



## Full Length Research Paper

# Comparison of CD10 expression in stroma of epithelial and mesenchymal tumors of the breast

Maha E. Salama<sup>1\*</sup>, Wael S. Ibrahim<sup>1</sup> and Dina A. Khairy<sup>2</sup>

<sup>1</sup>Pathology Department, Faculty of Medicine, Cairo University, Egypt.

<sup>2</sup>Pathology Department, Faculty of Medicine, Beni Sueif University, Egypt.

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The stroma surrounding a tumor is of utmost importance in deciding the tumor behaviour and progression thus studying its nature may contribute to finding new and effective methods of treatment. This study aimed at comparing the expression of CD10 in the desmoplastic stroma of breast carcinoma and the neoplastic stroma of phyllodes tumor. Paraffin blocks of 70 Egyptian female cases were collected, 36 cases with invasive duct carcinoma and 34 with phyllodes tumors. CD10 immunostaining was scored as *negative* when there was no tumor stromal staining, *weak* when there was diffuse weak staining or weak or strong focal staining in less than 30% of tumor stromal cells and *strong* when there was strong staining in 30% or more of tumor stromal cells. Positive CD10 expression was seen in 77.8% of invasive duct carcinoma and 32.4% of phyllodes tumor. In invasive duct carcinoma, most grade I cases showed weak CD10 immunostaining (66.7%), while most of grade III cases showed strong CD10 staining (71.4%) ( $p < 0.001$ ). In phyllodes tumors, most of benign cases were CD10 negative (83.3%), while strong CD10 staining was seen in 60% of malignant cases ( $p < 0.001$ ). In conclusion, CD10 was more widely expressed in the desmoplastic stroma of invasive duct carcinoma rather than the neoplastic stroma of phyllodes tumor. Moreover, stromal CD10 expression seemed to correlate with the tumor grades in invasive duct carcinoma as well as phyllodes tumors.

**Keywords:** CD10, breast, immunohistochemistry, phyllodes, duct carcinoma

## INTRODUCTION

The stroma surrounding any tumor plays a very important role in its progression. The interaction between cancer cells and surrounding tumor stromal cells is bidirectional and this mutual interaction allows for the progression of the tumor (Troester et al., 2009).

Desmoplasia is the growth of fibrous or connective tissue secondary to an insult, usually around a malignant

neoplasm. Cancer associated with a desmoplastic stroma is typically of poor prognosis (Liu et al., 2012). A desmoplastic response immunohistochemically shows transformation of fibroblastic-type cells to a myofibroblastic phenotype (Ayala et al., 2003).

Invasive duct carcinoma is a malignant epithelial tumor of the breast which often has a scirrhous, stellate appearance caused by desmoplastic reaction (Liu et al., 2012). While phyllodes tumor of the breast is a stromal tumor made up of both epithelial and stromal components that tend to behave in a benign fashion but may undergo sarcomatous transformation (Noguchi et al., 1993). The

\*Corresponding Author E-mail: [maha.elsayed@kasralainy.edu.eg](mailto:maha.elsayed@kasralainy.edu.eg);  
Phone Number: 0020-01001519184

stroma of invasive duct carcinoma differs from that of phyllodes tumors, while the former is desmoplastic that of the latter is monoclonal, and thus forms the neoplastic component of the lesion (Sawyer et al., 2000).

In invasive duct carcinoma the stroma was devoid of CD34+ fibrocytes but SMA-reactive myofibroblasts were detected. On the other hand, stromal CD34+ fibrocytes and additional SMA-reactive myofibroblasts were seen in phyllodes tumors. Therefore loss of CD34+ fibrocytes was specific for the desmoplastic stroma of invasive duct carcinoma (Barth et al., 2002).

CD10 is a matrix metalloproteinase that cleaves the protein components of extracellular matrix and thereby plays a central role in tissue remodeling. Several synthetic matrix metalloproteinases inhibitors have been developed and shown to be effective in blocking tumor growth in experimental animals, validating the concept of matrix metalloproteinases as therapeutic targets for cancer (Coussens et al., 2002).

