Virchows Arch (1997) 431:205–209

ORIGINAL ARTICLE

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Apocrine epithelium of the breast: does it result from metaplasia?

Received: 14 February 1997 / Accepted: 12 April 1997

Abstract Benign and malignant breast lesions may show an apocrine epithelium considered to be the result of metaplasia. In an attempt to clarify the histogenesis of the breast apocrine epithelium we searched for the presence of apocrine cells or cells with apocrine differentiation during human breast development. We analysed 10 autopsy specimens of female breasts from fetuses between 28 and 40 weeks of gestational age and tissue from 6 normal breasts, obtained after breast reduction in nulliparous young women between 22 and 28 years of age. Formalin-fixed, paraffin-embedded sections were stained with haematoxylin-eosin, PAS-diastase and a monoclonal antibody (BRST-2) anti-GCDFP-15, which is a specific apocrine marker. A 40-week fetal breast was analysed by electron microscopy. No cells with histological and ultrastructural apocrine features were found in the ducts of fetal breasts. All fetal breasts showed some ducts with sparse GCDFP-15-immunoreactive cells; the number of these cells increased with gestational age. PAS-diastase was negative. No cells with apocrine morphology were found in ducts and lobules of normal adult breasts. Scattered GCDFP-15-positive luminal epithelial cells and rare PAS-diastase-positive cells were observed in some lobules of all adult breasts. Cells with biochemical characteristics (GCDFP-15 expression) of apocrine differentiation are evident during human fetal breast development and persist in adult mammary glands. Unknown stimuli may induce these cells to take on an apocrine morphology. Apocrine epithelium of the breast may be a normal process of differentiation rather than metaplasia. We suggest the term "apocrine differentiation precursor cells" for GCDFP-15-positive breast epithelial cells with no apocrine morphology.

Key words Breast · Fetal breast · Apocrine differentiation · GCDFP-15

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Introduction

The apocrine epithelium, a normal constituent of apocrine glands of axillary and anogenital skin, consists of cells with eosinophilic cytoplasm containing PAS-positive diastase-resistant granules. This epithelium expresses a protein of 15 kDa, which is named gross cystic disease fluid protein-15 (GCDFP-15) since it was first isolated in the cyst fluid of fibrocystic breast disease [8]. This protein is considered to be a specific marker of apocrine differentiation. Immunohistochemical [12] and in situ hybridization [15] studies have shown that in normal tissues the secretion of GCDFP-15 appears to be restricted to apocrine glands and to some glands that have common phylogenetic features with apocrine glands, such as the salivary submandibular, lacrimal and bronchial glands. An apocrine epithelium frequently appears in benign breast lesions such as fibrocystic disease [9, 14, 17, 20], papillomas [17], and fibroadenomas [3]. Furthermore, cells with apocrine features are described in malignant breast tumours called apocrine carcinomas [1]. An apocrine epithelium is frequently observed in the breast lesions, but the biological significance and the mechanism by which this particular cellular feature is produced remain obscure.

Several explanations have been given for the presence of apocrine cells in the breast. The apocrine epithelium considered to be a metaplasia [2, 10, 18, 20], to be degenerate epithelium simulating the true apocrine epithelium [5], or to belong to cutaneous apocrine glands that remain entrapped in the breast during development. A further hypothesis is that apocrine epithelium is a normal constituent of the mammary gland.

The identification of apocrine cells or cells with apocrine differentiation during breast development could be a useful approach to clarifying the histogenesis of this type of cells. In this study the presence of cells with apocrine features was investigated in a series of human fetal breasts by the PAS-diastase, an immunohistochemical method using a monoclonal antibody (BRST-2) anti-GCDFP-15, and an ultrastructural approach. The pres-

ence of cells with apocrine differentiation was also investigated in normal ducts and lobules of adult breasts.

Materials and methods

Ten breasts were obtained from female fetuses. The breasts were fixed in formalin and embedded in paraffin. An average of five 4-μm sections were cut from each block. Four fetuses were nonmacerated spontaneous abortuses with gestational ages of 28, 29, 29 and 30 weeks. Chorioamnionitis and placental haemorrhage caused abortion in three of these cases, while no relevant cause of abortion was evidenced in the 28 weeks fetus. Six were newborn babies who died soon after birth (death after between 7 and 48 h). The gestational age of the newborns was from 31 to 40 weeks: two cases were 31 weeks, 1 was 32, 1 was 36, 1 was 37 and 1 was 40 weeks. Four cases had had pneumonia, one case, hyaline membrane disease, and another, massive cerebral haemorrhage. The newborns presented no malformations.

