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An Analysis of Correlation of Stromal CD10 Expression in Carcinoma Breast NOS Type with ER, PR and HER2/Neu

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Abstract : ***Background:*** *Breast carcinoma is one of the most common carcinoma among women in India. Stroma has an important pathogenetic role in carcinoma of breast. Stromal marker could be novel marker for assessing the prognosis of breast cancer.* ***Methods:*** *30 invasive ductal carcinoma of breast NOS type were selected. Hematoxylin and eosin staining was done. Immunohistochemistry was done with CD10, ER, PR, and HER2. CD10 expression in stroma was studied and statistically analyzed with ER, PR and HER2.* ***Results:*** *strong positivity for stromal CD10 was observed in 46% (14 out of 30) of cases. 13 out of 14(92%) CD10 positive cases were negative for ER and PR. 10 out of 14(71%) CD10 positive cases showed HER2/neu positivity. CD10 expression was significantly associated with ER and PR negativity(P value<0.05), HER2/neu positivity.* ***Conclusions:*** *Stromal CD10 expression is inversely correlated with hormonal receptors and directly correlated with HER2 positivity. CD10 could be used as novel prognostic marker in carcinoma of breast and used for drug development.*

Keywords - *breast carcinoma, prognostic marker, stromal CD10, stromal marker*

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I. Introduction

National cancer registry programme 2011 report shows that Breast cancer is the most common cancer among women in India.[1] Worldwide breast carcinoma is the most common non skin cancer in women.[2] Present the mortality rate for breast carcinoma in India is 11.1per 10,000.[3]

Breast parenchyma is composed of duct (epithelial origin) and stroma (mesenchymal origin). Growth of cancer breast depends partly on chemical mediators between tumour cells and stromal cells. [4] CD10 is a myoepithelial marker. [5] Aggressive invasive ductal carcinoma of breast is associated with loss of CD10 expression in myoepithelial cells and expression of CD10 in stroma.[4] Carcinogenesis is promoted by genetic changes in stroma.[6] At present only few studies highlight role of the stomal expression of CD10 in growth, tumor progression and prognosis of breast cancer.[7]

CD10 (common acute lymphoblastic leukaemia antigen, CALLA) is a cell surface protease. By acting as a stem cell regulator in the breast CD10 prevents uncontrolled proliferation of stem cells.[8] Apart from breast myoepithelial cells CD10 expressed in lymphoid stem cells, neutrophils, and other epithelial cells.[9] CD10 also expressed in stroma of prostate, lung and colorectal carcinoma. In stomach cancer, CD10 positive stromal cells are correlated with vascular invasion and metastasis. [10] Present chemotherapeutic drugs target the epithelial cells while stromal cells are spared which may result in recurrence. Stromal cells could be potential and novel therapeutic targets.

Present study intends to analyze the correlation of stromal expression of CD10 in breast carcinoma with ER, PR and HER2/neu

Aim and Objective

To analyze the correlation of stromal expression of CD10 in breast carcinoma with ER, PR and HER2/neu.

II. Methods

Study design

Prospective study

Study population

Specimen with invasive ductal carcinoma of breast received in the department of pathology, Coimbatore Medical College, during the period for one year.

Sample size

30 patients with invasive ductal carcinoma of breast diagnosed by histomorphological method.

Inclusion criteria

- Age from 18 to 75 years.
- Patients with Invasive ductal carcinoma of breast not otherwise specified (NOS) type, stage I, II and III diagnosed by histomorphological studies.
- Patients irrespective of whether axillary dissection done for lymph node status or not.

Exclusion criteria

- Age less than 18 and more than 75 years.
- Breast carcinoma other than invasive ductal breast carcinoma NOS type.
- Patients with Stage I tumour who received neoadjuvant chemotherapy.
- Patients with Stage IV tumour who received chemotherapy and radiotherapy.
- Male patients
- Ill fixed specimen

Data collection

Clinically diagnosed breast carcinoma patient were evaluated with complete blood count, blood urea, blood sugar, serum creatinine, X ray chest, ECG, and Echocardiogram for surgical fitness. The patients underwent modified radical mastectomy procedure after obtaining informed written understandable consent. Specimens were collected in 10% neutral buffered formalin.

