

The Role of CD10 Immunohistochemistry in the Grading Of Phyllodes Tumor of the Breast

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Abstract

Objective: To determine the relationship between the degree of CD10 expression in the stromal cells of phyllodes tumor and tumor grading.

Methods: A total of 61 cases of mammary phyllodes tumors over the past 11 years were searched from histopathology files, University Malaya Medical Centre. The paraffin blocks were retrieved and 4 µm thick slides were prepared and stained using an antibody against CD10 with the envision method. Fibroadenoma case was used as a control slide and breast myoepithelium as the internal control. Each stained slides was independantly and semiquantitatively analysed for the intensity and percentage of the stromal cells stained. The staining intensity was graded as negative (no staining), mild, moderate and strong if the staining was much weaker, slightly weaker and same intensity as that of the myoepithelium, respectively. The tumor was considered positive for CD10 if the staining intensity is moderate to strong in 20% or more of the stromal cells.

Results: 21 (44.7%) of 47 benign phyllodes tumor, 5 (83.3%) of 6 borderline phyllodes tumor and all 8 cases (100%) of malignant phyllodes tumor showed positive expression for CD10 immunostain.

Conclusions: There was a significant increase in CD10 expression in the stromal cells as the lesions progressed from benign to borderline and malignant phyllodes tumor.

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INTRODUCTION

Phyllodes tumor is an uncommon fibroepithelial neoplasm of the breast, occurring much less frequently than fibroadenoma. The reported incidence is 0.3 to 0.5% of female breast tumors [1-5]. There appears to be ethnical differences, with a higher incidence occurring in Asians. In Asian countries, phyllodes tumor occurs at younger age (average 25-30 years) [6]. Although in 2003, WHO has established a grading system based on semi-quantitative assessment of stromal cellularity, cellular pleomorphism, mitotic activity, margin appearance and stromal distribution [7], there is no standard interpretation of the histologic cut-point criteria and therefore, the diagnosis may vary by different pathologists [8].

The importance to properly diagnose other fibroepithelial neoplasm of the breast especially fibroadenoma is crucial since all grades of phyllodes tumor may recur. In the literature, the recurrence rate of phyllodes tumor ranges from 10-25% for benign phyllodes, up to 32% for borderline phyllodes and up to 40% for malignant phyllodes tumor [9-12]. One large study by Tan et al [10] has correlated the tumor grade with recurrent disease with contributions from degree of stromal atypia, stromal hypercellularity and nature of microscopic borders. It is also important to properly grade the phyllodes tumor since the borderline and frankly malignant phyllodes tumor can metastasize, the incidence is 4% and 22% respectively [9].

There are a few studies using immunohistochemical stains such as p53 [13, 14], Ki67 (MIB1) [15], CD31

[16, 17], Actin [3], c-kit (CD117) [18, 19] and CD10 [2-4] to correlate with the grading of phyllodes tumor. In the literature, evaluation of CD10 expression in phyllodes tumor has been reported in several studies. In one large study by Tse et al [2] has found a significant increase in stromal cell CD10 expression as the lesions progressed from fibroadenomas and benign phyllodes tumor to borderline and frankly malignant phyllodes tumor. A study done by Tsai et al [3] has showed that CD10, actin and vimentin can significantly differentiate between borderline and malignant phyllodes tumor. However, a small study by Zamecnik et al [4] has concluded that CD10 does not help in differentiation between fibroadenoma and phyllodes tumor.

In our current study, we further evaluated the role of CD10 immunohistochemical expression in the stromal cells of mammary phyllodes tumors with the aim of determining whether the degree of CD10 expression in the stromal cells is related to the grade of the tumor. We also investigated the correlation in between the grades of phyllodes tumor and patients' age, race, tumor size, and recurrent tumors.

