Title:

CD10 immunohistochemical expression in apocrine lesions of the breast

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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 cysts (11), apocrine adenosis (6), apocrine metaplasia or hyperplasia in papilloma (2), ductal carcinoma in situ (DCIS) (13), invasive carcinomas of ductal (14) or lobular (4) type.

Results: Benign apocrine lesions showed complete or partial luminal CD10 staining although most cases included parts without staining, and two lesions were completely negative. The MECs were often, but not always positive. 9/13 apocrine DCIS cases displayed no luminal staining, but 4 cases demonstrated very focal luminal positivity. The MECs around DCIS showed a spectrum of staining from nil to strong complete. Only 4 invasive carcinomas demonstrated luminal/membranous staining. Cytoplasmic CD10 positivity was seen focally in 4 invasive cancers and 3 DCIS.

Conclusion: CD10 positivity is luminal/membranous in most benign apocrine lesions, the staining being non universal and sometimes focal. Analogous 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.

Introduction

Cluster differentiation 10 (CD10) also known as neprilysin, enkephalinase, common acute lymphoblastic leukemia antigen (CALLA), membrane metallo-endopeptidase (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 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 expression of CD10 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), thus it is involved in the extracellular regulation of a number of signaling pathways of the mammalian nervous, cardiovascular, inflammatory and immune systems [1,3].

CD 10 is involved in the pathogenesis of numerous non-neoplastic diseases such as diabetic nephropathy [6] or Alzheimer's disease [7]. By immunohistochemistry (IHC) it can be detected in many hematological malignancies [8, 9, 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 (e.g. 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 the 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 MEC but can be detected on the surface of mammary stem cells, early common breast progenitor cells as well as 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 myoepithelial cells [25].

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

In diagnostic breast histopathology, CD10 IHC is used to identify myoepithelial cells (MECs). Although MECs around normal structures (ducts and lobules) are nicely highlighted by this marker, in pathologic conditions such as DCIS, CD10 has a relatively low sensitivity as a MEC marker [29], and its specificity seems also compromised by the fact that rarely tumor cells also stain with the antibody [30], although the pattern of staining in the neoplastic mammary epithelium has not been widely studied.

Apocrine epithelium has been described to be positive for CD10 [31] and Kalof and colleagues clearly stated the consistent luminal staining of apocrine metaplasia [29]. While studying apocrine breast lesions immunostained for CD10 as a MEC marker, we also recognized that paratumoral apocrine cysts demonstrated strong, predominantly apical reaction, and we also found traces of this staining pattern in the literature [29, 31]. To our

knowledge, no previous studies examined CD10 expression of apocrine lesions systematically before. 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 of these lesions with the aim of exploring 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, University of Szeged or Bács-Kiskun County Teaching Hospital were used. The antibody used was a mouse monoclonal antibody (clone 56C6, Dako, Glostrup, Denmark ready to use; or Cell Marque, Rocklin, California, 1:50 dilution).

Immunohistochemical stainings were carried out following the instructions of the manufacturer on four to five micrometer-thick whole tissue sections (44 cases) and tissue microarray (TMA) sections, using different chromogens in the two department (diaminobenzidine, Dako, Glostrup, Denmark / Nova RED or VIP, Vector Laboratories, Burlingame, California).

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

Results

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

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

As concerns malignant lesions,7/13 apocrine DCIS cases displayed no luminal staining (Figure 2B), but 4 cases (0.31; 95% CI: 0.13-0.58) demonstrated very focal luminal positivity. The MECs around DCIS showed a spectrum of staining from nil to strong complete. Only 4/18 (0.22; 95% CI: 0.09-0.46) invasive carcinoma demonstrated luminal / membranous staining (Figures 1 and 2C). Cytoplasmic CD10 positivity was seen focally in 4 invasive cancers (Figure 2D) and 3 DCIS, and more markedly in 1 invasive carcinoma of no special type (Figure 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 versus 8/31; $p<0.0001$, chi square test with Yates correction for continuity) and this was also true for any epithelial staining including aberrant cytoplasmic labeling too (17/19 versus 11/31; $p=0.0006$, chi square 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 and only focal labeling of luminal ductal epithelium was reported by Kalof and coworkers; the limited number of invasive and in