Stromal CD10 expression is also associated with biological aggressiveness in various epithelial malignancies including the breast as it is overexpressed in malignant, compared to borderline and benign tumors (Makretsov et al., 2007).

Since CD10 could be the target for therapeutic trials of breast tumors, it is important to know if its expression differs according to the type of stroma. Thereby, this study aims at comparing the immunohistochemical expression of CD10 in the desmoplastic stroma of malignant epithelial breast carcinoma versus the neoplastic stroma of phyllodes tumor.

## MATERIALS AND METHODS

This is a preliminary study that consisted of the paraffin blocks of 70 Egyptian female cases, 36 cases presented with invasive duct carcinoma while 34 cases presented with phyllodes tumors, both of different grades, obtained from the Pathology Department, Faculty of Medicine, Cairo University and private laboratories.

### ➤ Histopathological evaluation

#### ❖ Invasive duct carcinoma

Invasive duct carcinoma cases were histologically graded according to Elston/Nottingham modification of Bloom-Richardson system which depends on the percentage of tumor tubule formation, number of mitotic figures and the degree of nuclear pleomorphism (Elston and Ellis, 2002).

#### ❖ Phyllodes tumors

Benign phyllodes tumors were classified when there was no extensive stromal overgrowth, mitotic figures were 2 or less per 10 high power fields, well circumscribed border and absence of nuclear pleomorphism. On the other hand, malignant phyllodes tumors were diagnosed when they showed extensive stromal overgrowth, 5 or more mitotic figures per 10 high power fields, infiltrating border and marked nuclear pleomorphism. Borderline phyllodes tumors were more atypical than the benign but did not fulfill all the criteria of malignancy (Tse et al., 2005).

### ➤ Immunohistochemical evaluation

Each paraffin block was recut by rotatory microtome at 4 mm thickness then mounted on glass slides to be stained by hematoxylin and eosin for routine histopathological examination and on charged slides for immunostaining using standard immunoperoxidase method.

For the assessment of CD10 expression, a representative slide from each case was stained using an antibody against CD10 (CD10/CALLA, Ab-2, mouse monoclonal, antibody, clone 56C6, cat. sc-58939, dilution 1:50, SANTA CRUZ BIOTECHNOLOGY, INC., 2145 DELAWARE AVENUE, SANTA CRUZ CALIFORNIA 95060). As positive control for cases stained for CD10, a section of tonsil was employed. Every section was carefully examined at power magnification (x100) for the presence of tumor stromal immunostaining using Olympus microscope CX21.

The CD10 immunostaining was scored as **negative** when there was no tumor stromal staining, **weak** when there was either diffuse weak staining or weak or strong focal staining in less than 30% of tumor stromal cells and **strong** when there was strong staining in 30% or more of tumor stromal cells (Nikita et al., 2007).

### ➤ Statistical analysis

Data was collected, coded, and analyzed by SPSS software version (9) under windows XP. The Fisher exact test was used to compare stromal CD10 immunoeexpression between invasive duct carcinoma and phyllodes tumors. The Chi-square test was used to determine differences in CD10 expression between the different grades of tumors. One-way ANOVA test was used to determine whether the difference was significant. Significance was established at P<0.05.

**Table 1.** Comparison of stromal CD10 expression in invasive duct carcinoma and phyllodes tumor

Tumor type	CD10 expression			Negative
	Weak	Positive Strong	Total	
Invasive duct carcinoma	22 (78.6%)	6 (21.4%)	28 (100%)	8 (22.2%)
Phyllodes tumor	6 (54.5%)	5 (45.5%)	11 (100%)	23 (67.6%)

$P < 0.001$

**Table 2.** Correlation between the intensity of CD10 expression and tumor grade in invasive duct carcinoma

invasive duct carcinoma	Intensity of CD10 expression	
	Weak	Strong
Grade I	2 (33.3%)	1 (16.7%)
Grade II	18 (94.7%)	0 (0%)
Grade III	2 (18.2%)	5 (45.5%)

