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Spectrum of GCDFP-15 expression in human fetal and adult normal tissues

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Abstract GCDFP-15, a glycoprotein identified in the cyst fluid of cystic breast disease, is considered to be a marker of apocrine differentiation. Studies on GCDFP-15 localization in adult normal tissues are lacking, and no information on GCDFP-15 expression during fetal development has been reported. We investigated GCDFP-15 expression in a large series of formalin-fixed, paraffin-embedded normal human adult and fetal tissues using the monoclonal antibody BRST-2. In normal adult tissues GCDFP-15 expression was found in all apocrine, lacrimal, ceruminous and Moll's glands and in numerous serous cells of the submandibular, sublingual and minor salivary glands. The serous cells of nasal and bronchial glands were also positive; parotid and laryngeal glands showed rare immunoreactive cells. GCDFP-15-positive cells were observed in all cutaneous eccrine glands from different body sites. In fetal tissues immunoreactivity was observed in numerous acinous cells of all tracheal, bronchial and submandibular salivary glands. GCDFP-15 positivity was identified in numerous cells of all axillary sweat glands and in rare cells of some sweat glands of the thorax, abdomen, back, leg and arm. In both apocrine and nonapocrine glands GCDFP-15 was always localized in the secretory component. These data suggest that GCDFP-15 is a glandular differentiation marker associated with apocrine secretion; that it is expressed in glands that have phylogenetic origins in common with apocrine glands (submandibular salivary and submucosal bronchial glands); and that eccrine cutaneous glands express GCDFP-15 and thus might be referred to as mixed apocrine-eccrine glands. GCDFP-15 is expressed during fetal development and may represent a common marker of embryologically linked glandular structures.

Key words GCDFP-15 · Apocrine differentiation · Apocrine glands · Human fetal tissues

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Introduction

Human cystic breast disease is a common benign condition, which occurs most frequently before the menopause. This condition is characterized by the production in the cysts of a fluid secretion containing four major protein components named gross cystic disease fluid proteins (GCDFPs), with molecular weights of 70, 44, 24 and 15 kDa [7].

The main characteristics of GCDFPs are that they are immunologically similar to albumen and that GCDFP-44 is immunologically identical to the plasma zinc alpha$_2$ glycoprotein [3], but is present at concentrations 5–30 times that in plasma. This protein has been found by immunohistochemistry in apocrine cells of the breast and skin, some cells of parotid glands, a few acinar and ductal epithelial cells of the normal breast and in some breast carcinomas [2]. GCDFP-24 is the major component protein of breast cystic fluid [7], and it has a normal plasma analogue identified as apoliprotein D [1]; this protein has been found by immunohistochemistry [11] in apocrine cells, renal tubules, adrenal cortex and pituitary gland. GCDFP-15 is a protein that is also present at a very low concentration in the plasma of normal women, but its average concentration is 140,000 times as high in breast cyst fluid as in plasma [7].

GCDFP-15 is the most widely studied component protein of the cystic fluid. The presence of GCDFP-15 has been demonstrated by immunohistochemical [5, 10, 13, 16, 23], ultrastructural [14] and in situ hybridization [18] studies in normal apocrine glands, in apocrine epithelium of the breast and in some breast carcinomas. Detectable levels of GCDFP-15 are also present in salivary, lacrimal and bronchial glands [13]. Salivary gland tumours [21] and sweat gland tumours of probable apocrine origin [9, 12] also show GCDFP-15 immunoreactivity. The GCDFP-15 gene has recently been isolated and its chromosomal localization determined [17]. GCDFP-15 production in the breast appears to be under hormonal control [8]; furthermore, GCDFP-15 seems to exert a specific mitogenic activity on the breast cell lines [4].

On the basis of these data, GCDFP-15 could be considered to be a specific apocrine marker with a potential value in clarifying some features of apocrine differentiation in specific tissues and tumours, such as those of the breast and skin.

In order to confirm the apocrine specificity of GCDFP-15 we decided to evaluate the localization of this molecule in a broad series of normal adult and fetal tissues, using immunohistochemistry.

Materials and methods

The localization of GCDFP-15 was detected by immunohistochemistry in normal adult and fetal tissues.

