An investigation of cytokeratin expression in skin epithelial cysts and some uncommon types of cystic tumours using chain-specific antibodies

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Summary. The differentiation state of skin epithelial cysts and some uncommon types of epithelial skin tumours was investigated by immunohistochemical staining, mainly using cytokeratin (CK) polypeptide-specific monoclonal antibodies. Samples of interfollicular epidermis, hair follicles and eccrine sweat glands were included as reference tissues. The CK reactivity in epidermoid cysts and milia is not restricted to CKs involved in epidermal-type differentiation, i.e. CK1, 5, 10 and 14, but in addition CK16, a hyperproliferative keratinocyte marker is suprabasally expressed. CK1 and 10 are other prominent suprabasal markers, while CK14 seems to be preferentially expressed in the basal cell layer. Of the non-epidermal CKs, only CK4 was focally or more extensively detected in about 50% of the cases. In terms of CK reactivity, keratinization of trichilemmal cysts corresponds to the keratinization of the anagen-phase hair follicle in the isthmus. The CK reactivity is again restricted to CK1, 5, 10, 14 and 16. However, the CK1 as well as CK10 reactivity is subject to severe limitations, since both CKs were only convincingly observed in foci of terminal differentiation. Eccrine ducts express a complex CK set, including CK7, 8, 14, 18 and 19. This CK set perfectly corresponds to the CK composition observed in acini of eccrine sweat glands. In addition, a discontinuous CK4 and 16 reactivity was seen in about 50% of the sites, while CK1 and 10 displayed a strictly focal appearance. On the other hand, syringomas produces in its distinct structures, a CK pattern reminiscent of the one observed in eccrine sweat gland ducts and includes CK1, 5, 10, 14, 16 and 19. Finally, the CK expression pattern of pilomatrixoma includes CK1, 8, 10, 14 and 19, and is reminiscent of the CK staining of hair bulb matrix cells differentiating in the keratogenous zone in the direction of hair cortex. The reactivity of CK1 and 10 was mainly restricted to foci of squamoid differentiation and also to transitional cells bordering on shadow cells, as far as it concerns CK10. Occasionally, CK7 and 16 were observed in individual cells or small cell groups. In our view these CK reactivity patterns are useful to judge the differentiation state reached in pathological conditions, but so far do not allow us unequivocally to determine the origin of these lesions.

Key words: Cytokeratins — Epidermoid and trichilemmal cysts — Milia — Hydrocystoma — Syringoma — Pilomatrixoma

Cysts of skin are a large group of lesions properly considered benign epithelial tumours, since they increase in size more through accumulation of cystic contents than through proliferation of cells in their walls. Unfortunately, there is no uniformity as far as classification, nomenclature and histogenesis is concerned [1, 6, 10—12]. Furthermore, confusion is created since other skin tumours may locally or more generally develop a cystic consistency, giving rise to various mixed forms.

A direct connection of the cyst with normal-looking epidermis or epidermal appendages is helpful in diagnosis. In some instances, however, a pre-existing connection may be disrupted, if it ever existed before. Other hypotheses as to the histogenesis deserve consideration, but these are hampered by a lack of dynamic evidence (e.g. a pluripotent and/or equipotent epidermal and appendage matrix cell origin [6]). Otherwise, the exact mode of differentiation of cyst cells is sometimes hard to determine from routine histological examination. Various histochemical techniques including immunohistochemistry may yield more precise information and reveal the true nature of cystic lesions.

Cytokeratins (CKs), the structural polypeptides of intermediate filaments in epithelial cells, make up a family of 19 polypeptides in humans and are subject to differential expression [14]. A consistent and relatively simple
picture has emerged from the data on CK expression. Among other things, the expression of CKs largely depends upon the type of the epithelium and on the tissue compartment within complex epithelia. Referring to the epithelium in particular, the CK content depends on its localization and the stage of keratinocyte maturation. In addition, the CK composition may reflect the state of cellular growth (normal versus hyperproliferative). A comprehensive model emphasizing the CK subfamily and CK pair or co-expression concept has been constructed [18], and is still the guide in CK expression studies. The existence of these rules suggests that the CK composition may indeed be of prognostic and diagnostic value. During tumour development, these rules seem in general to be well-accepted and the CK composition consistently relates to the factors already mentioned [4]. Although this statement mainly concerns malignant neoplasms, there is good reason to presume that it is equally valid for benign tumours. This, together with the increasing availability of CK polypeptide-specific monoclonal antibodies, forms the basis for the current investigations.

