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Dermal cylindroma. Expression of intermediate filaments, epithelial and neuroectodermal antigens

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Summary. We report on immunohistochemical staining patterns in so-called apocrine tumors of skin with special emphasis on the dermal cylindroma. The results were compared with apocrine tubular adenoma, syringocystadenoma papilliferum and the normal eccrine sweat gland.

A relationship of dermal cylindroma to the apocrine gland is suggested by expression of lysozyme and alpha1-antichymotrypsin. The tumor shares keratin, epithelial membrane antigen (EMA) and EGF-receptor expresssion with eccrine and apocrine glands. The presence of intermingled cells with a coexpression of keratin and vimentin argues for a partial myoepithelia-like differentiation. Neuroectodermal antigens are missing.

Therefore, dermal cylindroma is classified as an adnexal tumor of skin with a variable rate of cells of apocrine secretory, myoepithelial and undifferentiated phenotypes.

Key words: Apocrine tumors, Immunohistology, Coexpression

Introduction

Current classification of adnexal tumors of skin differentiates eccrine, apocrine and pilosebaceous varieties. An outstanding matter of debate, however, is how one can distinguish between eccrine and apocrine glandular tumors. The question is of practical impact since there are several tumor entities with apocrine or eccrine differentiation: hydrocystoma or cystadenoma, syringocystadenoma papilliferum, basal cell epithelioma with glandular differentiation (Hashimoto et al., 1987). Even the morphological finding of secretion by decapitation seems not to be an absolute criterium excluding eccrine differentiation. Can immunohistochemistry provide additional information?

The dermal cylindroma has light and electron microscopic features of an apocrine secretion (Hashimoto et al., 1987; Massa and Medenica, 1987; Lever and Schaumburg-Lever, 1990). On the other hand, some recent investigators suggest an eccrine differentiation pathway based upon immunohistochemistry of keratins and epithelial antigens like CEA (Cotton and Braye, 1984). To possibly resolve this controversy, we have investigated dermal cylindromas and other apocrine tumors by immunohistochemistry using three groups of antibodies: against intermediate filaments; epithelial antigens; and neuroectodermal antigens. Comparing their staining patterns with those of the normal eccrine sweat gland of skin (Wollina, 1991), we hoped to gain a better insight into the differentiation of dermal cylindromas.

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Materials and methods

Tissues

Samples of human skin tumors were obtained from the tissue bank of the Department of Dermatology, University of Jena. These included 12 dermal cylindromas from five patients. In one case, snap-frozen tissue stored in liquid nitrogen was available. It was fixed in acetone for 10 min at 4° C. In all other cases, the tumors were fixed in formalin and routinely processed. Additionally, syringocystadenoma papilliferum (n=2) and apocrine tubular adenoma (n=1) were studied.

Antibodies

Primary antibodies used in this study are summarized in Table 1. Rabbit antimouse globulin (working dilution 1:50), mouse monoclonal alkaline phosphatase-antialkaline phosphatase complex (APAAP; 1:1000), peroxidase-labelled swine antirabbit and rabbit antimouse immunoglobulins (1:30) were purchased from Dakopatts, Amsterdam, Netherlands. Goat

Table 1. List of primary antibodies. Antibodies with an asterisk (*) were used only on frozen sections.

