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Immunohistochemical demonstration of keratins 8 and 14 in benign tumours of the skin appendage

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Summary. The expression of keratins 8 and 14 was investigated immunohistochemically by the avidin-biotin-peroxidase (ABC) method using formalin-fixed paraffin-embedded specimens from 42 tumours of human skin appendages. Results were compared with the staining of 34 specimens from normal skin and skin appendages adjacent to the tumours. Keratin 14 was detected by the monoclonal antibody (mAb) 312C8-1, and was found in the basal cells of the epidermis, the outer root sheaths of hair follicles, and the peripheral cells of sebaceous glands. It was also detected in the inner and outer layers of cells in the ductal portion and the myoepithelial cells in the secretory portion of apocrine and eccrine sweat glands. Keratin 8 was detected by mAb 35BH11, and was present in the secretory cells of eccrine and apocrine sweat glands but not in myoepithelial or ductal cells. The pilosebaceous apparatus and the epidermis were uniformly negative. In benign skin appendage tumours, the staining patterns for both keratins generally resembled their distribution in the corresponding normal tissues. The demonstration of keratins 8 and 14 may be useful in the recognition, classification and diagnosis of skin appendage tumours.

Key words: Keratin – Monoclonal antibody – Immunohistochemistry – Skin appendage tumour

Introduction

The classification of tumours of the skin appendages is based on their resemblance to the normal tissues from which these lesions are thought to arise. Tumours of uncertain histogenesis are mainly designated according to their most probable adnexal origin. Enzyme histochemistry and electron microscopic studies have partially clarified the histogenesis of such tumours (Hashimoto et al. 1987), but these methods are not readily applicable to routinely fixed paraffin-embedded materials. Immunohistochemistry has shed new light in this area by demonstrating specific antigens on routinely processed specimens. Various immunological tools have been used to detect antigens in the epidermis and adnexal structures for classifying tumours as well as for elucidating their histogenesis. Carcinoembryonic antigen (CEA) is present in eccrine and apocrine sweat glands but not in the pilosebaceous apparatus or the overlying epidermis (Penneys et al. 1981); thus the detection of CEA helps to distinguish sweat-gland-derived tumours from those arising from pilosebaceous units (Penneys et al. 1982). The expression of MBr1, MBr2, and GCDFP-15 has been reported to be specific for apocrine glands and apocrine-derived tumours (Menard et al. 1983; Mazoujian and Margolis 1988). Other antigens, such as MAM-3 and MAM-6 against human milk fat globule membranes (Tsubura et al. 1987), have also been used to investigate skin appendage tumours.

Keratins are a group of proteins consisting of several different polypeptides. Moll et al. (1982a) catalogued the major human keratins and identified them numerically as keratins 1–19. Different keratins are expressed specifically in different epithelial tissues and are considered to be good markers for epithelial differentiation; their study has allowed a number of advances in the classification of epithelial tumours. The generation of well-characterized monoclonal antibodies (mAbs) has facilitated progress in the field of immunohistochemistry and they have been used to demonstrate that different molecular weight keratins are localized to different domains within the human epidermis and skin appendages (Moll et al. 1982b; Gown and Vogel 1984; Cooper et al. 1985; Dairkee et al. 1985). Keratin expression in skin appendage tumours has been studied using mAbs that recognize keratin subsets (Noda et al. 1988; Zuk et al. 1988). However, mAbs that are specific for individual keratins provide more meaningful data. The present study investigated the keratin staining reactions in routinely processed tissue samples of benign skin appendage tumours and compared them with those in normal structures using

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two different mAbs, 35BH11 and 312C8-1, recognizing keratin 8 and 14, respectively.

Materials and methods

The 42 human skin appendage tumours employed in this study were retrieved from the files of the Pathology Division of Osaka Red Cross Hospital. Thirty-four normal skin and skin appendage tissue samples adjacent to the tumour specimens were also examined. All the tissues used had been routinely fixed in 10% formalin and embedded in paraffin. Sections were cut to 4 μm in thickness and stained with haematoxylin and eosin; other serial sections were subjected to the immunohistochemical studies. Specimens were classified from the haematoxylin and eosin stained sections using conventional histological criteria. The types of tumours and the numbers of cases investigated are listed in Table 2.

