Review article
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The histologic spectrum of apocrine lesions of the breast

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New data on apocrine carcinoma of the breast, especially on its unusual pathogenesis, are the facts that justify this study. The aim of this study was to describe the morphological features in both benign apocrine lesions and invasive apocrine carcinomas of the breast. The following apocrine lesions were pointed out: cysts, metaplasia, adenosis, adenoma, borderline malignant lesion, intraductal carcinoma and invasive apocrine carcinoma. Surgical specimens of breast benign and malignant lesions were fixed in formalin, embedded in paraffin blocks and the slides were stained with HE, PAS and immunohistochemical ABC complex methods, using primary antibodies against: p53, Ki-67, androgen receptor, and GCDFP-15. The criteria of apocrine lesions, as well as classification of apocrine carcinoma were pointed out also. In the discussion we cited literature data about incidence of apocrine lesions in the breast, immunohistochemical, ultrastructural and molecular characteristics of apocrine lesions focusing on differential diagnostic problems between apocrine and nonapocrine lesions, and benign versus malignant apocrine lesion. The authors have suggested that apocrine carcinoma represents unusual type of the breast carcinoma and which may origin from the following precancerous lesions: apocrine hyperplasia, apocrine adenosis, atypical apocrine adenosis and adenoma. Immunohistochemical markers for apocrine differentiation are: GCDFP-15 and androgen receptors. Ki-67 and p53 may be good markers for differentiation between benign and malignant breast apocrine lesions. Positively staining for androgen receptors, not only in apocrine carcinoma of the breast, but also in benign lesions, has led some authors to postulate a possible role of androgens in the stimulation of breast epithelium and the development of apocrine cells and apocrine carcinomas. However, in this stage the clinical significance remains uncertain and follow-up studies will be required to evaluate this issue.

KEY WORDS: *Breast Neoplasms; Apocrine Glands; Carcinoma; Carcinoma, Infiltrating Duct; Immunohistochemistry; Metaplasia; Cytodiagnosis*

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GENERAL ASPECT ON APOCRINE CELLS

Apocrine epithelial cells have abundant eosinophilic cytoplasm containing finely granular periodic-acid Schiff (PAS) positive diastase - resistant granules, with basically located nucleus and prominent nucleolus (1-4). Apocrine sweat glands are found in the skin of the axilla, groin, anogenital region, and other regions and other sites (1,2). A number of benign and malignant lesions in the breast contain epithelial cells, which are citologically identical to those that comprise the apocrine glands.

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Microscopic apocrine change is common in the female breast after the age of 30 years; it is rarely seen in women younger than 19, and increases with age, persisting postmenopausally (2,4). The presence of apocrine cells in the breast has generally been regarded as a metaplastic process. Now, several authors suggest that the presence of apocrine cells in the breast be termed a normal process of differentiation and that these cells are normal constituent of the glandular structure of the breast (1,2).

However, the data on the relationship of apocrine metaplasia to invasive breast cancer are controversial. Different authors have reported that apocrine differentiation in proliferative lesions may be a risk factor, a precursor lesion, or have no association with malignancy (2-9).

The aim of this review study was to describe the morphological features in both benign apocrine lesions and invasive apocrine carcinomas of the breast.

MATERIALS AND METHODS

Surgical material and fine needle aspiration cytology are taken from breast precancerous and malignant diseases. The methods we used are classic hematoxylin-eosin (H&E), for histological characteristics of apocrine cells and their lesions, histochemical PAS reaction, for identification of PAS-positive diastase-resistant granules, which gives finely granular cytoplasm of these cells, cytological Papanicolaou's (Pap) stain, for cytology specimens, and immunohistochemical method Avidine Biotine Complex (ABC, Dako, K 0377). Paraffin sections of 3 to 4 μm were dehydrated and rehydrated in graded alcohol, then placed in a pressure cooker in a closed plastic container filed with 10 mM citric acid (pH 6) for 10 min to do epitope retrieval, and then endogenous peroxide was blocked with 0.3% hydrogen peroxidase for 10 min. The sequence of reactants in the ABC method is typically in three steps: application of primary antibodies, application of biotinylated secondary antibody, and nuclei counterstained with hematoxylin. Positive staining was identified in the form of dark brown nuclear staining by Diaminobenzidine (DAB) of androgen receptors, Ki-67 antigen and p53 protein. The reaction was diffusely intracytoplasmic positive by Gross Cystic Disease Fluid Protein-15 (GCDFP-15). A tumor was considered positive when at least 10% of tumor cells were positively stained.

