

Role of CD10 Immunohistochemical Expression in Discriminating the Categories of Phylloides Tumor

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Abstract

Background and objectives: Phylloides tumors are categorized as benign, borderline and malignant on the basis of histological features which are subjected to significant inter-observer variability. Our aim was to study the role of immunohistochemical expression of CD10 in discriminating benign, borderline and malignant categories of phylloides tumor.

Methods: The expression of CD10 was studied in 85 phylloides tumors (27 benign, 25 borderline, and 33 malignant) using immunohistochemistry to see whether the expression differed between these histologic categories. Chi-square test was applied to determine the significance of difference in CD10 expression among tumor categories. Significance was established at $p < 0.05$.

Results: 5/27 (18.5%) benign, 16/25 (64%) borderline and 26/33 (78.8%) malignant cases expressed significant (2+ or 3+) staining. This expression of CD10 significantly varied among histological categories ($p < 0.001$). Histological features such as stromal atypia, stromal cellularity, tumor margins, mitotic activity and tumor size correlated significantly with tumor categories as well as with CD10 expression.

Conclusion: From these highly significant results, we believe that there is a strong correlation between CD10 expression and tumor grade and it can be used as an adjunct diagnostic tool for categorizing Phylloides tumors along with other histological features.

Introduction

CD 10, also known as CALLA (common acute lymphoblastic leukemia antigen), is a matrix metalloprotease which is responsible for stromal differentiation and tumor invasiveness. It has established diagnostic role in hematolymphoid malignancies such as acute lymphoblastic leukemia/lymphoma, follicular lymphoma, Burkitt's lymphoma and non-hematolymphoid malignancies such as endometrial stromal tumors, renal cell carcinoma and hepatocellular carcinoma [1-3]. It is also expressed in a variety of other non-hematopoietic malignancies as well as in normal myoepithelial cells in breast [4,5].

Phylloides tumors (PTs) are biphasic breast neoplasms of fibroepithelial origin, accounting for less than 1% of all the breast tumors [6]. These tumors are characterized by the overgrowth of stromal component with compression of breast ducts (epithelial component) and therefore impart a typical leaf like gross appearance [7,8]. Like other breast tumors, PTs also possess the potential of recurrence or metastasis [9,10]. These tumors are generally less aggressive and less frequently recur or metastasize as compared to the breast carcinomas.

In order to predict the possible behavior and outcome, these tumors are classified into benign, borderline and malignant categories [8]. Malignant PTs have the highest recurrence (36-65%) and metastatic frequency (35%) and benign PTs have the lowest recurrence (8-21%) and metastatic frequency (7%) [9,10]. The categorization of PTs is based on certain morphologic features including tumor borders, stromal overgrowth, stromal atypia, mitotic figures and presence of heterologous components [8]. Owing to the heterogeneity of these morphologic features and inter-observer variability, classifying individual tumors accurately into these categories is not always simple [11]. Furthermore, none of these morphologic features individually predicts the behavior of the tumor. Few researchers have attempted to determine the role of immunohistochemistry (IHC) as a predictive tool. Immunohistochemical markers like p53, CD 31, CD34, CD 117, vimentin, actin, VEGF and EGFR have been evaluated for differential

expression in the different categories of PT few have demonstrated a significant role in distinguishing between these categories [11-19].

The differential expression of CD10 has also been evaluated PTs' categories, with the results showing an increased expression in malignant cases [20-23]. As the studies conducted so far are still few and need to be validated with further studies on a larger number of cases. Information about the possible behavior and outcome of the tumor is one of the major concerns for oncologist, as it guides to the selection of appropriate treatment modalities. Surgery alone is the mainstay of treatment for benign PTs and malignant PTs may require further treatment such as radiotherapy and/ or chemotherapy [24-27].

The aim of our study was to evaluate the role of CD 10 immunohistochemical stain in categorizing phylloides tumors.

Materials and Methods

The study was approved by institutional "Ethical Review Committee". We retrieved 85 cases of phylloides tumor from the surgical pathology database of section of histopathology, Aga Khan University Hospital for cases reported between January 2006 and December 2013 through "Integrated Laboratory Management System (ILMS)" software.