The aim of the present study was to characterize and define on a molecular basis, the CK composition of different types of skin cysts and benign tumours with focal cystic appearance. A panel of monoclonal antibodies enabled screening of 10 out of 19 CK polypeptides. In addition, an antisemur which is monospecific for an 11th CK polypeptide was included in our study. It was our intention, using the CK profile, to relate the cystic wall or tumour epithelium to an epithelium of adnexa or to the epidermis, and thus to confirm the mode of differentiation and possibly the histogenesis of these lesions.

Materials and methods

Tissues

Forty-three benign epithelial skin tumours were excised during outpatient consultation, snap-frozen in liquid nitrogen immediately after excision and stored under the same conditions until frozen sections were cut. Parallel samples of normal epidermis and adnexae were used as internal controls.

Three types of keratinous cysts were examined: (1) 13 cases of epidermoid cysts, i.e. those with a lining identical in its stratification and keratinization with interfollicular epidermis and infundibulum; (2) 12 cases of trichilemmal or pilar cysts, i.e. those with a lining and keratinization analogous to the external root sheath of the follicle in direct contact with the hair; and (3) 10 cases of milia, i.e. small keratinous cysts of which the site of origin is less precisely described, but with an epidermoid lining.

In addition, 2 cases of eccrine hidrocystoma were investigated as an example of a non-keratinizing cyst with an inner lining of eccrine secretory cells, and 2 cases of syringoma, a sweat gland tumour considered to be eccrine and mainly presenting collections of convoluted and cystic sweat gland ducts, next to solid strands of epithelial cells. Finally, we included in our study 4 cases of pilomatrixoma (calcifying epithelioma of Malherbe), a hair follicle tumour with differentiation similar to the keratogenous zone. Detailed information on these tumours can be gathered from the authoritative textbooks cited previously [1, 6, 10–12].

Tissue sectioning

Serial cryostat 5 μm sections were obtained using a Leitz cryostat type 1720 (Leitz, Wetzlar, FRG) after mounting in Jung embedding medium (no. 08926). Sections were stuck onto ethanol-cleaned glass slides, coated with 1% gelatin—glycerin (1/1, v/v), air-dried (2 h) and fixed in secoton (10 min) at room temperature. A preliminary routine light microscopic examination was performed on haematoxylin and eosin stained sections to confirm the clinical diagnosis.

Immunohistochemical staining

Cryosections were processed for the indirect immunoperoxidase technique. Initially, sections were rehydrated in 10 mM phosphate buffer, pH 7.2, containing 0.85% NaCl (PBS) and incubated with the monoclonal antibodies (undiluted, 1:5 or 1:10, and 1:25 in PBS) for 30 min at 20°C in a moist chamber. Only LH8 and LL026 were systematically applied as an undiluted culture supernatant, while the EKH4 monoclonal antibody (IgG1 subclass) was purified from mouse ascites fluid using a Pharmacia protein A-Sepharose CL-4B column [5] and used at a 1:10 dilution range (1:40 to 1:200). Tissue culture medium from non-producing hybridomas was used as negative control. The rabbit anti-keratin AF87 was useful in the 1:120 to 1:240 dilution range. It is monospecific for CK1 and directed against a synthetic peptide corresponding to the carboxy-terminal amino acid sequence of the 67 kDa CK according to strategies developed before [16].

Slides were extensively washed in PBS (jet clean, and three washes, 10 min each, using a magnetic stirrer, the second wash containing in addition 0.1% Tween-20). Subsequently, incubation was performed with either peroxidase-conjugated rabbit anti-mouse Ig or peroxidase-conjugated goat anti-rabbit IgG(H+L) (both 1:40 in 10% normal human serum in PBS) for 30 min at room temperature, in monocolonal or polyclonal assays, respectively. The sections were washed for a further 30 min as described before, except that 0.05 M Tris-HCl, pH 7.6 (TB) or 0.05 M sodium acetate buffer, pH 4.85 (AB) was substituted for the third wash, depending on whether 3,3’-diaminobenzidine tetrahydrochloride (DAB) or 3-aminon-9-ethylcarbazole (AEC) was used as the electron donor substrate. DAB was dissolved in TB (0.45 mg/ml with 0.03% H2O2) and AEC in AB (0.2 mg/ml with 0.01% H2O2) and applied for 5–10 min. Finally, sections were washed in running tap water, counterstained with Mayer’s haematoxylin (15–30 s) and mounted in Kaiser’s glycerin—gelatin (Merck no. 9242).