MICROMORPHOLOGY

The definition of apocrine metaplasia was based on the criteria reported by O'Malley et al. (10) and summarized as follows: (1) markedly eosinophilic cytoplasm with fine granularity, (2) large and moderately vesicular nuclei with an occasional prominent red nucleoli, and (3) the occasional presence of apical snouts (Figure 1). Potential marker for apocrine differentiation, GCDFP-15, is consistently positive in all of the examined cases (11). However, the presence of CDFP-15 alone was not considered as apocrine metaplasia because of the reported presence of GCDFP-15 immunoreactivity in non apocrine breast epithelial cells (1) and, therefore, that finding was not included in the criteria in defining apocrine metaplasia. The multistep model of carcinogenesis in the breast suggests a transition from normal epithelium to invasive carcinoma via non-atypical and atypical hyperplasia and in situ carcinoma (1). Within the breast, these proliferations are heterogeneous in their cytological and architectural characteristics. Having in mind that morphological classification of breast disease remains controversial, we describe it.

1. Apocrine cysts. Benign epithelial cysts are associated with simple fibrocystic disease. Stratification of apocrine metaplastic cells without intervening fibrovascular stroma is detected in benign cases. Nuclei are usually small and uniform (4) (Figure 1).

2. Apocrine metaplasia. Apocrine metaplasia with smaller cysts lined with large polygonal cells having an abundant granular, eosinophilic cytoplasm, with small, round deeply chromatic nuclei, is seen focally in the lesions of intraductal papilloma or ductal adenoma (5,8,12). Epithelial overgrowth and papillary projections are common in cysts lined with apocrine epithelium (Figure 2).

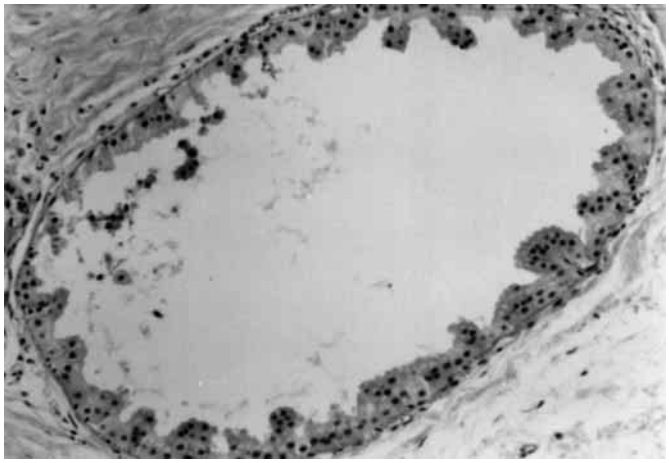

Figure 1. Apocrine cyst lined with apocrine epithelium HEx250

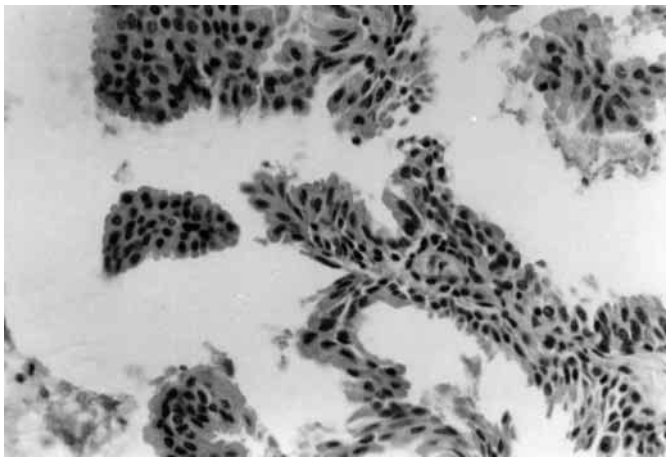

Figure 2. Epithelial overgrowth and papillary predictions in cysts, PASx300

3. Apocrine adenosis. Apocrine adenosis is the presence of apocrine metaplasia in adenosis in more than 50% of this change. It shows some degree of nuclear enlargement. However, the lobulocentricity of the lesion and the presence of a myoepithelial layer exclude the possibility of the invasive carcinoma. It is frequently found together with epithelial hyperplasia but not with cysts and fibrosis (8,13).

4. Atypical apocrine adenosis, borderline malignant or atypical ductal hyperplasia of apocrine type. Atypical apocrine cells are enlarged, often with signet ring morphology and with nuclear atypia, prominent nucleoli, and cytoplasmic granules (14) (Figure 3). The identification of preinvasive disease and, in particular, borderline lesions have highlighted deficiencies in understanding and classification of such lesions (8,10,14).