Normal adult tissues were retrieved from the files of our Institute and collected from biopsy and autopsy material. Three different specimens of each type of tissue were analysed. The tissues were taken from the respiratory system: nose, larynx, trachea (autopsy), bronchi, lung; the cardiovascular system: myocardium, endocardium, pericardium and aorta; the digestive system: tongue, parotid gland, submandibular and sublingual salivary glands, minor salivary glands, oesophagus, stomach, duodenum, small intestine, appendix, large intestine, anal canal, liver, exocrine pancreas, gallbladder, extrahepatic bile duct; the genitourinary system: kidney, ureter, bladder, prostate, seminal vescicles, deferent ducts, epididimous, testis, ovary, salpinx, proliferative and secretive endometrium, myometrium, uterine cervix and vagina; the endocrine glands: thyroid, parathyroid, adrenal gland, endocrine pancreas, hypophysis; the haemolymphatic tissues: lymph nodes, spleen, thymus, tonsil, bone marrow; the neuromuscular tissue: cerebral cortex, cerebellum, mesencephalon, pons, medulla oblongata, spinal cord (all autoptic tissues), peripheral nerve and skeletal muscle; bone and cartilage; the eye and its appendages: retina, uvea, cornea, conjunctiva, lacrimal gland, eyelid; and skin from different sites: scalp, axilla, thorax, back, abdomen, perianal, leg, arm, retroauricular, sole of the foot, palm of the hand and external acoustic meatus with ceruminous glands. Finally, the placenta was examined.

Normal fetal tissues were taken from a series of 8 fetuses of gestational age from 28 to 40 weeks sent to our Institute with autopsy requests. Fetuses were products of spontaneous abortions or newborns that had died soon after birth. They were not macerated and had died from pneumonia, cerebral haemorrhage and hyaline membrane disease. The specimens were taken from various systems: respiratory system: larynx, trachea, bronchi and lung; cardiovascular system: heart and aorta; digestive system: parotid and submandibular salivary glands, oesophagus, stomach, small and large intestine, appendix, liver, gallbladder, exocrine pancreas; genitourinary system: kidney, ureter, bladder, prostate, testis, ovary and uterus; endocrine system: thyroid, adrenal gland and endocrine pancreas; lymphatic tissue: lymph nodes, spleen, and thymus; neuromuscular tissue: cerebral cortex, cerebellum, mesencephalon, pons, medulla oblongata and skeletal muscle; and skin from the axilla, back, thorax, abdomen, leg, and arm.

The presence of GCDFP-15 was demonstrated by monoclonal antibody BRST-2 at a dilution of 1:60. The preparation of BRST-2 (D6 clone, Signet Laboratories, Mass.) has been described in detail elsewhere [19]. Among a series of monoclonal antibodies produced against GCDFP-15, BRST-2 showed the highest capacity to stain GCDFP-15 in formalin-fixed, paraffin-embedded apocrine tissues [15]. BRST-2 showed specificity for apocrine tissues in that all nonapocrine normal tissues were BRST-2 negative except for serous cells of bronchial glands and serous cells of salivary glands [15].

Sections of formalin-fixed, paraffin-embedded tissues 4 μm thick were used. After deparaffinization in xylene, the sections were hydrated in graded alcohol and incubated for 15 min in methanol with 1% H_2O_2 to eliminate endogenous peroxidase activity. The sections were then washed in phosphate-buffered saline (PBS, pH 7.4) and incubated in 10% normal horse serum for 15 min. This was diluted in PBS together with the following antibody solutions. After each incubation, the slides were washed for 10 min in PBS and all steps were performed at room temperature. Three successive incubations of 30 min each were performed using: (1) primary monoclonal antibody BRST-2 (2) biotinylated secondary antimouse antibody (1:500 Vector Laboratories, Burlingame, Calif.) (3) avidin–biotin peroxidase complex (Vector Laboratories). The reaction was developed for 1 min with 0.06% diaminobenzidine (DAB) and 0.02% H_2O_2 as substrate. Sections were counterstained with haematoxylin, then dehydrated in alcohol, cleared in xylene and mounted.

Negative controls were carried out by omitting the primary antibody in the procedure. Positive controls were apocrine cysts of the breast.

