microwave EDTA buffer pH- 8.0, 95ºC, 10 min

Table 2: Correlation of stromal CD10 with clinic-pathological parameters

Parameters		Total	Stromal CD10 positivity	Stromal CD10 negativity	P value	Significance
Age group (in years)	<40	40	28(70%)	12(30%)	0.5092	No
	40-60	102	72(70.6%)	30(29.4)		
	>60	16	9(56.3%)	7(43.7%)		
Tumor size	T1	10	5(50%)	5(50%)	0.0646	No
	T2	61	49 (80.3%)	12(19.7%)		
	T3	70	43(61.4%)	27(38.6%)		
	T4	17	12(70.6%)	5(29.4%)		
Tumor grade	1	16	5(31.2%)	11(68.8%)	0.0026	Yes
	2	83	60(72.3%)	23(27.7%)		
	3	59	44(74.6%)	15(25.4%)		
LN metastasis	Positive	95	75(78.9%)	20(21.1%)	0.0016	Yes
	Negative	63	34(54.0%)	29(46.0%)		
Tumor stage	1	4	2(50%)	2(50%)	0.0800	No
	2	87	55(63.2%)	32(36.8%)		
	3	67	52(77.6%)	15(22.4%)		
ER	Positive	86	49(56.9%)	37(43.1%)	0.0007	Yes
	Negative	72	60(83.3%)	12(16.4%)		
PR	Positive	71	44(62%)	27(38%)	0.1213	No
	Negative	87	65(74.7%)	22(25.3%)		
Her2/neu	Positive	76	60(79.0%)	16(21.0%)	0.0149	Yes
	Negative	82	49(59.8%)	33(40.2)		
NPI (Nottingham's prognostic index)	**EPG**	9	3(33.3%)	6(66.6%)	0.0061	Yes
	GPG	11	5(45.5%)	6(54.5%)		
	MPG1	44	26(15.9%)	18(43.2%)		

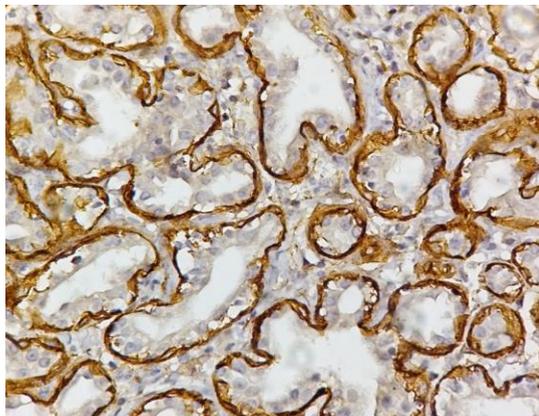|					
MPG2	40	31(77.5%)	9(22.5%)		
PPG	30	23(76.7%)	7(23.3%)		
VPG	24	21(87.5%)	3(12.5%)		
Triple negative	25	21(84.0%)	4(16%)	0.0994	No
Non Triple negative	133	88(66.2%0	45(33.8%)		

Fig. 1: **Control**: Non-neoplastic myoepithelial cells in normal breast tissue showing CD10 expression (x 400)
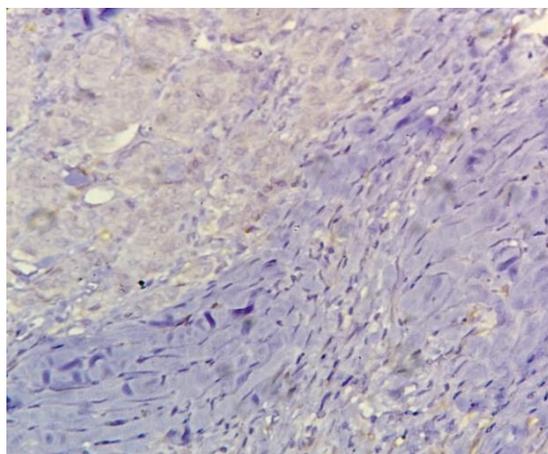

Fig 2: **Negative Staining**: Infiltrating Ductal Carcinoma exhibiting CD10 expression in <10% stromal cells (x 400)
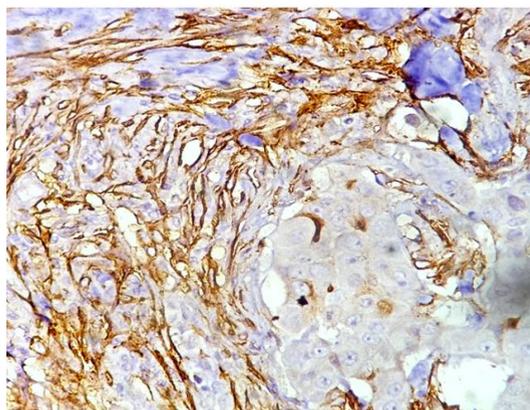

Fig 3: **Positive Staining**: Infiltrating Ductal Carcinoma exhibiting CD10 expression in >10% stromal cells (x 400)

Conclusion

Stromal CD10 in our study significantly correlated with well-known poor prognostic factors in breast cancer including higher tumor grade, nodal positivity, ER negativity, Her2/neu positivity and worsening prognosis (NPI), suggesting a definite role of the stromal CD10 expression on tumor aggressiveness in breast carcinoma and its possible use as an independent prognostic marker.

Conflict of Interest: None.

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