Co-expression of CKs and vimentin was verified using the double label indirect immunofluorescence technique previously described [2]. All secondary antibody conjugates were purchased from Nordic Immunological Labs. (Tilburg, The Netherlands).

Monoclonal antibodies

The monoclonal antibodies which were systematically used in this study are listed in Table 1. RCK106, CK18-2 and LE61, all reacting with CK18 and RV202 as a substitute for Vin 9, were used in a limited number of experiments to verify previous conclusions. References to antibody clones used in our previous paper [20].

Results on the expression of CK5 are based on RCK102 (CK5+8) staining patterns. Only in those cases where antibodies to CK8 (LE41, M20) were negative and RCK102 was positive, did we conclude that CK5 was present.

Results

Our statements are restricted to positive data and are summarized in Table 2. We have previously observed that broad spectrum clone 80 elicited positive staining in different tumour types. Langerhans’ cells, scattered among lining cells were the only vimentin expressing cell type,
Table 1. Specificities of the monoclonal antibody panel

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Antigen(s)*</th>
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<tbody>
<tr>
<td>6B10</td>
<td>CK4</td>
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<tr>
<td>RCK102</td>
<td>CK5 + 8</td>
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<tr>
<td>RCK105</td>
<td>CK7</td>
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<td>LE41</td>
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<td>CK18-2</td>
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<td>clone 80</td>
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<tr>
<td>Vim 9</td>
<td>Vimentin</td>
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<tr>
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<td>Vimentin</td>
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<td>RD301</td>
<td>Desmin</td>
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* CKs were numbered according to the catalogue of Moll et al. [14]; references dealing with the specificity of the antibodies can be found elsewhere [2]. In addition, we mention two recent papers [3, 20] describing the specificity of M20, LPH1, LH3 and LH8

b Unpublished result

besides melanocytes, which were, however, infrequently observed. Furthermore, co-expression of CK and vimentin was never observed.

Epidermoid cysts

Probes recognizing CK1, 5, 10 and 14 always showed staining of epidermoid cysts, compatible with the staining profiles found in epidermis and infundibulum as described in detail elsewhere [2], and here for the first time, as far as they concern CK1.

As concluded on the basis of a positive reaction for RCK102 (CK5 + 8) and a negative reaction for LE41 and M20 (CK8), CK5 was detected uniformly in basal keratinocytes and with decreasing strength throughout suprabasal layers, leaving a weak label in flattened keratinocytes. However, 9 out of 13 cases significantly re-expressed positive staining with RCK102 at multiple sites usually in the centre of the cystic cavity.

CK1 and 10 were absent in the stratum basale — either a single layer or hyperplastic — but were markedly expressed in all suprabasal layers of the cyst wall (Fig. 1). The CK10 stainability was significantly reduced in the cystic contents, which furthermore showed no CK1 reactivity, in accordance with the negative data obtained in the epidermal stratum corneum.

Antibodies which recognize CK14 produced a significant staining of the lower part of the cyst wall. LH5 left suprabasal layers unstained (Fig. 2) except in 1 case which revealed a persistent weak staining throughout. EKH4, however, showed a gradual loss of staining in living layers but revealed a residual labelling in cystic cavities.

In addition, reactivity of CK16, believed to be a prominent marker of hyperproliferative keratinocytes in general [21], was recurrently noticed in suprabasal keratinocytes. The antigenic reactivity, however, faded away in the stratum granulosum and cystic cavity, leaving a minor number of positive sites.

Of the non-epidermal CKs, only CK4 was frequently detected. It was focally or more extensively observed in 7 cases mainly suprabasally and during stages of terminal keratinization (Fig. 3). In 1 case, CK13 was focally co-expressed with CK4.

Finally, putative Merkel cells, observed with low frequency among basal keratinocytes in 7 cases, variably expressed CK7, 8, 18 and 19.