MATERIALS AND METHOD

Patient data and immunohistochemistry

The histopathology files from University Malaya Medical Centre (UMMC) were searched for phyllodes tumors of the breast over the past 11 years, yielding a total of 71 cases obtained from 63 patients. The cases were verified on the basis of patients' records and original histopathological reports. The original hematoxylin and eosin slides were reviewed and all the diagnoses were reconfirmed according to the following histological parameters: (1) stromal cellularity; (2) cellular pleomorphism; (3) mitotic activity; (4) margin appearance; (5) stromal distribution/overgrowth (stromal overgrowth has been defined as stromal proliferation to the point where the epithelial elements are absent in at least one low power field [x40]) and (6) malignant heterologous elements [9-11, 13, 20, 21]

A diagnosis of benign phyllodes tumor was made when there was low stromal cellularity, absence of cellular pleomorphism, low mitotic count (less than 3-4 per 10 high power fields), a rounded margin, absence of stromal overgrowth and absence of malignant heterologous elements (Figure 1). Malignant phyllodes tumor was diagnosed when there is stromal hypercellularity, stromal overgrowth, significant cellular pleomorphism, high mitotic count (more than 3-4 mitosis per 10 high power fields), infiltrative margins and the presence of malignant heterologous element (Figure 5). For borderline phyllodes tumor, it was diagnosed when the criteria for malignancy were not totally fulfilled (Figure 3).

For CD10 immunostaining, the paraffin blocks were retrieved (based on the most representative slide/slides) and 4µm thick slides was prepared and stained using an antibody against CD10 (Novocastra Laboratories, Newcastle upon Tyne, UK: 1:25 dilution; pressure cooker antigen retrieval, high PH solution) with the envision method. Fibroadenoma cases were used as a control slide and breast myoepithelium as the internal control (CD10 stained the cytoplasm of the myoepithelial cells) (Figure 2, 4 and 6). All the immunostained slides were assessed semiquantitatively using visual estimation according to both the intensity and percentage of the stromal cells stained. The staining intensity was graded as negative (no staining), mild, moderate and strong, if the staining was much weaker, slightly weaker and same intensity as that of the myoepithelium, respectively. The percentage of the stromal cells stained (regardless of the intensity) was graded into less than 20%, 20-50% and more than 50%. The tumor was considered positive for CD10, if the stromal cells were moderate to strong staining intensity in 20% or more of the stromal cells.

Statistical analysis

Statistical analysis was carried out using Fisher exact test (SPSS for Windows, release 13.0). The grades of the phyllodes tumor were correlated with CD10 stain, patient's age and race and tumor size. Statistical significance is established at $p < 0.05$.

RESULTS

Our study included 70 cases of phyllodes tumor, obtained from 61 patients. These 70 cases included 9 recurrent cases, 4 tumors from two patients who had two recurrences each and 5 tumors from five patients who had one recurrence each. Of the total 61 cases (excluding the recurrent cases), there were 47 (77%) benign, 6 (10%) borderline and 8 (13%) malignant phyllodes tumors. For the nine recurrent cases, there were 5 benign, 1 borderline and 3 malignant phyllodes tumors.

The patients' age ranged from 11 to 73 years (mean, 39 years). Stratification into age groups of younger than 30 years, 30 to 50 years and older than 50 years revealed the following: 21 (34.4 %), 24 (39.3%) and 16 (26.2%) women respectively. Twenty-two (36%) patients were Malays, 21 (34.4%) patients were Chinese and 18 (29.5%) patients were Indian. The race was correlated with the tumor grades and was statistically significant ($p = 0.02$) (Table 1).

For the 47 benign phyllodes tumor, the patient age range was 11-63 years (mean 35 years), and the tumor size range was 1.5 to 25.0 cm (mean 4.75 cm). For the 6 borderline phyllodes tumor, the patient age range was

42-61 years (mean 52 years), and the tumor size range was 2.5 to 14.0 cm (mean 8.42 cm). For 8 malignant phyllodes tumor, the patient age range was 34-73 years (mean 53 years), and the tumor size range was 1.5 to 24.0 cm (mean 6.81 cm). The mean age of patients increased with the increased of the tumor grades (Table 2). The age was correlated with the tumor grades and was statistically significant ($P = 0.007$). The mean size was not increased with the increased of the tumor grades and also was not statistically significant (Table 3).