$P < 0.001$

**Table 3.** Correlation between intensity of CD10 expression and tumor grade in phyllodes tumor

Phyllodes tumor	Intensity of CD10 expression	
	Weak	Strong
Benign	3 (12.5%)	1 (4.2%)
Borderline	2 (40%)	1 (20%)
Malignant	1 (20%)	3 (60%)

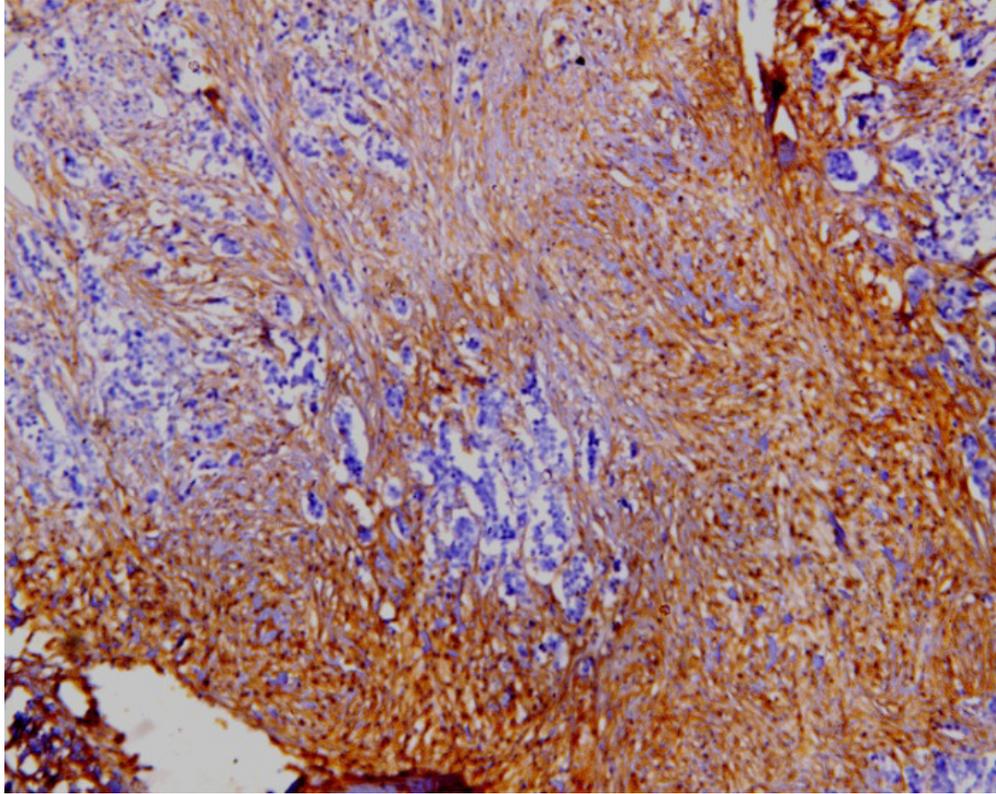
$P < 0.001$

## RESULTS

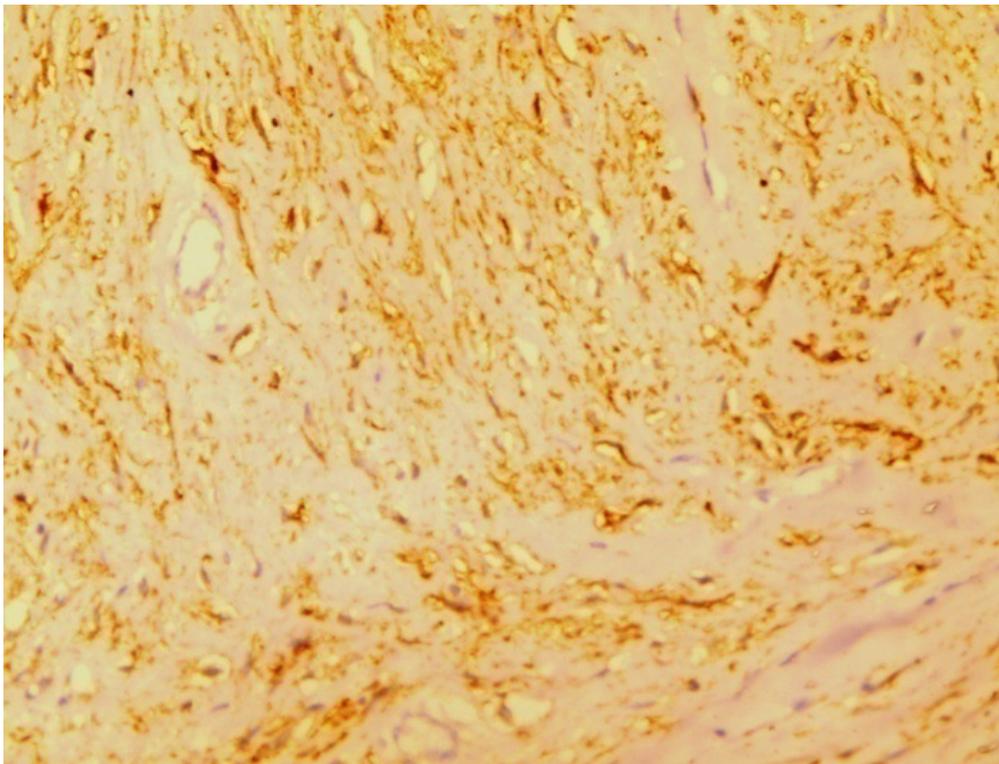
This study included 70 Egyptian female cases, 36 cases had invasive duct carcinoma with different grades representing 51.4% of cases. The cases were distributed as 16.6%, 52.8% and 30.6% respectively for grades I, II and III. On the other hand, 34 cases presented with phyllodes tumor with different grades representing 48.6% of total cases distributed as 70.6% benign, 14.7% borderline and 14.7% malignant.

Positive CD10 expression was seen in 28 cases of invasive duct carcinoma (77.8%) and 11 cases of phyllodes tumor (32.4%) (Table1). In invasive duct carcinoma, grade I cases showed negative CD10 expression in 50% of cases and 33.3% of cases showed weak intensity. On the other hand, most of grade III

