

Comparing the expression of myoepithelial cell markers CD10 and smooth muscle actin with the estrogen receptor status in the invasive carcinoma breast: An immunohistochemical study

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ABSTRACT

Background: Breast cancer is the most frequent cancer in females throughout the world. Around 20% of breast carcinomas are estrogen receptor alpha (ER)-negative. Thus, theoretically this negativity could be either the result of down-regulation of ER expression in the tumor cells, or the result of the tumor being derived from cells which normally lack that expression. Normal basal, including myoepithelial cells of the breast is ER-negative. CD10 and smooth muscle actin (SMA) are used as markers for the demonstration of these basal cells. **Aims:** To compare the expression of positive staining for CD10 and SMA in ER-negative and ER-positive invasive breast carcinomas. **Materials and Methods:** The study was performed on 40 paraffin-embedded tissues of already diagnosed cases of invasive breast carcinomas with known ER status, i.e., thirty ER-negative and ten ER-positive cases. Expression of CD10 and SMA was demonstrated using avidin-biotin-peroxidase complex (ABC) technique. Tumor was considered to be positive for both markers only when more than 10% of tumor cells were stained positive. **Results:** Overall, CD10 tumor cell staining was seen in eight, 23.3% (7/30) ER-negative cases and in 10% (1/10) ER-positive cases. Also the staining intensity was considered to be strong. SMA tumor cell staining was seen in only 6.7% (2/30) ER-negative cases and the staining intensity was considered to be moderate. Percentages of positively stained tumor cells varied between 13% to 72% and 23% to 45% for CD10 and SMA, respectively. **Conclusion:** CD10 is a better marker when compared to SMA, as it is expressed in more number of cases and gives strong positivity in tumor cells. Higher expression of CD10 and SMA is correlated with higher tumor grade and ER negativity.

Key words: Breast carcinoma, CD10, estrogen receptor, myoepithelial cells

INTRODUCTION

Breast cancer is the most frequent cancer in females throughout the world with 1.38 million new cases occurring every year and represents 23% of all malignancies among females.^[1] It is estimated that every year approximately 1,15,000 new breast cancer cases, and 53,000 deaths are detected in India.^[2] The mammary epithelium is composed

fundamentally of two cell types such as basal (myoepithelial) and luminal epithelium.^[3] The breast tumor mostly arises from the luminal epithelial cells and shows positivity for various luminal markers like estrogen receptor (ER), progesterone receptor (PR), Her-2/neu, and cytokeratin 8/18.^[4] Around 20% of the invasive breast carcinomas are estrogen receptor alpha (ER)-negative.^[5] Thus, theoretically, this negativity could be either the result of down-regulation of ER expression in the tumor cells, or the result of the tumor being derived from cells which normally lack that expression. Normal basal, including myoepithelial cells of the breast are ER-negative.^[6] At times, basal like carcinomas can be seen that have got more association with *BRCA* and have a poor prognosis.^[4] As myoepithelial cells have mixed epithelial and smooth muscle phenotypes, the distinction between epithelial cell layer and MEC layer is not always readily identifiable on routine HandE-stained sections. So

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most of the immunological markers are detected against smooth muscle related antigens. The immunological markers used are CD10, SMA, Schwann cell markers, calponin, smooth muscle myosin heavy chain, p63, high molecular weight (HMW) cytokeratin, h-caldesmon, and maspin. Normal luminal breast epithelial cells are usually negative for these three markers.^[7]

CD10 is a 100 kDa cell surface metalloendopeptidase which inactivates a variety of biologically active peptides. It was initially identified as a common acute lymphoblastic leukemia antigen (CALLA) and considered to be tumor specific. CD10 has been identified on the surface of various non-lymphoid cells and tissues such as breast myoepithelial cells, fibroblast cells, bile canaliculi cells, and gut epithelial cells.^[8] Amongst six isoforms of actin, SMA is abundantly present in the vascular and visceral smooth muscle cells. It is also present in the myoepithelial cells of the breast.^[9] This study was aimed at comparing the incidence of positive staining of CD10 and SMA in ER-negative and ER-positive invasive breast carcinomas, and also to investigate the possibility that differentiation along myoepithelial lines plays a role in imparting ER negativity on some breast carcinomas.