For the CD10 staining of all cases, 21 of the 47 benign phyllodes tumor cases were positive, 5 of the 6 borderline phyllodes tumor were positive and all 8 cases of malignant phyllodes tumor were positive (Table 4). There was a significant increase in CD10

expression in the stromal cells as the lesions progressed from benign to borderline and malignant phyllodes tumors ($p = 0.001$).

Among the recurrences, nine recurrent tumors (seven first recurrences and two second recurrence from seven patients) together with the initial tumors were reviewed. The initial seven cases of tumor were benign in three patients, borderline in two patients and malignant in two patients. The benign and borderline cases recurred as benign and the malignant cases recurred as malignant. Of the nine recurrent phyllodes tumors, CD10 expression was positive in eight tumors and one case was negative. There was no association in tumor grades between initial and recurrent tumors (Table 5) and no association was found between CD10 expression and the number of recurrence (Table 6).

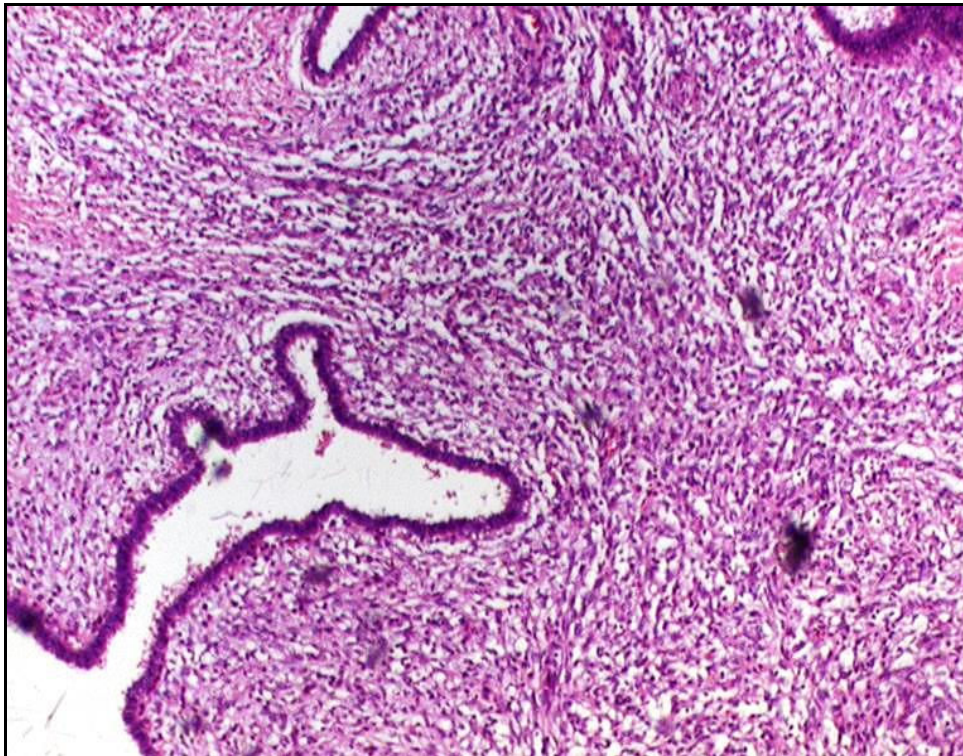


Figure 1. Photomicrograph showing a benign phyllodes tumor (H&E, x400).



Figure 2. Photomicrograph of the same benign phyllodes tumor (as shown in figure 1) showing positive cytoplasmic staining for CD10 in the stromal cells. Note the CD10 expression is more marked in subepithelial location (CD10, x400).

