Immunohistochemical Study of CD34 Expression in Fibroadenomas, Phyllodes Tumors and Spindle Cell Carcinoma of the Breast

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Abstract

Aims: Strong expression of CD34 has been described in many tumors. It has been proposed that these lesions arise from long-lived mesenchymal cells. We tested the hypothesis that spindle cell lesions of the breast arise from similar mesenchymal cells in mammary stroma, and to determine the potential diagnostic value of CD34 immunostaining in these lesions.

Methods and results: Sections of fibroadenomas (20), phyllodes tumors (7), spindle cell carcinoma (10) and normal breast tissues adjacent to these lesions (10) were stained immunohistochemically for CD34 in formalin fixed, paraffin embedded tissues. All the mammary stroma, particularly around lobules, stained for CD34. All cases of fibroadenoma showed strong CD34 immunostaining (median 100%). The median percentage of CD34 immunostaining in cases of phyllodes tumors were 65% (p=0.0002). In benign phyllodes tumors there was diffuse staining in the stroma and in half of the cases there was decreased staining adjacent to epithelium. However in the malignant phyllodes tumors the staining was patchy. All cases of spindle cell carcinoma were CD34 negative.

Conclusions: the expression of CD34 in fibroadenomas and phyllodes tumors suggests that these lesions may arise from long-lived CD34 positive mesenchymal cells in the breast stroma, and the absence of CD34 staining in spindle cell carcinoma is of potential diagnostic value in the distinction from malignant phyllodes tumors in difficult cases.

Key Words: CD34 – Fibroadenoma – Phyllodes – Tumors – Spindle cell carcinoma.

Introduction

CONNECTIVE tissues contain dendritic fibroblast-like cells which express the human progenitor cell antigen CD34. Van de Rijn and Rouse [1] showed that CD34 is a transmembrane glycoprotein which is involved in modulation of both pro- and anti-adhesive cellular behaviour and in signal transduction and regulation of cellular differentiation [2,3]. It was first described in haematopoetic progenitor cells of the bone marrow [4-8]. Since that time, the antigen has been detected in a number of normal tissues including vascular endothelium [9-11] and a variety of fibroblast-like cells of the dermis [10,12,13]. Several reports have demonstrated CD34 in various soft tissue neoplasms such as angiosarcoma [14-17], epithelioid sarcoma [17,18], Kaposi's sarcoma [15,19,20], leiomyosarcoma [17] gastrointestinal stromal tumors [21] and solitary fibrous tumor [22,23]. Several breast lesions are formed mainly of spindle cells, i.e. fibroadenoma, phyllodes tumor and spindle cell carcinoma. Fibroadenoma and phyllodes tumor arise by proliferation of mammary stroma and epithelial elements. However, it is the spindle cell stromal element that determines the biology of these biphasic tumors [24].

We tested the hypothesis that the spindle cell component of fibroadenomas, phyllodes tumors and spindle cell carcinoma of the breast arise from CD34 positive mesenchymal cells in the mammary stroma, and to determine the potential diagnostic value of CD34 immunostaining in these breast lesions.

Material and Methods

1-Histopathological study:

This study included 20 cases of fibroadenoma (12 cases pericanalicular and 8 cases intracanalicular fibroadenoma), 7 cases of phyllodes tumors (4 benign and 3 malignant, graded using the criteria of Maffat, et al. [25]), 10 cases of spindle cell carcinoma and 10 cases of normal breast tissues adjacent to these lesions.

The material was collected from the archives of the Pathology Department, Faculty of Medicine, Assiut University Hospital. All specimens were formalin-fixed and paraffin-embedded. Cases were selected by reviewing the corresponding haematoxylin and eosin (H & E) stained slides.
2. **Immunohistochemical staining:**

Sections from each case were stained using labelled streptavidin-biotin peroxidase technique (LSAB). Briefly, after being deparaffinized in xylene and rehydrated in ethanol, the sections were immersed in citrate buffer (pH6) and heated in a microwave oven for 10 minutes to retrieve antigen. The sections were incubated with the primary antibody following the procedure indicated by Moore & Lee [26]. The primary antibody used was QBEND/10 (CD34, Novocastra, Peterborough, UK).

- The blood vessels were used as internal positive control.
- The percentage of stained spindle cells was estimated semi-quantitatively using high power fields (x400).
- Statistical analysis was performed using chi-squared analysis.

**Results**

The results of our study are summarized in Table (1).

All the normal breast tissues showed strong CD34 immunoreactivity in the perilobular and to a lesser extent in the interlobular stroma (Fig. 1).

In fibroadenomas, nearly all the spindle cells component of the pericanalicular and intracanalicular types showed CD34 immunoreactivity (median 100%) (Fig. 2).