MATERIALS AND METHODS

Forty cases diagnosed as invasive breast carcinoma with known ER status were included in this study. The patients' records were reviewed to obtain clinicopathological characteristics like age, sex of the patient, size of the tumor and the lymph node status. For each case, hematoxylin and eosin stained sections were reviewed for determining the histological type and tumor grade (based on Nottingham modification of Bloom Richardson system).^[10] Sections stained for ER were also reviewed to confirm receptor status of tumor. Immunohistochemistry (IHC) was

performed using avidin-biotin-peroxidase complex (ABC) technique. New sections of 3 μ m-5 μ m thickness for each case were then cut from paraffin-embedded blocks and mounted on freshly prepared 0.01% poly-L-lysine-coated slides. Slides were dried overnight at 37°C, dewaxed in xylene, and then gradually rehydrated. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide in methanol for 10 min followed by three washings in phosphate buffered saline (PBS), and then antigen retrieval was achieved in a pressure cooker (4 times, 5 min each; 0.1 M citrate buffer, pH 6.0). For detection of CD10, we used mouse monoclonal antibody clone 56C6 procured. The sections were then incubated with primary antibody for CD10 at 1:50 dilution for 1 h at room temperature. Following this, sections were incubated with biotin-conjugated secondary antibody for 30 min and then incubated using streptavidin-biotin system for 30 min at room temperature. Each step was followed by PBS wash. The reactions became visible after the immersion of sections in 3,3'-diaminobenzidine hydrochloride solution. Sections were then counterstained with Mayer's hematoxylin stain for 2 min to 5 min, dehydrated, cleared and mounted with mounting media. For SMA, monoclonal antibody clone 1A4 was procured. The primary antibody was diluted 1:50 in PBS. Moreover, antigen retrieval was not performed for SMA. Sections of tonsil and leiomyoma were used as positive control for CD10 and SMA, respectively.

CD10 [Figure 1] and SMA positivity [Figure 2] was seen as a brown-colored reaction, staining the cell cytoplasm and the membrane. Negative control sections did not have any colored end product. Following this, CD10 and SMA scores were analyzed. Scoring was taken as either positive or negative for both CD10 and SMA. Staining present in more than 10% of the tumor cells was considered to be positive; and if present in less than 10% of the tumor cells was considered to be negative.

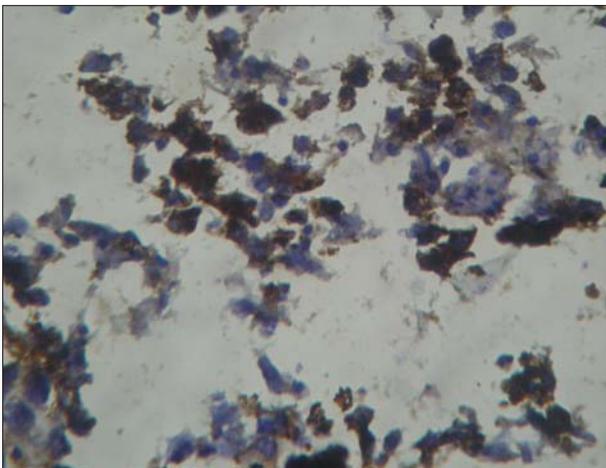


Figure 1: CD10 tumor cell positivity in invasive breast carcinoma (immunoperoxidase method, DAB \times 400)

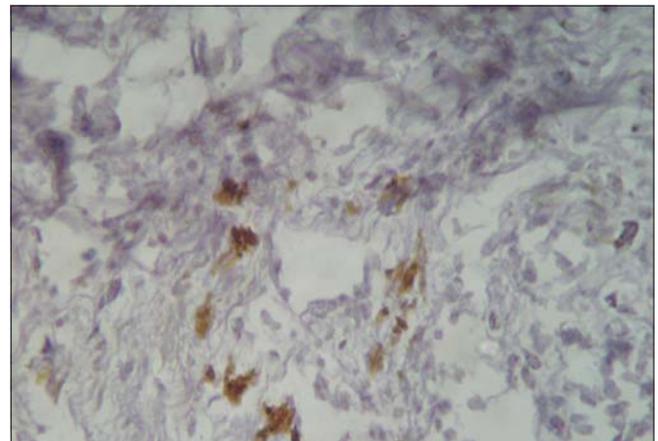


Figure 2: SMA tumor cell positivity in invasive breast carcinoma (immunoperoxidase method, DAB \times 400)

