RESEARCH ARTICLE

Role of CD10 Immunohistochemical Expression in Predicting Aggressive Behavior of Phylloides Tumors

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Abstract

Background: Phylloides tumors are rare breast neoplasms with a variable clinical course depending on the tumor category. Along with histologic features, the role of immunohistochemical staining has been studied in predicting their behavior. Objectives: Our aim was to evaluate the role of CD 10 immunohistochemical staining in predicting survival, recurrence and metastasis in phylloides tumor. We also evaluated correlations of other clinicopathological features with overall and disease-free survival. Materials and Methods: CD10 expression was studied in 82 phylloides tumors divided into recurrent/metastatic and non-recurrent/non-metastatic cohorts. The Chi-square test was applied to determine the significance of differences in CD10 expression between outcome cohorts. Uni and multivariate survival analyses were also performed using log-rank test and Cox regression hazard models. Results: All 3 metastatic cases, 5 out of 6 (83.3%) recurrent cases and 37 out of 73 (50.7%) non-recurrent and non-metastatic cases expressed significant (2+ or 3+) staining for CD10. This expression significantly varied between outcome cohorts (p<0.03). Tumor category and histological features including mitotic count and necrosis correlated significantly with recurrence and metastasis. A significant decrease in overall and disease free survival was seen with CD10 positivity, malignant category, increased mitoses and necrosis. Neither CD10 expression nor any other clinicopathologic feature proved to be an independent prognostic indicator in multivariate analysis. Conclusions: CD10 immunohistochemical staining can be used as a predictive tool for phylloides tumor but this expression should be interpreted in conjunction with tumor category.

Keywords: CD10 - phylloides tumor - benign - malignant - recurrence - metastasis - immunohistochemistry

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Introduction

Breast cancer is the most common malignancy in females globally and the incidence is higher in Pakistan as compared to neighboring countries and rest of the world (Moore et al., 2009; Shaukat et al., 2013; Asif et al., 2014). Phylloides tumor (PT) is a fibroepithelial neoplasm which characteristically exhibit proliferation of stromal component accompanied by compression of breast ducts and impart typical leaf life appearance (Noguchi et al., 1993; Tan et al., 2013). PT accounts for less than 1% of all breast neoplasm and like others, inherit the recurrent and metastatic potential (Rowell et al., 1993; Barth, 1999; Asoglu et al., 2004; Khurshid et al., 2013). Prediction of possible behavior and outcome of the disease helps oncologist to choose the most suitable treatment modality. Complete surgical resection with safe margins is the mainstay of treatment for all PTs and malignant PTs may require frequent follow-up visits and additional radiotherapy and/or chemotherapy (Burton et al., 1989, Hawkins et al., 1992; Barth, 1999; Khosravi-Shah, 2011).

On the basis of a constellation of histological features, these tumors are classified into benign, borderline and malignant categories (Tan et al., 2013). Malignant PTs have the highest recurrence (36-65%) and metastatic frequency (35%) and benign PTs have the lowest recurrence (8-21%) and metastatic (7%) frequency (Rowell et al., 1993; Asoglu et al., 2004). The histological features used to characterize PTs do not hold an individual predictive value. Few researchers have evaluated the role of immunohistochemistry (IHC) in predicting the recurrence and metastasis of PTs. Immunohistochemical markers such as Ki-67, p53, CD 31, CD34, CD 117, vimentin, actin, VEGF and EGFR have been evaluated for their possible predictive but none has yet demonstrated a significant role (Millar et al., 1999; Chen et al., 2000; Ortega et al., 2001; Tse et al., 2001; 2003; 2004; 2005; 2009; Tan et al., 2005).