In phyllodes tumors, the median percentage of CD34 positive spindle cells was lower (median 65% \(p=0.0002\)). The benign phyllodes tumors showed a median of 70% and the malignant phyllodes tumors showed a median of 30% \(p=0.05\). In the four benign phyllodes tumors there was diffuse CD34 staining in the stroma and two cases of them showed reduced staining of spindle cells adjacent to epithelial elements (Fig. 3). However in the malignant phyllodes tumors the staining was patchy, with areas of high proportion of CD34 positive spindle cells and areas of low proportion of CD34 positive spindle cells.

In all cases of spindle cell carcinoma, there was strong staining of blood vessels, but no staining of the tumor cells (Fig. 4).
Table (1): CD34 immunostaining in cases of fibroadenoma, phyllodes tumor and spindle cell carcinoma.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>No. of cases with immunoreactivity</th>
<th>Percentage of spindle cell staining</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal breast tissue</td>
<td>10</td>
<td>10</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pericanalicular</td>
<td>12</td>
<td>12</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Intracanalicular</td>
<td>8</td>
<td>8</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Phyllodes tumor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign</td>
<td>4</td>
<td>4</td>
<td>70%</td>
<td>70%</td>
</tr>
<tr>
<td>Malignant</td>
<td>3</td>
<td>3</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>Spindle cell carcinoma</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

In the embryo, CD34 deep dendritic cells first appear in a band of the reticular dermal-subcutaneous anlage and perimysial tissues. In adult, it is expressed on ubiquitous adventitial and interstitial fibroblasts and on endothelium [1,2]. The CD34 dendritic interstitial cells have been described in the mesenchyme of several tissues, e.g. skin [10], gastrointestinal tract Monihan et al. [27], uterus cervix, lindenmayer & Miottinen [28] and testis, Maher & lee [29]. In our study these cells were seen in the mammary stroma, they were detected in the perilobular and to a lesser extent in the interlobular stroma of normal breast tissues. This finding was in agreement with several studies i.e. Yamazaki & Eyden [30] Lee, et al. [31] and Moore & Lee [26].

Spindle cells of solitary fibrous tumor, Yokoi, et al., Damiani, et al., Suster, et al. [23,32,33], dermatofibrosarcoma protuberans, fibrous histiocytoma, Kutzner [13], Kaposi's sarcoma, Traweek, et al. and Regezi, et al., [15,20] some gastrointestinal stromal tumors Suster, et al. [21] and angiosarcoma Sirgi, et al. [17] all express CD34. To this diverse group of CD34 positive tumors we add mammary fibroadenomas and phyllodes tumors because all these cases in our study showed CD34 positive spindle cells. This result was in agreement with the result of Barth, et al. [34].

With increasing use of stereotactic core biopsy and aspiration cytology to triage mammary lesions for surgery, CD34 reactivity should be interpreted with caution, because a small sample that might be devoid of epithelial elements from say, a cellular fibroadenoma, might result in a diagnosis of a more aggressive CD34 positive spindle cell or vascular tumors if immunohistochemistry is applied.

Some CD34 positive lesions such as dermatofibrosarcoma protuberans in the skin Aiba, et al. [35] and intestinal fibroid polyps Wille & Borchard [36], arise at sites where there are normal CD34 positive fibroblast like cells. Thus it has been suggested that such lesions may arise from these CD34 positive fibroblast like cells. In this study we can also suggest that fibroadenomas and phyllodes tumors arise from perilobular or interlobular CD34 positive stroma in a manner similar to that proposed for dermatofibrosarcoma protuberans and intestinal fibroid polyps.

A smaller proportion of spindle cells stained for CD34 in phyllodes tumors than in fibroadenomas, and there was less staining in malignant than in benign phyllodes tumors. Similar results were seen in the studies of Silverman & Tamsen [24]. Chen, et al. [37] and Moore & Lee [26]. This reduced expression of CD34 may be a reflection of decreasing or altered differentiation. A similar loss or reduction of CD34 expression has been found in fibrosarcoma arising in dermatofibrosarcoma protuberans Mentzel, et al. [38] and in malignant solitary fibrous tumor arising in a benign solitary fibrous tumor [23].

In cases of phyllodes tumors there were decreased expression of CD34 in spindle cells adjacent to the epithelium, this finding suggests that CD34 expression can be controlled through epithelial-mesenchymal interactions. The concept of epithelial-mesenchymal interaction is supported by the finding that p53 expression in phyllodes tumors is particularly seen in stroma immediately adjacent to epithelium [39].
In cases of spindle cell carcinoma, no CD 34 expression could be detected, this result was in agreement with that of Moore & Lee [26]. The absence of CD 34 expression in spindle cell carcinomas, may be useful in separating spindle cell carcinoma from sarcoma arising in a phyllodes tumor, a distinction that is sometimes difficult.

In conclusion, the CD 34 expression in the present study suggest that fibroadenomas and phyllodes tumors may arise from CD 34 positive mesenchymal cells in the mammary stroma, and inmunohistochemistry for CD 34 can help in separating spindle cell carcinoma from sarcoma arising in phyllodes tumor.

References