Six breasts were collected in which the normal ducts and lobules could be identified. The tissues were obtained from nulliparous young women between 22 and 28 years of age during breast reduction. An average of ten formalin-fixed paraffin-embedded blocks were obtained from each breast, and ten slides were prepared from each block.

Four-micrometre sections of fetal and adult breasts were stained with haematoxylin-eosin and PAS-diastase. Additional 4-μm sections were used for immunohistochemical study.

The presence of GCDFP-15 was evidenced by monoclonal antibody BRST-2 (D6 clone, Signet Laboratories, Mass., USA) [16] diluted 1:60 with incubation for 30 min at room temperature. Reactions were carried out using the ABC method (avidin-biotin peroxidase complex by Vector Laboratories Burlingame, Calif.). Peroxidase was developed with diaminobenzidine tetrahydrochloride and hydrogen peroxide. Sections were counterstained with hematoxylin, dehydrated in alcohols, cleared in xylene and mounted.

A section of axillary skin with apocrine glands was included as positive control. Negative controls were carried out omitting the primary antibody.

Three fetal breasts of 32, 36, and 40 weeks of gestational age were processed for electron microscopy study. The specimens were fixed at 4°C for 90 min in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2, washed in several changes of the same buffer at 4°C and post-fixed in OsO_4 in 0.1 M cacodylate buffer pH 7.2 at 4°C for 60 min. The fragments were dehydrated with alcohols, washed in propylene oxide, infiltrated with a mixture of Epon-Araldite and propylene oxide and embedded in Epon-Araldite. Semithin sections were cut and stained with methylene blue and toluidine blue (1:1), and observed with an optical microscope. Breast gland was observed in a breast of 40 weeks of gestational age, while only fibrous tissue was found in the other breasts. Thin sections were cut from selected blocks with a LKB Ultrotome III, placed on uncovered 200-mesh copper grids, stained with uranyl acetate and lead citrate and observed with CM12 Philips electron microscope at 80 kV.

Results

The fetal breasts at gestational age between 28 and 32 weeks appeared at low magnification as a small, well circumscribed nodule in the dermis. In the nodule we observed round-oval ducts with intraluminal amorphous material surrounded by cellular mesenchymal tissue (Fig. 1). The ducts were lined with a double coat of epithelial cells. The outer cells showed frequently indistinct borders, clear cytoplasm, and roundish, vesicular nuclei (Fig. 2); the inner cells showed weakly eosinophilic cytoplasm and frequently irregular luminal cytoplasmic projections (Fig. 2). In the late phases of pregnancy the breasts appeared more enlarged and extended in the subcutaneous tissue. The ducts were branched with round end buds. The ducts were lined with two or three layers of cells with the same features described above. PAS-diastase was negative.

In normal adult breasts we observed lobules composed of ductules and loose stroma. The ductules were lined with luminal epithelial cells and outer myoepithelial cells. Some ductules contained intraluminal amorphous secretion. No apocrine cells were present. In all breasts some lobules showed rare PAS-diastase-positive cells. The intraluminal amorphous secretion was also PAS-diastase positive.

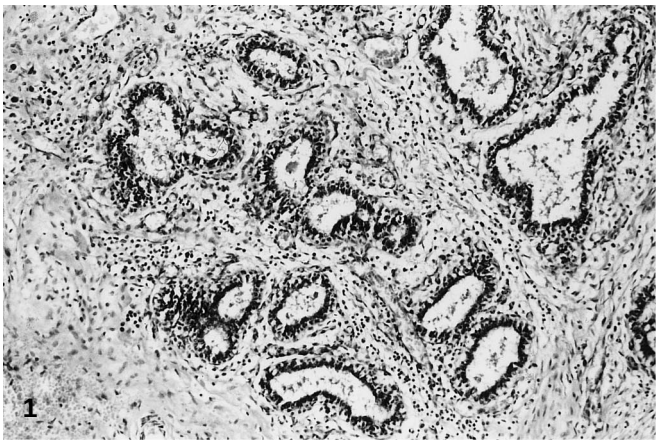
In all the fetal breasts we observed scattered epithelial cells with cytoplasmic, diffuse positivity for GCDFP-15. These positive cells were localized in some ducts with no particular distribution pattern, and their number increased with gestational age. A higher number of GCDFP-15-immunoreactive cells was observed in the breast at 40 weeks of gestational age (Figs. 3, 4). Sweat glands of the skin overlying the breasts showed numerous GCDFP-15-immunoreactive cells, while the squamous epithelium was negative for GCDFP-15.