CD 10 is a matrix metalloprotease which plays an important role in stromal differentiation and tumor invasion. It has an established diagnostic role in a variety of tumors especially follicular lymphomas, Burkitt’s lymphomas and endometrial stromal tumors (Stein et al., 1984; Gregory et al., 1987; Chu et al., 2001). Expression of CD10 has been demonstrated in a number of other
non-hematopoietic neoplasms as well as in normal myoepithelial cells of breast (Chu et al., 2000; Moritani et al., 2002). CD10 has also been evaluated in different categories of PTs and increased immunohistochemical expression in observed with increasing tumor category (Tse et al., 2005; Tsai et al., 2006; Al-Masri et al., 2012; Hussin et al., 2013). The role of CD10 has been evaluated in predicting possible outcome of PTs and no study has described any correlation of recurrence with CD10 immunohistochemical expression. Two studies have described significantly increased expression in metastatic cases (Tsai et al., 2006; Al-Masri et al., 2012). As the studies conducted so far are few in number, therefore, validation with further studies on a larger number of cases is required.

The aim of our study was to evaluate the role of CD10 immunohistochemical stain and clinicopathological features in predicting the survival, recurrence and metastasis in phyllodes tumors.

Materials and Methods

The study was approved by institutional “Ethical Review Committee”. We retrieved 82 cases of Phyllodes Tumor from the surgical pathology database of Section of Histopathology, Aga Khan University Hospital for cases reported between January 2006 and March 2014 through “Integrated Laboratory Management System (ILMS) software”. We included the excisional biopsy, wide local excision, mastectomy and modified radical mastectomy (MRM) specimen. Trucut biopsies, incisional biopsies and blocks received (from outside) for second opinion were not included. Moreover, specimen with clear margins i.e. presence of normal breast tissue around the entire periphery of tumor, were included. Verbal informed consent and follow up information regarding recurrence and metastasis 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, mitotic counts and distance from resection margin was obtained. These cases were divided into three categories including benign, borderline, and malignant according to WHO criteria (Tan et al., 2013). Representative block of the tumor with maximum cellularity and internal control of myoepithelial cells was selected for prospective staining with CD10 immunohistochemical staining. In recurrent cases, blocks of initial tumor were selected. Immunohistochemical staining was performed on the selected slides (as per kit manufacturer’s instructions) by a technologist, utilizing commercially available monoclonal (ready to use) CD10 antibody (code 56C6, Dako) on automated immunostainer. Immunostaining was then be assessed by at least two pathologists. 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 (Tsai WC et al., 2006).

Statistical analysis

Pearson Chi-Square test was applied to examine the correlation of CD10 expression and clinicopathological features with recurrence and metastasis. Disease-free survival (DFS) and overall survival (OS) periods were calculated from the dates of pathologic diagnosis to the dates of recurrence or metastasis and death, respectively. Univariate survival curves were plotted using the Kaplan-Meier method, and statistical differences were determined by using the log-rank test. Multivariate analysis was performed using the stepwise backward LR Cox regression hazards model. A p value of less than .05 was considered significant.

Results

All of the 82 retrieved cases were females. Out of these, 60 (73.2%) were breast lumps (excisional biopsy and wide local excision specimen), followed by 9 (11%) simple mastectomy specimen, 12 (14.6%) were MRM specimen and 01 breast lump with axillary lymph nodes. Age of presentation ranged from 16-69 years with mean age of 37.2±11.9 SD. When stratified in age groups, 25 (30.5%) cases were 30 years or below, majority 48 (58.5%) were between 31 to 50 years and 9 (11.8%) were above 50 years of age. Tumor size ranged from 2.2 to 23 cm with average tumor size of 8.5±5.1 cm SD. When stratified into groups, 28 (34.1%) cases were below 5 cm, 29 (35.4%) cases were between 5-10 cm and 25 (30.5%) were above 10 cm in size. Skin ulceration was observed in 04 (4.9%) cases. Distance from tumor margin ranged from 4-50 mm with median of 1 mm. 46 (56.1%) cases had safe (≥1 cm) margin while 36 (43.9%) cases has <1 cm but clear margin. When categorized according to WHO criteria, 26 (31.7%) cases were benign, 25 (30.5%) were borderline and 31 (37.8%) were malignant. Positive (2+ or 3+) staining for CD10 immunohistochemical stain was observed in 46 (54.1%) cases (Figure 1A-D). Out of 6 (7.3%) recurrent cases, 4 cases were malignant and 2 cases were borderline. All of 3 (3.7%) cases with lymph node metastasis were malignant. Out of 8 (9.8%) cases which died of disease, 7 were malignant and 1 was borderline. In addition to surgical treatment, 3 malignant cases received chemotherapy, 3 malignant and 1 borderline cases received radiotherapy and 1 malignant case received both chemotherapy and radiotherapy. Follow up duration and Disease free survival (DFS) ranged from 1-88 months (median=32 months and 29.3 months respectively).