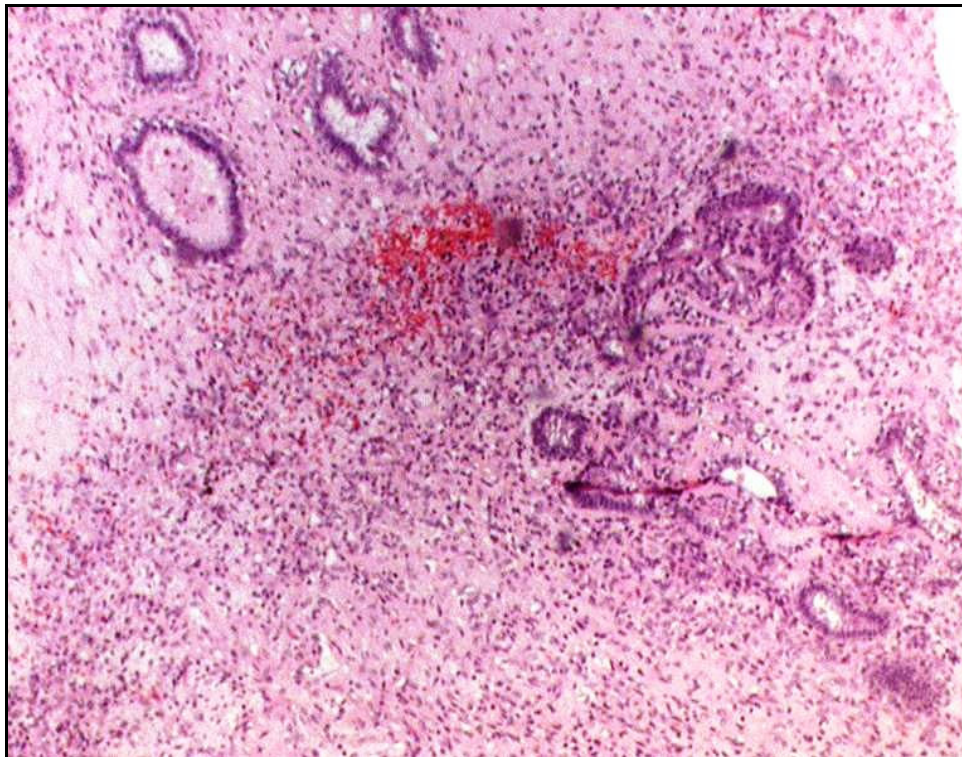


Figure 3. Photomicrograph showing a borderline phyllodes tumor (H&E, x400).