Results

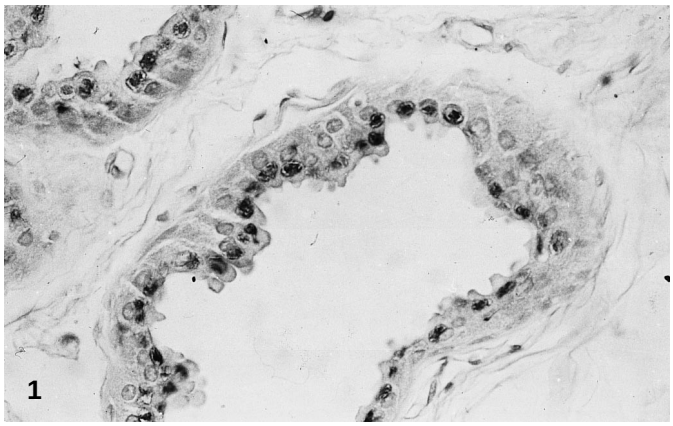
The results of GCDFP-15 expression in normal adult and fetal tissues are summarized in Tables 1–4.

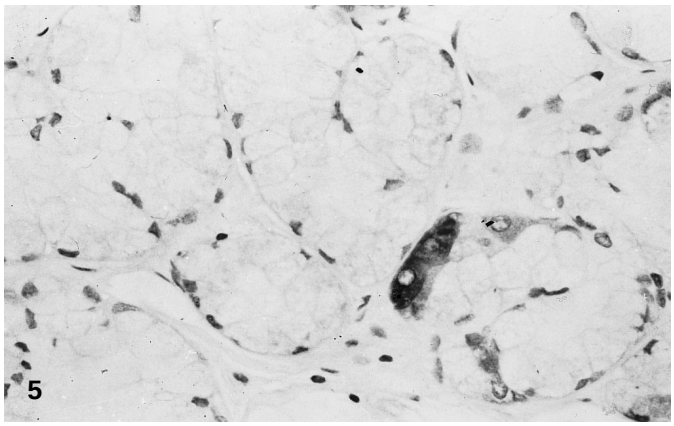
In normal adult tissues, examination of the skin revealed that all acinous cells of axillary (Fig. 1) and perianal apocrine glands showed intense, supranuclear or, more rarely, diffuse cytoplasmic GCDFP-15 positivity. All axillary (Fig. 2) and perianal eccrine glands showed 80% positive cells in the secretory coil. In other cutaneous sites all eccrine glands showed about 50% of GCDFP-15 immunoreactive cells in the secretory coil (Fig. 3), while the ductal segments resulted negative. An intense, cytoplasmic GCDFP-15 immunoreactivity was observed in all cells of the ceruminous glands. Other skin structures, such as epidermis, sebaceous glands and hair follicles, were negative.

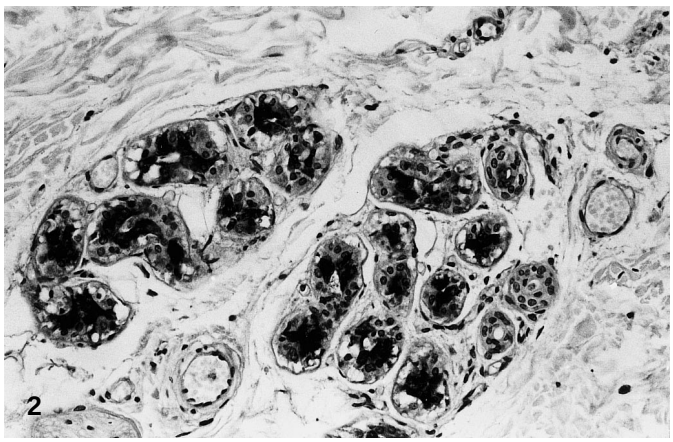
In the respiratory system a large number of GCDFP-15 immunoreactive serous cells was found in all glands of the nose, trachea and bronchi (Fig. 4). The mucous cells and ducts were negative. In the larynx the positive cells were rare and localized in some glands. The other structures of these organs were negative.