Antibody	Origin	Characterization and working dilution
1. *Antibodies against intermediate filaments*		
AHK	Dakopatts	rabbit wide spectrum antikeratin, 1:100
RPN 1161*	Amersham Buchler	IgG2a broad spectrum antikeratin, 1:10
RKSE 60*	Euro-Diagnostics	IgG1 against keratin #10, 1:100
K 8.12*	Bio-Yeda	IgG1 against keratins #13/16, 1:100
Cam 5.2	Becton-Dickinson	IgG2a against keratins #8/18/19, 1:2
CK 2*	Boehringer	IgG1 against keratin #18, 1:5
PVIM	Dakopatts	rabbit antivimentin, 1:50
VIM 9 (1)	Monosan	IgG1 against vimentin, 1:10
NE 14*	Boehringer	IgG1 against neurofilament, 1:2
G-A-5*	Biochrom	IgG1 against GFAPP, 1:5
2. *Antibodies against epithelial antigens and Ki67*		
Filaggrin	Paesel	IgG1 against (pro-)filaggrin, 1:100
EMA	Dakopatts	IgG2 against epithelial membrane antigen (EMA), 1:50
ACAM	Dr. I. Wez, Jena	rabbit anticalmodulin, 1:50
CEA	Dakopatts	rabbit anti-carcinoembryonal antigen (CEA), 1:300
29.11*	Sigma	IgG1 against EGF-receptor, 1:2000
Ki67*	Dakopatts	IgG1 against Ki67 proliferation antigen, 1:5
Lysozyme	Dakopatts	rabbit antilysozyme, 1:400
AACT	Dakopatts	rabbit antibody to alpha1-antichymotrypsin (AACT), 1:500
3. *Antibodies against neuroedtodermal antigens*		
S-100	Dakopatts	rabbit anti-S-100, 1:20
NSE*	Dakopatts	rabbit antibody to neuron-specific enolase (NSE), 1:25
NKI/C-3	Monosan	IgG1 against melanoma-associated antigen, 1:20
LS 59	Dr. L.M. Jerry (Calgary/Canada)	IgG2a against neuroglandular antigen, 1:100

antimouse bridging immunoglobulin (1:25) from Tago, Burlingame/CA, was used in double staining experiments.

Staining procedures

Serial sections were cut at 8 µm from formalin-fixed, paraffin-embedded blocks, dewaxed by Histosol (Merck, Darmstadt, FRG) and rehydrated through decreasing concentrations of ethanol, followed by washing in deionized water for 10 min. All incubation steps except otherwise noted were performed at room temperature. Endogenous peroxidase was inactivated with methanol-acetone (20 min). Subsequently the slides were rinsed in phosphate-buffered saline (PBS) for 10 min.

Sections were trypsinized (0.1% in distilled water) for 30 min at 37° C, rinsed in PBS and overlaid with the appropriate normal swine or rabbit serum (1:3 diluted in PBS) for 30 min at room temperature. Trypsinization was omitted before staining with antibodies against neuron-specific enolase (NSE), calmodulin (ACAM), NKI/C-3 and LS59.

Primary antibodies were diluted in PBS supplemented with bovine serum albumin, supplemented with 0.2M glucose and 1.5IU/ml glucose oxidase (Boehringer Mannheim, FRG) and incubated for one hour. Peroxide oxidase (POX) staining was performed according to Mason and Sammons (1987) with 3-amino-9-ethylcarbazole (AEC), purchased from EGA-Chemie, Steinheim, FRG. The sections were counterstained with Mayer's hemalun (Merck).

Frozen sections were processed to 4 µm thick, and acetone-fixed specimens stained with POX or APAAP as described elsewhere (Mason and Sammons, 1987). Naphtol-As-phosphate (Sigma) served as coupler.

For double-staining on both formalin- and acetone-fixed sections we combined the APAAP technique with Fast Blue BB salt (Sigma) and POX with AEC (Mason and Sammons, 1987). TRIS buffer was used instead of PBS. The following antibody combinations were performed: PVIM plus Cam 5.2; Vim 9 (1) with AHK; K 8.12 or RPN 1161; AHK and Ki67. In these protocols, counterstained was omitted.

In controls, primary antibodies were substituted by buffer. No staining was observed.