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We included the excisional biopsy, wide local excision, mastectomy and modified radical mastectomy (MRM) specimens. Trucut biopsies, incisional biopsies and blocks received (from outside) for second opinion were not included. Moreover, specimen with poor fixation and processing artifacts were also excluded. Verbal informed consent was obtained from the patients via telephonic conversation on their contact numbers mentioned at the requisition slips. Pathology reports and slides of the cases were reviewed and data regarding the patient's age, and pathological features such as tumor size, tumor borders, resection margin status, stromal cellularity, stromal overgrowth, nuclear atypia, necrosis, heterologous element and mitotic counts was obtained. These cases were divided into three categories including benign, borderline, and malignant according to WHO criteria [8].

Representative block of the tumor with maximum cellularity and internal control of myoepithelial cells was selected for prospective staining with CD10 immunohistochemical staining. 3-4 µm thick sections of paraffin embedded tissue were placed on poly-L-Lysine coated slides and kept overnight at 37°C. For deparaffinization, the slides were dewaxed with xylene and then rehydrated with 80%,90% and 100% alcohol and distilled water. Antigen retrieval was done by incubating the slides for 20 minutes in microwave at 450 watts. Peroxidase activity was blocked by placing hydrogen peroxidase on slides. Commercially available monoclonal (ready to use) CD10 antibody (code 56C6, Dako) was placed on slide for 30 minutes followed by washing with Xylene, distilled water & further addition of secondary antibody. After washing DAB (chromogen) was added. Slides were counter stained with Haematoxlin and subsequently washed. Immunohistochemical staining was performed on the selected slides (as per kit manufacturer's instructions) by a technologist, utilizing Immunostaining was assessed by at least two pathologists who were unaware of the histological diagnosis. In case of discrepancy, the staining was reassessed by both pathologists and if the discrepancy remained, third pathologist assessed staining. The percentage of stromal cells staining positive was scored from 0% to 100%. Staining intensity was scored as 0, 1+, 2+ and 3+ (no staining, weak, moderate and strong staining, respectively). IHC was considered positive for CD10 if more than 20% stromal cells exhibit moderate (+2) to strong (+3) expression [22].

Statistical analysis

Mean, median and ranges were calculated for age, tumor size and mitotic count, whereas percentages of different histological features and CD 10 positive cases in benign, borderline, malignant categories were calculated. Pearson Chi-Square test was applied to examine the correlation of CD10 expression and histological features with tumor categories.

Results

All of the 85 retrieved cases were females. Out of these, 62 (72.9%) were breast lumps (excisional biopsy and wide local excision specimens), followed by 10 (11.8%) simple mastectomy specimens, 12 (14.1%) were MRM specimens and 1 breast lump with axillary lymph nodes. Overall summary of clinicopathological features of phylloides tumor is given in Table 1.

When categorized according to WHO criteria, 27 (31.8%) cases were benign, 25 (29.4%) were borderline and 33 (38.8%) were malignant. Comparison of various clinical features and histopathological characters among the three categories of phylloides tumors is given in Table 2. Necrosis was present in 5 (15.2%) out of 33 malignant

cases and 2 (6.1%) out of 33 malignant cases showed sarcomatous transformation. Skin ulceration was observed in 2/25 (8%) borderline and 3/33 (9.1%) malignant cases. Positive CD 10 staining (2+ or 3+) was observed in 5/27 (18.5%) benign cases, 16/25 (64%) borderline cases and 26/33 (78.8%) malignant cases (Figures 1-4). Apart from stromal proliferation, epithelial proliferative lesions were also observed including intraductal papilloma (1 case), usual ductal hyperplasia (6 cases), atypical ductal hyperplasia (1 case) and ductal carcinoma in situ (2 cases) (Table 3).

CD10 positive expression and increase in tumor size significantly correlated with increase in tumor category ($p < 0.0001$). Among histological features, stromal atypia, stromal cellularity and mitotic count differed significantly in the tumor categories ($p < 0.0001$). Patient's age, necrosis, sarcomatous transformation and skin ulceration did not significantly correlated with tumor category (Table 3). We also observed histological features such as increase in stromal atypia, stromal cellularity, tumor margin and mitotic activity to correlate significantly with increase in CD10 expression ($p < 0.0001$) (Table 4).