Trichilemmal cysts

Trichilemmal cysts again systematically expressed CK1, 5, 10, 14 and 16, however in a distinctive manner. CK1 and 10 reactivity was limited in trichilemmal cysts. CK10 was never noticed in peripheral cells and intermediate cell layers, but was observed in cells close to the cavity, either focally (in 11 out of 12 cases; Fig. 4) or more generally (1 case). On the other hand, an extremely weak CK1 staining was observed in suprabasal cells of the wall in 6 cases. Sites with an occasional manifestation of keratohyalin granules were obligatorily CK1 and CK10 positive. CK1- and CK10-positive foci were further noticed inside the cavity, but never included parakeratotic areas which were, however, frequently present.

CK5 reactivity, as observed using RCK102, was rather variable throughout the entire cyst wall. In 3 out of 12 cases, the immunostaining decreased markedly as the lining cells matured (intermediate and superficial cells). In the transient area, the corresponding epitope was temporally masked, but became increasingly available in central depositions, in all except 2 samples.

CK14, judged from EKH4 staining, was consistently observed from the peripheral to the superficial layer of the wall (Fig. 5), except in CK10-positive areas where the EKH4 signal was seriously reduced (9 out of 12 cases) or absent (3 other cases). CK14 reactivity was irregular in central accumulations of cystic debris. Generally, LH8 only provided a patchy staining of the wall. Recurrently, CK16 reactivity was evident in suprabasal keratinocytes, and even restricted to the upper half of the wall in 50% of the cases. In the cystic cavity, positive foci were restricted to wall-associated areas.

Expression of non-epidermal CKs was only evident as a minor event. In 1 case a focal but impressive reactivity of CK4 was observed in all layers of the wall, and in another case, the presence of CK19 in a cell group along the peripheral side of the wall was seen. Merkel cells were rare and only noticed in 4 lesions (2 cases expressed CK18, 1 case expressed CK19, and 1 case co-expressed CK8 and 18).

The keratinization of trichilemmal cysts, judged from these CK reactivity patterns, resembles the trichilemmal
keratinization of the anagen phase hair follicle, i.e. the keratinization of the external root sheath directly facing the hair shaft in the isthmus. This conclusion is not only based on CK5, 14 and 16 reactivity patterns, but most convincingly refers to the reactivity of CK10, which was restricted to foci in intermediate layers of the outer root sheath (Fig. 6). Moreover, the isthmus was characterized by a significant decrease of the suprabasal CK1 reactivity.

Milia

CK5, in these cases, is a prominent marker of all cell lining layers, although local differences concerning reactivity levels must be mentioned. RCK102 elicited a conspicuously heterogeneous labelling of cystic contents in 8 out of 10 cases and a minimal labelling in the remaining two cases. The suprabasal onset of the CK1 and 10 reactivity fully corresponded to the established epidermal pattern. CK14, screened only with LH8, was a basal marker whose staining intensity decreased to vanishing point in transitional areas in 6 out of 10 cases. Nevertheless, in 4 cases a faint residual staining of keratinous debris persisted. CK16, again, was suprabasally expressed. The corresponding determinant, however, was only focally shown in the cavity.

From our search for the presence of non-epidermal CKs, it appeared that in 4 out of 10 cases, CK4-positive foci were present in the accumulated keratinous mass. Finally, in 1 case, an inflammatory rupture of the wall led to leakage of lamellated keratinous debris into the connective tissue. In these abscess cavities, only CK5 correspondant determinants were detected.

Eccrine hydrocystoma

Secretory cells of the simple epithelial lining and papillary projections or solid buds always expressed CK7, CK8 (M20 positive, though LE41 negative), CK14, CK18 (positive for M9 and RGE53) and CK19 (Fig. 7). Usually, superficial cells, i.e. those facing the cavity, were more intensely stained than deeply seated reserve cells, except in the case of the CK14 staining profiles. In addition, a discontinuous CK16 correspondant staining was observed throughout the epithelial lining, with maximal appearance in focal stratified configurations. Elongated myoepithelial cells, running with their long axis parallel to the cyst wall, only displayed CK14 staining. This complex CK set resembled the CK composition observed in eccrine sweat gland acini (see also results described elsewhere [2]). Finally, a discontinuous labelling of CK4 along the cystic lining — roughly in 50% of the sites — and a strictly focal localization of CK1 and 10 (RKSE60 and LH3 positive, LPH1 negative) were observed in hydrocystoma.