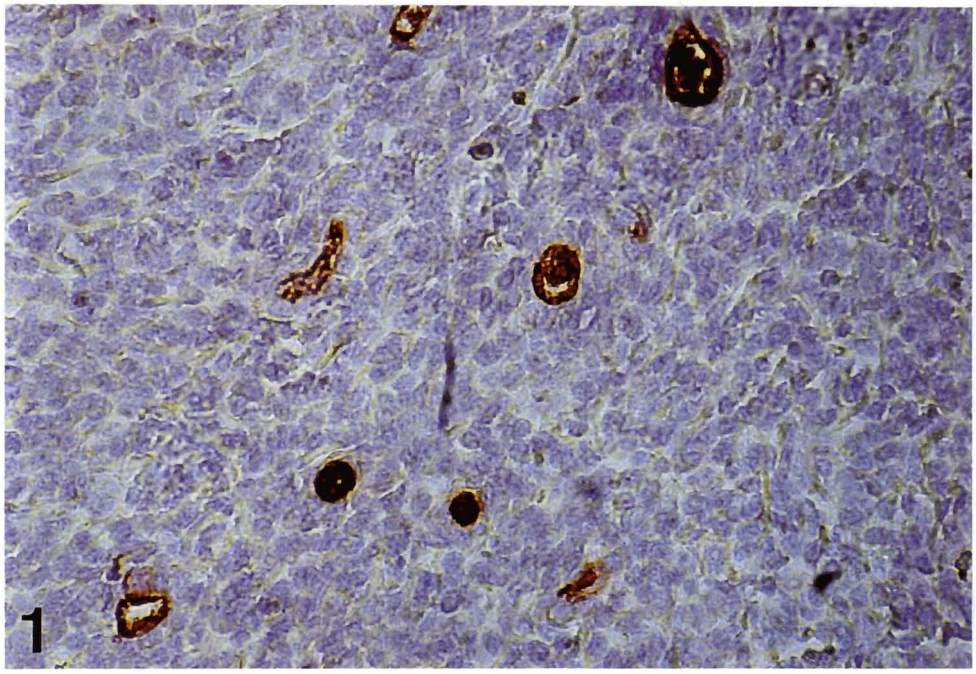


Fig. 1. Dermal cylindroma; staining with monoclonal antibody RPN 1161 against keratin. Note the different stages of lumen formation. x 250

Results

Dermal cylindroma (Table 2)

In dermal cylindromas, the majority of cells were non-reactive with antibodies against keratins. Cell forming duct-like structures or squamous whorls and scattered single, in part ring-like cells were labelled with AHK, Cam 5.2, RPN 1161, and CK2 (Fig. 1). This particular cell type also expressed a number of epithelial antigens like EMA, EGF-receptor, lysozyme and alpha1-antichymotrypsin (Fig. 2).

Scattered cells of solid areas were vimentin-positive (Fig. 3). Double-staining revealed that some keratin-positive cells simultaneously expressed vimentin (Fig. 4). There was no additional evidence for coexpression of intermediate filaments. The tumors were completely negative for neurofilaments and glial fibrillary acidic protein (GFAP). Both filaggrin, and high-molecular keratins were missing.

None of the neuroectodermal antigens (NSE, NKI/C-3, LS 59) were observed. Occasionally, ACAM disclosed a nucleolar or perinucleolar staining in tumor cell clusters. Within solid tumor masses scattered Ki67-positive cells were seen in the frozen specimen. As demonstrated by double-staining, the Ki67-positive, proliferating cells expressed keratin.

Syringocystadenoma papilliferum and apocrine tubular adenoma of skin

Most of the columnar, lumen-lining epithelium was strongly positive for Cam 5.2, but only a minority for CEA (Fig. 5). The amorphous luminal content was strongly reactive for CEA and moderately reactive for AMA or S-100 (Fig. 5c). Vimentin was not demonstrated. Occasionally, scattered LS 59-positive cells were found within the epithelium. The apocrine tubular adenoma was lysozyme-positive.

Discussion

Immunohistochemistry comes to the forefront in the diagnosis of adnexal cutaneous neoplasms. The problem of their classification is exemplified by dermal cylindroma, which was morphologically defined as apocrine, whereas histochemistry suggested an eccrine phenotype (Cotton and Braye, 1984; Hashimoto et al., 1987; Massa and Medenica, 1987; Lever and Schaumburg-Lever, 1990).

Dermal cylindroma is composed of solid and lumen-forming cells. The lumen formation shows a variable degree ranging from ring-like single cells, resembling embryologic pre-luminal cells of the dermal sweat duct (Hashimoto et al., 1966), to tall columnar lumina showing decapitation secretion like the apocrine secretory segment (Hashimoto et al., 1987). From an immunohistochemical point of view, these cells are the most mature expressing a number of different antigens but missing Ki67-labelling.