When separately analyzed, rate of recurrence and metastasis increased with tumor category but statistical significance was not observed. However, when collectively analyzed as a cohort, combined recurrence and metastatic rate increased significantly with tumor category (p=0.021). Death rate also correlated significantly with tumor category (p=0.008). OS and DFS insignificantly decreased with tumor category.

Similarly, when CD10 expression was separately
analyzed in cases with recurrence and metastasis statistical difference was not observed. However, when collective cohort of recurrent and metastatic cases was analyzed, statistical significance was observed (p=0.03) (Table 1). We also correlated clinicopathologic and histological features with recurrence and metastasis and found positive correlation of mitotic count and necrosis with recurrence and metastasis.

Tumor categories were also showed an association with a combined increase in recurrence and metastatic rate (p=0.021). Among histological features, mitotic activity and necrosis demonstrated positive correlation with recurrence and metastasis (p=0.008 and p=0.016 respectively). Stromal atypia, stromal cellularity, tumors margins, sarcomatous component, patient’s age, tumor size and clear (<1cm) margins were not significantly correlated with these adverse events (Table 2).

Overall survival (OS) and DFS (DFS) significantly varied with significant CD10 staining (p=0.04 & p=0.018), malignant tumor category (p=0.009 & p=0.02),

Table 1. Comparison of CD10 Immunohistochemical Staining between Outcome Cohorts (n=82)

| Outcome Cohorts | CD 10 Positive | CD 10 Negative | P value |
|-----------------|----------------|----------------|---------|
| Recurrent       | 05 (83.3%)     | 01 (16.7%)     | 0.152   |
| Non-recurrent   | 36 (52.6%)     | 40 (47.3%)     |         |
| Metastatic      | 3              | 0              | 0.160   |
| Non-metastatic  | 42 (52.3%)     | 37 (47.7%)     |         |
| Combined cohort |                |                |         |
| - Recurrent or metastatic | 08 (88.9%) | 01 (11.1%) | 0.030 |
| - Non-recurrent and non-metastatic | 37 (50.7%) | 36 (49.3%) |         |
| Dead            | 07 (87.5%)     | 01 (12.5%)     | 0.08    |
| Alive           | 38 (51.4%)     | 36 (48.6%)     |         |

Table 2. Comparison of Clinicopathologic and Histological Features with Poor Outcome (Recurrence and Metastasis) (n=82)