5. Apocrine adenoma. Apocrine adenoma is a rare benign epithelial tumor of the breast that can radiologically be presented as a

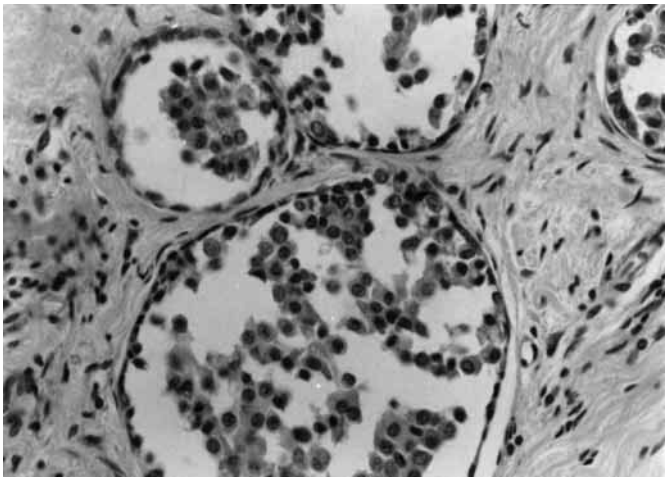

Figure 3. Atypical apocrine adenosis enlarged cells with nuclear atypia, HEx300

well-defined opacity. Pathologically, tumor is characterized by a circumscribed proliferation of metaplastic apocrine cells, which may contain calcifications (4,15,16).

6. Apocrine ductal carcinoma in situ (ADCIS). ADCIS is special type of ductal carcinoma in situ. Each case is assigned to 1 of 3 histological grades (low, intermediate, high), based on nuclear morphology and the presence of necrosis (6). However, histologically, it is occasionally difficult to interpret the malignant potential of intraductal lesions with diffuse apocrine features because benign apocrine metaplastic cells may be associated with various degrees of nuclear atypia or the presence of macronucleoli. Therefore, the authors use immunohistochemistry in the study of various apocrine breast lesions (12).

7. Apocrine carcinoma. Apocrine carcinomas are carcinomas that show cytological and immunohistochemical characteristic of apocrine cells in more than 90% timorous cells (3). This variant accounts for 1% of all mammary carcinomas (1,3) and most of its clinicoplathological features are still unknown (17-19). It could be classified into three subtypes according to predominant histopathological growth pattern: type I (intraductal spreading type), type II (adenosis associated type), and type III (infiltrating type) (Figure 4). Types I and II are usually without lymph node metastasis at the moment of discovering and have an excellent prognosis, whereas the infiltrating type is associated with lymph node metastasis and death from cancer. However, the tumors are heterogeneous with regard to pattern and local spread, including papillo-tubular, solid or trabecular. Nuclei grade is also moderate to severe. Apocrine carcinoma of the breast is characterized by higher patient age and tumor shadows (19).

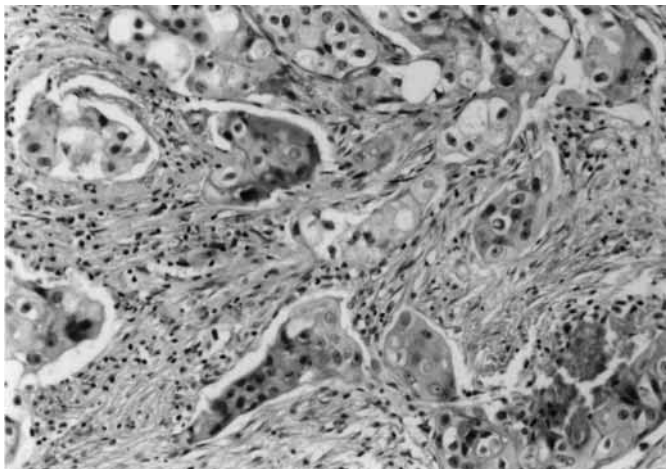

Figure 4. Figure 4. Apocrine carcinoma-infiltrating type, PASx250

FINE NEEDLE ASPIRATION CYTOLOGY

Apocrine metaplastic cells are frequently encountered in fine needle aspirates of breast lesions. Atypical apocrine metaplastic cells with signet ring features can also occur, and their presence may present a diagnostic dilemma in the differentiation of benign versus malignant lesions. Also present are clusters of cells that are enlarged and showed nuclear atypia, prominent nucleoli, and cytoplasmic granules. Papillary cohesive clusters of ductal cells are also identified. Atypical apocrine cells can be misinterpreted as mucinous carcinoma or ductal carcinoma not otherwise specified (20,21).