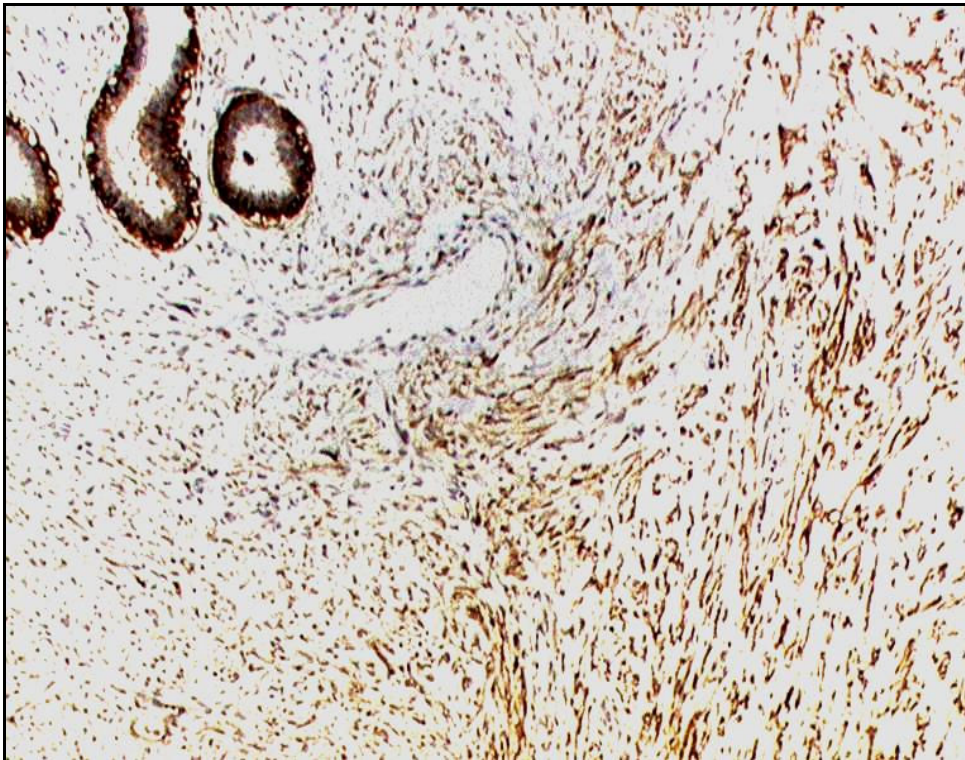


Figure 4. Photomicrograph of the same borderline phyllodes tumor (as shown in figure 3) showing stromal CD10 expression. Note the myoepithelial cells are also positive as an internal control (CD10, x400).

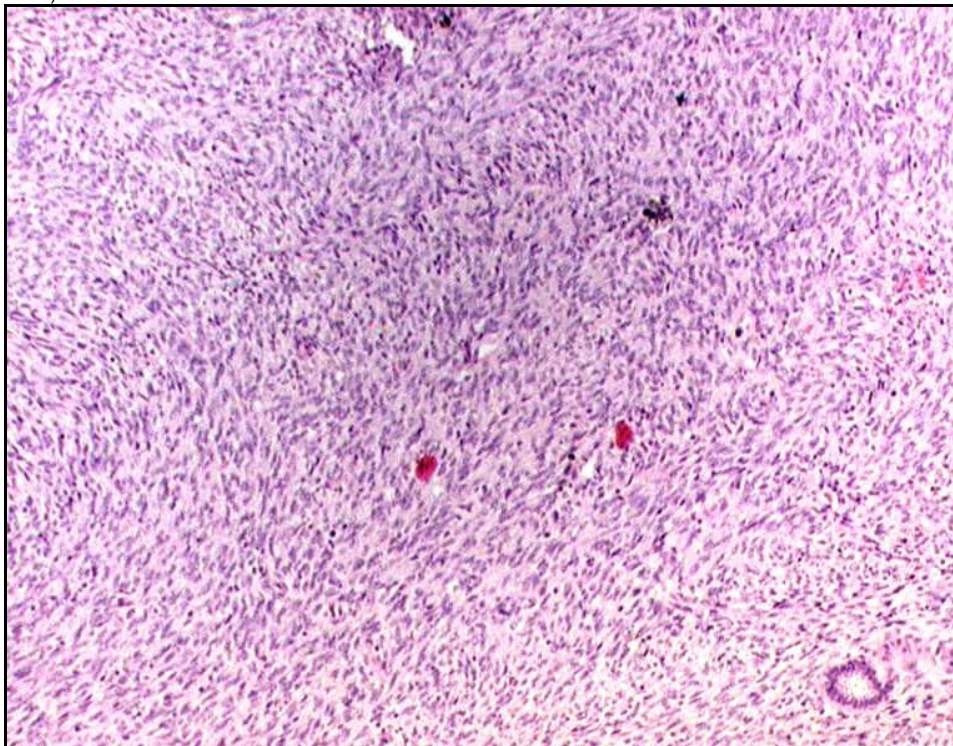


Figure 5. Photomicrograph showing a malignant phyllodes tumor (H&E, x400).

