Expression of epidermal growth factor receptor (EGFR) in phyllodes tumor

Introduction

Phyllodes tumor has been considered as a rare fibroepithelial neoplasm that comprises 0.3 to 1.5% of all breast lesions. The tumor was first fully characterized by Johannes Müller in 1838. It is a biphasic breast tumor composed of epithelium and spindle cell stroma. It usually occurs in middle-aged women, but can rarely occur in younger-aged group and in men. The median and mean ages of patients are both 45 years, and the average size is four to five centimeters. Although phyllodes tumor resembles fibroadenoma clinically, mean age of phyllodes tumor patients is approximately 15–20 years older than that of fibroadenoma, and the tumor presents with a history of rapidly enlarging breast lump. Macroscopically, if appears as lobulated round or oval masses with well-circumscribed border and rarely contains calcification. Sit-like spaces may be visible on the cut surface, which may appear slightly granular. Microscopically, tumor shows prominent stromal proliferation, so that the stroma abuts into the epithelial lined spaces, forming the sit-like spaces or leaf-like pattern, hence the name phyllodes.

Currently, phyllodes tumor is classified into benign, borderline malignant, and frankly malignant subtypes based on histological features including stromal cellular atypia, mitotic activity, stromal overgrowth, and tumor margin. Various grading systems have been proposed, but none is universally accepted. The vast majority is benign, while the incidence of the malignant subtype has been reported to range from 26% to 39%. Local recurrence can occur in all phyllodes tumors and systemic recurrence may also develop in cases of borderline or frankly malignant phyllodes tumor. The clinical behavior of phyllodes tumor of the breast is difficult to predict. Although histological features of phyllodes tumor have been traditionally used as predictors of clinical outcome, specific parameters that define recurrence and prognosis are not universally established yet, including any other factors required for proper treatment strategies. Previous studies have been conducted to investigate the usefulness of biologic tumor markers to predict the clinical outcome and classification of phyllodes tumors. The evaluated markers include p53 expression, Ki-67 index, microvascular density, vascular endothelial growth factor (VEGF) expression, HER2/neu, stromal CD10, CD34 expression, and c-kit expression. However, these studies did not contribute substantially to the information already provided by standard histopathological analysis. EGFR (ErbB-1) in human is a cell-surface receptor for members of the epidermal growth factor family (EGF-family). EGFR is a member of the ErbB family of receptors and closely related with the subfamily of four receptors of tyrosine kinase: EGFR (ErbB-1), HER1/c-neu (ErbB-2), HER 3 (ErbB-3) and HER 4 (ErbB-4). Now EGFR is also established as a significant prognostic marker in many human cancers. For example, in non–small cell lung cancer and some head and neck cancers, EGFR has been used as a tumor specific marker. Targeted therapy against this marker has been established in these cancers. However, the EGFR expression has not been established as a tumor-specific marker in mammary phyllodes tumor. The aim of present study is to evaluate histopathologic characteristics of phyllodes tumor and to access the IHC expression of EGFR in phyllodes tumors. And most importantly, it aims to determine if the degree of its expression in the stromal cells is related to the grade of the tumor in order to ascertain that malignant progression is associated with EGFR expression, which may be diagnostically useful.

Materials and Methods

A. Case collection

The study population is consisted of all patients with phyllodes tumor of the breast diagnosed at our institution between December 1995 and July 2010. Clinicopathologic data of patients, slides and tumor blocks were obtained by reviewing the medical records and histopathology archives. The histological sections were re-reviewed by a single pathologist for diagnosis. A total of 82 phyllodes tumors of the female breast were retrieved from the record.

B. Histology and grading

The diagnosis of benign and malignant phyllodes tumor was based on the criteria of stromal cellularity, cellular atypia and mitotic activity by a hematoxylin & eosin (H&E)–stained slide by one pathologist. All slides were reviewed for the following histological parameters: (1) stromal cellularity, (2) nuclear pleomorphism, (3) stromal overgrowth, (5) mitotic rate, and (5) margin of the tumor (whether infiltrative or rounded). Parameters (2) and (3) were graded as low/mild, moderate or severe; stromal overgrowth was graded as present or absent: the mitotic count was expressed as the number of mitotic figures per 10 high-power fields (400, Nikon Labophot, field area 0.13mm²). The diagnosis of benign phyllodes tumor was made when there was low cellularity, non–stromal overgrowth, mild pleomorphism, a rounded margin and a mitotic count of 2 or less per 10 high-power fields. Malignant phyllodes tumor was diagnosed when the mitotic count was 5 or more per 10 high-power fields together with stromal overgrowth and an infiltrative margin. Phyllodes tumor of borderline malignancy was diagnosed when the criteria for malignant phyllodes tumor were not fully fulfilled.