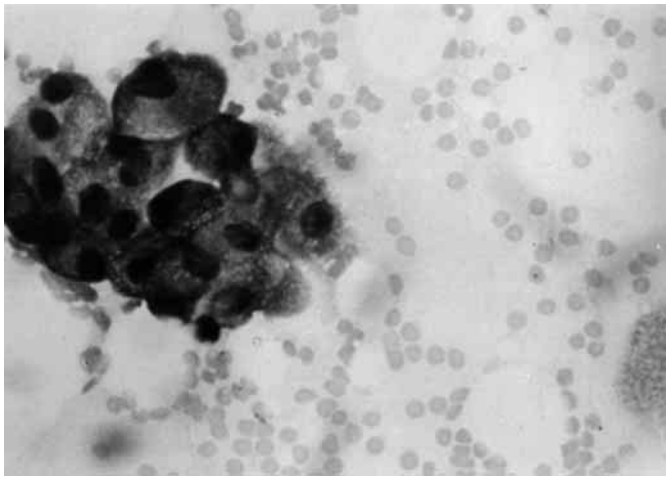

Figure 5. Fine needle smear - prominent nuclear pleomorphism. Papaniocolau's stain x 400

Apocrine carcinoma: the smears are of moderate to high cellularity, consisting of predominantly dispersed or loosely cohesive tumor cells in a focally granular background. The carcinoma cells contain abundant dense to granular cytoplasm; round or oval and sometimes eccentrically located nuclei; smooth nuclear outline evenly dispersed chromatin; solitary macronucleoli. The cell borders are mostly discrete. In contrast to benign apocrine cells, the malignant cells show nuclear overlapping, more frequent nuclear pleomorphism, increased nuclear/cytoplasmic ratios and occasional mitotic figures (21,22) (Figure 5).

DISCUSSION

The relationship between apocrine change and breast carcinoma, despite numerous studies, remains controversial. A number of conflicting reports using a variety of approaches have been published that have resulted in a confused picture regarding the pathogenic nature of these lesions in the breast. Some hypotheses regarding a possible relationship between apocrine epithelium and carcinoma have been proposed (9). The apocrine epithelium may be precursor of malignant transformation; it may reflect a response to the same stimulus that promotes carcinoma or it could indicate instability of the breast epithelium, which causes the development of alterations with a higher propensity for cancer.

