CD10 Immunohistochemical Expression in Apocrine Lesions of the Breast

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Key Words

Breast cancer · CD10 · Apocrine adenosis · Ductal carcinoma in situ

Abstract

Objective: In the breast, CD10 is expressed by myoepithelial cells (MECs), and apocrine metaplasia has also been mentioned as being positive with this marker. Apocrine lesions have been explored for the expression of CD10. **Methods:** The apocrine lesions studied included 11 cysts, 6 cases of apocrine adenosis, 2 of apocrine metaplasia or hyperplasia in papilloma, 13 ductal carcinomas in situ (DCIS) and invasive carcinomas (14 ductal and 4 lobular). **Results:** Benign apocrine lesions showed complete or partial luminal CD10 staining, although most cases included parts without staining, and 2 lesions were completely negative. The MECs were often but not always positive. Nine of the 13 cases of apocrine DCIS displayed no luminal staining, but 4 demonstrated very focal luminal positivity. The MECs around the DCIS showed a spectrum of staining from nil to strong and complete. Only 4 invasive carcinomas demonstrated luminal/membranous staining. Cytoplasmic CD10 positivity was seen focally in 4 invasive cancers and in 3 DCIS. **Conclusion:** CD10 positivity is luminal/membranous in most benign apocrine lesions, the staining being nonuniversal and sometimes focal. Anal-

gous staining in apocrine malignancies seems rarer in DCIS and even rarer in invasive apocrine carcinomas, but atypical cytoplasmic positivity may also occur. CD10 is not an ideal myoepithelial marker in apocrine lesions.

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Introduction

Cluster differentiation 10 (CD10) also known as neprilysin, enkephalinase, common acute lymphoblastic leukemia antigen (CALLA), membrane metalloendopeptidase (MME) or neutral endopeptidase (NEP) is a membrane-bound zinc-dependent metalloprotease enzyme that degrades a number of small secreted peptides [1, 2].

It is a fairly ubiquitous enzyme found on the surface of many different cell types including pre-B cells, germinal-center B cells, neutrophils, T-cell precursors and epithelial cells of the kidney, stomach, colon, prostate and liver canaliculi as well as in stromal cells of the endometrium and myofibroblasts [3]. In humans, CD10-related DNA sequences are found on chromosome 3 [4]. Three different splice variants of CD10 have been identified, suggesting that CD10 expression may be controlled in a tissue-specific manner [5].

Physiologically, CD10 plays an important role in the metabolism of signaling peptides like natriuretic peptides, angiotensins, bradykinin, endothelin, enkephalins, oxytocin, tachykinins, substance P, calcitonin gene-related peptide (CGRP) and vasoactive intestinal polypeptide (VIP), involving it in the extracellular regulation of a number of signaling pathways of the mammalian nervous, cardiovascular, inflammatory and immune systems [1, 3].

CD10 is involved in the pathogenesis of numerous nonneoplastic diseases such as diabetic nephropathy [6] and Alzheimer's disease [7]. With immunohistochemistry (IHC), it can be detected in many hematological malignancies [8–10], soft tissue neoplasia (e.g. pleiomorphic undifferentiated sarcoma, fibrosarcomas, leiomyosarcomas and malignant peripheral nerve sheath tumors) [11] as well as in carcinomas of different organs, like the skin [12, 13], lung [14], pancreas [15], liver [16], stomach [17], cervix [18], kidney [19], bladder [20] and prostate [21]. Such a wide spectrum of expression may suggest a limitation in the usefulness of CD10 immunostaining in routine diagnostic pathology.

As concerns the breast, CD10 has an important role in its development through modulation of cell growth and differentiation, and by having effects on epithelial-mesenchymal morphogenesis [22, 23]. CD10 is not only expressed by myoepithelial cells (MECs) but can also be detected on the surface of mammary stem cells, early common breast progenitor cells and in myoepithelial progenitors. CD10 protease maintains the early progenitor population in the human mammary lineage by degrading signaling proteins that would otherwise promote maturation [24]. A study using a mouse model has shown the involvement of oxytocin, a peptide cleaved by CD10, in the differentiation of MECs [25].