The two mAbs used have been described previously. mAb 312C8-1 identifies a keratin protein of molecular weight 51000 daltons that is catalogued as keratin 14 (Dairkee et al. 1985). mAb 35BH11 (ICN Immunobiologicals, Lisle, Ill., USA) identifies a keratin of molecular weight 52500 daltons and is catalogued as keratin 8 (Gown and Vogel 1984). The immunoglobulin subclass of both mAbs is IgM.

Immunoperoxidase staining was performed as described previously (Tsubura et al. 1988). Following deparaffinization, sections were hydrated and incubated for 20 min in 0.3% H_2O_2 to reduce endogenous peroxidase activity. Then the sections were digested with 0.1% (w/v) trypsin (Merck, Darmstadt, FRG) diluted with 0.01 M phosphate-buffered saline (pH 7.6) for 15 min at 37° C to enhance the antigenic exposure (Pinkus et al. 1985; Battifora and Kopinski 1986). After digestion, sections were rinsed with 0.01 M Tris-buffered saline (pH 7.2) and incubated with normal horse serum to block non-specific staining. Sections were then incubated with the primary antisera. Both mAbs (ascitic fluid) were used at a 1:500 dilution and incubation was performed for 1 h at room temperature. Biotinylated anti-mouse immunoglobulin and avidin-biotin complex (ABC staining kit, Vector Laboratories, Burlingame, Calif., USA) were used according to the manufacturer's instructions. Peroxidase activity was visualized with diaminobenzidine (Wako Pure Chemicals, Osaka, Japan) and counterstaining was carried out with haematoxylin. Preliminary trypsinization was necessary for demonstrating the immunoreactivity of keratin proteins in the formalin-fixed paraffin-embedded specimens, and after this preparatory step, similar staining was obtained to that seen with the more optimal alcohol-fixed or frozen materials. For negative controls, serial sections were incubated with non-immune

Table 1. Distribution of keratins 8 and 14 in normal skin and skin appendages

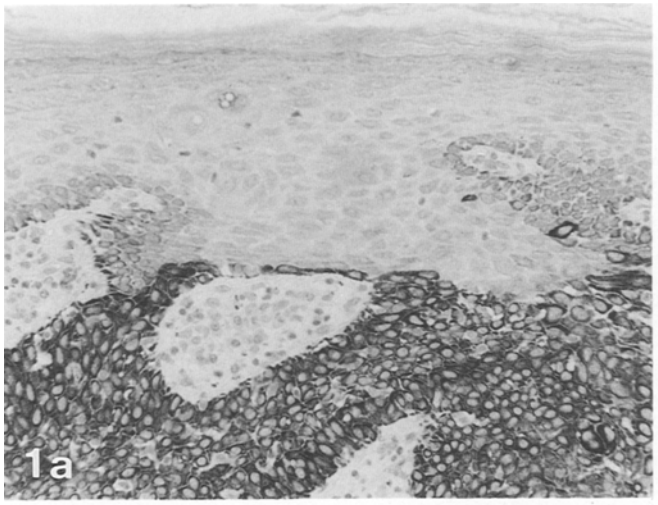
Antibody	35BH11	312C8-1
Specificity	Keratin 8	Keratin 14
Epidermis	−	+[a]
Eccrine sweat gland		
Duct cells		
Inner layer cells	−	+
Outer layer cells	−	+
Secretory coil		
Dark cells	+	−
Clear cells	+	−
Myoepithelial cells	−	+
Apocrine sweat gland		
Duct cells	−	+
Acinar cells		
Luminal cells	+	−
Myoepithelial cells	−	+
Sebaceous gland		
Duct cells	−	+[a]
Secretory portion		
Sebocytes	−	−
Seboblasts	−	+
Hair follicle	−	+[b]