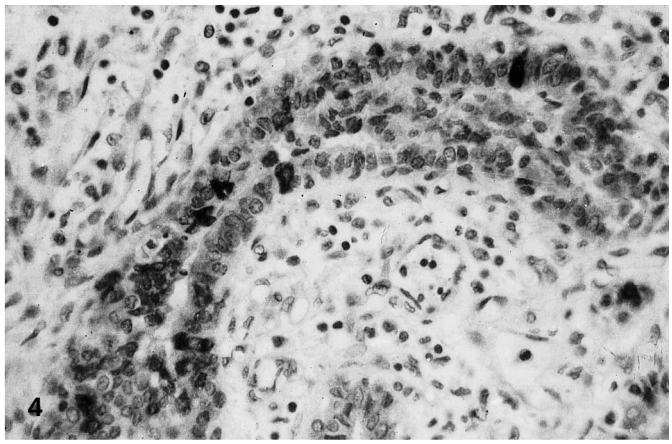
Scattered GCDFP-15-immunoreactive cells with no apocrine feature were observed in some normal lobules (Fig. 5).

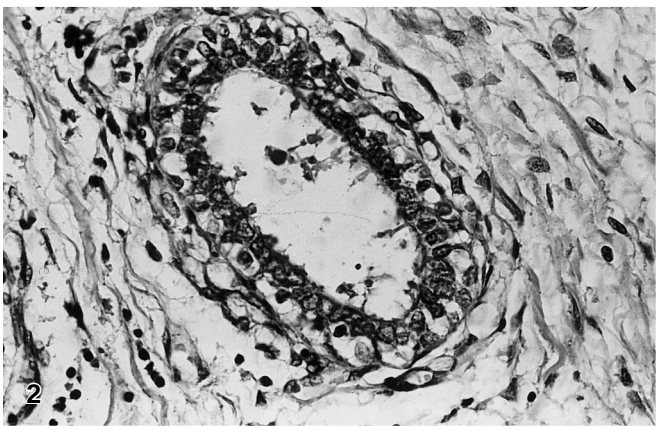
In the fetal breast at 40 weeks we observed well-defined ducts with lumina. The lumina contained finely granular electron-dense material and fragments of cytoplasm, which detached from the epithelial cells. The ducts were lined with two or three cell layers (Fig. 6). The inner cells showed roundish nuclei with finely granular chromatin and distinct nucleoli. The cytoplasm showed large mitochondria and moderately abundant rugose endoplasmatic reticulum with dilated cisternae. Intracellular lumina with microvilli were sometimes observed. The outer cells were smaller and showed nuclei with chromatin condensed in clusters. The cytoplasm showed similar features to those of the inner cells. The cells were connected by well-developed desmosomes. No apocrine cells were observed.

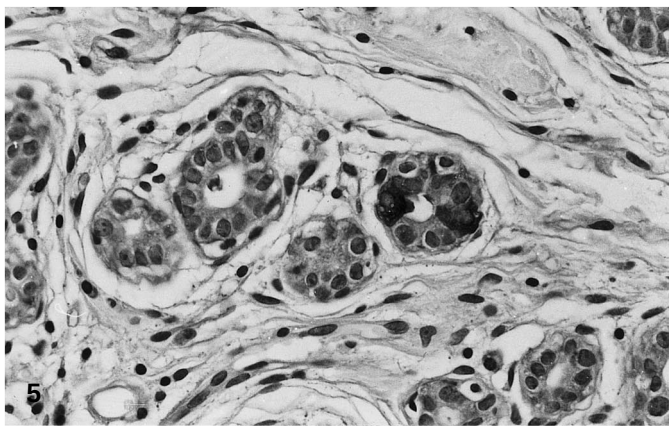
Discussion

The apocrine epithelium of the breast is considered to reflect a metaplastic alteration in epithelial structures of the terminal duct-lobular units and has been intensely studied for a possible relationship to cystic disease of the breast and breast carcinoma. Apocrine cells are considered to be the progenitors of breast cystic disease [20]. The apocrine epithelium associated with secretion accumulation may favour the progressive unfolding of lobules, formation of microcysts and finally the appearance of macrocysts [20]. Another interesting point is the relationship between apocrine cystic changes and breast car-