cases were CD10 positive (63.7%), most of the cases showed strong CD10 staining (45.5%) (Figure1). By studying phyllodes tumors, most of the benign cases were CD10 negative (83.3%), while 12.5% of cases showed weak CD10 staining. On the contrary, majority of malignant phyllodes cases were CD10 positive (80%), strong CD10 staining was seen in 60% of cases (Figure 2). We noticed a highly significant difference in stromal CD10 expression between invasive duct carcinoma and phyllodes tumors, CD10 was more widely expressed in the desmoplastic stroma of invasive duct carcinoma rather than the neoplastic stroma of phyllodes tumor. Moreover, stromal CD10 expression seems to correlate with the tumor grades in invasive duct carcinoma as well as phyllodes tumors, these results were also highly significant (Tables 2 and 3 respectively).



**Figure 1.** CD10 expression in invasive duct carcinoma grade III  
Most of the desmoplastic stromal cells of the tumor show diffuse strong CD10 expression (CD10x200).



**Figure 2.** CD10 expression in malignant phyllodes tumor  
There is diffuse strong CD10 expression in most of the neoplastic stromal cells of the tumor (CD10x200).

## DISCUSSION

The stroma surrounding a tumor is of utmost importance in deciding the tumor behavior and progression and studying its nature may contribute to finding new and effective methods of treatment of tumors.