Discussion

CD10 immunohistochemical expression in myoepithelial cells of the breast duct is unique in the sense that the epithelial cells and

Age in years	
(Range)	16-69
(Mean)	37.4 ± 11.8
(Median)	38
Age groups	
• 30 years or below	25 (29.4%)
• 31 to 50 years	50 (58.8%)
• 51 years or above	10 (11.8%)
Specimen type	
• Breast lump only	62 (72.9%)
• Simple mastectomy	10 (11.8%)
• MRM	12 (14.1%)
• Breast lump and lymph nodes	01 (1.2%)
Tumor size (cm)	
(Range)	2-23
(Mean)	8.5 ± 5.1
(Median)	7
Tumor size (groups)	
• Below 5 cm	29 (34.1%)
• 5 to 10 cm	30 (35.3%)
• Above 10 cm	36 (30.6%)
Tumor category	
• Benign	27 (31.8%)
• Borderline	25 (29.4%)
• Malignant	33 (38.8%)
CD 10 staining	
• 0 (no staining)	9 (10.6%)
• 1+ (weak)	30 (35.3%)
• 2+ (moderate)	24 (28.2%)
• 3+ (strong)	22 (25.9%)
Necrosis	06 (7.1%)
Sarcomatous transformation	02 (2.4%)
Skin ulceration	05 (5.9%)
Tumor margin distance	1-50 mm

Table 1: Overall summary of clinicopathological features of phylloides tumor cases.

	Benign (n=27)	Borderline (n=25)	Malignant (n=33)	p Value
Age in years				
(Range)	16-54	19-55	19-69	p = 0.585
(Mean)	36 ± 12	37.6 ± 9.8	38.7 ± 13.2	
(Median)	35	38	38	
Age groups				
• 30 years or below	10 (37%)	05 (20%)	10 (30.3%)	p = 0.633
• 31 to 50 years	15 (55.6%)	17 (68%)	18 (54.4%)	
• 51 years or above	02 (7.4%)	03 (12%)	05 (15.2%)	
Tumor size (cm)				
(Range)	2-18	2.2-19	2.7-23	P <0.0001
(Mean)	5.2 ± 3.3	9.1 ± 4.7	10.6 ± 5.3	
(Median)	4.5	8.5	9	
Tumor size (groups)				
• Below 5 cm	19 (70.4%)	04 (16%)	06 (18.2%)	p < 0.0001
• 5 to 10 cm	06 (22.2%)	12 (48%)	12 (36.4%)	
• Above 10 cm	02 (7.4%)	09 (36%)	15 (45.5%)	
CD 10 staining (score)				
• 0 (no staining)	04 (14.8%)	03 (12%)	02 (6.1%)	p < 0.0001
• 1+ (weak)	19 (70.4%)	06 (24%)	05 (15.2%)	
• 2+ (moderate)	02 (7.4%)	10 (40%)	12 (36.4%)	
• 3+ (strong)	02 (7.4%)	06 (24%)	14 (42.4%)	
Significant CD10 staining*				
• CD10 Positive	05 (18.5%)	16 (64%)	26 (78.8%)	p < 0.0001
• CD10 Negative	22 (81.5%)	09 (36%)	07 (21.2%)	
Skin ulceration	00	02 (8%)	03 (9.1%)	p = 0.286

*Based upon CD10 staining scores, the cases were termed CD10 positive and CD10 negative. Cases with score of 0 and 1+ were considered negative while cases with score of 2+ and 3+ were considered positive.

Table 2: Comparison of clinicopathological features in categories of phylloides tumor.

	Benign (n=27)	Borderline (n=25)	Malignant (n=33)	p Value
Stromal Atypia				
• Mild	26 (96.3%)	09 (36%)	0	p <0.0001
• Moderate	01 (3.7%)	14 (56%)	03 (9.1%)	
• Marked	0	02 (8%)	30 (90.9%)	
Stromal Cellularity				
• Mild	20 (74.1%)	03 (12%)	0	p <0.0001
• Moderate	07 (25.9 %)	19 (76%)	04 (12.1%)	
• Marked	0	03 (12%)	29 (87.9%)	
Tumor Margins				
• Pushing	27 (100%)	21 (84%)	03 (9.1%)	p <0.0001
• Infiltrative	00	04 (16%)	30 (90.9%)	
Mitoses (per 10 HPF)				
Range	1-4	2-18	5-42	p <0.0001
Average	2.6 ± 1	7.6 ± 3	20.5 ± 9.5	
Mitotic Count				
• 0-4 / 10HPF	27 (100%)	01 (4%)	00	p <0.0001
• 5-9 / 10HPF	00	18 (72%)	03 (9.1%)	
• >10 /10HPF	00	06 (24%)	30 (90.9%)	
Sarcomatous Component	00	00	02 (6.1%)	p =0.199
Necrosis	00	01 (4%)	05 (15.2%)	p =0.058
Additional epithelial lesions				
• Papilloma	01	00	00	
• Usual Duct Hyperplasia	01	03	02	
• Atypical Ductal Hyperplasia	00	01	00	
• Du ductal carcinoma in situ	00	01	01	