| Clinical & Histological features | Non-recurrent & Non-metastatic | Recurrent or Metastatic | p value |
|----------------------------------|--------------------------------|-------------------------|---------|
| Age groups                       |                                |                         |         |
| 30 years or below                | 22 (30.1%)                     | 03 (33.3%)              | 0.464   |
| 31 to 50 years                   | 44 (60.3%)                     | 04 (44.4%)              |         |
| 51 years or above                | 07 (9.6%)                      | 02 (22.2%)              |         |
| Tumor size (groups)              |                                |                         |         |
| Below 5 cm                       | 26 (35.6%)                     | 02 (22.2%)              | 0.223   |
| 5 to 10 cm                       | 27 (37%)                       | 02 (22.2%)              |         |
| Above 10 cm                      | 20 (27.4%)                     | 05 (55.6%)              |         |
| Tumor category                   |                                |                         |         |
| Benign                           | 26 (35.6%)                     | -                       | 0.021   |
| Borderline                       | 23 (31.5%)                     | 02 (22.2%)              |         |
| Malignant                        | 24 (32.9%)                     | 07 (77.8%)              |         |
| Stromal Atypia                   |                                |                         |         |
| Mild                             | 33 (45.2%)                     | 01 (11.1%)              | 0.08    |
| Moderate                         | 17 (23.3%)                     | 02 (22.2%)              |         |
| Marked                           | 23 (31.5%)                     | 06 (66.7%)              |         |
| Stromal Cellularity              |                                |                         |         |
| Mild                             | 22 (30.1%)                     | -                        | 0.073   |
| Moderate                         | 27 (37%)                       | 03 (33.3%)              |         |
| Marked                           | 24 (32.9%)                     | 06 (66.7%)              |         |
| Tumor Borders                    |                                |                         |         |
| Pushing                          | 47 (64.4%)                     | 03 (33.3%)              | 0.077   |
| Infiltrative                     | 26 (35.6%)                     | 06 (66.7%)              |         |
| Mitotic Count                    |                                |                         |         |
| 0-4 / 10HPF                      | 27 (37%)                       | -                        | 0.008   |
| 5-9 / 10HPF                      | 20 (27.4%)                     | 01 (11.1%)              |         |
| >10 / 10HPF                      | 26 (35.6%)                     | 08 (88.9%)              |         |
| Necrosis                         |                                |                         |         |
| Present                          | 03 (2.6%)                      | 03 (33.3%)              | 0.016   |
| Absent                           | 70 (95.9%)                     | 06 (66.7%)              |         |
| Skin ulceration                  |                                |                         |         |
| Present                          | 02 (2.7%)                      | 02 (22.2%)              | 0.168   |
| Absent                           | 71 (97.3%)                     | 07 (77.8%)              |         |
| Sarcomatous component            | 1                              | 1                        | 0.209   |
| Tumor margin distance            |                                |                          |         |
| <1cm                              | 01 (16.7%)                     | 35 (46.1%)              | 0.168   |
| ≥1cm                              | 05 (83.3%)                     | 41 (53.9%)              |         |

Figure 1. A) No Staining (0) in Benign Phylloides Tumor. CD10 stain highlights a continuous layer of Myoepithelial cells (Internal Control). B) Weak (1+) staining in borderline phylloides tumor. Stromal cells faintly stain with CD10 stain. The intensity is slightly weaker than myoepithelial cells; C) Moderate (2+) staining in borderline phylloides tumor. Stromal cells strongly stain with CD10 stain but the intensity is slightly weaker than myoepithelial cells; D) Strong (3+) staining in malignant phylloides tumor. Stromal cells strongly stain with CD10 stain. The intensity is similar to the staining intensity of myoepithelial cells.

Figure 2. Kaplan-Meier Curves for Overall Survival (OS) showing significant reduction with A) CD10 positivity (Log-Rank significance=0.04), B) Malignant tumor category (Log-Rank significance=0.009), C) Mitotic count ≥10/10HPF (Log-Rank significance=0.002) and D) Necrosis (Log-Rank significance=0.032)
Table 3. Mean Follow Up Durations and Disease-free Survival Durations of Factors with Significant Difference. (n=82)

|                        | Follow up duration (months) | p value (Log-Rank test) | Disease-free Duration (months) | p value (Log-Rank test) |
|------------------------|----------------------------|-------------------------|--------------------------------|-------------------------|
| CD 10 staining         |                            |                         |                                |                         |
| Positive               | 28.4                       | 0.04                    | 28.2 (1-80)                    | 0.018                   |
| Negative               | 40.3                       |                         | 37.8 (2-88)                    |                         |
| Tumor category         |                            |                         |                                |                         |
| Benign                 | 40.3                       | 0.009                   | 36.8                           | 0.02                    |
| Borderline             | 33.6                       |                         | 33.9                           |                         |
| Malignant              | 28.5                       |                         | 27.3                           |                         |
| Mitotic Count          |                            |                         |                                |                         |
| 0-4 / 10HPF            | 39.8                       | 0.002                   | 36.4                           | 0.008                   |
| 5-9 / 10HPF            | 33.7                       |                         | 33.1                           |                         |
| >10 / 10HPF            | 29                         |                         | 28.6                           |                         |
| Necrosis               |                            |                         |                                |                         |
| Present                | 18.7                       | 0.032                   | 16                             | p<0.0001                |
| Absent                 | 35                         |                         | 33.6                           |                         |

