

Role of CD10 Immunoexpression in Grading Phyllodes Tumour of the Breast

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ABSTRACT

Introduction: Fibroepithelial tumours are a heterogeneous group of biphasic neoplasms consisting of a proliferation of both epithelial and stromal components. Fibroadenoma (FA) and Phyllodes Tumour (PT) constitute the major entities. It is crucial to distinguish benign from borderline PT (low grade malignant PT), because the former do not metastasize, have a lesser risk of local recurrence and initial local recurrences are histologically benign in almost all instances. Multiple Immunohistochemical (IHC) markers are being studied to find their utility in grading the PT accurately for planning proper treatment.

Aim: To study, the IHC expression of CD10 in the stromal cells of a series of PTs and FA, with the aim of determining whether the degree of CD10 expression in the stromal cells is related to the grade of the tumour.

Materials and Methods: Records of 28 cases of PT and 35 cases of FA received in the Department of Pathology in a tertiary care hospital were obtained. Histopathology reports and slides of all the cases were reviewed and clinical data such as age and histomorphological features such as tumour cellularity, stromal overgrowth, mitotic count and nuclear atypia were noted. Representative block of the tumour with maximum cellularity was subjected to CD10 staining. For FA and benign

PT a technique of tissue microarray was used. For borderline and malignant PT, representative section was used. Stromal cell staining was assessed, using cytoplasmic staining of the breast myoepithelium as internal control.

Results: Present study included 35 cases of FA, 20 cases of benign PT, five cases of borderline PT and three cases of malignant PT. The mean age of the patients increased with the increasing tumour grade of PT and this was also observed for FA and benign PT. The mean age increased with increase in tumour grade of PT and was statistically significant ($p < 0.05$). The mean size did not increase with the increasing tumour grade of PT and was statistically insignificant ($p = 0.0429$). Mean tumour size was more in benign PT as compared to FA and was highly statistically significant ($p < 0.01$). CD10 staining was diffuse (Grade-3) and strong in malignant PT. The staining intensity was strong but patchy (Grade-2) in borderline PT. Weak and patchy (Grade-1) CD10 staining was seen in four benign PT and six FA. Other cases of benign PT and FA were negative for CD10 immunoreactivity.

Conclusion: Our study showed that CD10 expression strongly correlates with the PT grade, which can help in the differentiation between benign and malignant variants of PT.

Keywords: Benign, Borderline, Fibroadenoma, Malignant

INTRODUCTION

Fibroepithelial tumours are a heterogeneous group of biphasic neoplasms consisting of a proliferation of both epithelial and stromal components [1]. FA and PT constitute the major entities. FA is a common benign biphasic tumour. PT are classified into benign, borderline and malignant categories on the basis of a combination of histological features, including the degree of stromal hypercellularity, mitosis and cytological atypia, stromal overgrowth and nature of the tumour borders/margins [1]. Borderline and frankly malignant PT can metastasize, the incidence being 4% and 22% respectively [2,3]. Histological criteria used for grading PT lack standard interpretation and there is an inter-observer variability among pathologists [4]. Multiple IHC markers are being studied to find their utility in grading the PT accurately for planning proper treatment [5-9]. The fundamental principle of therapy is complete excision with safe margins to prevent local recurrence [2]. Malignant PT may require frequent follow up visits and additional radiotherapy and/or chemotherapy [10].

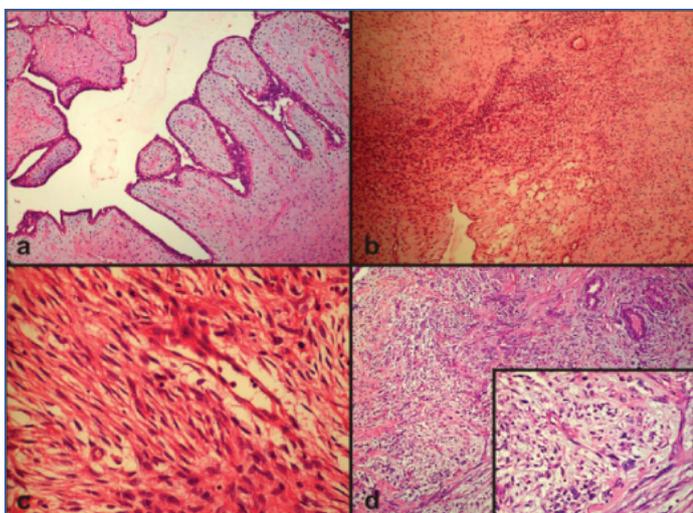
The utility of various IHC markers to distinguish benign from malignant PT has been studied. CD10 is zinc-dependent peptidase (metalloproteinase), which degrades a variety of bioactive peptides. CD10 has been evaluated and increased expression has been observed with increasing tumour grade of PT. Few such studies

have been documented in literature so far which need validation [5,7,9-12].