Histopathological examination

30 breast carcinoma specimens were fixed in 10% neutral buffered formalin for twenty four hours. Specimens were grossed and representative bits from carcinomatous areas were sampled. Hematoxylin and Eosin stained microscopic slides of the primary tumours were reviewed to confirm the diagnosis, to define tumour subtype and to standardize grading of invasive ductal carcinoma.

Immunohistochemistry (IHC) for CD10

Four micron sections were cut. Sections were deparaffinized in xylene followed by hydration in descending ethanol grades. Antigen retrieval was performed by heating sections at 95°c 4 cycles of 5 min each for CD10 in Tris–EDTA buffer (pH 9.0), for HER2/neu,ER and PR in citrate buffer(Ph 6.0). Sections were then incubated with power block for 10 min, followed by incubation with primary antibodies for 1 hour. Mouse monoclonal antibody against human CD10 was used. After two washes with TBS (trisphosphate buffer solution) secondary antibody was added for 30 min. After two washes with TBS, 3, 3'-diaminobenzidine substrate (DAB tetra hydrochloride) was applied to the sections for 10 min and sections were counterstained with Ehrlich Hematoxylin, dehydrated with ethanol and xylene and mounted permanently with DPX.

Quality control

As part of quality control positive control slide from fibro adenoma (Periductal cells) were used for CD10. Negative control slides were also used to enhance the accuracy of the results.

Evaluation of staining

CD10 scoring was done as per the following table (TABLE 1). [11] Pattern of staining for CD10 is cytoplasmic and membranous positivity in stromal cells. Both negative and weak expressions were considered as negative. Only strong CD10 expression was considered as positive for statistical purpose (Figure 1 to 3). ER and PR scoring was done as per Allred/Quick scoring method (TABLE 2). HER2/neu scoring was done as per TABLE 3.

Statistical analysis:

The collected data was tabulated and analyzed. Statistical correlations between stromal expression of CD10 and ER, PR & HER2/neu were performed as per Chi square test. P values of less than 0.05 were considered as significant.

Human participant protection

Study was undertaken after obtaining institutional ethical committee clearance. The procedures were carried out with written understandable informed consent from the patients.

III. Results

Thirty cases were studied and the following results were obtained. Most of invasive ductal carcinoma of breast cases belong to 41- 50 age group (36.7%).

73% (22 out of 30) of the cases showed positivity for CD10 in the stroma, of which 46 %(14) cases were strongly positive and 27 %(8) were weakly positive (TABLE 4), (Figure 1to 3).

92% (13 /14) of the strong stromal CD10 positive Invasive ductal carcinoma of breast showed ER negativity. The association is statistically significant, p value is less than 0.05(p value 0.002, Chi- square test)(TABLE 5),(Figure 4). 92% (13 /14) of the strong stromal CD10 positive Invasive ductal carcinoma of breast showed PR negativity. The association is statistically significant, p value is less than 0.05 (p value 0.0005, Chi- square test) (TABLE 6), (Figure 5). 71% (10/14) of the stromal CD10 positive Invasive ductal carcinoma of breast showed HER2/neu expression. The association is statistically significant, p value is less than 0.05 (p value 0.0009, Chi- square test)(TABLE 7),(Figure 6).

TABLE 1: CD10 scoring

Score	Result	CD10 staining
0	Negative	<10% stromal positive cells(cytoplasmic and membrane positivity)
1	Weak	10%-30% stromal positive cells
2	Strong	>30% stromal positive cells

TABLE 2: Allred/ Quick Score System. ER And PR Markers Were Considered Positive When The Combined Score For Proportion And Intensity Is 3 Or More.

Score	Score for proportion	Score for intensity
0	No staining	No staining
1	<1% Nuclei staining	Weak staining
2	1%-10% Nuclei staining	Moderate staining
3	11%-33% Nuclei staining	Strong staining
4	34%-66% Nuclei staining	
5	67%-100% Nuclei staining	

TABLE 3 : HER 2-neu scoring

Staining pattern	Score	HER2 neu Overexpression
No staining or membrane staining<10% tumor cells	0	Negative
Faint/perceptible membrane staining in >10% cells	1+	Negative
Weak to moderate complete membrane staining in >10% cells	2+	Weak
Strong complete membrane staining in >30% cells	3+	Strong

TABLE 4: Stromal expression of CD1O in breast carcinoma

Stromal CD10 expression	Negative	Weak positive	Strong positive	Total
Breast carcinoma	8(27%)	8(27%)	14(46%)	30

TABLE 5: Correlation Of Stromal CD10 Expression With ER. 92% (13 /14) Of The Strong Stromal CD10 Positive Invasive Ductal Carcinoma Of Breast Showed ER Negativity.