[a] Basal cells were positive
[b] The outer root sheath cells were positive

Table 2. Staining patterns of benign tumours of the skin appendages with the monoclonal antibodies 35BH11 and 312C8-1

	No. of tumours	Antibody 35BH11	312C8-1
		Specificity Keratin 8	Keratin 14
Eccrine sweat gland tumours			
Eccrine poroma	4	Duct lining cells positive	Diffusely positive
Syringoma	4	Negative	Outer layer cells positive
Chondroid syringoma	11	Luminal cells positive	Basal cells positive
Apocrine sweat gland tumours			
Apocrine naevus	1	Same as normal	Same as normal
Papillary hidradenoma	3	Luminal cells positive	Negative
Sebaceous gland tumours			
Sebaceous naevus	4	Same as normal	Same as normal
Sebaceous adenoma	2	Same as normal	Same as normal
Hair follicle tumours			
Trichoepithelioma	3	Negative	Most cells positive
Pilomatrixoma	5	Negative	Negative
Trichilemmal cyst	2	Negative	All cells positive
Proliferating trichilemmal cyst	1	Negative	All cells positive
Epidermal cyst[a]	2	Negative	Basal cells positive

[a] Included for comparison



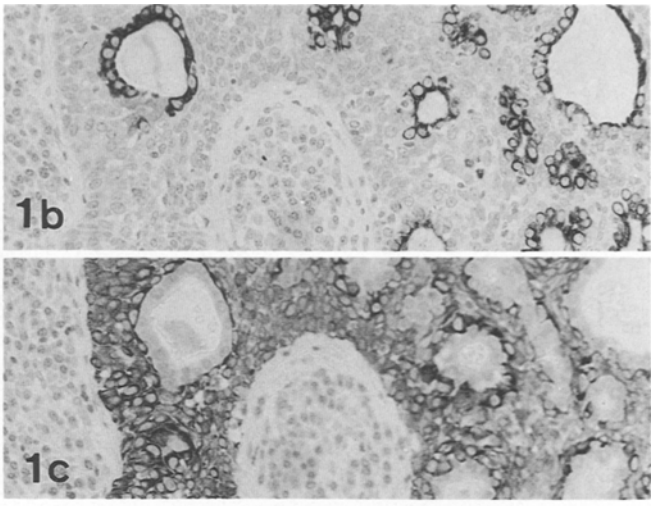
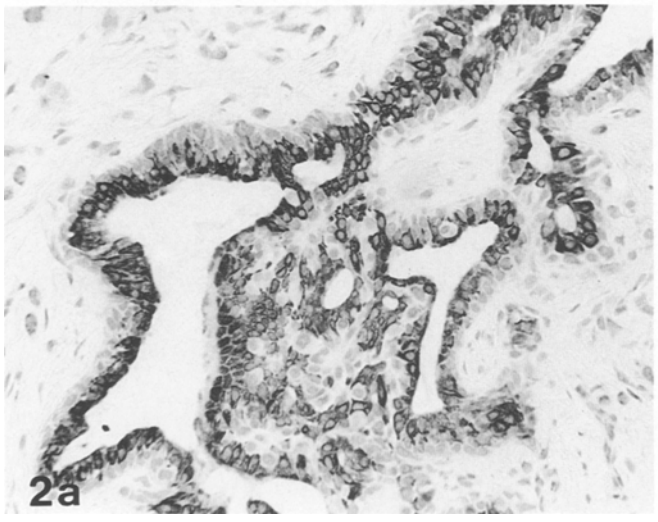


Fig. 1. a Eccrine poroma stained with 312C8-1. The poroma cells proliferating down towards the dermis are diffusely stained. ×200. **b, c** Eccrine poroma stained with 35BH11 and 312C8-1, respectively. The ductal lumens in the tumour cell nests are positive for 35BH11, while these cells are negative for 312C8-1. ×200