In present study, the IHC expression of CD10 was evaluated in the stromal cells of a series of PTs and FAs, with the aim of determining whether the degree of CD10 expression in the stromal cells is related to the grade of tumour.

MATERIALS AND METHODS

This is a cross-sectional study. Records of 28 cases of PT and 35 cases of FA received over a period of five years (2012-2016) in the Department of Pathology in a Smt. Kashibai Navale Medical college and General Hospital, Pune, Maharashtra, India, were obtained. Histopathology reports and slides of all the cases were reviewed and clinical data such as age and histomorphological features such as tumour cellularity, stromal overgrowth, mitotic count and nuclear atypia were noted. PT cases were divided into three categories including benign [Table/Fig-1a], borderline [Table/Fig-1b,c] and malignant [Table/Fig-1d] as described previously [2]. Representative block of the tumour with maximum cellularity was subjected to CD10 staining. For FA and benign PT a technique of tissue microarray was used [13]. For borderline and malignant PT representative section was used. Stromal cell staining was assessed, using cytoplasmic staining of the breast myoepithelium as internal control. The staining



[Table/Fig-1]: Photomicrograph showing: a) Benign PT with no stromal overgrowth and no mitosis (H&E, 100x); b) Photomicrograph showing borderline PT (H&E, 100x); c) Photomicrograph showing borderline PT (H&E, 400x); d) Photomicrograph showing malignant PT with marked stromal overgrowth, nuclear atypia, mitosis (H&E, 100x), inset (H&E, 400x).

intensity was graded as 0 (no staining), weak, moderate or strong if the staining was much weaker, slightly weaker, or of the same intensity as that of the myoepithelium respectively. The percentage of stained cells was also assessed as negative (when there was no stromal staining), Grade-1 (<10% stromal cells were positive), Grade-2 (10 to 30% stromal cells were positive), and Grade-3 (>30% stromal cells were positive) [9].

STATISTICAL ANALYSIS

The statistical software named Primer software version 5.0 (manufactured by McGraw-Hill) was used for analyzing the data. The groups were compared using the Pearson's chi-square test (PCT) or Fisher's-Exact-test (FET). ANOVA test was used to compare the difference in mean between multiple groups and t-test was used to compare the difference in mean between two groups. A p-value of 0.05 or less was considered statistically significant.

RESULTS

Present study included 35 cases of FA, 20 cases of benign PT, five cases of borderline PTs and three cases of malignant PT. Age range of FA was 18 to 45 years with mean age of 25 years. Mean tumour size of FA was 2.92 ± 1.26 cm. Relation between patients age and tumour size with tumour grade of PT is compared in [Table/Fig-2,3] respectively.

Relation between CD10 expression in PT and FA and Ki67 labeling index in PT with tumour grade is given in [Table/Fig-4,5] respectively.

The mean age increased with increase in tumour grade of PT and was statistically significant ($p < 0.05$). The mean size did not increase with the increasing tumour grade of PT and was statistically

Tumour type	Youngest age (years)	Oldest age (years)	Mean \pm SD
Benign PT	18	56	34 \pm 11.25
Borderline PT	40	50	45 \pm 5
Malignant PT	45	55	49.67 \pm 5.03

[Table/Fig-2]: Relation between patients age and tumour grade of PT. $F=4.76$, $p < 0.05$ (ANOVA)

Tumour type	Smallest size (cm)	Largest size (cm)	Mean \pm SD
Benign PT	2	13	5.2 \pm 3.17
Borderline PT	3	11	7.2 \pm 3.34
Malignant PT	4	10	6.17 \pm 2.36

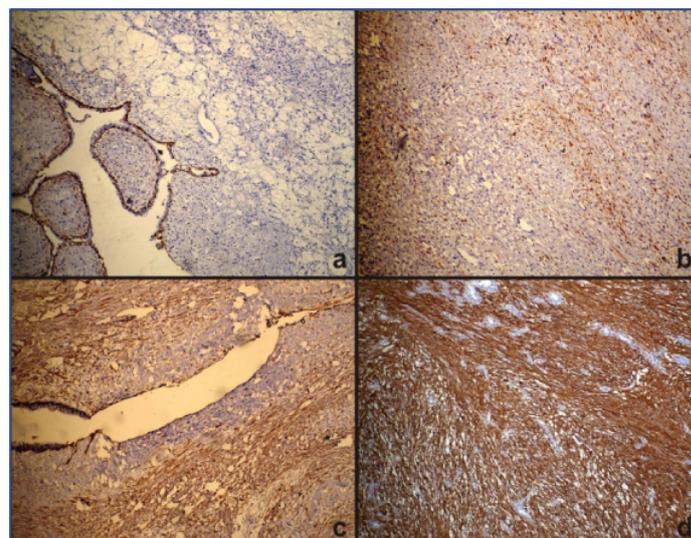
[Table/Fig-3]: Relation between tumour size and tumour grade of PT. $F=0.87$, $p=0.429$ (ANOVA)

insignificant ($p=0.0429$). Mean tumour size was more in benign PT as compared to FA and was highly statistically significant ($p < 0.01$). Mean age of patients with benign PT was more than that in FA and this was highly statistically significant ($p < 0.01$).