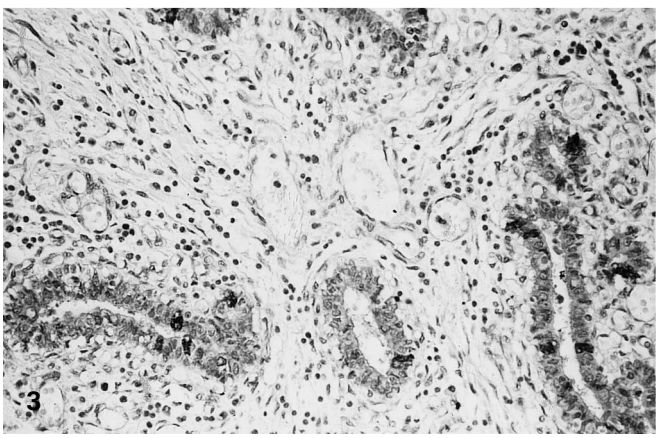


Fig. 1 Fetal breast at 32 weeks of gestational age. Some roundish mammary ducts surrounded by cellular connective tissue. Haematoxylin-eosin, ×100

Fig. 2 Fetal breast at 32 weeks of gestational age. A mammary duct lined with two layers of cells. Inner cells show weakly eosinophilic cytoplasm; outer cells are with clear cytoplasm. Haematoxylin-eosin, ×250

Fig. 3 Fetal breast at 40 weeks of gestational age. Some mammary ducts with scattered GCDFP-15-positive cells. BRST-2 monoclonal antibody, ×100

Fig. 4 Fetal breast at 40 weeks of gestational age. A duct with scattered GCDFP-15-immunoreactive cells; the positivity is intense and diffusely cytoplasmic. BRST-2 monoclonal antibody ×250

Fig. 5 Normal adult breast. Acini showing two luminal cells with immunoreactivity for GCDFP-15. Myoepithelial cells are negative. BRST-2 monoclonal antibody, ×250

cinoma. Clinical follow-up studies of women with breast apocrine cystic disease evidenced an increased risk of subsequent breast carcinoma [19]. Cystic apocrine metaplasia was more common in cancer-associated breasts than in the normal breast and also the number of foci of apocrine cysts was more numerous in breasts with cancer [19]. Some hypotheses regarding the relationship between apocrine epithelium and carcinoma have been proposed [7]. First, the apocrine epithelium may be a precursor of malignant trasformation; secondly, it may reflect a response to the same stimulus which promotes carcinoma; and thirdly the apocrine epithelium could indicate an instability of the breast epithelium, which causes the development of alterations with a higher propensity for cancer. However, the data regarding the relationship between apocrine epithelium, cysts and cancer are unclear, and the stimuli regulating these processes are unknown. In any case, the apocrine epithelium is frequently observed in the breast tissue. In the histological reviews of normal breasts, apocrine epithelium was found in a higher percentage of women [4, 19] than was cancer. Furthermore, apocrine cells are not always of lobular origin, as evidenced by their presence in gross duct papillomas [17].

