Diagnostic significance of the immunoexpression of CD34 and smooth muscle cell actin in benign and malignant tumors of the breast

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Abstract

Purpose. Our aim was to investigate the distribution of CD34 and smooth muscle cell actin positive myofibroblasts in the stroma of the normal mammary gland, benign and malignant tumors. The observations were especially focused on the diagnostic value of the cumulative results obtained with these immunoreactions. Methods. Our study included 112 female patients with suspect breast masses obtained by surgery or biopsy. We performed morphological study and immunohistochemistry for CD34 and SMA. Results. We have found normal breast tissue, sclerosing adenosis, fibroadenomas, fibrocystic diseases, phyllodes tumor, DCIS, ductal invasive, lobular, squamous, medullary, mucinous, and papillary carcinomas. We also found apocrine metaplasia, florid ductal hyperplasia, atypical hyperplasia, papilloma and LCIS associated with the malignant tumors. All the normal breast tissues and most of benign lesions were positive for CD34 and negative for SMA. The exceptions were represented by a case of fibroadenoma and the phyllodes tumor, with CD34 positivity and a focal acquisition of SMA; fibrocystic disease with associated apocrine metaplasia adjacent to a squamous carcinoma with loss of CD34 expression and focal acquisition of SMA. All our DCIS and invasive carcinomas have lost CD34 expression and gained SMA positivity. Some particular behavior had the mucinous and squamous carcinomas. Conclusions. Although there were some exceptions especially when one of the two markers was interpreted separately and in some cases associated with sclerotic stroma, we conclude that the combined expression of CD34 and α-SMA is of potential diagnostic value in the distinction between benign and malignant tumors in some difficult cases.

Keywords: CD34, actin, breast, fibroblast, myofibroblasts.

Introduction

Mammary cancer is the most frequent and one of the deadliest neoplasms in women. The recent trend toward improvement in breast cancer mortality rate is largely due to increased diagnosis of early stage disease, while our therapeutic options for advanced stage breast carcinomas are still fairly limited. Thus, there is a need to better understand the cellular and molecular basis of breast cancer initiation and progression and to use this knowledge for the design of targeted, molecular based therapy [1].

The diagnosis is based mainly on the pathological findings. Despite its accuracy, the conventional microscopic examination does not always discriminate between a benign and a malignant tumor or, between an invasive versus non-invasive.

Myoepithelial cells are contractile structures found in mammary, salivary and sweat glands. They have a combined smooth muscle and epithelial phenotype. An intact myoepithelial layer is seen in both benign and in situ lesions, whereas loss of them is considered the gold standard for the diagnosis of invasive breast cancer.

CD10 and smooth muscle cell actin are well-known markers for myoepithelial cell [2] and it has been suggested that α-SMA acts as a tumor suppressor when overexpressed in luminal epithelial cells.

Normal and tumor derived myoepithelial cells differ in their ability to interact with luminal breast epithelial cells for polarity and basement membrane deposition [3].

The importance of stromal interaction with epithelial cells is well established in embryonic development and tumorigenesis [4-6]. The concept of a link between stromal cell maturation and adjacent epithelial cell proliferation was introduced more than 20 years ago [7].

During tumorigenesis some data suggest that precancerous epithelial cells acquire multiple genetic mutations and the associated stroma become “activated”, commonly expressing myofibroblastic markers [8, 9].

The growth, differentiation, invasive behavior and polarity of normal mammary epithelial cells and breast carcinomas are influenced by stromal cells including fibroblasts, myoepithelial cells, myofibroblasts and leucocytes. The stroma of normal mammary gland and some benign tumors contains many CD34 positive fibroblasts/fibrocytes. To date the histogenesis and function of this cell type is largely unknown. CD34 positive cells were also found in prostate, urinary bladder, fallopian tubes, thyroid gland, pancreas, colon, uterine cervix and testis. In malignant tumors developed in most of aforementioned organs it was noticed a loss of CD34 positive cells and gain of smooth muscle cell actin positive myofibroblasts [10, 11].

Our purpose was to investigate the presence and distribution of CD34 and smooth muscle cell actin positive cells in the stroma of the normal mammary gland, benign and malignant tumors.