C. Tissue Microarray Construction

A tissue microarray of 82 phyllodes tumors of the breast was constructed according to standard protocols using a dedicated tissue microarray instrument (Beecher...
D. Immunohistochemistry

For IHC detection of EGFR, endogenous peroxidase activity was blocked for 30 min in a methanol solution containing 0.3% hydrogen peroxide after deparaffinization and dehydration. After antigen retrieval, specimens were allowed to cool for 30 min, and then incubated overnight at 4°C with primary antibody. EGFR expression was detected using biotinylated rabbit anti–mouse antibody. The signal was amplified by avidin–biotin complex formation and developed with diaminobenzidine followed by haematoxylin counterstaining. Before the slides were mounted, all sections were dehydrated in alcohol and xylene. For evaluation of EGFR expression, all slides were examined by light microscopy. Membranous and cytoplasmatic staining of tumor cells was scored from 0 to 31 weak; at least 10% of tumor cells with a faint staining intensity, 2 moderate; at least 10% with a moderate staining intensity, 3 strong; at least 10% with a strong staining intensity. Regional stromal cells showing ≥1 score were counted as positive for EGFR over-expression. But, intensity of EGFR expression was not correlated with proportion of EGFR staining. Therefore, we were also scored for percentage of stained stromal cells. The percentage ranged from 0% to 100%. The sections stained with more than 50% of area with moderate to strong intensity are counted as strong positive.

### RESULTS

A. Clinical manifestation

82 patients with breast phyllodes tumors, including 57 benign, 11 borderline and 14 malignant, were evaluated. All the patients were women, with the mean age of 36.59 ± 10.81 years (range 11–49 years). The mean tumor size was 46.93 ± 36.49 mm (range 2.42–260mm). Among these, seven tumors recurred after initial diagnosis. Initially, two were benign, three were borderline and the other two were malignant. One of the malignant phyllodes tumors developed distant metastases. In the benign group, the mean age was 34.33 ± 9.8 years (range 11–49 years) and the mean tumor size was 40.59 ± 25.78mm (range 2.42–130mm). In the borderline group, the mean age was 41.36 ± 11.33 years (range 12–50years) and the mean of tumor size was 44.45 ± 29.69mm (range 9–120mm). In the malignant group, the mean age was 42 ± 12years (range 19–60years), and the mean of tumor size was 74.71 ± 61.17mm (range 20–260mm) (Table 1).

### Table 1. Clinical features in phyllodes tumor according to degrees of malignancy.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Benign</th>
<th>Borderline</th>
<th>Malignancy</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(year)</td>
<td>36.59</td>
<td>34.33</td>
<td>41.36</td>
<td>42 ± 12</td>
<td>0.015</td>
</tr>
<tr>
<td>Size(mm)</td>
<td>46.93</td>
<td>40.59</td>
<td>44.45</td>
<td>74.71 ± 61.17</td>
<td>0.160</td>
</tr>
<tr>
<td>Recurrence n(%)</td>
<td>7.8(5)</td>
<td>2(3.5)</td>
<td>3(10.7)</td>
<td>2(11.8)</td>
<td>0.022</td>
</tr>
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</table>

Significant difference could be seen in the age and the degree of malignancy (p = 0.015). The recurrence showed significant correlation with the degree of malignancy (p = 0.022).