Figure 3. Kaplan-Meier Curves for Disease-free Survival (DFS) showing significant reduction with A) CD10 positivity (Log-Rank significance=0.018), B) Malignant tumor category (Log-Rank significance=0.02), C) Mitotic count ≥10/10HPF (Log-Rank significance=0.008) and D) Necrosis (Log-Rank significance<0.0001).

Discussion

CD10 immunohistochemical staining is specific to myoepithelial cells as this staining is neither shared by other stromal components nor by epithelial components (Gillette et al., 1990; Guelstein et al., 1993). Hence, it is efficiently used as internal control. In English literature, so far 4 studies have been conducted to evaluate the role of CD10 in predicting recurrence and/or metastasis of phyllodes tumors. In the earliest study, Tse et al. (2005) evaluated 14 recurrent and 2 metastatic cases in a cohort of 181 phyllodes tumors. He performed CD10 staining on both initial as well as second (recurrent/metastatic) cases and did not find any association of staining pattern with recurrence or metastasis. Tsai et al. (2006) selected a small cohort of 22 phyllodes tumor with 6 recurrent and 3 metastatic cases. He evaluated CD10, ASMA and Vimentin immunohistochemical stains and did not report any association of these with recurrence or metastasis. Al Marsi et al. (2012), in his study of CD10 expression in 46 cases, observed metastasis in 6 cases which positively correlated with increased immunohistochemical staining. Hussin et al. (2013) also failed to prove any association of CD10 immunoeexpression with recurrence in their 9 recurrent cases out of total 61 cases. Our findings are also in concordance with the other studies as we do not find association with recurrence (p=0.152) or metastasis (p=0.16) separately. However, when we combine these cases with features of adverse behavior, we find a positive correlation (p=0.03). In our opinion, both recurrence and metastasis are indicators of poor behavior and association of CD10 expression with this cohort should be interpreted as association with poor behavior i.e either recurrence or metastasis. Moreover, death rate which is another indicator of poor behavior, was also associated with increased CD10 expression (p=0.008).

Among various other immunohistochemical markers like Ki-67, p53, CD 31, CD34, CD 117, vimentin, actin, VEGF and EGFR which have been evaluated in phyllodes tumor, only Tan PH et al. (2005) has described a significant association of CD117 staining with recurrence.

Tan PH et al. (2005), in her another study of phyllodes tumor, have evaluated the predictive role of histologic parameters in detail and found stromal atypia, stromal cellularity, tumor margins and necrosis to be significant. According to Al Marsi et al. (2012), tumor size and tumor grade was also significantly correlated with metastasis. We also analyzed clinicopathologic and histologic features and observed tumor category, mitotic activity and necrosis to correlate with recurrence and metastasis.

Complete excision of with wide margins is the mainstay of treatment for phyllodes tumor. The recurrent rate for negative margins is 10% while it is 18% for excision with positive margins (31). Except for Tan PH et al. (2005), we did not find any study which mentioned the margin status in recurrent cases at the time of initial excision. As tumor margin status is the sole predictor of recurrence, in other studies, the assessment of other features in recurrent cases could have been affected by this confounding factor. The strength of our study lies in avoiding this confounding factor by selecting cases with clear margin (at least 4mm) of clearance. When analyzed, 5 (10.9%) out of 41 cases with safe margins (>1cm) showed recurrence while only 1 (2.8%) out of 35 cases with clear but <1cm margin showed recurrence. The difference in recurrence rates of these two groups and recurrence free survival did not differed significantly (p=0.223 and p=0.52).

Although CD10 positivity, malignant tumor category, increased mitoses and necrosis showed a significant
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