RESULTS

Thirty patients with ER-negative tumors varied in ages between 28 years and 70 years with mean age of 49 years. For ten patients with ER-positive tumors, the ages varied between 30 years and 70 years with mean age of 50 years. The ER-negative tumors varied in size between 20 mm and 100 mm with mean of 60 mm, while ER-positive tumors varied in size between 15 mm and 60 mm with mean of 37.5 mm, the difference is statistically insignificant ($P = 0.097$). 97.5% cases were female patients and only 2.5% cases were male patients. Histologically, all cases were of invasive ductal carcinoma of not otherwise specified (NOS) type. Amongst ER-negative tumors, 66.7% (20/30) were of grade III, 33.3% (10/30) were of grade II, and none of the cases were of grade I. In ER-positive tumors, 60% (6/10) were grade III, 30% (3/10) were grade II, and 10% (1/10) were of grade I. Lymph nodes were recovered in 33 cases (24 ER-negative and 9 ER-positive). Secondary metastatic deposits were seen in 85% (28/33) cases, rest 15% (5/33) showed reactive hyperplasia of lymph node. Overall, 91.6% (22/24) of ER-negative tumors and 66.7% (6/9) of ER-positive tumors were presented with secondary metastatic deposits in lymph nodes.

Comparison of CD10 and smooth muscle actin immunostaining with estrogen receptor status of patient CD10 positivity was indicated by a dark brown cell membrane and cytoplasmic staining [Figure 1]. Seven out of thirty (23.3%) ER-negative cases and one out of ten (10%) ER-positive cases showed CD10 tumor cell staining [Table 1]. The percentage of positively stained tumor cells varied between 13% and 72%. Staining intensity was strong in tumor cells and weak to moderate in stromal cells. Blood vessels were not stained in any of the cases.

SMA tumor cell staining was seen in 6.7% (2/30) ER-negative cases. None of the ER-positive tumors stained with SMA. Tumor cells in these two cases also showed positivity for CD10 [Table 1]. With SMA, the percentage of positively stained tumor cells varied between 23% to 45%. The staining intensity was moderate in tumor cells, strong in stromal cells and blood vessels. The difference between staining in two groups is statistically significant (Yates corrected Chi-square = 3.98, $P = 0.046$).

Comparison of CD10 and SMA immunostaining with lymph node status

For cases, positive for CD10 tumor cells, 87.5% (7/8) showed presence of metastatic deposits and 12.5% (1/8) showed reactive hyperplasia in lymph nodes [Table 2]. When compared with the ER status, all ER-negative tumors showed metastatic deposits while the ER-positive tumor showed reactive hyperplasia of the lymph node. In both

cases (100%) positive for SMA, tumor cells showed the presence of metastatic deposits in lymph nodes and both were ER-negative.

Thus, on comparing CD10 and SMA immunoeexpression with the grade of tumor [Table 3], it was found that tumor cell positivity was seen maximum in grade III tumors for both CD10 and SMA.

DISCUSSION

Carcinoma breast incidence rates begin to rise in early thirties and reached a peak in 50 years to 64 years of age group people. The current overall risk of developing breast cancer during an 80 year life span is about 10%.^[11]

A substantial proportion of primary breast cancers contain estrogen receptors and/or progesterone receptors. The hormone receptor status of breast tumors and their

Table 1: Comparison of immunohistochemistry expression of CD10 and smooth muscle actin with estrogen receptor status

	Cases positive for immunomarker	ER-negative cases (%)	ER-positive cases
CD10 tumor cells	8	7 (23.3)	1 (10%)
SMA tumor cells	2	2 (6.7)	0

IHC: Immunohistochemistry, SMA: Smooth muscle actin, ER: Estrogen receptor

Table 2: Comparison of immunohistochemistry expression of CD10 and smooth muscle actin with lymph node status

Lymph nodes	No. of cases	CD10 tumor cells positive cases	SMA tumor cells positive cases
Secondary deposits	28	7 (87.5%)	2 (100%)
Reactive pathology	5	1 (12.5%)	0
Not recovered	7	0	0
Total	40	8	2

IHC: Immunohistochemistry, SMA: Smooth muscle actin

Table 3: Comparison of Immunohistochemistry expression of CD10 and smooth muscle actin with grade of tumor

Tumor grade	No. of cases	CD 10 tumor cell positive	SMA tumor cells positive
Grade I	1	0	0
Grade II	13	1	0
Grade III	26	7	2
Total	40	8	2