All these features demonstrate that the apocrine epithelium of the breast is still an enigma. A variety of hypotheses have been postulated to explain the presence of apocrine epithelium in the breast: that it is a metaplastic

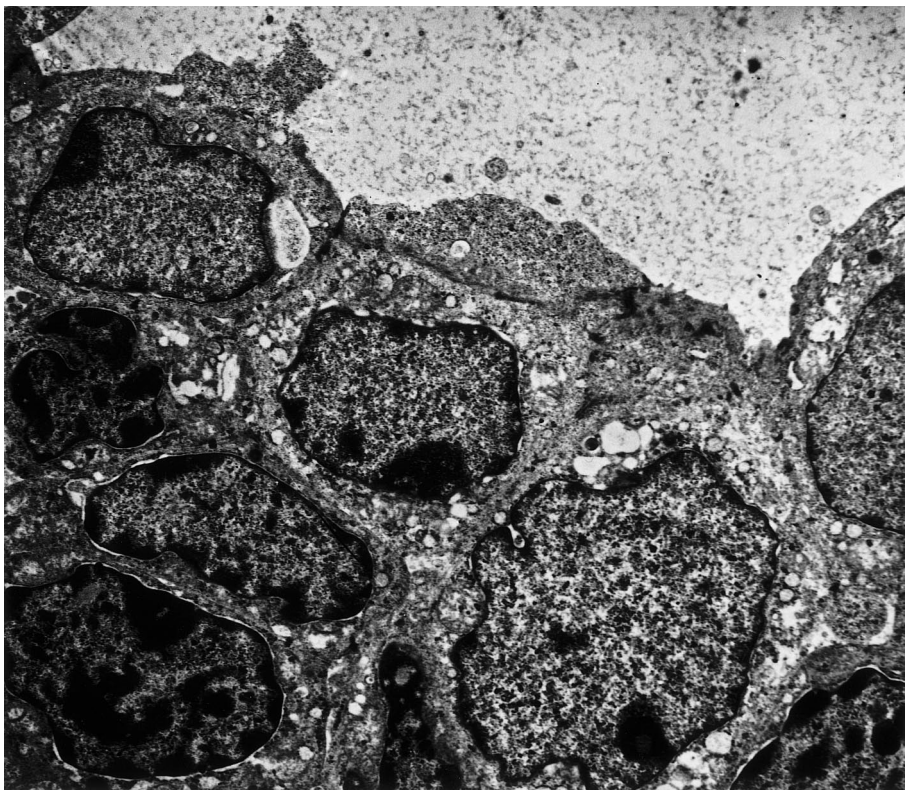


Fig. 6 Fetal breast at 40 weeks of gestational age (electron microscopy). A mammary duct lined with three layers of cells, with no apocrine feature. ×7000

phenomenon, [20]; that it is a degenerate epithelium simulating true apocrine epithelium [5], and that it derives from apocrine glands entrapped in the breast during development. A further hypothesis is that apocrine epithelium is a normal constituent of the breast.

We have examined apocrine differentiation during fetal breast development by immunohistochemistry. A series of monoclonal antibodies against GCDFP-15 has been developed [16], and among these BRST-2 (D6) monoclonal antibody shows the highest capacity to stain GCDFP-15 in formalin-fixed, paraffin-embedded apocrine tissues [13]. BRST-2 also showed specificity for apocrine tissues, in that non-apocrine normal tissues were BRST-2 negative except for serous cells of salivary glands and serous cells of bronchial glands [13]. An immunohistochemical study we performed on a broad series of normal adult tissues using monoclonal antibody BRST-2 (personal communication) has confirmed that GCDFP-15 is only expressed in glandular structures and, in particular, it is associated with apocrine differentiation. Furthermore, we observed that GCDFP-15 is expressed in fetal tissues, as evidenced by its presence in glandular structures at 28 weeks of gestational age.

We have found that GCDFP-15-positive cells are present in all fetal breasts at different gestational ages and their number increases during breast fetal development. Electron microscopy study of a fetal breast at 40 weeks failed to show cells with apocrine features. Scattered GCDFP-15-positive and rare PAS-diastase-positive cells were observed in some lobular structures of normal adult breasts.

These data suggest that cells with functional features of apocrine differentiation are present during fetal breast development and persist in adult normal breast parenchyma. However, the presence of cells without apocrine morphology but with a molecular signal of apocrine differentiation has been highlighted by in situ hybridization studies [11]. These GCDFP-15-positive cells are probably the precursors of apocrine epithelium; unknown stimuli may cause them to mature, take on the complete apocrine morphology, and proliferate. Rare apocrine carcinomas of the breast and GCDFP-15-positive breast carcinomas without apocrine morphology [6, 11] might originate from these cells. It may be that apocrine epithelium of the breast is not a metaplastic event, but a normal process of differentiation of the breast gland. We propose the term “apocrine differentiation precursor cells” for those GCDFP-15-positive cells with no apocrine morphology observed in fetal and adult normal breasts.