Syringoma

Both syringoma cystic sweat ducts and tail-like strands of cells projecting into the stroma (known as tadpole-like or comma structures), as well as small solid epithelial nests and cystic ducts near the epidermis (filled with keratinous debris) produced identical CK reactivity patterns. Besides staining with broad spectrum CK antibodies, expression of CK1, 5, 10 (Fig. 8), 14, 16 and 19 was always observed. CK14 staining accumulated above peripheral cells in the respective structures, while CK19 was preferentially located in internal epithelial cells and particularly in periluminal areas. In addition, a weak and patchy CK7 staining was observed in the intraductal debris. This CK picture, with the exception of CK7, corresponds to the CK set of eccrine sweat ducts of which multiple analyses have been reported [2]. The expression of CK1 and 16 in the ducts of eccrine sweat glands was additionally shown.
Fig. 1. Immunohistochemical staining profile of an epidermoid cyst with the monoclonal antibody RKSE60. It is obvious that CK10 is expressed uniformly in suprabasal layers and in addition it is weakly expressed in the cystic cavity. b, Stratum basale; c, cystic content; d, dermis; g, stratum granulosum; s, stratum spinosum. Scale bar, 20 µm

Fig. 2. Immunohistochemical staining profile of an epidermoid cyst with the monoclonal basal cell marker LH8 which detects CK14. The cyst wall is lined by a single layer of labelled basal cells. In addition, some associated suprabasal cells express this CK14 epitope. Abbreviations are explained in the legend to Fig. 1. Scale bar, 12 µm

Fig. 3. Immunohistochemical staining of an epidermoid cyst with the monoclonal antibody 6B10 which detects a CK4-specific determinant. CK4 reactivity is restricted to terminal differentiation stages, either as a weak to moderate or strong, but strictly focal staining (arrows). Abbreviations are explained in the legend to Fig. 1. Scale bar, 12 µm

Pilomatricoma

Basaloid cells along the periphery of tumour islands irregularly expressed CK14 and 19 (Fig. 9). Occasionally, individual cells or small cell groups also displayed CK7-positive staining. Maturing and transitional cells displayed CK1 (focally), CK8 (LE41 positive, though M20 negative), CK10 (focally, preferentially shown by the LH3 antibody), CK14 and CK16 (focally). CK1- and CK10-positive foci mainly concerned clustered or scattered squamoid cells, though the latter CK was also detected in transitional cells bordering on typical shadow cells. Typical shadow or ghost cells were weakly and patchily stained by RCK102 and the clone 80 antibody, while polypeptide-specific antibodies produced no staining at all. These staining characteristics are reminiscent of the CK staining patterns observed in matrix cells of the hair bulb, differentiating in the keratogenous zone in the direction of the hair cortex. The Vim 9 antibody to vimentin only stained tumoral stroma and a minor number of mesenchymal cells scattered within epithelial tumour islands.

Discussion

In addition to histological criteria [1, 6, 10–12], we have shown that CK1 and 10 are useful markers to discriminate epidermal cysts, more cautiously denoted as epidermoid cysts, and trichilemmal cysts or pilar cysts. In epidermoid cysts, CK1 and 10 are obligatory and permanent constituents of living suprabasal layers, similar to what is seen in the interfollicular epidermis and infundibulum [2] and references therein). However, in trichilemmal cysts, the intense CK1 and 10 reactivity is restricted to foci in transitional areas and accumulated debris. Apart from this, these foci occasionally reveal — though in minimal amounts — cytological features reminiscent of epidermal-type keratinization, e.g. keratohyalin granules. Our three probes directed against CK10 revealed identical immunostaining patterns, eliminating doubts that epitope masking may cause these restricted staining areas. Moreover, trichilemmal keratinization of the anagen follicle in the isthmus is also associated with a severe decline in CK1 and 10 expression competence, as shown here. This loss of the CK10 reactivity is, however,
Fig. 4. Immunohistochemical staining of a trichilemmal cyst with monoclonal antibody RKSE60. CK10 staining is subject to serious limitations and postponed to terminal stages of cyst-wall differentiation (focal staining, arrows). b, Stratum basale; c, cystic content; d, dermis; ic, intermediate cells; sc, superficial cells. Scale bar, 12 μm