Simple-type keratins (# 8, 18, 19) and epithelial antigens of both secretory coils and inner duct cells of eccrine glands, like EMA and EGF-receptor have been detected in the lumen-lining epithelium of cylindromas (Cotton and Braye, 1984; Nanney et al., 1984; Noda et al., 1987, 1988). Almost all of these antigens are present

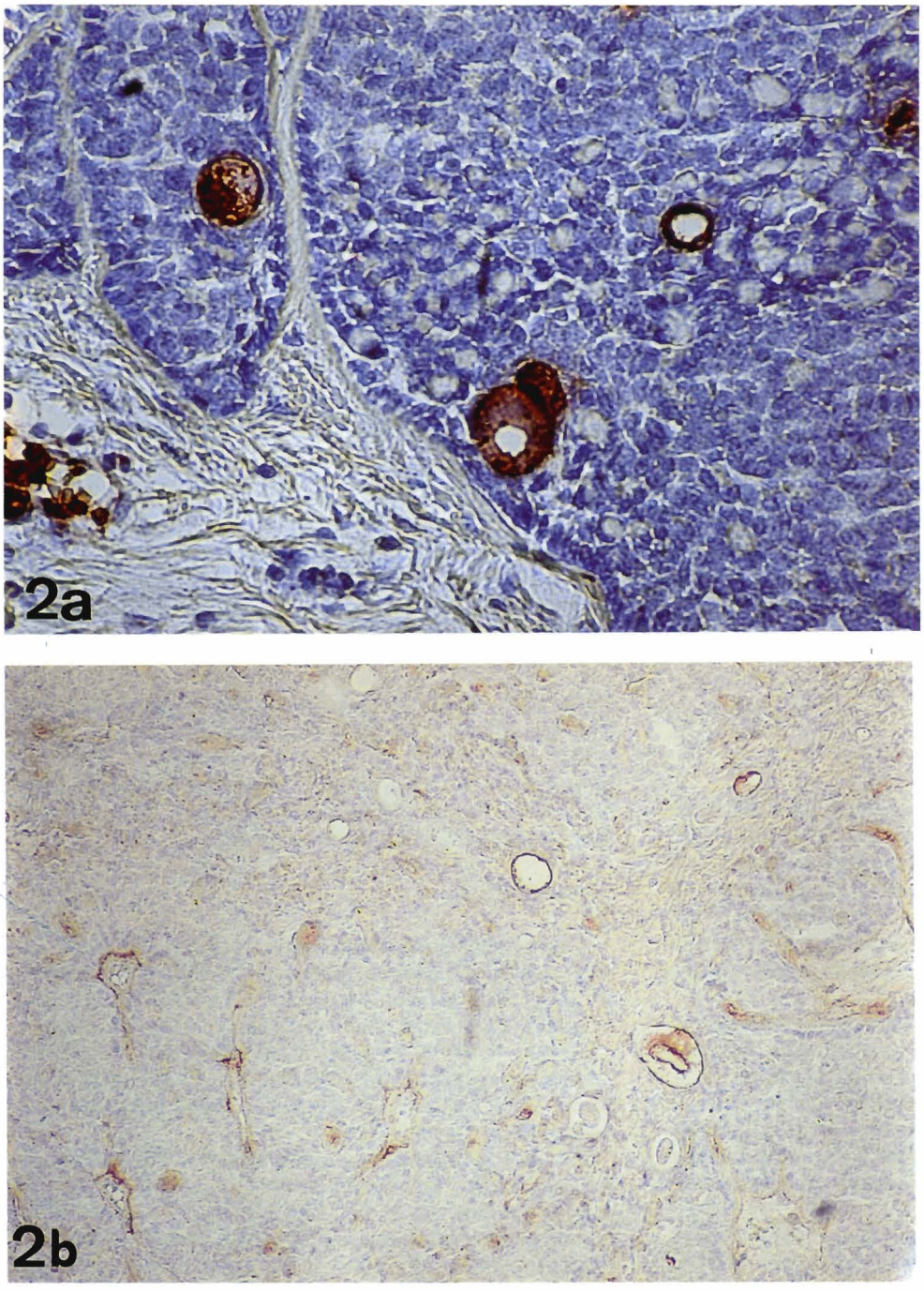


Fig. 2. Dermal cylindroma. **(a)** Staining with monoclonal antibody 29.11 against EGF-receptor. Decoration of «preluminal», ring-like cells. x 250. **(b)** Predominant luminal staining with a polyclonal antibody against alpha1-anti-chymotrypsin. x 100

in normal eccrine sweat glands (Cotton, 1986; Iki, 1990; Wollina et al., 1990, 1991; Wollina, 1991). Definitive conclusions could not be drawn from the keratins, CEA and EMA, since they have also been detected in apocrine glands (Penneys, 1984, Noda et al., 1987, 1988). Otherwise, a secretory phenotype is a reasonable deduction from these findings.

