Original Research Article

A study on stromal CD10 expression in invasive breast carcinoma

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Abstract

Introduction: Breast cancer is one of the most common cancers among Indian women. Although breast cancer is an epithelial malignancy, stroma plays a key role in modulating tumor invasion and metastasis. Stromal markers are now emerging as novel markers in assessing the prognosis of invasive breast cancer and have not been studied extensively till date.

Aim and objectives: To estimate the frequency of expression of stromal CD10 in invasive breast carcinomas, to assess prognostic significance of stromal CD10 expression and its correlation with other clinicopathological factors like age, menopausal status, tumor size, grade and lymph node status.

Materials and methods: A total of 59 cases of breast cancer were included in the study. Representative sections were taken and hematoxylin and eosin staining was done. Immunohistochemistry was performed with CD10. Stromal expression of CD10 (>10% stromal positivity was considered positive) in invasive breast carcinoma was noted and was statistically analyzed with different known prognostic markers of breast carcinoma.

Results: Stromal expression of CD10 was found to be significantly associated with increasing tumor size (P = 0.03), increasing tumour grade (P =0.001), worsening prognosis (P = 0.002) and lymph node status (0.0005). No correlation was found between CD10 over expression and age, menopausal status.

Conclusion: 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.

Key words

Invasive Breast Carcinoma, Stroma, CD10.

Introduction

Breast cancer is one of the most common cancers among Indian women. Although breast cancer is an epithelial malignancy, stroma plays a key role in modulating tumor invasion and metastasis. A better understanding of stromal contribution to cancer progression will identify specific signals that promote growth, dedifferentiation, invasion, and ectopic survival of tumor cells and may eventually result in the identification of new therapeutic targets for future treatment [1]. Stromal markers are now emerging as novel markers in assessing the prognosis of invasive breast cancer and have not been studied extensively till date. This justifies the current study of new stromal marker CD10 for prognosis in invasive breast carcinoma.

CD10 is a zinc-dependent metalloproteinase that has been called common acute lymphoblastic antigen (CALLA). It is frequently expressed in bone marrow lymphoid stem cells, pro-B lymphoblasts, mature neutrophils, various lymphoma subtypes, renal cell carcinoma and endometrial stromal sarcoma. Several reports indicated that stromal CD10 expression is associated with biological aggressiveness in various epithelial malignancies [2–6].

Aim and objectives

The aim and objectives of this study

- To estimate the frequency of expression of stromal CD10 in invasive breast carcinomas
- To assess prognostic significance of Stromal CD10 expression and its correlation with other clinicopathological factors like age, menopausal status, tumor size, grade and lymph node status.

Materials and methods

A prospective study was conducted on 59 Modified Radical Mastectomy specimens which were sent to the Department of Pathology, MGM Hospital, Warangal from Aug 2014 to July 2015. Relevant history like age, menopausal status was taken.

All specimens were formalin fixed, representative sections taken and H & E staining was done. The grading of breast carcinoma was done according to the Nottingham's combined histologic grade (Elston–Ellis modification of Scarff–Bloom–Richardson grading system). Nottingham's Prognostic index (NPI) was calculated, and patients were divided in six NPI groups (as advocated by Blamey, et al.) [7].

(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 to 6.8

Immunohistochemistry for CD10

3 µ sections were taken on poly-L-Lysine coated slides. Sections were deparaffinized in xylene followed by hydration in descending ethanol grades. Antigen retrieval was performed by heating sections at 95°C, 3 cycles of each 5 min in Tris–EDTA buffer (pH 9.0). Sections were then incubated with power block for 10 min to reduce the non-specific antibody binding followed by incubation at 4°C with mouse monoclonal antibody against human CD10 (Dako AntiHuman CD10) for 1hour. After three washes with TBS, secondary antibody was added for 30 min. After three washes with TBS

(trisphosphate buffer solution), 3,3'-diaminobenzidine substrate (DAB tetrahydrochloride) was applied to the sections for 10 min and sections were counterstained with hematoxylin, dehydrated with ethanol and xylene and mounted permanently with DPX. Negative control section was processed by omitting primary antibody. The myoepithelial cells lining the normal acinar and ductal structures in normal breast parenchyma were considered as the positive control for CD10 expression. CD10 scoring was done as negative, weak positive and strong positive (**Table - 1**).

Table - 1: CD10 scoring.

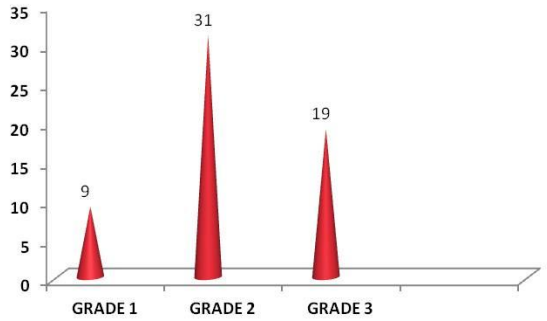
Score	CD10 staining
Negative	<10% stromal positive cells/core
Weak positive	10–30% stromal positive cells/core
Strong positive	>30% stromal positive cells/core

Statistical analysis was performed by using Graph pad software. The Correlation between stromal cells CD10 expression and clinicopathological features was evaluated using the chi-square test. A P-values <0.05 was considered as statistically significant.