The observations were especially focused on the diagnostic value of the cumulative results obtained with these immunoreactions.
Material and methods

Our study included 112 female patients aged between 23 and 88 years, with suspect breast masses discovered by clinical and/or mammography exams. The specimens obtained by open surgery were fixed in 10% neutral buffered formalin for 48 hours, and then processed into paraffin blocks using standard histological procedures. Five micrometers serial sections were obtained from each case and mounted on silanized slides. For every case we performed routine hematoxylin and eosin stain method for morphologic study.

Immunohistochemical study for specific highlight of fibroblasts, myofibroblasts and myoepithelial cells used monoclonal antibodies specific for CD34 antigen and smooth muscle cell actin. Before immunostaining, sections were pretreated by microwave heating in citrate buffer, pH 6, 20 minutes for antigen retrieval. Then, sections were immersed in 3% hydrogen peroxide in distilled water for 5 minutes at room temperature to block endogenous peroxidase activity.

After incubation with primary antibody for 30 minutes, we applied LSAB2 working system and bound antibodies were visualized with 3, 3’diaminobenzidine. Nuclei were counterstained with modified Lillie’s Hematoxylin (Table 1).

Table 1 – Details of primary monoclonal antibodies used

<table>
<thead>
<tr>
<th>Antibody against</th>
<th>Source</th>
<th>Clone</th>
<th>Code No.</th>
<th>Dilution</th>
<th>Working system</th>
<th>Antigen retrieval</th>
<th>Incubation period with primary antibody</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 34</td>
<td>Dako</td>
<td>QBEEnd</td>
<td>NP036</td>
<td>Ready-to-use</td>
<td>LSAB2</td>
<td>Microwave HIER* pH=6.0; 20 minutes</td>
<td>30 minutes</td>
<td>blood vessels endothelium</td>
</tr>
<tr>
<td>SMA</td>
<td>Dako</td>
<td>1A4</td>
<td>U 7033</td>
<td>Ready-to-use</td>
<td>LSAB2</td>
<td>Microwave HIER* at 90-99ºC, 20 minutes</td>
<td>30 minutes</td>
<td>normal glands myoepithelial layer, blood vessels smooth muscle cells</td>
</tr>
</tbody>
</table>

Then, the slides were examined in optic microscopy using Nikon Eclipse E600 Microscope. We observed distribution, density and immunostaining pattern of three cell types.

As a positive internal control, the staining of blood vessels was used for CD 34 and for SMA, the staining of normal myoepithelial cells. We reported the fibroblast reactivity for CD 34 and αSMA as positive, negative or reduced, where there was only focally positive.

Results

On hematoxylin and eosin routine stain we have found 5 normal breast tissue, 7 cases of sclerosing adenosis, 13 fibroadenomas, 11 fibrocystic disease, 1 case of phyllodes tumor, 5 DCIS, 46 ductal invasive carcinoma, 10 lobular carcinoma, 5 squamous cell type, 2 medullary carcinoma, 2 mucinous, 5 papillary carcinomas.

We also found apocrine metaplasia, florid ductal hyperplasia, atypical hyperplasia, papilloma and LCIS associated with malignant tumors.

In normal breast, both intralobular and interlobular stroma was positive for CD34 immunostain. Intralobular stroma had a stronger intensity of positive reaction whereas interlobular stroma showed a lower density of positive CD34 positive cells and positive stromal vessels endothelium.

Myoepithelial cells were positive for SMA as a continuous layer around components of TDLU. We detected no SMA positive myofibroblast in normal breast. This pattern was maintained in the presence of other pathology, including adjacent carcinoma.

CD34 positive stromal cells in fibroadenomas had a dendritic or stellate morphology.

The density of such types of cells was higher in fibroadenomas than normal breast stroma. Also, we found a central concentration of fibroblasts in fibroadenomas and progressive loss of positive reaction around collapsed ducts.

Except 3 cases of fibroadenoma with rare positive cells for SMA in the stroma, all others had no stromal cells stained for this antibody.

Around the ducts with florid hyperplasia we found a concentration of CD34 positive stromal cells with circular, concentric pattern.

The same feature of the stroma was also found between cystic dilatation. Some ducts with apocrine metaplasia were surrounded by reduced stroma without positive cells for CD34, whereas SMA was focally positive.

The hypercellular stroma of the phyllodes tumor contained fibroblasts and myofibroblasts that were positive for both CD34 and SMA antibodies. The density of CD34 positive cells was higher than those for SMA.