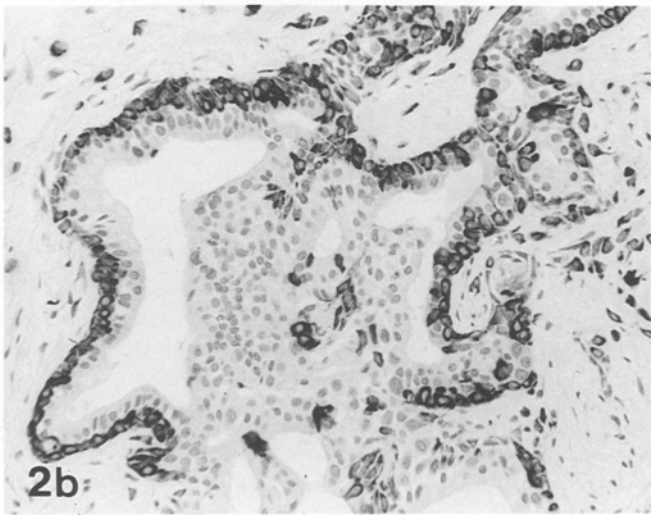


Fig. 2. a Chondroid syringoma stained with 35BH11. Positivity is restricted to the luminal cells. ×200. **b** Chondroid syringoma stained with 312C8-1. Positive reaction is seen in the basal cells and in cells intermingled with the cartilagenous matrix. ×200

mouse serum instead of the primary antibodies (at a comparable dilution). In addition, the ubiquity of the normal epidermis and skin appendages served as a built-in positive control.

Results

The sites of staining with 312C8-1 and 35BH11 in the normal human skin are summarized in Table 1. In general, the expression of the keratin 8 and 14 epitopes was constant and no differences in the labelling patterns were observed among the normal tissue components. A completely different pattern of reactivity was seen for the two mAbs. In the epidermis, 312C8-1 stained the basal cell layer, whereas no 35BH11-positive cells were demonstrated. In eccrine sweat glands, 312C8-1 stained the inner and outer layers of the intradermal duct epithelium as well as the cells of the acrosyringium and the myoepithelial cells of the secretory portion. 35BH11 invariably stained most of the dark cells and clear cells of the secretory portion. Myoepithelial cells and ductal cells were completely negative for 35BH11. In apocrine sweat glands, 35BH11 stained luminal cells positively and 312C8-1 stained the basal myoepithelial cells of the secretory portion. Ductal cells revealed a similar pattern of reactivity as seen in the eccrine ducts. In the pilosebaceous apparatus, 312C8-1 reacted with the peripherally located seboblasts as well as the basal cells of the sebaceous ducts. It also reacted with cells of the outer root sheath but not with any other hair components. With 35BH11, the pilosebaceous apparatus was invariably unstained.

As summarized in Table 2, the pattern of reactivity was similar in each type of lesion. In eccrine poromas, the proliferating cells were generally attached to the epidermis reaching downwards to the dermis. Tumour cells were diffusely positive for 312C8-1 in the solid tumour areas (Fig. 1 a) and were negative for 35BH11. However, a single row of cells around the ductal lumens was posi-