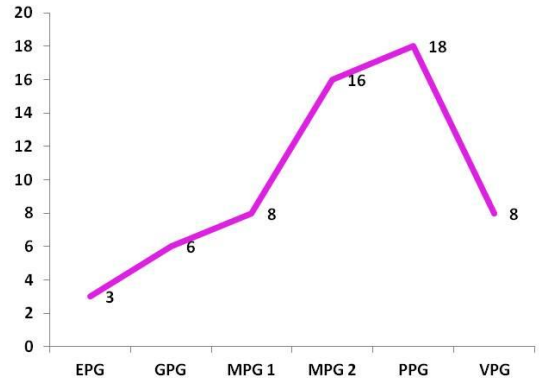
Results

Most of our patients belong to 41 -50 years of age group. Infiltrating ductal carcinoma, not otherwise specified (NOS), the comprised majority of our study population (93%) followed by three cases (5%) of medullary carcinomas and one case of lobular carcinoma (2%). Modified Bloom and Richardson grading was performed on all cases. Most patients in our study belonged to grade 2 (52%), while 19/59 (32%) belonged to grade 3 and 9/59 (15%) belonged to grade 1 (**Graph - 1**). Nottingham's prognostic index was calculated and patients were divided into six groups as described previously (**Graph - 2**).

Graph – 1: Representing distribution of cases – Modified Bloom Richardson's grading system.



Graph – 2: Representing distribution of cases – Nottingham's prognostic index.



CD10 immune staining was done on all 59 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 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. CD10 was found to be positive in 81% (48 cases), out of which 54% (32 cases) showed weak immunoreactivity (**Figure - 3**) and 27% (16 cases) showed strong immunoreactivity (**Figure - 4**).

Correlation between stromal CD10 expression and clinicopathological data of the cases was as per **Table - 2**. Age and menstrual status had no significant correlation with stromal CD10 expression. Meanwhile, larger tumor size (p=0.003), higher tumor grade (p=0.0016), the

number of positive nodes ($p<0.001$), worsening prognosis ($p=0.002$) have associated with stronger stromal CD10 staining.

Figure – 1: Highlighted non neoplastic myoepithelial cells by CD10 positive control (400X).

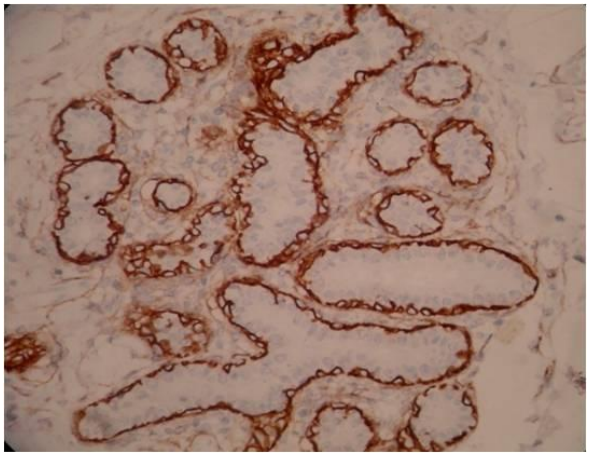


Figure – 2: Negative CD10 Stromal staining (immunohistochemical staining, 400X).

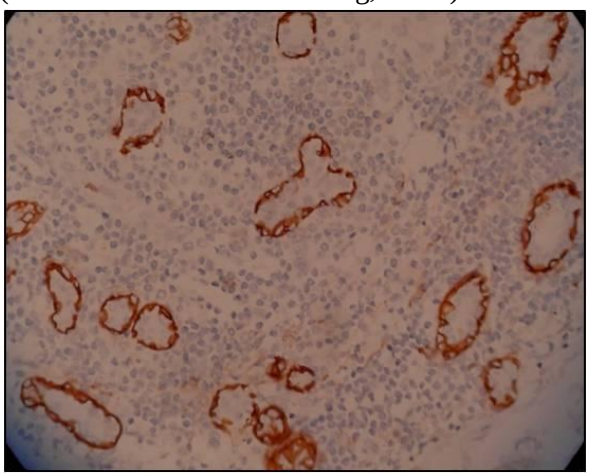


Figure – 3: Weak CD10 Stromal staining (immunohistochemical staining, 400X).