CD10 is a zinc-dependent peptidase (metalloproteinase), which degrades a variety of bioactive peptides in the stroma. Earlier studies suggested that CD10 expression in tumor stroma is associated with biological aggressiveness of the tumor. So CD10 constitutes a clinically important prognostic marker and a potential target for development of novel therapies (Makretsov et al., 2007). Studies of CD10 expression in stroma of invasive breast cancer and its use as a possible predictor of clinical outcome are not so many. Similarly, CD10 expression in mammary stromal neoplasms, most notably phyllodes tumors, had not been well documented especially among the African, nevertheless the Egyptian population.

In this study we collected 70 paraffin blocks of Egyptian women suffering from breast tumors, 36 were diagnosed as invasive duct carcinoma mostly of grade II followed by grade III, while 34 were diagnosed as phyllodes tumors mostly benign (24 cases) and equal number of borderline and malignant cases (5 cases each).

We tried to compare between stromal CD10 expression in invasive duct carcinoma and phyllodes tumor. We found that CD10 expression was seen in 77.8% of invasive duct carcinoma cases but in only 32.4% of phyllodes tumor cases. This was surprising as we expected more CD10 positive cases in the neoplastic stroma of phyllodes tumor.

When we tried to correlate the intensity of CD10 expression with the different grades of each tumor we observed a highly significant correlation denoting that CD10 expression intensity increases as the tumor grade and aggression increase.

In invasive duct carcinoma grade I half of the cases were negative for CD10 immunostaining and most of the positive cases showed weak intensity, while most of grade III cases were CD10 positive, of which the majority showed strong staining. As for grade II, there was an overwhelming CD10 positivity of the weak intensity type. Close results were observed by Makretsov et al., 2007 who observed that strong CD10 immunostaining was found in 59% of invasive duct carcinoma grade III cases. Regarding invasive duct carcinoma grades I and II, most of their cases showed weak staining. Their results were also statistically significant. This statistically significant correlation between CD10 immunostaining intensity and tumor aggressiveness may be attributed to the fact that CD10, being one of the matrix metalloproteinases, cleaves the protein components of extracellular matrix

and thereby plays a central role in tissue remodeling. Therefore, in mammary duct carcinoma, CD10 helps the tumor cells to invade the surrounding tissue stroma (Coussens et al., 2002).

Phyllodes tumors did not differ much from invasive duct carcinoma, as CD10 intensity also correlated with the tumor grade in a highly significant manner, which could claim that CD10 can be used to differentiate between benign and malignant phyllodes tumors. Benign phyllodes tumor cases were mostly CD10 negative and most of the positive cases showed weak intensity. As expected, in malignant phyllodes tumor the overwhelming majority of cases were CD10 positive not to mention the high percentage of cases showing strong intensity. These results go with those obtained from a small series study done by Mechttersheimer et al. 1990 in which three benign phyllodes tumors were studied showing weak CD10 staining, while the only malignant phyllodes tumor case conducted showed very intense staining.

This study observed a significant increase in the immunostaining of CD10 in the desmoplastic stroma of invasive duct carcinoma versus the neoplastic stroma of phyllodes tumor. But before rushing to conclusions, when we also observed a highly significant correlation between intensity of CD10 staining and tumor grades in both tumors, a question arose: Is it about the nature of the stroma or the tumor aggression? Our study had a drawback and that is the small number of malignant phyllodes tumor in comparison to invasive duct carcinoma cases (5 Vs 36 respectively). This is of course due to the higher prevalence of the latter over the former. So, maybe this wide difference in number between the cases and the prevalence of benign phyllodes tumor over the malignant, caused CD10 staining to be more in the latter than the former. Other studies are encouraged to complete in this field hoping to collect more of the malignant phyllodes cases.

## CONCLUSION

- This study suggests that CD10 immunoexpression is higher in the desmoplastic stroma of invasive duct carcinoma compared to the neoplastic stroma of phyllodes tumor.
- We conclude that CD10 immunostaining intensity significantly correlates with tumor grade and aggression in both invasive duct carcinoma as well as phyllodes tumors.
- Our findings may be an important observation that may have diagnostic and prognostic implications as well as promising potential target for development of novel therapies, a subject for further investigations.

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