The apocrine cysts showing papillary hyperplasia have long been controversial lesions, and numerous studies have investigated their association with breast carcinoma (4,7). The molecular data show that they can exhibit a range of genetic alterations and that at least a proportion of these lesions may be clonal neoplasms, representing a nonobligatory precursor of ADCIS and invasive apocrine carcinoma (1). At this stage, the clinical significance remains uncertain and follow-up studies will be required to evaluate this issue. Microglandular adenosis, apocrine adenosis, and tubular carcinoma of the breast should be recognized to prevent confusion during the diagnostic work. Microglandular adenosis is characterized by an absence of myoepithelial cells, epithelial membrane antigen (EMA) and GCDFP-15 (13). The absence of EMA in microglandular adenosis makes it unique among benign glandular hyperplasias of the breast. Apocrine adenoma contains myoepithelial cells and a distinct basal lamina. It is characterized by the presence of GCDFP-l5, which is not present in microglandular adenosis (13). Tubular carcinoma lacks both myoepithelial cells and a basal lamina. It is negative for GCDFP-15. Periductal and vascular elastosis are common and usually prominent, whereas they are not found in either microglandular adenosis or apocrine adenosis. Apocrine carcinoma of the breast is an unusual and special category of predominantly androgen receptor+, estrogen receptor-, and progesterone receptor-breast cancer (12,23,24) characterized by cells with abundant, eosinophilic cytoplasm and nuclei with often-prominent nucleoli. Apocrine carcinoma must be differentiated from oncocytic carcinoma (25,26). All three reported cases of oncocytic carcinoma were composed mostly of cells with low-grade nuclei and abundant granular eosinophilic cytoplasm (25). More than 70% of the neoplastic patients in each case were immunoreactive with an antimitochondrial antibody. Apocrine cells and oncocytes share similar morphologic features at HE level. Oncocytic cells were not positive at the immunohistochemical and molecular levels for GCDFP-15/PIP (prolactin-inducible protein) mRNA, which are typical markers of apocrine differentiation (11,13). Apocrine and oncocytic carcinomas have following ultrastructural differences (30): mitochondria in apocrine cells usually are in perinuclear location; not so numerous and diffusely dispersed, as in oncocytes; apocrine cells display features of the active secretory elements: prominent microvilli, well-developed Golgi complex, and electron dense secretory granules polarized toward the luminal pole. The genetic alterations in benign apocrine lesions, ADCIS and invasive apocrine carcinoma are the target of many authors (12,27). The most common alterations in apocrine hyperplasia were gains of 2q, 13q and 1p, and losses of 1p, 17q, 22q, 2p, 10q and 16q (12,28). ADCIS and invasive carcinoma show gains of 1q, 2q, 1p, and losses of 1p, 22q, 17q, and 16q as their most common DNA copy number changes (27, 28). Immunohistochemical analysis of Ki-67, p53, p21 and p27 were shown the following results (12): Ki-67 positive cases are significantly higher in malignant than in benign apocrine lesions (12). None of the benign or borderline cases is immunohistochemically positive for p53, but many malignant cases demonstrate p53. p53 immunoreactivity is also positively correlated with nuclear grade of carcinoma cases. Neither p21 nor p27 demonstrate any correlation with histological parameters or findings of the apocrine lesions. It means that Ki-67 and p53 may be good markers for differentiation between benign and malignant breast apocrine lesions (12). GCDFP-15, a 15-kd glycoprotein, which was isolated in the cystic fluid of fibrocystic breast disease, represents an immunocytochemical marker of apocrine differentiation. The gene has been localized to chromosome 7, and is identical to that of the prolactin-inducible protein (11). Immunihistochemical studies of benign and malignant breast lesions showing apocrine differentiation report that the cells lack estrogen and progesterone receptors, but stain positive for androgen receptor (Figure 6), contrasting with the normal breast epithelium (23,24). These intriguing observations reflect the fact that apocrine cells differ from nonapocrine normal cells

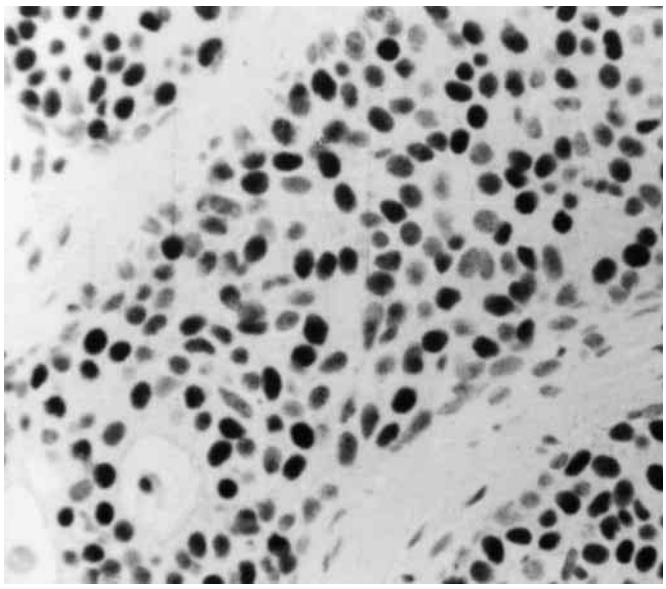

Figure 6. Androgen receptor (NCL-AR-2F, dilution 1:50 Novocastra): high degree of nuclear activity, ABCx300

not only morphologically but also biologically (24,29). Staining positively for androgen receptor in benign apocrine lesions has led some authors to postulate a possible role of androgens in the stimulation of breast epithelium and the development of apocrine cells (23,24). To further investigate these lesions, loss of heterozygosity was evaluated at multiple chromosomal loci, including several loci commonly mutated in breast cancer (30). This molecular evidence supports immunohistochemical data that apocrine carcinomas of the breast may possess unique mechanisms of carcinogenesis, compared with ordinary ductal carcinomas. However, further study is needed to support this assertion and to determine if the loss of heterozygosity detected is truly etiologic or if it is the result of genetic progression.

CONCLUSION

Apocrine carcinoma is an unusual type of breast carcinoma, which develops from apocrine precancerous lesions. Immunohistochemical markers for both benign and malignant apocrine differentiation are the expression of PIP/GCDFP-15 located to chromosome 7, and positive staining for androgen receptors. The clinical significance of apocrine differentiation remains uncertain and follow-up studies will be required to evaluate this issue.

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