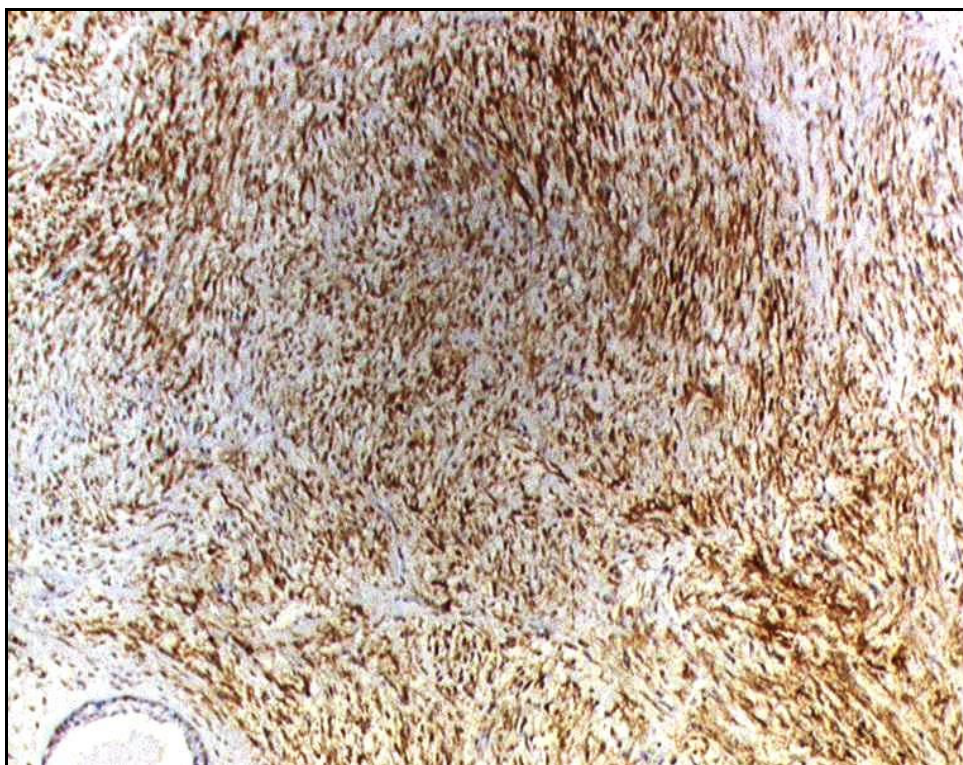


Figure 6. Photomicrograph of the same malignant phyllodes tumor (as shown in figure 5) showing diffuse and high intensity of stromal CD10 staining (CD10, x400).

Table 1. Correlation between race and grades of phyllodes tumor.

Grades of phyllodes tumor	Race			Total	p-value
	Malay	Chinese	Indian		
Benign phyllodes tumor	19(40.4%)	13(27.6%)	15(40%)	47	0.02
Borderline phyllodes tumor	3(50%)	3(50%)	0	6	
Malignant phyllodes tumor	0	6(75%)	2(25%)	8	

Table 2. Correlation between patients' age and grades of phyllodes tumor

Grades of phyllodes tumor	Age (years)			Total	p-value
	30	30-50	>50		
Benign phyllodes tumor	21(44.7%)	19(40.4%)	7(14.9%)	47	0.007
Borderline phyllodes tumor	0	2(33.3%)	4(66.7%)	6	
Malignant phyllodes tumor	0	3(37.5%)	5(62.5%)	8	

Table 3. Correlation between tumor size and grades of phyllodes tumor

Grades of phyllodes tumor	Tumor size			Total	p-value
	<5 cm	5-10 cm	>10 cm		
Benign phyllodes tumor	34(72.3%)	9(19.1%)	4(8.5%)	47	0.2
Borderline phyllodes tumor	2(33.3%)	2(33.3%)	2(33.3%)	6	
Malignant phyllodes tumor	6(75%)	1(12.5%)	1(12.5%)	8	

Table 4. Correlation between CD10 expression and grades of phyllodes tumor

Grades of phyllodes tumor	CD10 positive	CD10 negative	Total	p-value
Benign phyllodes tumor	21 (44.7%)	26 (55.3%)	47	0.001
Borderline phyllodes tumor	5 (83.3%)	1 (16.7%)	6	
Malignant phyllodes tumor	8 (100%)	0	8	

Table 5. Correlation between initial and first recurrent tumor and grades of phyllodes tumor

Grades of phyllodes tumor	Initial tumor	First recurrent tumor	Total	p-value
Benign phyllodes tumor	3 (37.5%)	5 (62.5%)	8	0.4
Borderline phyllodes tumor	2 (100%)	0	2	
Malignant phyllodes tumor	2 (50%)	2 (50%)	4	