Fig. 5. Immunohistochemical staining of a trichilemmal cyst with monoclonal antibody EKH4. CK14 staining is observed throughout the entire cystic epithelium, in addition the cystic content displays a faint staining. Abbreviations are explained in the legend to Fig. 4. Scale bar, 12 μm

Fig. 6. Immunohistochemical staining of a hair follicle with RKSE60. Trichilemmal keratinization of the external root sheath in the isthmus is associated with a drastic decrease of the CK10 reactivity, now restricted to some groups of cells in an intermediate position (arrows). In addition, CK10 is a prominent marker of sebum glands. Abbreviations are explained in the legend to Fig. 4; in addition: sg, sebum gland. Scale bar, 12 μm

not due to a permanent block, and can be triggered in vivo as well as in vitro [17].

Outer root sheath facing inner root sheath, i.e. below the isthmus, was obligatorily CK1 and 10 negative, but increasingly gained expression of CK19 in the outermost layer towards the bulbar portion. CK19, however, except for a local presence in outermost cells in one case, did not belong to the pilar cyst CK set, which so far is the perfect counterpart of the CKs expressed in the trichilemmal keratinization area of the anagen hair follicle. The remarkable stability in phenotypic expression of outer root sheath cells in pathological conditions not only applies to CK1 and 10, but also to CK5, 14 and 16, whose presence has been immunohistochemically demonstrated, and which is in accordance with previous biochemical data [17].

The reactivity of CK16 in epidermoid cysts, in view of its absence in the normal epidermal counterpart and according to its qualification [4, 21], is considered a manifestation of the hyperproliferative nature of the lesion. The presence of CK16 in trichilemmal cysts, however, is open to speculation mainly because CK16 represents a major component of the putative recipient tissue as shown here and elsewhere [7, 14, 17]. Another significant difference between epidermoid and trichilemmal cysts concerns the frequency of expression of CK4, which is, however, a minor member of the CK set. Epidermoid cysts regularly showed a tendency towards focal or more extensive reactivity of CK4, while the presence of CK4 in our trichilemmal cyst series was restricted to a single observation. In agreement with the normal distribution of CK4 and its status as a marker of terminal differentiation in non-cornified stratified squamous epithelia [18], these observations may suggest that epidermoid cysts are frequently, at least focally, defective in cornification.

Milia, a third type of miniature keratinous cyst, the site of origin of which is less precisely defined [1, 10, 11], is considered a true epidermoid cyst [6, 12]. Our immunohistochemical CK study fully confirms this histological typing (positive for CK1, 5, 10, 14) even if one considers the expression of the hyperproliferative keratinocyte marker CK16 and the frequency of the focal reactivity of the non-epidermal CK4.

The CK composition of eccrine hydrocystoma, apart from a few anomalies, largely corresponds to the CK set of eccrine sweat gland acini [2], which is in complete
accordance with current views on the nature of this tumour [6, 10–12]. Eccrine hydrocystoma and eccrine sweat gland acini express a complex CK set, including CK7, 8, 14, 18 and 19. The focal presence of CK1 and 10 in some tumour areas, with reduced CK8 and 18 reactivity, may reflect a commitment to a ductal epithelium CK pattern, as discussed below. Completely unexpected, however, was the rather impressive staining of CK4 not previously observed in sweat gland acini or ducts [2]. CK4 is also a major CK of the (sero)mucous gland differentiation programme [2]. However, it is not clear at the moment whether the hydrocystoma has embarked on this differentiation programme, and further experiments are needed to establish this. The discontinuous reactivity of CK16 in hydrocystoma, contrary to the minor and focal reactivity of CK16 in normal glands, may reflect the state of cellular growth (i.e. hyperproliferative versus normal).

The various parts of syringoma manifest an identical CK composition, obviously related to the CK composition of sweat ducts, i.e. CK1, 5, 10, 14, 16 and 19 as shown here and elsewhere [2]. This provides new and reliable molecular evidence for its nature. The weak intraductal staining corresponding to CK7 may belong to debris of glandular origin, where CK7 is consistently expressed as discussed above. Remarkably, syringoma cells do not acquire any new potency of CK reactivity as compared to eccrine hydrocystomas and thus seem phenotypically stable.