CD10 also has prognostic implications; its expression in breast tumor stromal cells is correlated with estrogen receptor (ER) negativity, a higher grade and poor prognosis [26, 27]. CD10 has been shown to discriminate between benign, borderline and malignant phyllodes tumors of the breast and its expression has been found on IHC to correlate significantly with the occurrence of distant metastasis [28].

In diagnostic breast histopathology, CD10 IHC is used to identify MECs. Although MECs around normal structures (ducts and lobules) are nicely highlighted by this marker, in pathologic conditions such as ductal carcinoma in situ (DCIS), CD10 has a relatively low sensitivity as an MEC marker [29], and its specificity also seems compromised by the fact that, rarely, tumor cells also stain with the antibody [30], although the pattern of staining in

Fig. 1. Proportion of benign lesions, in situ and invasive cancers showing luminal/membranous CD10 positivity. The bars represent 95% CIs.

the neoplastic mammary epithelium has not been widely studied.

Apocrine epithelium has been described to be positive for CD10 [31] and Kalof et al. [29] clearly documented the consistent luminal staining of apocrine metaplasia. While studying breast lesions immunostained for CD10 as an MEC marker, we also recognized that paratumoral apocrine cysts demonstrated a strong, predominantly apical reaction, and we have also found traces of this staining pattern in the literature [29, 31]. To our knowledge, no previous studies have systematically examined CD10 expression of apocrine lesions. In this study, we analyzed a series of breast lesions with apocrine differentiation for the expression of CD10, both in the epithelial and the myoepithelial components. The aim was to explore how the immunostaining varied in benign, in situ and invasive malignant lesions.

Materials and Methods

In this retrospective study, tissue blocks of 50 breast lesions from the archives of the Department of Pathology at the University of Szeged and the Bács-Kiskun County Teaching Hospital were used. The antibody used was a mouse monoclonal antibody (clone 56C6, ready to use; Dako, Glostrup, Denmark or Cell Marque, 1:50 dilution; Rocklin, Calif., USA).

Following the instructions of the manufacturer, IHC stainings were carried out on 44 sections of whole tissue (thickness: 4–5 µm) and tissue microarray. Different chromogens were used in the two departments (diaminobenzidine, Dako or Nova RED or VIP, Vector Laboratories, Burlingame, Calif., USA).

Statistical calculations were made with GraphPad QuickCalcs, (San Diego, Calif., USA).

Fig. 2. Examples of CD10 positivity in different lesions. **a** Apocrine cysts with areas of papillary hyperplasia. Note focal to near-complete luminal epithelial and strong myoepithelial positivity. **b** DCIS with a lack of luminal/membranous staining in foci of lumen formation and weak myoepithelial labeling. **c** Luminal and strong cytoplasmic staining in invasive carcinoma of no special type. **d** Very focal cytoplasmic labeling in invasive carcinoma. $\times 10$.

Results

Fifty apocrine lesions were included in the study: 10 cysts with or without papillary hyperplasia, 1 cyst without an MEC layer [32], 6 apocrine adenoses, 2 papillomas, 13 DCIS, 14 invasive carcinomas of no special type (formerly ductal carcinomas) and 4 invasive lobular carcinomas.

17/19 [0.89; 95% confidence interval (CI) 0.68–0.97] benign apocrine lesions (fig. 1) showed complete or partial luminal CD10 staining (fig. 2a), although most cases included parts without staining and 2 lesions (an apocrine adenosis and a cyst with papillary hyperplasia) were completely negative (fig. 1). The MECs were often but not always positive.

As concerns malignant lesions, 8/13 apocrine DCIS cases displayed no luminal staining (fig. 2b), but 4 (0.31; 95% CI 0.13–0.58) demonstrated very focal luminal positivity. The MECs around the DCIS showed a spectrum of staining from nil to strong and complete. Only 4/18 (0.22; 95% CI 0.09–0.46) invasive carcinomas demonstrated luminal/membranous staining (fig. 1, 2c). Cytoplasmic CD10 positivity was seen focally in 4 invasive cancers (fig. 2d) and in 3 DCIS, and more markedly in 1 invasive carcinoma of no special type (fig. 2c); 2 of these invasive cancers and 1 in situ carcinoma with ‘aberrant’ cytoplasmic staining demonstrated no membranous staining. Benign lesions showed luminal/membranous staining more

commonly than malignant ones (17/19 vs. 8/31; $p < 0.0001$, χ^2 test with Yates correction for continuity) and this was also true for any epithelial staining including aberrant cytoplasmic labeling (17/19 vs. 11/31; $p = 0.0006$, χ^2 test with Yates correction for continuity).