	CD10			
ER	NEGATIVE	WEAK POSITIVE	STRONG POSITIVE	TOTAL
NEGATIVE	2	3	13	18
POSITIVE	6	5	1	12
TOTAL	8	8	14	30

TABLE 6: Correlation Of Stromal CD10 Expression With PR. 92% (13 /14) Of The Strong Stromal CD10 Positive Invasive Ductal Carcinoma Of Breast Showed PR Negativity.

	CD10			
ER	NEGATIVE	WEAK POSITIVE	STRONG POSITIVE	TOTAL
NEGATIVE	3	1	13	17
POSITIVE	5	7	1	13
TOTAL	8	8	14	30

Table 7: Correlation Of Stromal CD10 Expression With HER2/neu. 71% (10/14) of the stromal CD10 positive Invasive ductal carcinoma of breast showed HER2/neu expression.

	CD10			
ER	NEGATIVE	WEAK POSITIVE	STRONG POSITIVE	TOTAL
NEGATIVE	8	7	4	19
POSITIVE	0	1	10	11
TOTAL	8	8	14	30

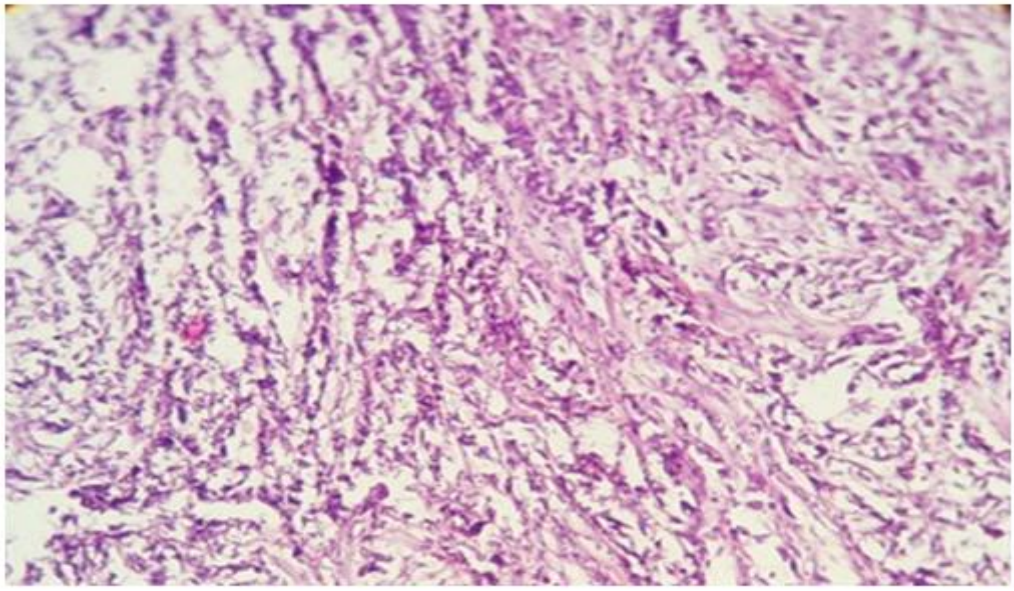

Figure1:Stromal CD10 negativity in breast carcinoma (10 x)