In the most majority of cases with benign lesions, including atypical ductal hyperplasia and papilloma, smooth muscle cell actin was positive only in myoepithelial cell layers and media of the vessels, and CD34 was positive.

The cases of DCIS had a pattern similar to malignant tumors with loss of CD34 expression and gain of SMA expression, but LCIS that we have found associated to lobular invasive carcinoma had a pattern with CD34 positivity and without acquisition of SMA.

For ductal invasive carcinoma our findings showed loss of positive stain for CD34 in the stroma and keep it only for the endothelium of tumoral vessels. There was an isolated zone of the stroma between the tumor and surrounding adipose tissue that shared positive cells for CD 34.
Diagnostic significance of the immunoexpression of CD34 and smooth muscle cell actin in benign and malignant tumors.

**Figure 1** – Ductal invasive carcinoma. Only the blood vessel endothelium is CD34 positive. CD34 expression is lost in the stroma (CD34, ×200).

**Figure 2** – Squamous cell carcinoma. In the stroma there are fibroblasts/fibrocytes strong positive for αSMA (alfa SMA, ×400).

**Figure 3** – Fibrocystic disease. CD34 is strong positive in the stroma (CD34, ×200).

**Figure 4** – Fibrocystic disease. αSMA is negative in the stroma. In the normal mammary lobule, the myoepithelial layer is SMA positive (SMA, ×200).

**Figure 5** – Fibroadenoma. Stroma with strong positive reaction for CD34 (CD34, ×400).
In contrast, stromal bands that contain a lot of positive cells for SMA and negativity for CD34 separated tumoral areas of invasive ductal carcinoma. In one case of ductal invasive carcinoma situated beneath the skin, the dermis had a lot of CD34 positive fibrocytes and fibroblasts comparative with the stroma from carcinoma which loss CD34 staining and gain SMA positivity.

Another case associated a zone of mucinous type carcinoma that was negative for CD34 and weakly positive for SMA. The benign lesions associated with invasive ductal carcinoma were negative for SMA; myofibroblast proliferation was stopped at the demarcation between them.

We had 5 cases of metaplastic carcinoma of the breast, squamous cell type with particular behavior for CD34 and SMA. They lacked stromal fibroblasts positive for CD34. For this type of breast carcinoma CD34 was useful only for counting microvessel density in tumoral area. Focally, tumor cells were positive for smooth muscle cell actin with granular pattern; the stroma was reduced and moderate positive for SMA. In some places immunostain for SMA had a linear pattern, surrounding squamous areas (Table 2).

Adjacent to both benign and malignant lesions, adipocytes situated immediately near tumoral area shared intense positive stain for CD34 with focal pattern.

<table>
<thead>
<tr>
<th>Histology</th>
<th>CD34</th>
<th>α-SMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (16)</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Fibroadenoma (15)</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Phyllodes tumor (1)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Fibrocystic Disease (17)</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Sclerosing adenosis (11)</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Florid ductal hyperplasia (23)</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Papilloma (2)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Atypical ductal (6) hyperplasia</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Apocrine metaplasia (15)</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>DCIS (8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LCIS (2)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Ductal carcinoma (46)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lobular carcinoma (10)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Squamous carcinoma (5)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mucinous carcinoma (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medullary carcinoma (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Papillary carcinoma (5)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

+/− = reduced
*The normal mammary tissue and benign adjacent lesions were included

Table 2 – Summary of CD34 and α-SMA expression in stromal cells, in relation to histopathological features*

Discussions

The tissue homeostasis depends upon the strict maintenance of a complex spatial and temporal dialogue between the epithelium and the cellular and acellular components of the tissue stroma. Epithelial tissues are multicellular, three-dimensional structures that interact dynamically with multiple cell types, such as fibroblasts, adipocytes, infiltrating immune cells and endothelial cells, within the context of a proteinaceous microenvironmental network called extracellular matrix.

Although growth factors and extracellular matrix are recognized as important contributors to breast epithelial growth, morphogenesis, hormone responsiveness and neoplastic progression, the influence of functional interactions between stromal and epithelial cells are far to be defined [12-14].

Breast fibroblasts are mesenchymal cell types that confer morphogenic and mitogenic induction of epithelial cells, enhancement of tumor growth and progression requiring active angiogenesis. Bissel et al. hypothesized and demonstrated that desmoplastic tumor stroma or wounded microenvironment is a tumor promoter and the activated stroma acts as an auxiliary factor against a pre-existing genetically mutant target tumor cell [15]. On the other hand, the tumors with desmoplastic stroma were characterized by the presence of alpha-smooth muscle actin reactive myofibroblasts.