Acknowledgments This work was supported by the A.I.R.C. (Italian Association for Cancer Research). The authors thank Mrs. Rosetta Biondi and Federico Soldani for their technical assistance.

References

1. Abati AD, Kimmel M, Rosen PP (1990) Apocrine mammary carcinoma: a clinicopathological study of 72 cases. Am J Clin Pathol 94:371–377
2. Ahmed A (1975) Apocrine metaplasia in cystic hyperplastic mastopathy. Histochemical and ultrastructural observations. J Pathol (Lond) 115:211–214

3. Azzopardi JG (1979) Problems in breast pathology. Saunders, Philadelphia
4. Cowan DF, Herbert TA (1989) Involution of the breast in woman aged 50 to 104 years: a histopathologic study of 102 cases. Surg Pathol 2:323–333
5. Dawson EK (1932) Sweat gland carcinoma of the breast. A morphohistological study. Edinburgh Med J 39:409–438
6. Eusebi V, Millis RR, Cattani MG, Bussolati G, Azzopardi JG (1986) Apocrine carcinoma of breast. Am J Pathol 123:532–541
7. Haagensen DE (1991) Is cystic disease related to breast cancer? Am J Surg Pathol 15:687–694
8. Haagensen DE, Mazoujian G, Dilley WG, Pedersen CE, Kisters SJ, Wells SA (1979) Breast gross cystic disease fluid analysis. I. Isolation and radioimmunoassay for a major component protein. J Natl Cancer Inst 62:239–247
9. Higginson JF, McDonald JR (1949) Apocrine tissue, chronic cystic mastitis and sweat gland carcinoma of the breast. Surg Gynecol Obstet 88:1–10
10. Krompecher EU (1913) Über Schweiszdrüsen der Brustdrüse und deren Krebse. Verh Dtsch Ges Pathol 16:365–384
11. Losi L, Lorenzini R, Eusebi V, Bussolati G (1995) Apocrine differentiation in invasive carcinoma of the breast. Comparison of monoclonal and policlonal gross cystic disease fluid protein-15 antibodies with prolactin-inducible protein mRNA gene expression. Appl Immunohistochem 3:91–98
12. Mazoujian G, Pinkus GS, Davis S, Haagensen DE (1983) Immunohistochemistry of a breast gross cystic disease fluid protein (GCDFP-15): a marker of apocrine epithelium and breast carcinomas with apocrine features. Am J Pathol 110:105–112
13. Mazoujian G, Parish TH, Haagensen DE (1988) Immunoperoxidase localization of GCDFP-15 with mouse monoclonal antibodies versus rabbit antiserum. J Histochem Cytochem 36:377–382
14. Murad TM, Hamm E von (1968) The ultrastructure of fibrocystic disease of the breast. Cancer 22:587–600
15. Pagani A, Eusebi V, Bussolati G (1994) Detection of PIP/GCDFP-15 gene expression in apocrine epithelium of the breast and salivary glands. Appl Immunohistochem 2:29–35
16. Parish TH, Wu YJ, Rittenhaus C, Davis S, Haagensen D, Ip SHC (1985) Generation of monoclonal antibodies reactive with glycoprotein marker of apocrine epithelium and metastatic breast carcinoma. In: Peeters H (ed) Protides of the biological fluids. Pergamon Press, Oxford, pp 517–521
17. Pier WJ, Garancis JC, Kuzma JF (1970) The ultrastructure of apocrine cells in intracystic papilloma and fibrocystic disease of the breast. Arch Pathol 89:446–452
18. Tremblay G (1968) Histochemical studies of oxidative enzymes in apocrine like cells of the breast and in axillary apocrine glands. J Invest Dermatol 50:238–243
19. Wellings SR, Alpers CE (1987) Apocrine cystic metaplasia: subgross pathology and prevalence in cancer-associated versus random autopsy breasts. Hum Pathol 18:381–386
20. Wellings SR, Jensen HM, Marcum RG (1975) An atlas of subgross pathology of the human breast with special reference to possible precancerous lesions. J Natl Cancer Inst 55:231–243