Table 6. Correlation between CD10 expression and all recurrent phyllodes tumors

Grades of phyllodes tumor	CD10 positive	CD10 negative	Total	p-value
Benign phyllodes tumor	4 (80%)	1 (20%)	5	1.0
Borderline phyllodes tumor	1 (100%)	0	1	
Malignant phyllodes tumor	3 (100%)	0	3	

DISCUSSION

CD 10 is a 90-110 kDa cell surface, zinc-dependent metalloprotease that has been called “common acute lymphoblastic leukemia antigen (CALLA)”. This antigen has also been referred to as “neutral metalloendopeptidase” in the kidney and “enkephalinase” in the brain. CD10 has been very useful for classifying acute leukemias and subclassifying malignant lymphomas [22]. CD10 is expressed at high rates in renal cell carcinoma [23], hepatocellular carcinoma [24], trophoblastic tumor [25] and carcinoma of the urinary bladder and prostate and at much lower rates in breast cancer, stomach cancer and müllerian epithelial tumors of the female genital tract.

CD10 is clearly and constantly detectable in myoepithelial cells and it is not expressed in other cells such as luminal epithelial cells, fibroblasts, myofibroblasts, smooth muscle or endothelial cells as compared to other markers of myoepithelial cells such as alpha smooth muscle actin (α -SMA), high molecular weight cytokeratin (HMWCK) and S100 protein [26, 27]. Thus, CD10 is more specific than those conventional markers of myoepithelial cells and clearly highlights the distribution of myoepithelial cells. However, there is no stromal expression of CD10 is detected in normal breast tissue.

Apart from positive expression of CD10 immunostaining in proliferating stromal cells in

fibroepithelial breast lesion that has been performed in several studies [2, 3, 4], CD10 was also expressed in stromal cells in invasive ductal carcinoma [28] and more intense in the stromal cells close to the cancer cells. Similarly, in our current study, we found that in most of the cases, CD10 expression is more marked in subepithelial location (Figure 2) where the stromal condensation is present, which is a focus of high proliferative activity. CD10 expression is also has strongest staining in the hypercellular stromal area.

Two studies done evaluating CD10 expression in phyllodes tumor included limited number of cases, 22 and 6 cases respectively [3, 4]. The latest study has included 20 cases of fibroadenoma and has concluded that CD10 cannot assist in the differential diagnosis between fibroadenoma and phyllodes tumor. One large study was more comprehensive and included 181 cases of phyllodes tumor and 33 cases of fibroadenoma [2]. Comparing this large study and our study, the percentage of phyllodes tumors with CD10 expression differed between different grades, 50% and 100% in malignant grade, 31.4% and 83.3% in borderline grade and 5.9% and 44.7% in benign grade. However both studies showed significant increase in CD10 expression as the lesions progressed from benign phyllodes tumors to borderline and frank malignancy.

Our results demonstrated several findings. Firstly, there was a significant increase in CD10 expression in the stromal cells as the lesions progressed from benign to

borderline and malignant phyllodes tumor, similar findings to a few studies before [2, 3]. We also demonstrated that there was a progressive increase in the patients' age with the increased of the tumor grades and was statistically significant ($p=0.007$).

In predicting recurrences, there was no significant relationship between CD10 expression and tumor recurrence, probably due to small number of recurrent cases and adequate treatment given for the initial tumors. Stromal CD10 expression is a new and strong predictor of tumor recurrence and death. Multivariate analysis by Cox's proportional hazards regression model revealed that stromal CD10 expression is an independent prognostic factor for patient outcome [29]. In the literature, a few cytogenetic studies by comparative genomic hybridization (CGH) showed 1q gain, 3p loss, 7q gain, 6q loss and 3q loss are the most common changes detected and there is also evidence that 1q gain may be associated with recurrent disease in phyllodes tumors [30, 31]. In that case, more molecular studies should be done especially to help in predicting recurrent tumors.

Based on our study, CD10 immunostain may be a useful adjunct in the diagnosis of frankly malignant phyllodes tumor since all 8 cases were positive for the stain. All the cases showed diffuse and strong intensity for CD10 immunostain. In future, more malignant cases shall be obtained to support this current study.

DISCLOSURE

Authors declare that there is no any conflict among them.

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