On the other hand, lysozyme and alpha1-antichymotrypsin found in cylindromas were absent in the normal eccrine sweat glands (Ezoe and Katsumata, 1990; Wollina, 1991) as well as in benign eccrine tumors of skin (Katsumata and Ezoe, 1990). Adnexal tumors of the apocrine type express lysozyme (Katsumata and Ezoe, 1990). We also observed lysozyme-positive epithelial cells in apocrine tubular adenoma.

Among the dermal cylindromas, there was evidence for coexpression of vimentin and keratin in a minority of cells localized within solid tumor masses. Some of them

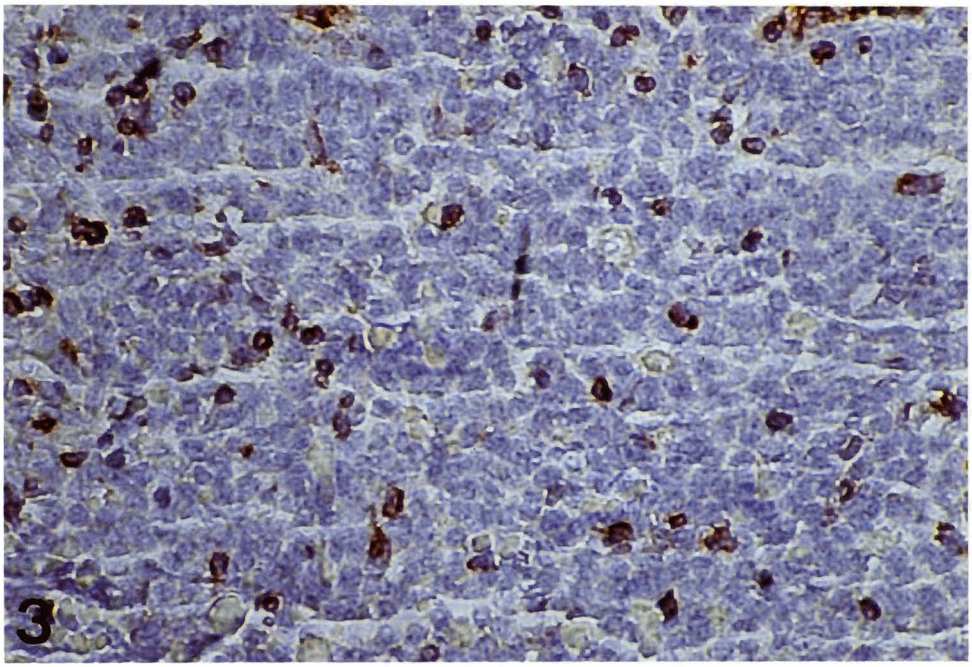


Fig. 3. Dermal cylindroma; staining with polyclonal antivimentin. Scattered cells within the solid tumor mass are decorated. x 250