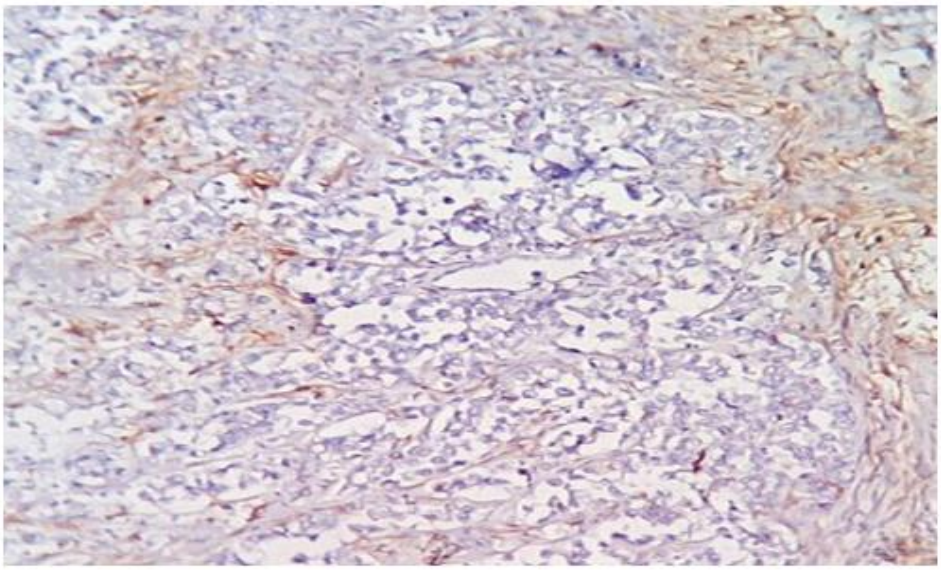

Figure 2: Stromal CD10 positivity in IDC Breast 1+ (10%-30% stromal positive cells) (10x)

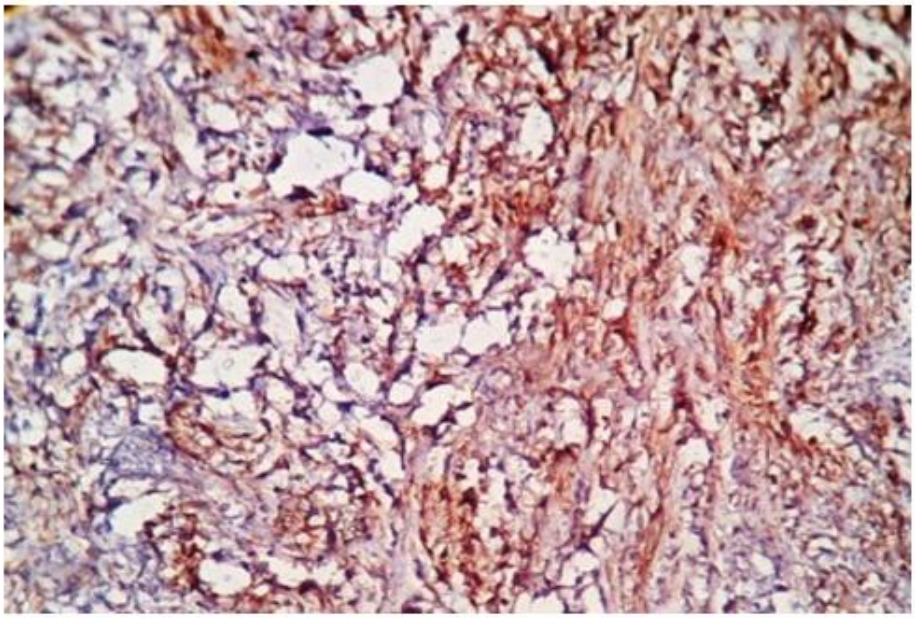

Figure 3: stromal CD10 positivity in IDC Breast 2+ (>30% stromal positive cells) (10 x)

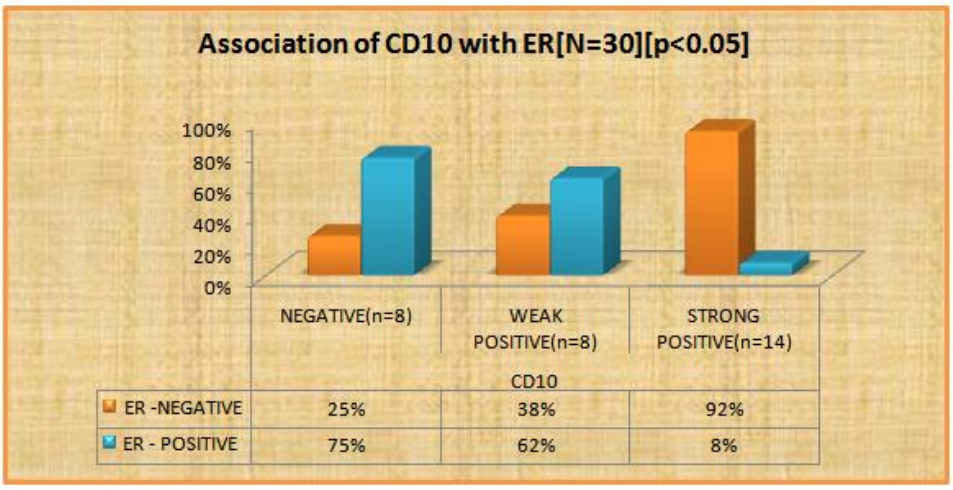

Figure 4: The Correlation Of Stromal CD10 Expression With ER is statistically significant, p value is less than 0.05(p value 0.002, Chi- square test).