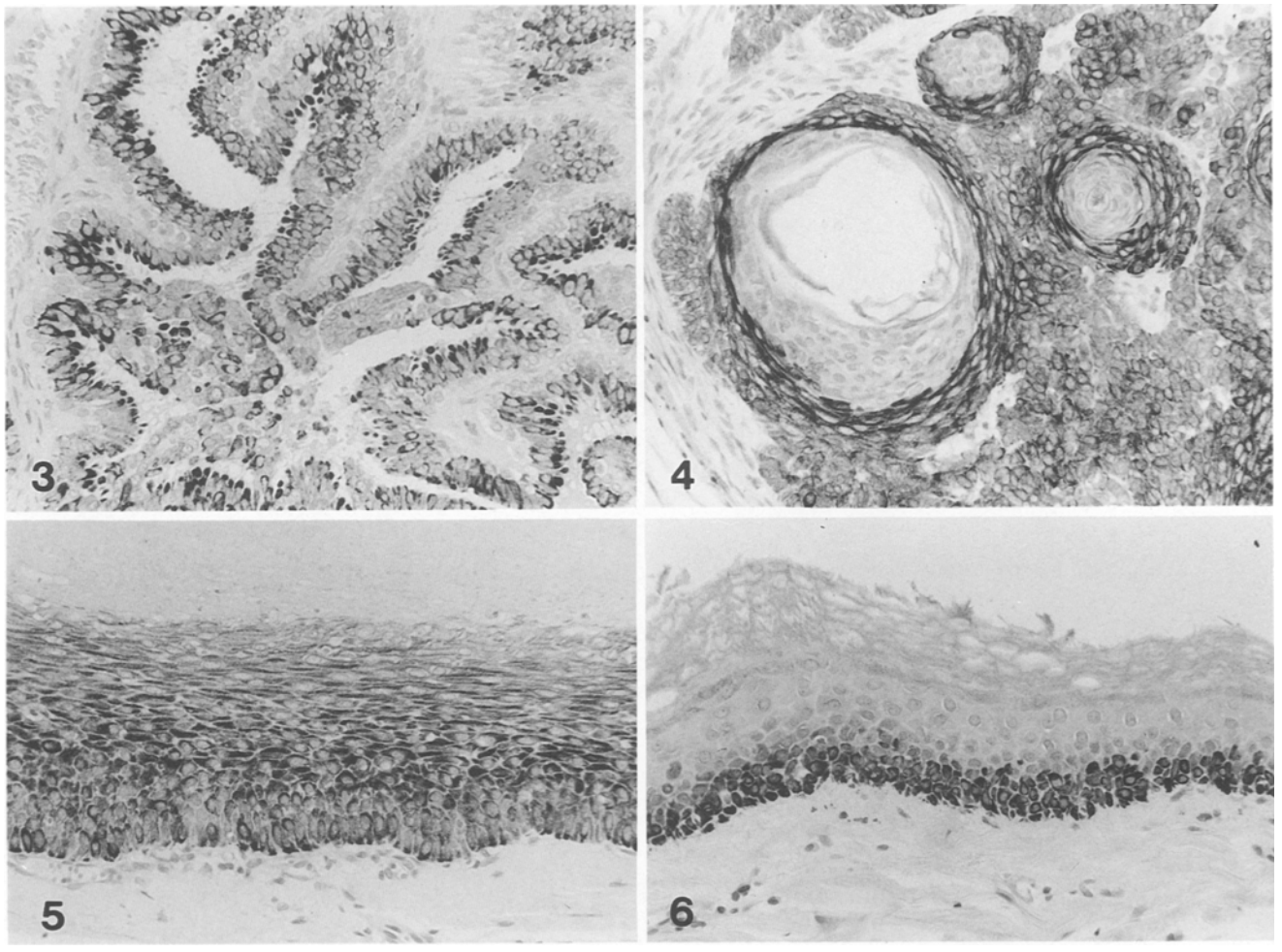


Fig. 3. Papillary hidradenoma stained with 35BH11. Luminal cells are selectively stained while basal cells are unstained. × 200

Fig. 4. Trichoepithelioma stained with 312C8-1. Positive staining of the tumour cells is seen, while several layers of cells surrounding the horn cyst are non-reactive. × 200

Fig. 5. Trichilemmal cyst stained with 312C8-1. Palisading basal cells and ballooning cells are all positively stained. × 200