Pilomatrixoma is a benign tumour expressing trichocyte-type Cks as well as certain epithelial Cks [13]. The epithelial CK characteristics of basaloid cells (so-called basophilic "undifferentiated" cells), maturing cells and shadow cells of pilomatrixoma are similar to those of normal hair bulb matrix cells differentiating into cortex cells. Indeed, that region of the follicle contained cells positive for both trichocyte and epithelial Cks [7]. Previous studies of the lectin-binding capacities already pointed to a similar pattern of differentiation [9]. Recently, CK5, 6, 14, 16, 17 and 19 were biochemically identified in pilomatrixoma [13]. Our screening of CK14 and 19, and to a lesser degree of CK16, confirm these biochemical results. The staining patterns produced for CK19 were identical to those presented before [13], despite using different antibodies. Furthermore, negative results for CK4, 13 and 18 were independently obtained in both cases. The presence of CK8, however, is shown here for the first time. In addition, CK1 and 10 are shown in some tumour regions where maturing cells are suspected to enter a pathway of squamous cell differentiation, a phenomenon also recognized before, using a
CK10/11-specific probe [13]. Apart from this, CK10 is also displayed in some tumour sites where squamous cells and synchronous deposition of keratohyalin granules are not discerned. In these areas, achievement of the CK10 reactivity is a differentiation-associated phenomenon, as we observed in hair cortex cells above the keratogenous zone.

Concerning the CK pair or co-expression concept [18], though it is a guide, it does not lay down strict rules. For instance, CK4 and 13 are the major CK pair expressed in non-cornified stratified squamous epithelia of various internal organs [4, 14, 18]. Uncoupling of these differentiation-related markers is, however, a frequent observation in certain complex epithelia, in fetal epidermis, in some cultured cell lines and in pathological conditions (e.g. [2, 14, 19, 20]). In carcinomas, this may reflect differences in the state of differentiation related to the clonal origin of tumour cells [19]. Here, the uncoupling of CK4 and 13 is immunohistochemically documented in epidermoid and trichilemmal cysts, in milia and eccrine hydrocystoma. We do not estimate that it stands for a loss of the normal state of differentiation since CK4 and 13 are absent in the healthy counterpart. Rather, we are dealing with a disturbance of terminal differentiation, but the intrinsic and external factors that may contribute to this defect remain unexplained. This CK4/13 potency is gained imperfectly in certain lesions, suggesting that normal physiological triggers are not at hand.

On the other hand, concerning the CK1/10 pair, we observed either a strict co-expression, a combined decrease or a focal acquisition of reactivity. The apparent uncoupling of the CK1/10 reactivity revealed in non-living layers referred to the failure of the CK1 specific antiserum to generate positive staining. However, the antiserum AF87 was directed against the C-terminus of CK1, which is supposed to be removed from the polypeptide during terminal stages of epidermal keratinization [16]. Seemingly, a similar modification occurs in pathological conditions. Altogether, our CK1/10 data on cystic tumours indicate no discrepancies suggesting an increasing loss of normality as, for example, observed in certain squamous cell carcinomas [8, 15].

Finally, when interpreting our immunohistochemical data, one should be aware of the varying availability of the respective epitope when different antibodies against one target polypeptide are applied (see also [2, 4, 13]). Obviously, this remark concerns CK8 and 10 in eccrine hydrocystoma and pilomatrixoma. Similar problems appear apparently arise for the CK14 reactivity in epidermoid and trichilemmal cysts. In all these cases, differential masking and conformational or proteolytic alterations of the corresponding epitope seem to be involved and should be considered explicitly. In particular, this is the case with LH3, a conformationally sensitive CK14 antibody, and with LPH1, which is directed against CK10 and presumably also against complexes of CK1 and 10 (I. M. Leigh, E. B. Lane, in preparation).

In conclusion, our immunohistochemical data on CK reactivity using polypeptide-specific antibodies allow us to decide conclusively on the state of differentiation reached in skin epithelial cysts and some uncommon types of epithelial tumours. Strictly speaking, however, this study does not allow us to decide whether these cysts and tumours are derived from cells already committed to a similar differentiation pathway, or whether they are produced from a certain equipotent and/or pluripotent cell population, susceptible to some, as yet unknown, stimulus.

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References

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