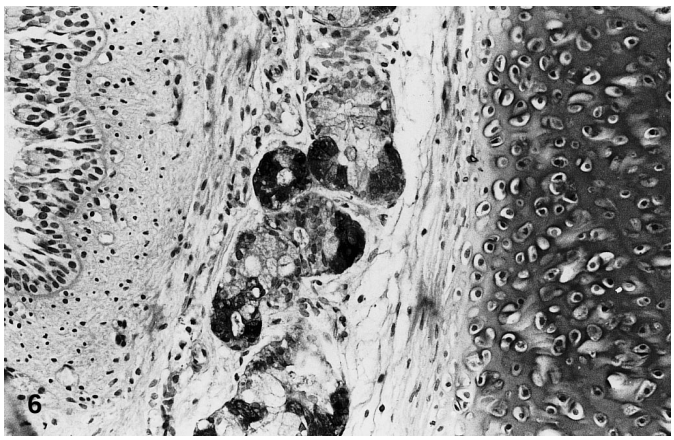
Table 1 GCDFP-15 immunoreactivity in normal human adult skin

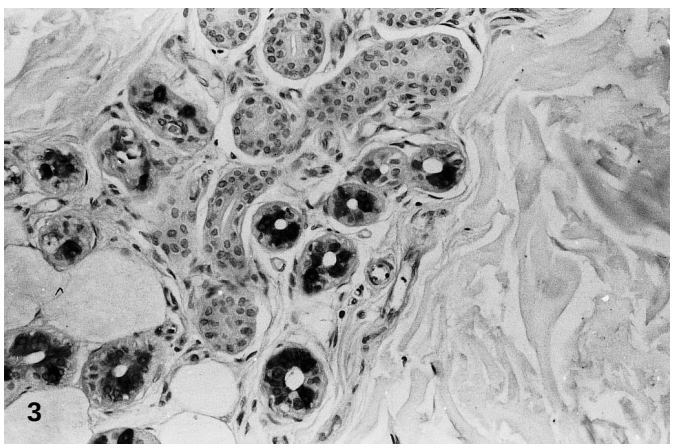
Structures	Immunoreactive cells (%)	Site of positivity
Epidermis	0	–
Hair follicles	0	–
Sebaceous glands	0	–
Stromal cells	0	–
Apocrine glands		
Axilla	100	Acini
Perianal	100	Acini
Moll's glands	100	Acini
Ceruminous glands	100	Acini
Eccrine glands		
Axilla	80	Secretory coil
Perianal	80	Secretory coil
Abdomen	60	Secretory coil
Back	50	Secretory coil
Thorax	50	Secretory coil
Sole of the foot	40	Secretory coil
Scalp	40	Secretory coil
Palm of the hand	40	Secretory coil
Leg	40	Secretory coil
Arm	40	Secretory coil











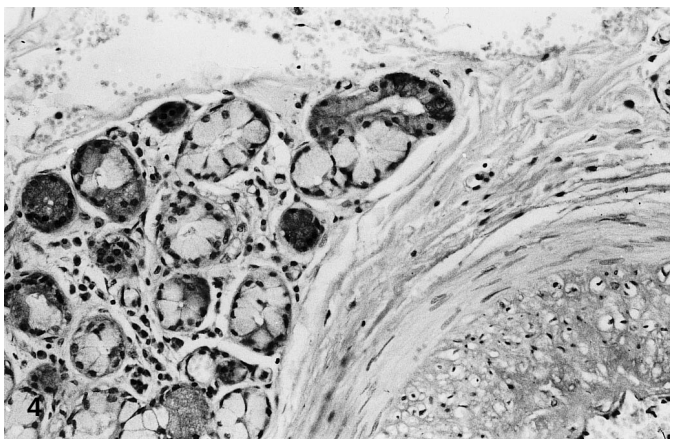


Fig. 1 Adult human skin, axilla. Apocrine gland lined with cells with intense supranuclear immunoreactivity for GCDFP-15. BRST2 monoclonal antibody, X250

Fig. 2 Adult human skin, axilla. Eccrine glands with numerous cells showing diffuse cytoplasmic positivity for GCDFP-15. BRST2 monoclonal antibody, X250

Fig. 3 Adult human skin, leg. Eccrine gland with some cells of the secretory coil showing strong cytoplasmic GCDFP-15 immunoreactivity. BRST2 monoclonal antibody, X250

Fig. 4 Adult human bronchus. Submucosal glands with serous cells showing a diffuse, moderately intense, GCDFP-15 positivity. BRST2 monoclonal antibody, X300

Fig. 5 Adult human minor salivary gland. Two serous cells show diffuse, intense, cytoplasmic immunoreactivity for GCDFP-15; all mucous cells are negative. BRST2 monoclonal antibody, X300