Fig. 6. Epidermal cyst stained with 312C8-1. Only the basal cells are stained. × 200

tive for 35BH11 (Fig. 1b) and negative for 312C8-1 (Fig. 1c). Syringomas are composed of several rows of epithelial cells. A reaction for 312C8-1 was restricted to the outermost layer of the epithelial cords, while 35BH11 was completely negative. In chondroid syringomas, glandular formations were usually composed of double layers of cells in which the luminal cells were cuboidal and the outer cells were spindle-shaped. These glands were embedded in a homogeneous cartilage-like connective tissue matrix. 35BH11 only reacted with the luminal cells (Fig. 2a), while the outer layer cells including those streaming into the stroma exhibited a positive reaction for 312C8-1 (Fig. 2b). The staining observed in apocrine naevi resembled that seen in normal apocrine glands. Papillary hidradenoma is composed of apocrine-type luminal cells accompanied by basal cells. 35BH11 stained the luminal epithelial cells (Fig. 3), but 312C8-1 failed to stain the basal cells.

In pilosebaceous tumours, 35BH11 was uniformly non-reactive. In the case of sebaceous naevi and sebaceous adenomas, the staining pattern with 312C8-1 was similar to that observed in normal sebaceous glands. For trichoepitheliomas, 312C8-1 stained proliferating cells, but no labelling was seen in a few layers of cells surrounding the horn cyst wall (Fig. 4). No 312C8-1 staining was seen in pilomatrixomas. For trichilemmal cysts (including proliferating trichilemmal cyst), all the layers of cells were stained by 312C8-1 but not the horny material (Fig. 5), whereas only basal cells were labelled in epidermal cysts (Fig. 6).

Discussion

Our immunohistochemical studies have clearly shown that human skin and skin appendages had different patterns of staining with the two mAbs, 35BH11 and 312C8-1. Epithelial-derived tumours maintain the same expression of keratin as the tissue of origin, so such tumours can be identified and classified by the use of mAbs for the different keratins.

Expression of keratin proteins in sweat gland tumours may reflect their origin from different segments of the normal sweat gland. Electron microscopic and enzyme histochemical studies of eccrine poromas have shown that the predominant cells of these tumours have features similar to those of the acrosyringium (Hashimoto and Lever 1964), and the ductal lumens seen in such tumours have the characteristic features of eccrine acrosyringeal ducts (Hashimoto et al. 1987). The 312C8-1-positive staining that we observed in solid tumour cell nests corresponded to that observed in the acrosyringium, while the 35BH11 positivity of the duct lining cells may reflect the embryonic character of the acrosyringium. Syringomas may arise from the eccrine ducts, since the staining pattern of several eccrine-specific mAbs (EKH4, EKH5, and EKH6) (Hashimoto et al. 1987) as well as our current results support previous ultrastructural and enzyme histochemical findings (Lever and Schaumburg-Lever 1983). In chondroid syringomas, duct-like structures usually consist of two rows of cells. Our findings indicate that the 35BH11-positive luminal cells and the 312C8-1-positive outer layer cells may arise from the secretory portion of sweat glands. Papillary hidradenomas consist of luminal cells and myoepithelial cells. Although myoepithelial cells have been identified by the Alkaline phosphatase reaction and by the detection of myofibrils (Tappeiner and Wolfe 1969), the reason why 312C8-1 did not stain the basal cells remains unclear.

In trichoepitheliomas, several layers of cells around the horn cyst were negative for 312C8-1. These probably represented cells similar to the inner root sheath. Keratinization in trichilemmal cysts is analogous to the keratinization that takes place in the outer root sheath of the hair (Pinkus et al. 1981). The 312C8-1-positive cells seen in all layers of the cyst wall indicate its outer root sheath origin, in contrast to epidermal cysts which originate from the follicular infundibulum or from epidermal inclusions where basal cells were only labelled by 312C8-1. 312C8-1 staining is thus useful in differentiating trichilemmal cysts from epidermal cysts. The lack of 312C8-1-positive cells in pilomatrixomas is in accordance with the differentiation of this tumour in the direction of the hair cortex cell (Lever and Schaumburg-Lever 1983).

Keratins 8 and 14 can be demonstrated in routinely processed tissue specimens, and the findings may be of assistance in the diagnosis or assessment of the histogenesis of benign tumours of the skin appendages.