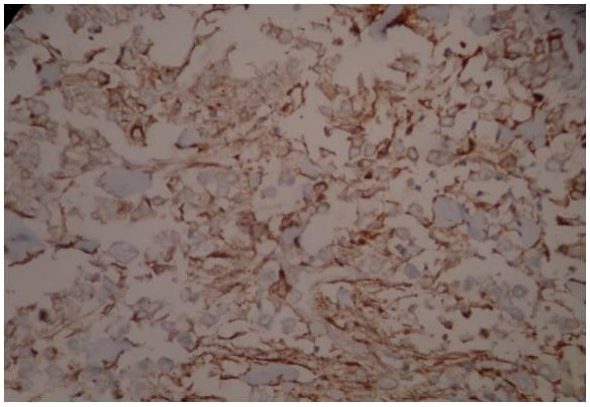
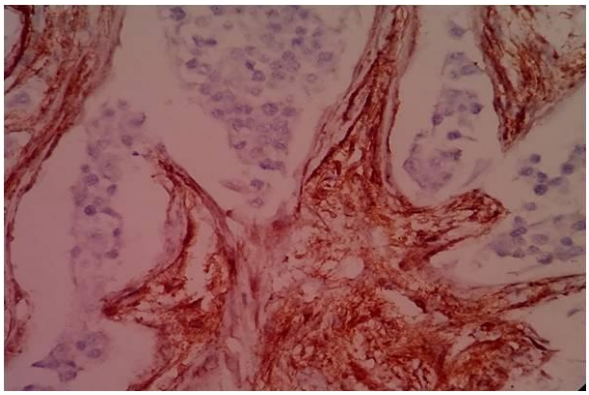


Figure – 4: Strong CD10 Stromal staining (immunohistochemical staining, 400X).



Discussion

Although breast cancer is an epithelial malignancy arising in the epithelial cells of the terminal ductal lobular unit, stromal microenvironment plays an important role in breast cancer evolution and metastasis. Proliferation of stromal cells is commonly seen when cancer cells invade and metastasize. The invasive and/or metastatic potential of several types of cancer cells is regulated by interactions with stromal cells, which involve stimulatory and inhibitory factors that regulate such functions as cellular adhesion, migration, and gene expression [8, 9, 10]. Although the proliferation of stromal cells is thought to be induced by the cancer cells, the morphological phenotype distinguishing cancer-specific stromal proliferation from normal mesenchymal cells has not been studied extensively. We immunohistochemically demonstrated that CD10 is expressed by the stromal cells within the area of invasive ductal carcinoma, but not in the stromal cells of normal breast. The fact that CD10-positive stromal cells are frequently seen at the invasive front suggests that tumor- stromal interaction exists between breast cancer cells and CD10-positive stromal cells. Because CD10 belongs to the group of metalloprotease family, CD10 may be induced by the cancer cells through soluble factors similar to other members of metalloprotease family. Makretsov, et al. found stromal CD10 expression in 79% of invasive breast carcinomas [4] that is close to the frequency observed in our study. Like Iwaya, et al. [3], we observed no

statistically significant relationship between stromal CD10 expression and age. Our result showed that stromal CD10 expression is positively correlated with increasing tumor size, nodal metastasis, tumor grade and worsening prognosis. Mohammadizadeh, et al. [14], and Ali Taghizadeh kermani, et al. [15] also showed similar significant correlation between CD10 expression and increasing tumour grade, lymph node status and tumour size.

Table – 2: Correlation between stromal CD10 expression and clinicopathological data of the cases.

		Total No.	**CD10 staining**			**P value**
			Negative	**Weak positive**	**Strong positive**	
Age	<40 years	22	10	8	4	
	41-60 years	26	14	7	5	0.52
	>61 years	11	6	3	2	
Menstrual status	Premenopausal	33	14	11	8	0.21
	Postmenopausal	26	7	10	9	
Tumor size	<2 cm	20	15	4	1	0.003
	2-5 cm	25	7	9	7	(<0.05)
	>5 cm	14	8	7	5	
Lymph node status	Negative	28	18	8	2	0.0005
	1-3	15	4	7	4	(<0.05)
	>4	16	2	9	5	
Tumor grade	Grade I	8	7	1	0	0.0016
	Grade II	32	12	13	7	(<0.05)
	Grade III	19	3	10	6	
NPI	EPG	3	2	1	0	0.0023
	GPG	6	3	2	1	(<0.05)
	MPG I	8	5	2	1	
	MPG II	16	2	9	2	
	PPG	18	3	8	7	
	VPG	8	2	4	2	

Kim, et al. have also found a significant correlation between stromal CD10 expression and tumor size, [12] while Makretsov, et al. [4], Iwaya, et al. [3], Masaki, et al. [11], and Puri, et al. [13] failed to show a significant correlation between the two parameters. Iwaya, et al. [3], Masaki, et al. [11], and Kim, et al. [12] suggested stromal CD10 expression to be significantly correlated with nodal involvement. However, Makretsov, et al. found no correlation between stromal CD10 expression and lymph no de status [4]. While Makretsov, et al. [4], and Kim, et al. [12] detected a statistically significant positive correlation between stromal CD10 expression and tumor grade, Iwaya, et al. [3], and Puri, et al. [13] reported the absence of a significant correlation between the two parameters. Since tumor size and nodal status are important determinants of tumor stage in brea st carcinoma, our data concerning the positive co rrelation 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.

Conclusion

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

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