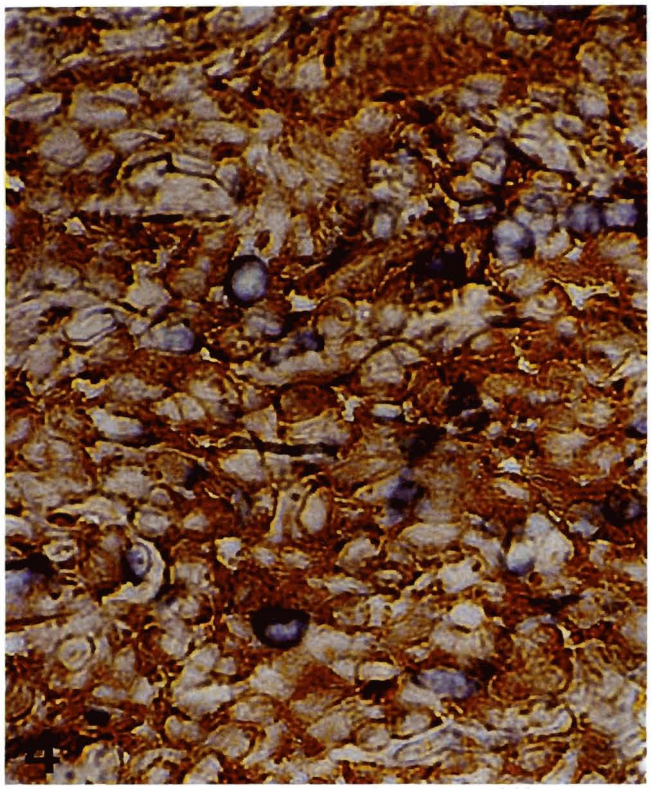
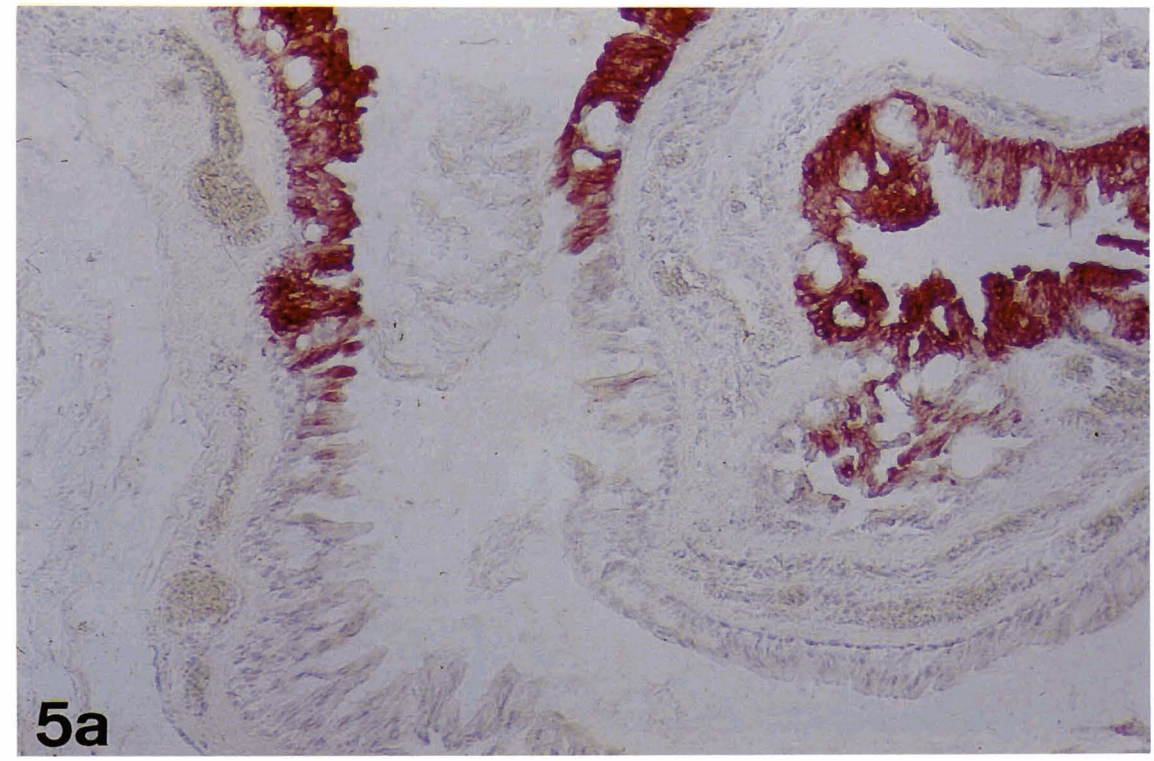


Fig. 4. Dermal cylindroma; double-staining with polyclonal antikeratin and monoclonal Vim 9 (1) demonstrates cells coexpressing both keratin and vimentin . x 250

Table 2. Overview of immunohistochemical staining patterns of dermal cylindroma and the normal eccrine sweat gland (according to Wollina, 1991).