References

Battifora H, Kopinski A (1986) The influence of protease digestion and duration of fixation on the immunostaining of keratins. A comparison of formalin and ethanol fixation. J Histochem Cytochem 34:1095–1100

Cooper D, Schermer A, Sun T-T (1985) Classification of human epithelia and their neoplasms using monoclonal antibodies to keratins: strategies, applications, and limitations. Lab Invest 52:243–256

Dairkee SH, Blayney C, Smith HS, Hackett AJ (1985) Monoclonal antibody that defines human myoepithelium. Proc Natl Acad Sci USA 82:7409–7413

Gown AM, Vogel AM (1984) Monoclonal antibodies to human intermediate filament proteins. II. Distribution of filament proteins in normal human tissues. Am J Pathol 114:309–321

Hashimoto K, Lever WF (1964) Ecrine poroma; histochemical and electron microscopic studies. J Invest Dermatol 43:237–247

Hashimoto K, Mehregan AH, Kumakiri M (1987) Tumors of skin appendages. Practical dermatopathology series. Butterworths, Boston

Lever WF, Schaumburg-Lever G (1983) Histopathology of the skin, 6th edn. Lippincott, Philadelphia

Mazoujian G, Margolis R (1988) Immunohistochemistry of gross cystic disease fluid protein (GCDFP-15) in 65 benign sweat gland tumors of the skin. Am J Dermatopathol 10:28–35

Menard S, Tagliabeu E, Canevari S, Fossati G, Colnaghi MI (1983) Generation of monoclonal antibodies reacting with normal and cancer cells of human breast. Cancer Res 43:1295–1300

Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982a) The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24

Moll R, Franke WW, Volc-Platzer B, Krepler R (1982b) Different keratin polypeptides in epidermis and other epithelia of human skin: a specific cytokeratin of molecular weight 46,000 in epithelia of the pilosebaceous tract and basal cell epitheliomas. J Cell Biol 95:285–295

Noda Y, Kumasa S, Higashiyama H, Mori M (1988) Immunolocalization of keratin proteins in sweat gland tumours by the use of monoclonal antibody. Pathol Res Pract 183:284–291

Penneys NS, Nadji M, McKinney EC (1981) Carcinoembryonic antigen is present in human eccrine sweat. J Am Acad Dermatol 4:401–403

Penneys NS, Nadji M, Morales A (1982) Carcinoembryonic antigen in benign sweat gland tumors. Arch Dermatol 118:225–227

Pinkus GS, O'Connor EM, Etheridge CL, Corson JM (1985) Optimal immunoreactivity of keratin proteins in formalin-fixed, paraffin-embedded tissue requires preliminary trypsinization. An immunoperoxidase study of various tumors using polyclonal and monoclonal antibodies. J Histochem Cytochem 33:465–473

Pinkus H, Iwasaki T, Mishima Y (1981) Outer root sheath keratinization in anagen and catagen of the mammalian hair follicle. A seventh distinct type of keratinization in the hair follicle: trichilemmal keratinization. J Anat 133:19–35

Tappeiner J, Wolfe K (1969) Hidradenoma papilliferum. Eine enzymhistochemische und electronenmikroskopische Studie. Hautarzt 19:101–109

Tsubura A, Morii S, Ueda S, Sasaki M, Zotter S, Watzig V, Mooi W, Hageman PC, Hilkens J, Van den Tweel J, Meijer C, Hilgers J (1987) Immunohistochemical demonstration of MAM-3 and MAM-6 antigens in normal human skin appendages and their tumors. Arch Dermatol Res 279:550–557

Tsubura A, Shikata N, Inui T, Morii S, Hatano T, Oikawa T, Matsuzawa A (1988) Immunohistochemical localization of myoepithelial cells and basement membrane in normal, benign and malignant breast lesions. Virchows Arch [A] 413:133–139

Zuk JA, West KP, Fletcher A (1988) Immunohistochemical staining patterns of sweat glands and their neoplasms using two monoclonal antibodies to keratins. J Cutan Pathol 15:8–17