Table 3: Comparison of histological features in categories of Phylloides Tumor.

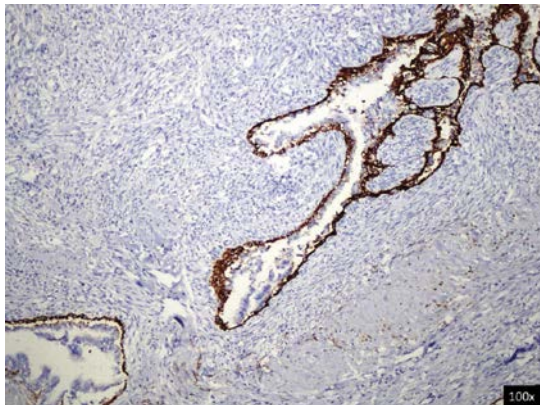


Figure 1: No staining (0 score) in benign phylloides tumor. CD10 stain highlights a continuous layer of myoepithelial cells (Internal Control) while stromal cells do not show any staining (100x).

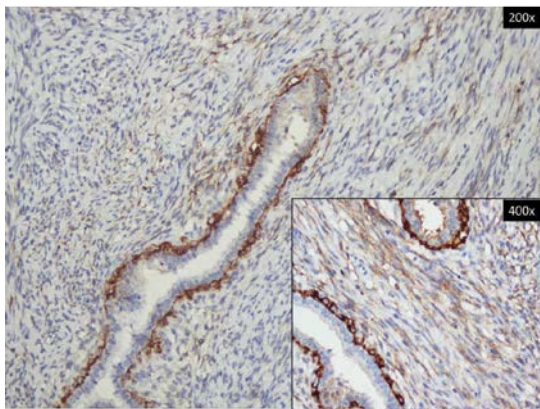


Figure 2: Weak (1+ score) staining in borderline phylloides tumor. Stromal cells faintly stain with CD10 stain. The staining intensity of the stromal cells is much weaker than myoepithelial cells which show strong CD10 staining (200x, inset: 400x).

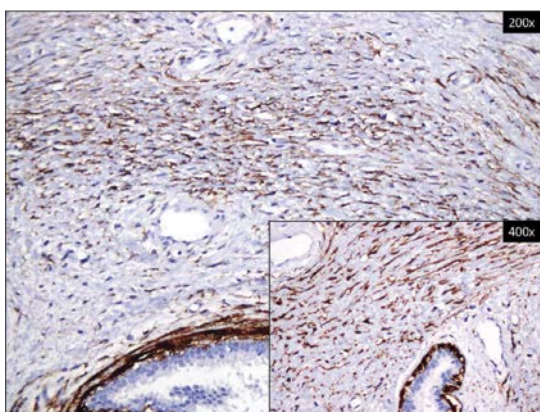


Figure 3: Moderate (2+ score) staining in borderline phylloides tumor. Stromal cells strongly stain with CD10 stain but the staining intensity of the stromal cells is slightly weaker than staining intensity of myoepithelial cells (200x, inset: 400x).

other components of normal breast stroma such as fibroblasts, myofibroblasts, and smooth muscle do not stain for CD10 while myoepithelial cells also demonstrate positivity for cytokeratins, actin and S100. Therefore, expression of CD10 in myoepithelial cells serves

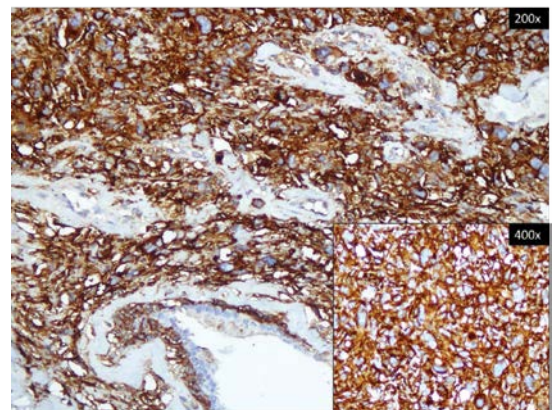


Figure 4: Strong (3+ score) staining in malignant phylloides tumor. Stromal cells strongly stain with CD10 stain. The intensity is even stronger than myoepithelial cells (200x, inset: 400x).

as a good internal control [28,29].