situ carcinomas (n=46) they studied were all negative [29]. Bains and Sidhu have 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 [33]; although no mention of receptor status was included in the description, on the basis of the figures, none of these lesions demonstrated the characteristic apocrine morphology. The authors concluded that CD10 positivity in metastatic tumors cannot rule out breast as primary, and related the phenotype to the CD10 positive progenitor cells capable of differentiating towards luminal epithelial and myoepithelial cells 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: none of 40 estrogen receptor (ER)-positive tumors 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 delineating the basal-like gene-expression profile based subgroup of breast cancers by IHC [36]. Not surprisingly, some of these carcinomas may also express CD10, a MEC marker in a substantial number of cases (16/20 of spindle cell metaplastic carcinomas and carcinosarcomas) [37], similarly to the rare cases demonstrating straight forward 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 and coworkers 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 [40], in contrast to the CD10 staining of the stromal myofibroblast reported to indicate worse prognosis.

CD10 positivity was described in benign apocrine epithelium [29, 31], but no previous data was available about CD10 expression in various other types of apocrine breast lesions. 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 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 the present study, our findings are in keeping with earlier works on the subject, and suggest that the sensitivity of CD10 as a MEC marker is lower compared to other markers like p40 and smooth muscle actin and its proportional sensitivity is even further diminished in certain lesions like benign sclerosing lesions [41] and DCIS [42] which are 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 to be benign even without the presence of a myoepithelial layer [32, 44].