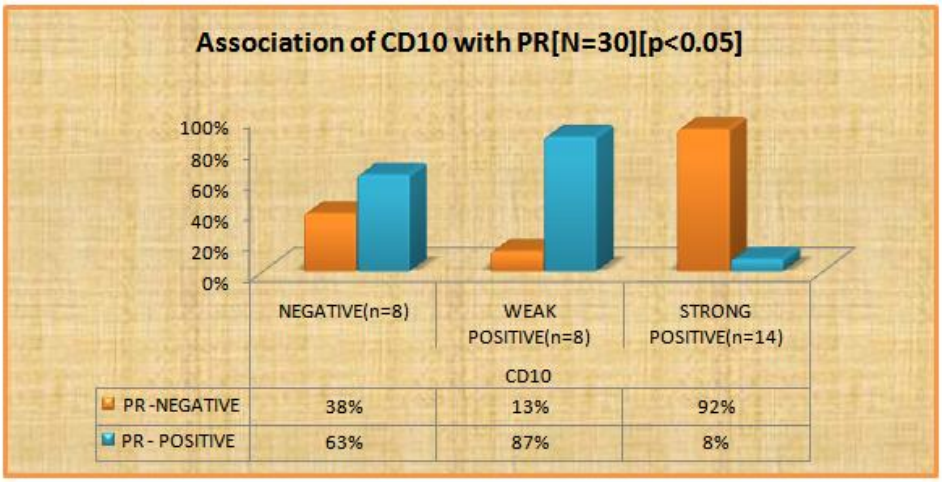

Figure 5: The Correlation Of Stromal CD10 Expression With PR is statistically significant, p value is less than 0.05(p value 0.0005, Chi- square test).

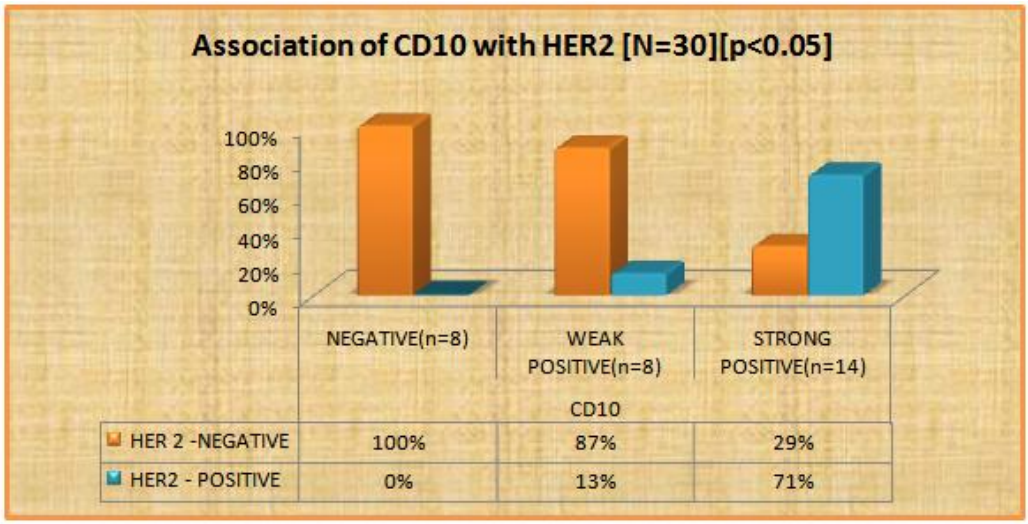

Figure 6: The Correlation Of Stromal CD10 Expression With HER2/neu is statistically significant, p value is less than 0.05(p value 0.0009, Chi- square test).