The stroma around invasive breast tumors differs from normal breast with alterations in stromal protein synthesis and expression of matrix metalloproteinases [16] and many of these features are attributed to activated fibroblasts, termed myofibroblasts, reflecting their acquisition of SMA expression.

Myofibroblasts through the secretion of chemokines, cytokines, growth factors, inflammatory mediators, as well as extracellular matrix proteins and proteases, play an important role in organogenesis, oncogenesis, inflammation, repair and fibrosis in most organs and tissues [17].

Recent studies demonstrated the feasibility of targeting tumor stroma for cancer prevention and treatment especially with some fibroblast derived factors such as a matrix metalloproteinase inhibitor and Tenascin [18].

CD34 is a transmembrane glycoprotein expressed by haematopoietic stem cells, endothelial cells and mesenchymal cells in different tissues including breast that is thought to be involved in the modulation of cell adhesion and signal transduction. CD34+ fibrocytes/fibroblasts derive from myeloid precursors, invade sites of tissue damage and are capable of connective tissue matrix synthesis. Besides its function as a matrix-production cell, the CD34 fibrocyte/fibroblast is a potent antigen-presenting cell and therefore it has been claimed that CD34+ may play
a role in host response to tissue damage [19, 20].

Different studies have shown that the presence or absence of this population of cells might be useful in distinguishing benign from malignant lesions of the skin [21] and gastrointestinal tract [22, 23].

The presence of stromal positive CD34 fibroblasts has been shown to be associated with benign lesions [20, 24-30] and this is in keeping with our findings. In our study, CD34 positive fibroblasts were found in normal mammary gland and benign lesions of the breast whereas staining for smooth muscle cell actin was negative in stromal cells of these cases.

The same aspects we have found in the normal mammary tissue and benign lesions adjacent to carcinomas. The staining intensity of SMA and CD34 in myofibroblasts was lower than the intensity that we have found in the myoepithelial layer and in the endothelium.

In most majorities of our benign cases, smooth muscle actin was positive only in myoepithelial layer and media of the vessels, with the exception of phyllodes tumor, and some cases of fibroadenoma and apocrine metaplasia that were focally positive for SMA. A particular case was represented by the apocrine metaplasia associated with fibrocystic disease, where CD34 was negative and SMA was focally positive.

We evaluate this finding as a phenomena associated with areas of fibrosis that we have found in this case and not as a strong criterion for premalignant differentiation.

Although most benign lesions, including ductal florid hyperplasia and sclerosing adenosis retain CD34 expression, loss of CD34 was a consistent finding in the fibroblasts associated with fibrosis following core biopsy and radial scars [31]. Some studies suggest that radial scars are associated with an increased risk of development of malignancy and the stroma of invasive breast cancer and radial scars share some similarities concerning the expression of factors associated with stroma formation [32].

Ramaswamy et al. [33] studied the fibroblast CD34 and SMA expression in radial scars and tubular carcinomas and concluded that although in a minority of cases radial scars show a pattern similar to malignancies, CD34 and α-SMA immunohistochemistry are valuable adjunctive tools in distinguishing radial scars from tubular carcinomas and the presence of CD34 exclude malignancies.

It has been suggested that the loss of CD34 is specific for malignancies, but others authors show that it is not always available [28]. They show that fibroblasts CD34 expression is lost in invasive carcinomas, including microinvasion, but also in a proportion of atypical ductal hyperplasia and in a high proportion of DCIS of high grade, but not around LCIS. Loss of CD34 expression is also seen in radial scars, whether they were associated with malignancy or not.

This lesions gain focal positivity for SMA and raises the possibility that loss of CD34 may be related to invasive potential. Particularly in sclerotic lesions, CD34 might be a less valuable marker of malignancies.

On the other hand, fibrosis is seen as a negative prognostic marker for breast carcinomas Yazhou et al. compared the clinicopathological parameters between invasive ductal carcinomas with and without stromal myofibroblasts and revealed significant differences in lymph node metastasis, grade of differentiation and microvessel density and showed that carcinomas with CD34 negative stromal myofibroblasts and positive SMA are associated with a poor prognosis [30].