Antibody	Cylindroma	Eccrine Sweat gland
1. *Intermediate filaments*		
AHK	+ (P/D), - (most S)	+
RPN 1161	+ (P)	-
RKSE 60	-	-
K 8.12	-	+ (SC)
Cam 5.2	+ (P/D)	+ (SC/M)
PVIM	+ (P/D)	+ (M)
Vim 9(1)	+ (very few P/few D)	+ (M)
NE 14	-	-
G-A-5	-	-
2. *Epithelial antigens and Ki67*		
Filaggrin	-	+ (A)
EMA	+ (P/D)	+ (SC/ID)
ACAM	- (most S)	-
CEA	-	+ (SC/ID)
29.11	+ (P/D)	+ (ID)
Ki67	+ (few S)*	+ (M/ID)*
Lysozyme	+ (P/D)	-
AACT	+ (P/D)	-
3. *Neuroectodermal antigens*		
S-100	-	+ (M/SC/ID)
NSE	-	(+) (SC)
NKI/C-3	-	(+) (SC)
LS59	-	(+) (SC)

*: on frozen sections; P: preluminal cells; D: duct-lining cells; S: solid areas of cylindromas; A: acrosyringium; ID: inner duct epithelium; OD: outer duct epithelium; SC:secretory coils; M: myoepithelial cells.



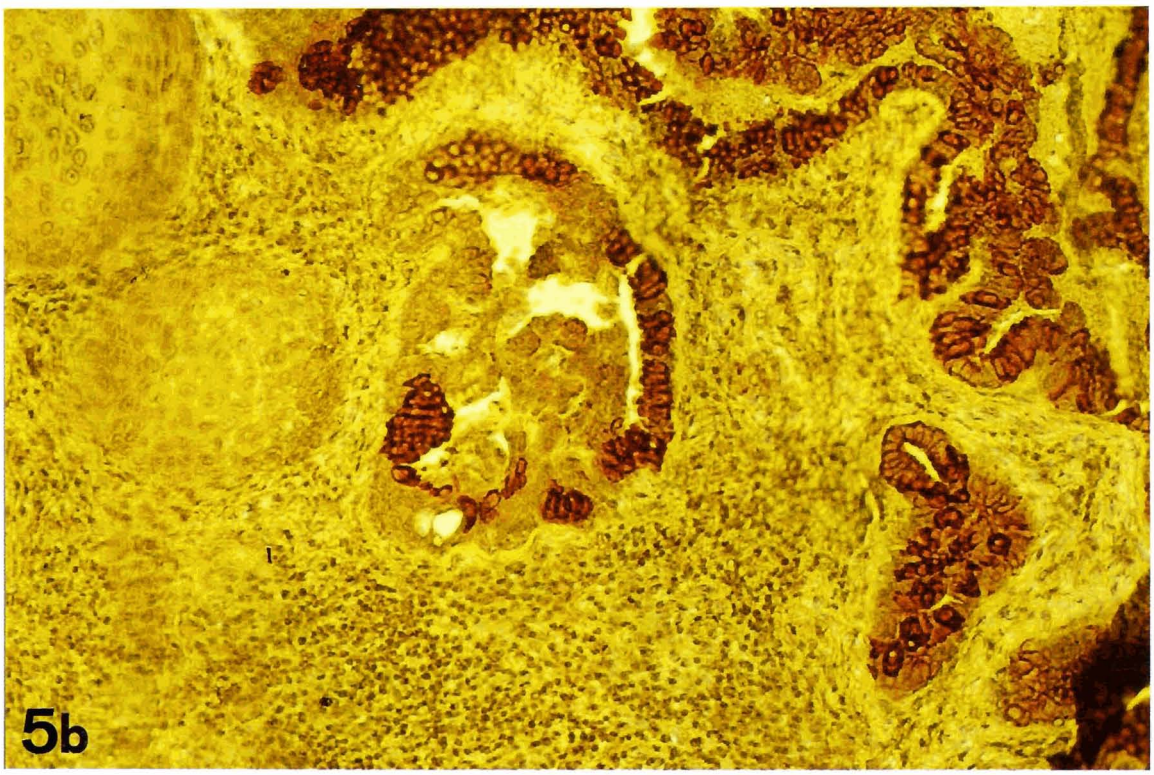
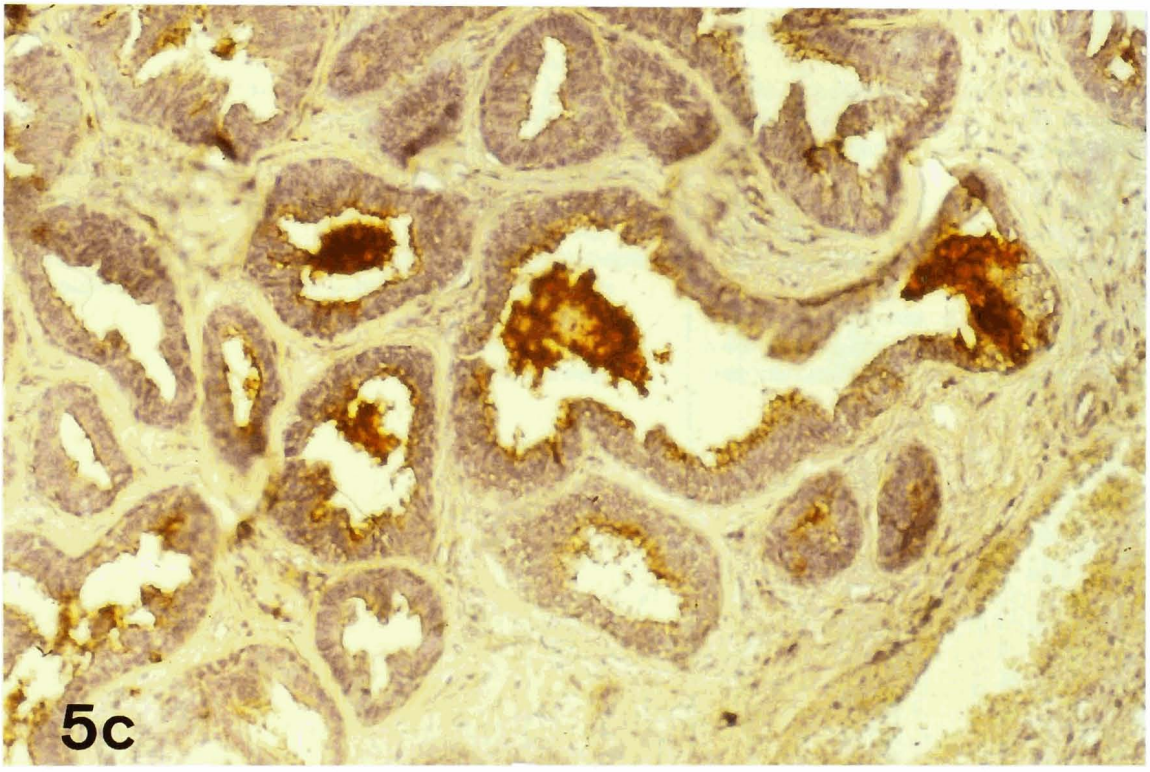