B. IHC expression for EGFR

In the entire series, IHC expression of EGFR was noted in total of 13 cases (13.55%) : Seven of 57 benign cases (12.3%) in which five cases were strong positive (8.8%), one case of 11 borderline (9.1%) which was strong positive, and five cases of 14 malignant (35.7%) which were all strong positive. There was a significant difference between benign and malignant phyllodes tumors (p = 0.027) significant difference was noted in strong positivity between benign and malignant phyllodes tumors (p = 0.027) (Table 2). Also, difference was noticed between borderline and malignant cases (p = 0.014) (Fig. 3). However, benign and borderline tumors showed no significant difference in IHC expression of EGFR (p<0.05).

There was an increasing trend of stromal EGFR staining with increasing degree of malignancy. The differences in the percentage of stromal cells with EGFR expression among benign, borderline and malignant tumors were significant (p = 0.001 between benign and borderline tumors, p = 0.021 between borderline and malignant tumors, p < 0.001 between benign and malignant tumors). The relationship between percentage of stromal cells with EGFR expression and each of the five histological parameters were also significant. In cases with infiltrative margins, EGFR expressions were higher than in cases with round margins (p = 0.013). Also, cases with low cellularity showed lower EGFR expression than cases with intermediate or severe cellularity (p < 0.001). For stromal overgrowth status, cases with presence of overgrowth showed higher EGFR expression than cases without overgrowth (p < 0.001). Similarly, cases with severe pleomorphism and high mitotic index also showed higher EGFR expression than cases with low pleomorphism and low mitotic index (p = 0.006, p < 0.001, respectively) (Table 2).

In seven recurrent phyllodes tumors were included in our study, the stromal expression of EGFR was variable: two
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**Table 2. Correlation with histologic parameters and EGFR expression**

<table>
<thead>
<tr>
<th>Histologic parameters</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
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<tbody>
<tr>
<td>Stromal overgrowth</td>
<td>p = 0.011</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>Mitotic index</td>
<td>p = 0.001</td>
<td>p = 0.001</td>
</tr>
</tbody>
</table>

**Table 3. IHC expression of EGFR in stromal cells in phyllodes tumors of differing degrees of malignancy**

<table>
<thead>
<tr>
<th>Type of PTa</th>
<th>No. of cases (%)</th>
<th>Benign</th>
<th>Borderline</th>
<th>Malignant</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>102 (100)</td>
<td>82</td>
<td>57</td>
<td>11</td>
<td>0.000</td>
</tr>
<tr>
<td>EGFR+ (HCC positive, %)</td>
<td>13 (25.5)</td>
<td>7 (11.8)</td>
<td>1 (9.4)</td>
<td>5.957</td>
<td>0.027</td>
</tr>
</tbody>
</table>

**Table 4. Characteristics of primary phyllodes tumors in recurrent cases**

<table>
<thead>
<tr>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td>35</td>
<td>39</td>
<td>49</td>
<td>48</td>
<td>49</td>
<td>47</td>
<td>43.0</td>
</tr>
<tr>
<td>Margin</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>EGFR+</td>
<td></td>
<td></td>
<td></td>
<td></td>
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**DISCUSSION**

Although phyllodes tumors typically behave with benign features, up to 35% of patients die from histologic malignancy. In malignant phyllodes tumors, standard treatment includes mastectomy or wide local excision. Unlike invasive ductal carcinoma, axillary lymph node dissection is usually not performed because the rate of lymph node metastases is less than 2%. However, phyllodes tumors have a high recurrence rate, overall of 22%, and the percentage breakdown of recurrence by grade of primary tumor is 17%, 25%, and 22% for benign, borderline and malignant phyllodes tumors respectively, reported by the World Health Organization (WHO). In addition, distant metastasis may also be preceded by local recurrence. And the role of postoperative radiotherapy and chemotherapy has not been fully established in the treatment of phyllodes tumors. Therefore, it is important to classify the tumors according to the grades to predict recurrence and prognosis. However, there was no definite relationship between the histologic grades of the primary tumor and the recurrent lesion, although most of the recurrent cases were similar to higher histologic grade. Some studies have shown that stromal overgrowth, infiltrating margin, high mitotic rate, and degree of stromal atypia are important predictors of recurrence and prognosis. Among these histologic parameters, margin status is the most consistent indicator of local recurrence and our study also shows the same result: recurrence has a close correlation with infiltrative margin significantly (p < 0.001).