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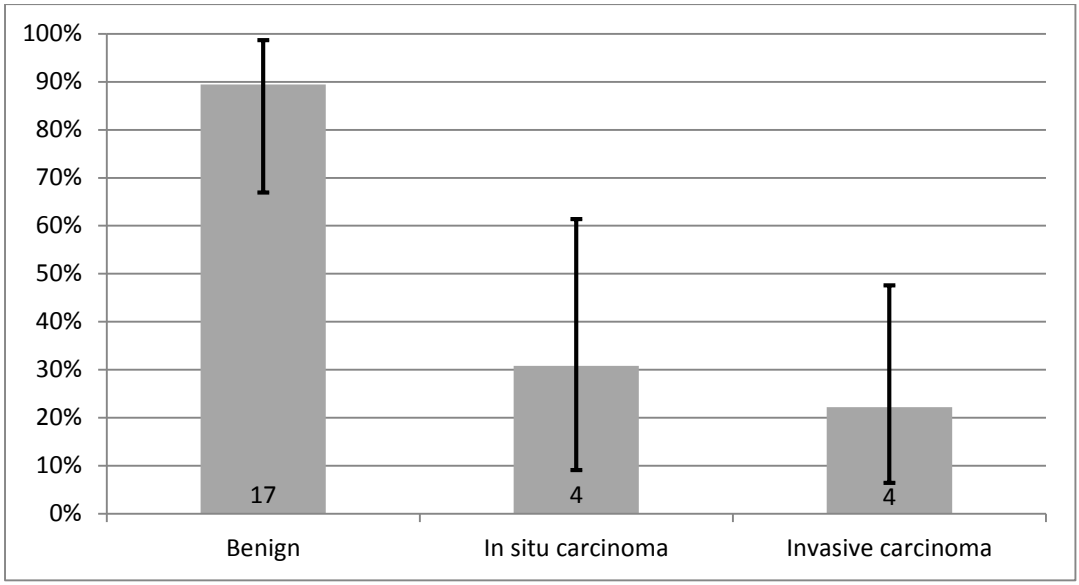
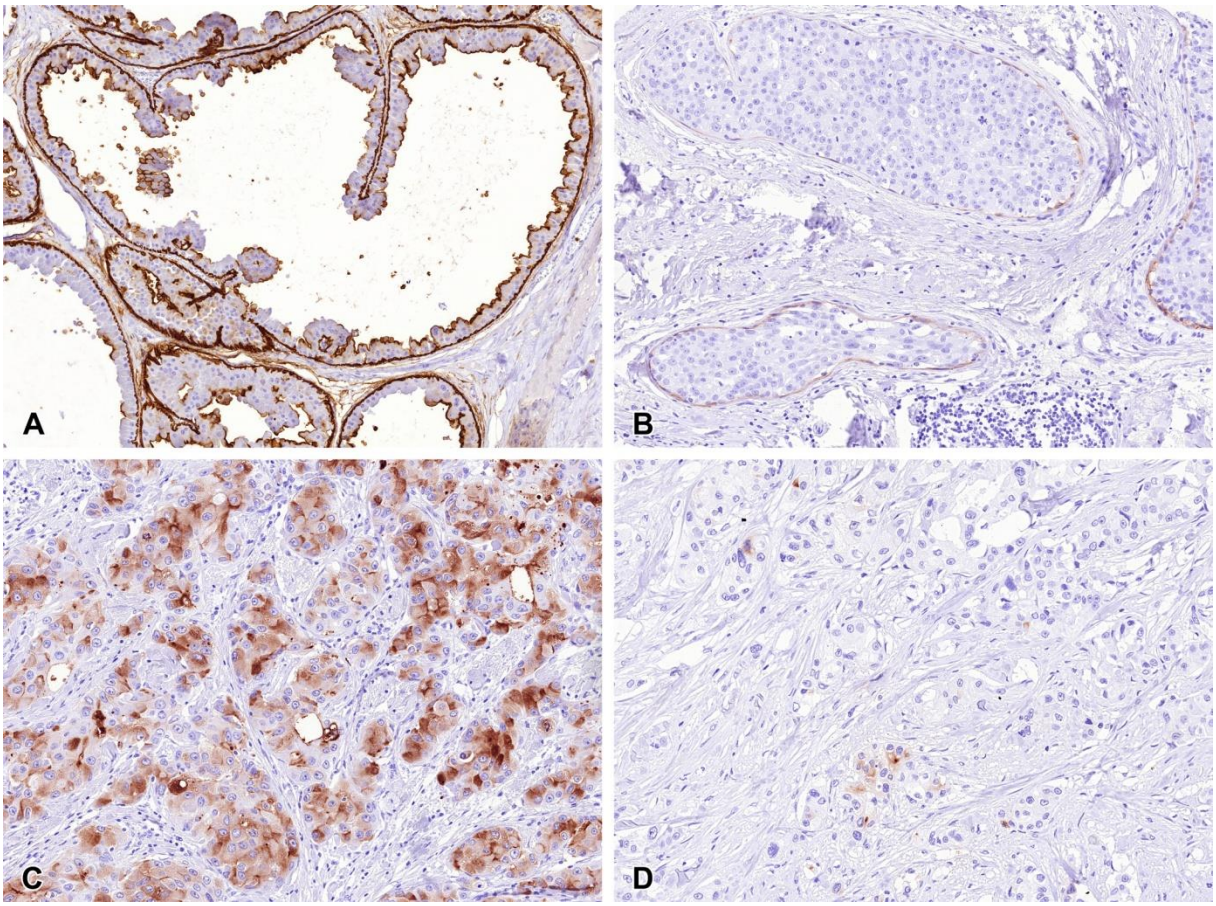
Figure 1
Proportion of benign lesions, in situ and invasive cancers showing luminal / membranous CD10 positivity.



The bars represent 95% confidence intervals.

Figure 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: Ductal carcinoma in situ with 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. (A-D: x10 objective magnification)