Discussion

The fact that CD10 is a ubiquitous enzyme found on the surface of many different normal cell types and pathologic lesions has a negative impact on its specificity and thus on its possible utility in routine histopathological differential diagnosis. Therefore, CD10 IHC reactions should be only used to answer specific differential diagnostic questions in well-known circumstances.

Overall, breast epithelium rarely expresses CD10. Only focal labeling of luminal ductal epithelium was reported by Kalof et al. [29]; the limited number of invasive and in situ carcinomas ($n = 46$) that they studied were all negative. Bains and Sidhu [33] reported on a case of invasive breast carcinoma, showing cytoplasmic CD10 staining associated with an in situ component and intraductal papilloma, demonstrating the same type of labeling. Although no mention of receptor status was included in their description, on the basis of the figures, none of these lesions demonstrated the characteristic apocrine mor-

phology. The authors concluded that CD10 positivity in metastatic tumors cannot rule out the breast as primary, and related the phenotype to the CD10-positive progenitor cells capable of differentiating towards luminal epithelial cells and MECs described by Stingl et al. [34].

Ductal and lobular carcinomas are rarely positive for CD10 [31], but some subsets may be different in this respect: of 40 ER-positive tumors, none demonstrated CD10 positivity (defined with a cut-off of 10% staining) and only a single case showed <10% labeling, whereas 12 of 77 ER-negative carcinomas (16%) showed cytoplasmic or membranous staining in 30–100% of the cells [35]. A subset of ER-negative breast cancers is also negative for progesterone receptors (PR) and human epidermal growth factor receptor-2 (Her2), and is therefore labeled as triple-negative. Some triple-negative carcinomas express basal (i.e. MEC) markers (cytokeratin 5 and/or EGFR) and this feature has been suggested for the delineation of the basal-like gene-expression profile-based subgroup of breast cancers on IHC [36]. Not surprisingly, some of these carcinomas may also express CD10, an MEC marker in a substantial number of cases (16/20 of spindle-cell metaplastic carcinomas and carcinosarcomas) [37], similarly to the rare cases that demonstrate straightforward myoepithelial differentiation [38]. Apocrine carcinomas are also generally ER- and PR-negative [39], and might have been included in previous studies of ER-negative carcinomas, but without distinct identification of this subset. Smollich et al. [40] identified cytoplasmic (and occasional membranous) CD10 (neprilysin) staining of tumor parenchymal (epithelial) cells in 33/126 (26%) of breast cancers and found this labeling to be associated with better prognosis, in contrast to the CD10 staining of the stromal myofibroblast reported to indicate worse prognosis.

CD10 positivity has been described in benign apocrine epithelium [29, 31], but no data on CD10 expression in various other types of apocrine breast lesions have been available until now. Our results indicate that benign apocrine epithelium (metaplasia) is typically positive for CD10 with a luminal staining pattern, although there are exceptions to the rule. Malignization or apocrine differentiation in malignant lesions seems to be associated with a partial or complete loss of this staining pattern, which is therefore rarer in in situ carcinomas and even rarer in invasive ones, and cytoplasmic (aberrant) staining may also occur in this subset.

Although the staining of MECs was not the primary aim of our study, our findings are in keeping with earlier works on the subject, and suggest that the sensitivity of CD10 as an MEC marker is lower than other markers like p40 and smooth-muscle actin. Its proportional sensitivity is even further diminished in certain lesions like benign sclerosing lesions [41] and DCIS [42], known for their reduced expression of MEC markers [43]. Based on the literature [29, 30, 42] and supported by our experience, CD10 is not an ideal myoepithelial marker. However, occasional CD10 staining of epithelial cells should be kept in mind, as its occurrence may interfere with the identification of some cells as epithelial or myoepithelial, especially in apocrine lesions, some of which may turn out to be benign, even without the presence of a myoepithelial layer [32, 44].

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