Fig. 5. Immunostaining of apocrine tumors. Syringocystadenoma papilliferum (a) and apocrine tubular adenoma (b - c). **(a, b)**: Demonstration of simple-type keratins with antibody Cam 5.2 in the lumen-lining epithelium; a) APAAP, x 100; b) peroxidase technique, x 250. **(c)** Immunostaining for CEA; peroxidase technique, x 250



showed a ring-like appearance. Interestingly enough, the coexpression of both intermediate filaments by myoepithelial cells of normal eccrine sweat glands was described recently (Wollina, 1991). Myoepithelial cells of several other exocrine glands are also capable of coexpressing vimentin and keratin (McGuire et al., 1989). Nanney et al. (1984) reported a strong immunoreactivity of myoepithelial cells but not secretory segments with antibodies against EGF-receptor. Expression of EGF-receptor by cylindromas provides additional support for the presence of a myoepithelial phenotype.

In conclusion, the dermal cylindroma has much in common with apocrine secretory segments. Cells of a myoepithelial phenotype can be found within solid areas and among ring-like cells with co-expression of keratin and vimentin. And a second practical conclusion can be drawn: CEA, EMA and simple-type keratins are not reliable «markers» that distinguish eccrine from apocrine sweat glands, as assumed earlier (Cotton, 1986).

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