Few cases of benign PT and FA were negative for CD10 immunoreactivity [Table/Fig-5a], Weak and patchy (Grade1) CD10 staining was seen in four benign PT and six FA [Table/Fig-5b]. The

Tumour type	CD10 positive (%)	CD10 negative (%)	Total (%)	Statistical analysis
Benign PT	4(20%)	16(80%)	20(100)	$\chi^2=14.933$, $p < 0.001$
Borderline PT	5(100%)	0	5(100)	
Malignant PT	3(100%)	0	3(100)	
Fibroadenoma	6(17%)	29 (83%)	35(100)	$\chi^2=0.0698$, $p=0.7915$

[Table/Fig-4]: Relation between CD10 expression and tumour grade of PT and FA. Chi-square test



[Table/Fig-5]: Photomicrograph showing: a) Negative CD10 staining (100x); b) Photomicrograph showing weak and patchy CD10 (Grade-1) staining in benign PT (100x); c) Photomicrograph showing strong and patchy CD10 (Grade-2) staining in borderline PT (100x); d) Photomicrograph showing strong and diffuse CD10 (Grade-3) staining in malignant PT (100x).

staining intensity was strong but patchy (Grade-2) in borderline PT [Table/Fig-5c], CD10 staining was diffuse (Grade-3) and strong in malignant PT [Table/Fig-5d].

DISCUSSION

The median age and mean age of PTs are known to be about 45 years, approximately 20 years older than the median age of patients with FA [2]. Relatively younger age group is observed in Asian and Latin patients [2]. In our study, the age correlated with the tumour grade and was statistically significant. Average size of PT is reported as 4 to 5 cm with a range of 1 to 20 cm in literature [2]. Although malignant PTs tend to be larger than benign variants, there are many exceptions with high grade malignant lesions smaller than 2 cm and largest lesions histopathologically benign [2]. In present study, mean size did not increase with increase in tumour grade and was not statistically significant. Similar observation was found in study done on 67 cases of PT [11].

Earlier studies suggested that CD10 expression in tumour stroma was associated with aggressive biological behaviour [9]. CD10 expression seen in the stromal cells of invasive breast carcinoma was associated with an increased incidence of lymph node metastasis [7]. The CD10 expression in the stromal cells of fibroepithelial lesions of the breast has been reported in very few studies. [5,7,9-12].

In one large study, it was observed that CD10 expression was low in both FA and benign PT and was much higher in borderline and malignant PT [7]. This was in correlation with clinical evidence

that both borderline and frankly malignant PT can metastasize, whereas, benign PT and FA do not metastasize [7]. We found similar observations in our study. CD10 expression does not assist in the differential diagnosis between FA and PT. Because of the limited data available, the percentage of benign versus malignant PT was not well defined. Reports suggest that about 85-90% of PT are benign and about 10-15% are malignant [14]. Our study shows that CD10 expression strongly correlates with the PT grade, which can help in the differentiation between benign and malignant variants. This is evidenced by the fact that CD10 immunoreactivity was seen in 20% of the benign PT, 100% of the borderline PT and 100% of malignant PT. This may be attributed to the fact that CD10 belongs to the metalloprotease family, so it provides tumours with the capacity to invade blood vessel walls, facilitating its metastatic potential [7].

There was significant increase in CD10 expression as the lesions progressed from benign PT to borderline and frank malignancy. All the malignant cases showed strong and diffuse intensity for CD10. All borderline cases showed patchy but strong immunoreactivity. Benign cases showed patchy and weak immunoreactivity. Similar observations were found in study done on 25 cases of PT [12].

In breast, CD10 is often adopted as a marker for myoepithelial cells in differential diagnosis. Not Otherwise Specified (NOS) type of sarcoma with CD10 expression (NSCD10) is a variant of sarcoma with myoepithelial differentiation. The expression of CD10 in NSCD10 is known to be very extensive and intensive than those of malignant PTs. CD34 is valuable marker to differentiate between the two as PTs are CD34 immunoreactive. Nevertheless meticulous histopathological evaluation for the presence of benign glandular elements helps confirm the diagnosis of malignant PT [15]. Sarcomatoid metaplastic carcinomas have spindle component which is positive for high molecular weight keratin or p63 and thus can be differentiated from malignant PT [1].

LIMITATION

The limitation of the present study is the small sample size of the borderline and malignant PT owing to their rarity.

CONCLUSION

Our study shows that stromal CD10 expression pattern and intensity strongly correlates with the PT grade, which can help in

the differentiation between benign and malignant variants of PT. Lesions with ability to metastasise (borderline and malignant PTs) show much greater CD10 expression than those without this ability (benign PT and FA). Thus, CD10 immunoreactivity can assist the meticulous histopathological examination to accurately grade the PTs and help in planning careful follow up and adequate treatment.

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