IV. Discussion

Stromal cells play an crucial role in breast cancer and its metastasis. Tissue microenvironment plays an important role in controlling cell survival, proliferation, migration, polarization, and differentiation. [12], [13]

CD10, zinc dependant protease, is a cell surface marker. CD10 act as a stem cell regulator in the breast and controls proliferation of stem cells. [8] In the normal breast tissue only a small population of stromal cells express CD10.[14], [15] In gastric carcinoma, CD10 expression in stromal cells is associated with vascular invasion and metastasis.[10] In nasopharyngeal carcinoma, expression of CD10 in stroma correlates with tumor progression.[16]

The bilateral interaction between normal epithelial cells and stromal cells is influenced by several factors secreted by the tumor cells or by stromal cells. [12], [17], [18] The matrix metalloproteinase (MMP) is one of the molecular factor. MMP plays an crucial role in tumor progression, tumor invasion and metastasis.[19] Increased MMP activities correlate with bad prognosis and promotes tumourigenesis, angiogenesis, invasion and metastasis.[20]

CD10 is a MMP which prevents excess proliferation of stem cells by cleaving signaling proteins. [21] In carcinoma breast loss of CD10 in myoepithelial cells leads to proliferation of malignant cells and invasion of in situ cancer. In invasive cancer stromal expression of CD10 might prevent differentiaon of cancer cells and helps in maintaining the cancer stem cells. [21] It also explains enhanced expression of CD10 in stroma of high grade breast carcinomas. [8]

In the present study 73% (22 out of 30) of the cases showed positivity for CD10 in the stroma, of which 46%(14) cases were strongly positive and 27%(8) were weakly positive. Only two cases of strong positivity for CD10 were noted in the adjacent normal breast parenchyma. Stromal expression of CD10 had a statistically significant association with breast cancer than in parenchymal tissue, p value is 0.002.

In a study done by Makretsov et al 79 %(205 out of 258) of invasive ductal carcinoma of breast showed expression of CD10 in stroma.[4] Thomas S et al study shows stromal CD10 positivity in 55% (16out of 29) of cases.[23]

In the present study inverse correlation between stromal CD10 expression and hormonal receptors expression was observed.

92%(13/14 cases) of the stromal CD10 positive cases of invasive ductal carcinoma of breast not expressed both ER and PR. This inverse correlation was found to be statistically significant with the p value less than 0.05(0.002 for ER and 0.0005 for PR). Puri et al found negative correlation between stromal CD10 expression and hormonal receptors. But their results are statistically not significant. Makretsov et al study shows statistically significant correlation between stromal CD10 expression and ER negativity.[4] Jana SH et al study shows no correlation between stromal CD10 expression and PR.[8]

In the present study we obtained direct correlation between stromal CD10 expression and HER2/neu over expression. 71% (10/14 cases) of stromal CD10 positive invasive ductal carcinoma of breast cases showed HER2/neu positive. This correlation is statistically significant with the p value less than 0.05(p value 0.0009). Jana SH et al study also shows correlation between stromal CD10 expression and HER2/neu over expression.[8] Puri et al study shows statistically significant correlation between stromal CD10 expression and HER2/neu over

expression.[24] Makretsov et al does not find statistically significant correlation between stromal CD10 expression and HER2/neu over expression.[4]

CD10 could be a therapeutic target for treating carcinoma breast since it cleaves doxorubicin and results in resistance to chemotherapeutic agent. Experimental studies show CPI0004Na, a CD10 cleavable peptide prodrug of doxorubicin, improves antitumor efficacy and reduces the toxicities of chemotherapeutic agents.[25]

V. Conclusion

To conclude, expression of CD10 in stroma of invasive ductal carcinoma of breast is directly correlated with HER2/neu over expression and higher tumour grade. It inversely correlates with ER and PR expression. Thus analyzing stromal CD10 expression in all invasive ductal carcinoma of breast especially in triple negative patients may help in choosing optimal treatment option. Increased level of stromal CD10 activity leads to inhibition of epithelial cell differentiation. Thus cancer stem cells are maintained and may result in recurrence of malignancy. Since CD10 cleaves the drug doxorubicin thereby causes chemo resistance. Thus inhibiting the activity of CD10 may have an increased response to chemotherapeutic agents and decreases the recurrence. Experimental studies show CPI0004Na improves antitumor efficacy.

Further studies are needed to identify the source of stromal CD10 expression, its role in epithelial to mesenchymal transition, its role in tumerogenesis of breast cancer, effect of chemotherapeutic agents on CD10, to develop newer drugs targeting CD10 and to correlate with chemotherapeutic response and overall prognosis.

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