In English literature, so far five studies have been conducted with an objective of evaluating CD10 expression in phylloides tumors' categories. In the most recent study of 71 phylloides tumor cases, Hussein et al. [30], has reported significantly increasing CD10 expression when moving from benign to malignant categories. He also found age groups (<30, 30-50 and >50) and race to correlate significantly with tumor categories while Tumor size and recurrence rates were not significantly different among tumor categories. Al-Masri M et al. [23], in his study of 43 cases, demonstrated significantly increasing CD10 expression with increasing malignancy in tumor categories. He also reported a significant difference in expression of CD10 in metastatic and non-metastatic cohorts. Tumor size, but not patient's age correlated with tumor grade. Tsai et al. [22], also found CD10 along with ASMA to be discriminating between Phylloides tumor categories. Although the study was conducted only on 22 cases but the results were statistically significant. Tse et al. [20], conducted the largest study of 181 cases to evaluate CD10 expression in Phylloides as well as including 33 fibroadenoma. He found an increasing immunoeexpression trend with increasing malignancy trend. Age and tumor size also correlated significant with tumor grades in his study. In contrast, Zamenick et al. [21], did not observed significant difference of CD10 staining in fibroadenoma and Phylloides tumor categories.

We observed an increase in percentage of CD 10 positive cases from benign to malignant cases. Since it is a metalloprotease, its increased expression reflects the metastatic potential of the tumors in general so like other authors [16], we also think that this staining pattern is attributed to metalloprotease nature of the molecule which contributes to the malignant potential of tumor by allowing the tumor cells to invade surrounding stroma.

We also found the histological features such as stromal atypia, stromal cellularity and mitotic count to hold their significance in categorizing Phylloides tumor, as they differ significantly in tumor categories along with tumor size. Moreover, increase in stromal atypia, stromal cellularity, mitotic count and tumor margins correlated with increase in CD10 expression also. In our opinion, this correlation is attributed to the increase in proliferative nature of tumor cells which further explains the aggressive behavior of higher tumor grades.

Conclusion

On the basis of the significant results of this study conducted on a

	CD10 Negative (n=38)	CD10 Positive (n=47)	p Value
Tumor size (groups)			
• Below 5 cm	21 (55.3%)	08 (17%)	$p < 0.0001$
• 5 to 10 cm	13 (34.2%)	17 (36.2%)	
• Above 10 cm	04 (10.5%)	22 (46.8%)	
Stromal Atypia			
• Mild	25 (65.8%)	10 (21.3%)	$p < 0.0001$
• Moderate	05 (13.2%)	14 (56%)	
• Marked	08 (21.1%)	23 (8%)	
Stromal cellularity			
• Mild	18 (47.4%)	05 (10.6%)	$p < 0.0001$
• Moderate	13 (34.2%)	17 (36.2%)	
• Marked	07 (18.4%)	25 (53.2%)	
Tumor margins			
• Pushing	31 (81.6%)	20 (42.6%)	$p < 0.0001$
• Infiltrative	07 (18.4%)	27 (57.4%)	
Mitotic count			
• 0-4 / 10HPF	23 (60.5%)	05 (10.6%)	$p < 0.0001$
• 5-9 / 10HPF	07 (18.4%)	14 (29.8%)	
• >10 /10HPF	08 (21.1%)	28 (59.6%)	
Necrosis			
• Present	01 (2.6%)	05 (10.6%)	$p = 0.218$
• Absent	37 (97.4%)	42 (89.4%)	
Sarcomatous component	00	02 (4.3%)	$p = 0.500$

Table 4: Correlation of histological features with CD10 staining.

large cohort, we conclude that CD10 immunohistochemical staining is helpful in discriminating Phylloides tumors into histologic categories but CD10 expression should always be used as an adjunct tool along with histologic features.

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