Fig. 6 Human fetal trachea (32 weeks). Submucosal glands show numerous cells with intense cytoplasmic immunoreactivity for GCDFP-15. BRST2 monoclonal antibody, X300

Table 2 GCDFP-15 immunoreactivity in normal human adult noncutaneous tissues

Structures	Immunoreactive cells (%)	Site of positivity
Epithelium[a]	0	–
Stromal cells	0	–
Glands		
Nose	Serous cells (60)	Acini
Larynx	Serous cells (10)	Acini
Trachea	Serous cells (80)	Acini
Bronchi	Serous cells (80)	Acini
Salivary glands		
Submandibular	Serous cells (70)	Acini
Sublingual	Serous cells (70)	Acini
Minor	Serous cells (70)	Acini
Parotid	Serous cells (10)	Acini
Lacrimal	Serous cells (100)	Acini

[a] Epithelium of nose, larynx, trachea and bronchi

Table 3 GCDFP-15 immunoreactivity in normal human fetal skin

Structures	Immunoreactive cells (%)
Epidermis	0
Hair follicles	0
Stromal cells	0
Sweat glands	
Axilla	40
Perianal	40
Abdomen	10
Back	10
Thorax	10
Leg	10
Arm	10

In the digestive system GCDFP-15 immunoreactivity was identified in the serous cells of minor salivary glands (Fig. 5), sublingual and submandibular salivary glands. The parotid gland showed rare, weakly positive, alveolar cells. In all salivary glands the extra-alveolar ducts were negative. Oesophagus, stomach, intestine, liver, gallbladder, extrahepatic bile duct and pancreas were also negative.

From the examination of the eye and its appendages, lacrimal and Moll's glands showed that 100% of acinous cells had diffuse cytoplasmic positivity for GCDFP-15. The other ocular structures were negative. All the genitourinary organs, endocrine glands, cardiovascular, haemolymphatic and neuromuscular tissue, bone, cartilage and placenta, were negative.

When normal fetal tissues were examined all axillary sweat glands were found to have about 40% of GCDFP-15-positive cells in the secretory coil. In other cutaneous sites GCDFP-15 was expressed in rare cells localized in some of the sweat glands. In the respiratory system a large number of GCDFP-15-positive cells was observed in the acini of all tracheal (Fig. 6) and bronchial glands, while rare immunoreactive cells were found in some glands of the larynx. The other structures of these organs were negative. In the digestive system numerous GCDFP-15-positive cells were observed in the acini of the submandibular salivary gland, while the extra-alveolar ducts were negative. Oesophagus, stomach, intestine, gallbladder, appendix, liver and pancreas were negative. The endocrine glands, genitourinary organs, lymph nodes, thymus, cardiovascular and neuromuscular tissue and umbilical cord were negative.

Table 4 GCDFP-15 immunoreactivity in normal human fetal noncutaneous tissues

Structures	Immunoreactive cells (%)	Site of positivity
Epithelium[a]	0	–
Stromal cells	0	–
Glands		
Larynx	10	Acini
Trachea	40	Acini
Bronchi	30	Acini
Salivary glands		
Submandibular	40	Acini
Parotid	10	Acini

[a] Epithelium of larynx, trachea and bronchi

Discussion

Apocrine cells derive their name from the way their secretion appears on light microscopy, where the release of secretory products is associated with loss of the apical cellular portion. The cells have a columnar eosinophilic cytoplasm containing PAS-positive diastase-resistant granules, and ultrastructurally they display osmiophilic granules and empty vesicles at the apical cellular pole [5].

These cells are a normal constituent of the apocrine glands of axillary and anogenital skin and are frequently observed in the breast, where they may display an eosinophilic granular or foamy cytoplasm [6]. The apocrine breast epithelium is considered to reflect a metaplastic alteration of the native epithelial cells. Recently Eusebi et al. [6], in an extensive and detailed review of apocrine mammary cells, have suggested that the apocrine epithelium is a normal constituent of the breast, and they use the term apocrine differentiation to define apocrine mammary cells.