Fibroadenomas and mammary phyllodes tumor arise by proliferation of mammmary stroma and epithelial elements. In these cases, stroma is the element that determines the biology of these biphasic tumors.

Fibroadenomas and some phyllodes tumors are composed of CD34+ fibroblasts that show varying myxoid, collagenous or myofibroblastic differentiation. Fibroadenomas variants show prominent collagenous actin myofibroblastic differentiation, especially the hypercellular ones [27]. In our cases of fibroadenomas, the density of CD34+ stromal cells was higher than in normal breast and in the case of phyllodes tumor, the CD34 density was higher than in fibroadenomas.

In the phyllodes tumor, although both CD34 and SMA were positive, the density of CD34+ cells was higher than those for SMA. We associate these findings with the presence of hypercellular stroma that was present in these situations.

Malignant phyllodes tumors of the breast exhibit lower levels of CD34 expression than benign phyllodes or fibroadenomas. It has been suggested that expression of CD34 is associated with benign phyllodes tumors, while actin and CD117 are preferentially expressed in malignant phyllodes tumors and these immuno-histological markers might be useful for the histopathological grading of phyllodes tumors [29].

The absence of CD34 staining in spindle cell carcinomas is of potential diagnostic value in distinction from malignant phyllodes tumors in some difficult cases [34].

The assumption that the loss of CD34 expression is a feature of stromal alterations was underlined by the present study. In most of our cases of carcinoma whether there were of ductal, lobular, metaplastic, papillary, medullary or mucinous type, the loss of CD34 expression was accompanied by the acquisition of SMA with only few exceptions.

The five cases of DCIS that we had were of the same fibroblast phenotype with loss of CD34 and gain of SMA, but the two cases of LCIS that we have found associated to lobular carcinomas, had a pattern similar to benign lesions, with retained CD34 expression and no SMA expression. That was in line with the studies of Chauhan et al.

Regarding the exceptions, we had a case of a fibroadenoma with malignant changes, where the CD34 was lost but we did not observed acquisition of SMA. The maintenance of CD34 expression and gain of focal SMA positivity was observed in 3 cases of fibroadenoma and in the phyllodes tumor.

Another case with fibrocystic disease and apocrine metaplasia adjacent to a squamous carcinoma was focally positive for actin in the area of benign lesions. Particular behavior had the mucinous and squamous
cells carcinomas. In the mucinous carcinomas, CD34 expression was low, but it was associated with reduced SMA expression.

The metaplastic carcinoma with squamous cell type had a loss of CD34 expression and reduced stroma with focally positive reaction for SMA. The squamous areas were surrounded by stromal positive cells for SMA with a linear pattern.

When a carcinoma had adjacent normal tissue or benign lesions, the demarcation between them was very clear while the myofibroblasts proliferation was obviously stopped at that level.

The fact that the adipocytes situated immediately near the tumors shared CD34 positivity could be related to the paracrine interactions between microvascular endothelial cells and preadipocytes within adipose tissue depots. The adipose tissue has a hyperplastic capacity that resides in the fibroblast-like preadipocyte pool through the factor produced by microvascular endothelial cells [35].

The mechanism leading to a loss of CD34+ fibrocytes in the stroma of carcinomas are far to be understood. Valenti et al. have shown that medium conditioned by the breast cancer cell line MCF-7 induces SMA expression in stromal cells obtained from normal breast tissue [36], and Abe et al. found that CD34+ fibrocytes gain SMA expression when exposed to transforming growth factor β [37]. However, the absence of CD34 expression together with SMA expression is more valuable than each separately for the diagnosis of malignancies.

Conclusions

Although there were some exceptions when one of the two markers was interpreted separately, and in some cases associated with sclerotic stroma, we conclude that according to our findings, the combined immunohistochemical expression for CD34 and α-SMA can be of potential diagnosis value in the distinction between benign and malignant tumors, especially in some difficult cases when eosin-hematoxylin staining is not relevant.

Acknowledgements

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References

[36] VALENTI M.T., AZZARELLO G., BALDUCCI E. et al., Conditioned medium from MCF-7 cell line induces myofibroblasts differentiation, decreased cell proliferation, and increased apoptosis in cultured normal fibroblasts but not in fibroblasts from malignant breast tissue, Histochem J, 2001, 33:499-509.

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