However, many authors have found no correlation between histologic characteristics and tumor recurrence. Such results highlight the need for markers that can predict patient outcome more reliably. So far, correlations between expressions of p53, Ki67, c-kit, EGFR and stromal CD10, CD34 with tumor grade have been described. Among the markers, we investigated EGFR. The EGFR is a cell-surface tyrosine kinase receptor for members of the epidermal growth factor family of extracellular protein ligands. Specific mutations causing over-expression of EGFR have been correlated with activation of the receptor that could lead to uncontrolled cell division and subsequent cancer. In breast cancer, EGFR appears to be involved in the pathogenesis and progression. About twenty years ago, one study investigated expression of epidermal growth factor receptor (EGFR) in 213 breast tumors immunohistochemically. They concluded that, in contrast to the normal state, EGFR-expression is rather a rare phenomenon in breast cancer cells, positively correlated with a declining grade of differentiation (p = 0.023). Yet, evaluation of EGFR as a potential biomarker in phyllodes tumors has not been well established. In a large study analyzing 433 phyllodes tumors, expression of EGFR in phyllodes tumors increased from benign to malignant tumors. The results showed that the overall positive rate for EGFR was 36.2%, 51%, and 86.6% for benign, borderline and malignant respectively. And FISH demonstrated EGFR gene amplification in 8% of immunohistochemically positive cases. Other authors’ EGFR over-expression was detected in 32% in 12.5% of benign, 10% of borderline and 63% of all malignant phyllodes tumors. There are significant correlations between tumor grade and EGFR over-expression. Other study investigated expressions of Ki67, p53 and EGFR family members in neoplastic cells with malignancy and its relation to unfavorable clinical course. The study suggested that expression of EGFR increased with increasing malignancy. In current study, there was a significant difference in the IHC expression of EGFR between benign, borderline and malignant except in the comparison between benign and borderline. The differences in the percentage of stromal cells with EGFR expression among benign, borderline and malignant tumors were all significant. These findings are similar to the recent results seen in above studies. However, we did not investigate the amplification of EGFR in FISH. If the results of FISH would be added, our study could clarify usefulness of EGFR in phyllodes tumors. Therefore further studies are needed. In addition, all five histological parameters showed significant correlation with EGFR expression and the degree of tumor malignancy in our study, they could support further evidence about the role of EGFR in phyllodes tumor formation. From this study, EGFR have significant efficacy for discriminating between benign and malignant breast phyllodes tumors although they were not related to recurrence rate. Moreover, combination with the histological features of the tumors aids establishment of the correct diagnosis and evaluates diagnostic sensitivity in questionable cases.

**CONCLUSION**

In our study, there was an increasing trend for EGFR IHC expression in relation to the degree of malignancy. And the overall percentage of EGFR+ stained phyllodes tumors is related to the degree of malignancy and histological parameters. Additionally, tumor margin of five histological parameters had close relationship with tumor recurrence. However, this study did not show the correlation between recurrence and expression of EGFR. Despite the small number of cases included in this study, we believe that our findings show that these biological markers and histological parameters can be used for evaluation of breast phyllodes tumors.
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Key Words : Phyllodes tumor, EGFR, Breast

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Received : August 31, 2012 Accepted : January 6, 2013

Abstract

Background : Phyllodes tumor of the breast is a rare stromal-epithelial neoplasm with the potential for invasion and metastatic spread, but the clinical behavior of phyllodes tumor is difficult to predict. Specific parameters that define recurrence and prognosis are not universally established yet. We aim to reveal the relationship of EGFR expression and type of phyllodes tumor.

Methods : This study investigated 82 phyllodes tumors (57 benign, 11 borderline, 14 malignant) for EGFR expression using immunohistochemistry (IHC). The staining was correlated to tumor margin status, degree of malignancy, stromal cellularity, mitotic activity, nuclear pleomorphism and stromal overgrowth.

Results : There was an increasing trend of stromal EGFR staining with increasing degree of malignancy (p < 0.001 between benign and borderline tumors, p = 0.021 between borderline and malignant tumors, p < 0.001 between benign and malignant tumors).

Conclusion : We believe that our findings show that EGFR expression and histological parameters can be used for evaluation of breast phyllodes tumors.

REFERENCES


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