Apocrine cells express GCDFP-15, which is a glycoprotein identified in the cyst fluid aspirated from patients with cystic breast disease [7]. An immunohistochemical and in situ hybridization study [18] showed that GCDFP-15 was expressed in all apocrine cells and in serous cells of normal salivary and bronchial glands, while it was absent from other tissues. Some differences in the staining pattern were observed with the two methods. In the apocrine cells immunohistochemistry identified diffuse cytoplasmic GCDFP-15 positivity, although the stain predominated in the apical portion where the granules are localized. In contrast, in situ hybridization staining was predominantly around the nuclei, perhaps indicating that this method is more closely related to genetic GCDFP-15 synthetic machinery. In the breast both methods identified GCDFP-15 expression in some acinar cells not

showing structural apocrine features. These data suggest that GCDFP-15 may be regarded as a marker of apocrine cells. In particular, GCDFP-15 may make it possible to identify those cells without apocrine morphology but with apocrine genealogy or biochemical characteristics. These cells could be designated “cells with apocrine differentiation”.

A major problem regarding the specificity of GCDFP-15 for apocrine tissues is that detailed studies of GCDFP-15 localization in normal tissues are lacking and that no information is provided regarding the expression of this marker during fetal development.

We carried out this study with the aim of confirming the specificity of GCDFP-15 to apocrine tissues. In normal adult tissues GCDFP-15 positivity was found in all apocrine, lacrimal, ceruminous and Moll’s glands and in numerous serous cells of submandibular, sublingual and minor salivary glands; serous cells of nasal and bronchial glands were positive. Parotid and laryngeal glands showed rare immunoreactive cells. In skin from different body sites, different percentages of GCDFP-15-positive cells were observed in all eccrine glands. In apocrine and non-apocrine glands GCDFP-15 positivity was restricted to the secretory component of the gland, while the ductal system was negative. In fetal tissues GCDFP-15 immunoreactivity was observed in numerous acinous cells of all tracheal, bronchial and submandibular salivary glands. GCDFP-15 positivity in the skin was demonstrated in numerous cells of most axillary glands and in rare cells of some sweat glands of the thorax, abdomen, back, leg and arm. Our data suggest that GCDFP-15 is a glandular differentiation marker, in that its expression has only been found in glandular structures and, in particular, in the secretory component of the glands. It is expressed in all apocrine glands and in glands that have phylogenetic origins in common with apocrine glands, including submandibular salivary glands and bronchial glands [13]; alternatively, in these nonapocrine serous glands GCDFP-15 immunoreactivity could be the product of a nonspecific reaction. Pagani et al. [18], using an in situ hybridization procedure, showed that in these serous cells there is a synthesis of GCDFP-15 and immunoreactivity therefore could not be the result of a cross-reaction of the antibody with other proteins.

GCDFP-15 is also expressed in a variable percentage of cells in adult eccrine glands; this suggests that GCDFP-15 is not a specific apocrine marker. However, GCDPF-15-positive cells in the eccrine glands may be cells with biochemical features of apocrine differentiation, and consequently the eccrine glands could be mixed apocrine-eccrine glands; in fact in the axilla a distinct type of sweat gland with apocrine and eccrine features (apo-eccrine glands) [20] has been described. Furthermore, a study on sweat gland tumours has shown that GCDFP-15 is localized in tumours of probable apocrine origin, while tumours of eccrine origin are negative [12]. In sweat gland tumours of uncertain histogenesis, such as chondroid syringoma (mixed tumours), GCDFP-15 positivity is always localized in areas with apocrine-like morphology [9]. However, GCDFP-15 immunoreactivity in eccrine glands should be confirmed by the in situ hybridization procedure in an attempt to confirm the presence of specific GCDFP-15 mRNA.

GCDFP-15 is expressed during fetal development, as shown by its presence in sweat, salivary, tracheal and bronchial glands from at least 28 weeks of gestational age. The number of GCDFP-15-positive cells in sweat glands is lower than in adults, and this could be due to the immaturity of the apocrine compartment during fetal development.

We also evaluated GCDFP-15 expression during breast development [22] in an attempt to explain the real nature of breast apocrine epithelium. We found GCDFP-15-immunoreactive cells without apocrine morphology in fetal and adult mammary glands. These results seem to confirm the hypothesis of Eusebi et al. [6], according to which development of the apocrine epithelium of the breast is a normal process of differentiation rather than a metaplasia. We suggest the term “apocrine differentiation precursor cells” for GCDFP-15-positive breast epithelial cells with no apocrine morphology.

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