IHC: Immunohistochemistry, SMA: Smooth muscle actin

responsiveness to endocrine therapy are highly correlated, with receptor-positive tumors responding favorably. The incidence of ER-negative invasive breast carcinoma has been reported to be as high as 67.6% in India, as compared to 30% to 33% in the Western literature.^[12] Theoretically, this negativity could be either due to the result of down-regulation of ER expression in the tumor cells, or the result of the tumor being derived from or differentiating toward cells which normally lack that expression.^[13]

Until recently, myoepithelial cells of the breast were thought to be rather inert with no active role in neoplasia. It is now accepted that myoepithelial cells can be involved in a variety of breast neoplasms. The best recognized are the myoepithelioma, the adenomyoepithelioma, and the myoepithelial carcinomas. Myoepithelial carcinomas are thought to be extremely rare malignant tumors composed entirely of neoplastic myoepithelial cells which can be spindle shaped^[14] or clear^[15] and are ER- and PR-negative.^[16] There are probably other invasive breast carcinomas that are currently included within the group of "invasive ductal, not otherwise specified" which are ER-negative and show immunohistochemical evidence of myoepithelial, or other basal cell differentiation. Such tumors may be worth identifying separately as they probably need a different therapeutic approach.^[5] Thus, CD10 and SMA are the markers of these basal cells that can be used for their demonstration in routinely processed sections.^[7]

In the present study, seven out of 30 (23.33%) ER-negative tumors were CD10-positive. One out of 10 (10%) ER-positive tumors were CD10-positive. In other words, of total eight cases in which tumor cells were CD10-positive, 87%(7/8) were ER-negative, and only 13%(1/8) were ER-positive. The difference was statistically significant ($P = 0.03$) (Chi-square test applied). Similarly, Rachel Kesse-Adu *et al.*, showed that the expression of both CD10 and SMA was more in the ER-negative tumors (29%) than in the ER-positive tumors (2.5%).^[13]

In the study by Rachel Kesse-Adu *et al.*, in CD10-positive cases, the patients varied in ages between 37 years and 84 years. In SMA-positive cases, the patients varied in ages between 33 years and 70 years^[13] Similarly in the present study, in CD10- and SMA-positive cases, the patients varied in ages between 35 years and 58 years and 40 years and 50 years, respectively. No correlation was found between the expression of CD10 and SMA with age of the patients. This could be because of the fact that age of majority of the patients in the present study were in these two decades.

All CD10-positive ER-negative cases were of grade III and none was of grade II. The difference was statistically

significant ($P = 0.03$) (Chi-square test applied). Similarly, in concordance with our study, Nikita Makretsov *et al.*, quoted that CD10 expression in invasive breast carcinoma associated with ER negativity, higher tumor grade, and decreased survival.^[17] Regarding SMA, in all, two cases showed positive staining in more than 10% of the tumor cells. Both of these were ER-negative tumors of grade III and none was ER-positive. The percentage of positively stained tumor cells varied between 23% and 45%. Both SMA positive cases were also positive for CD10, i.e., none of our case was independently positive for SMA.

In our study, in 87.5% of CD10 and 100% of SMA positive cases, the recovered lymph nodes showed secondary deposits and further all these cases were ER-negative. Cui Yazhou *et al.*, quoted similar findings and showed that the cases showing positive staining with myoepithelial cell markers were more commonly associated with lymph node metastasis.^[18]

Thus, it was observed that both CD10 and SMA were more commonly expressed in the ER-negative tumors (23%) than in the ER-positive tumors (10%). Furthermore, in the tumor cells, expression of CD10 was more than that of SMA.

Thus, while comparing the expression of CD10 with SMA, it was seen that CD10 stained the tumor cells strongly and blood vessels were not highlighted. Furthermore, it was also seen that numbers of cases, positive for CD10 were more than that of SMA positive cases. Cases positive for SMA were also stained for CD10. Hence, it was concluded that CD10 is a better marker than SMA for finding out non-conventional type of breast carcinomas. Thus, it was suggested that all the ER-negative cases should be subjected to CD10 and SMA, the myoepithelial cell markers, and if positive, they can be derived from or differentiating along the direction of basal non-conventional luminal epithelial breast cells that normally lack the expression of ER but totally or partially express various myoepithelial markers. Such tumors have an aggressive outcome and might need a different therapeutic approach.

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