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

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ABSTRACT Cytokeratin polypeptides of human epidermis, of epithelia microdissected from various zones of the pilosebaceous tract (outer root-sheath of hair follicle, sebaceous gland), and of eccrine sweat-glands have been separated by one- and two-dimensional gel electrophoresis and characterized by binding of cytokeratin antibodies and by peptide mapping. The epithelium of the pilosebaceous tract has three major keratin polypeptides in common with interfollicular epidermis (two basic components of mol wts 58,000 and 56,000 and one acidic polypeptide of mol wt 50,000); however, it lacks basic keratin polypeptides in the mol wt range of 64,000-68,000 and two acidic keratin-polypeptides of mol wts 56,000 and 56,500 and contains an additional characteristic acidic cytokeratin of mol wt 46,000. Another cytokeratin polypeptide of mol wt 48,000 that is prominent in hair-follicle epithelium is also found in nonfollicular epidermis of foot sole. Both epidermis and pilosebaceous tract are different from eccrine sweat-gland epithelium, which also contains two major cytokeratins of mol wts 52,500 and 54,000 (isoelectric at pH 5.8-6.1) and a more acidic cytokeratin of mol wt 40,000. A striking similarity between the cytokeratins of human basal-cell epitheliomas and those of the pilosebaceous tract has been found: all three major cytokeratins (mol wts 58,000; 50,000; 46,000) of the tumor cells are also expressed in hair-follicle epithelium. The cytokeratin of mol wt 46,000, which is the most prominent acidic cytokeratin in this tumor, is related, by immunological and peptide map criteria, to the acidic keratin-polypeptides of mol wts 48,000 and 50,000, but represents a distinct keratin that is also found in other human tumor cells such as in solid adamantinomas and in cultured HeLa cells.

The results show that the various epithelia present in skin, albeit in physical and ontogenic continuity, can be distinguished by their specific cytokeratin-polypeptide patterns and that the cytoskeleton of basal-cell epitheliomas is related to that of cells of the pilosebaceous tract.

The cytoskeleton of epithelial cells is characterized by the presence, frequently abundance, of intermediate-sized filaments (tonofilaments) containing α-keratinlike proteins (10-25, 41, 50, 53-57). Comparisons of tonofilament proteins from different epithelial cells and tissues have shown that, unlike many other cytoskeletal proteins, these keratinlike proteins ("cytokeratins"; 20) are not identical in diverse epithelia, but show differences of polypeptide composition. Different patterns of keratin polypeptides have been reported for epidermal tissue from different regions of the body surface (1-3, 7, 13, 25, 37, 51). Different keratin patterns have also been found in different layers of epidermis (1-3, 24, 25; for review see 30) as
well as in other stratified squamous epithelia (6, 13, 25, 41, 64) and in simple epithelia such as hepatocytes, intestinal cells, and early embryonal epithelia (5, 11, 13, 21, 32). Differences of cytokeratin polypeptide composition have also been found in various cultured cells, including keratinocytes (12–14, 17, 18, 23–26, 30, 54, 55). The data available suggest that cytokeratins are characteristic of epithelial cells but are expressed in different epithelia in tissue- or cell-type specific patterns of different polypeptides of the keratin family of proteins (13, 17, 22, 64).

Analyses of the epidermal keratinlike proteins have so far focused on the vertical aspects of epidermal differentiation, i.e., cells derived from the stratum basale of interfollicular regions (e.g. 1–3, 24, 25, 30). We have examined the cytokeratins of epithelial cells that are in continuity with, and are ontogenetically derived from, epidermal basal cells but have developed into functionally and structurally different domains. These include the upper and lower portions of the outer root sheath epithelium of hair follicle, the secretory and the ductal portions of the sebaceous gland, and the secretory and the ductal portions of the eccrine sweat gland. We show that in the skin functionally defined epithelial mosaics exist that are characterized by different patterns of cytoskeletal polypeptides.

MATERIALS AND METHODS

Tissues and Cells

Specimens of normal human skin were obtained in one of the following ways: (a) Total skin was obtained during surgical removal of basal-cell epitheliomas or melanomas located on scalp, arms, face, and breast. The skin samples were taken in a distance of at least 3 cm from the edge of the tumors, immediately frozen in isopentane cooled in a liquid nitrogen bath, and histologically documented. (b) Nonsimilar-basement epithelium obtained during surgery was prepared by taking thin slices parallel to the surface using a cryocut knife as described (13). (c) Slices of thigh skin and foot-sole skin were taken from human corpses a few hours post mortem and were frozen.

For preparation of epidermis, pieces of skin obtained as described under (a) were immersed in ice-cold phosphate-buffered saline (PBS, pH 7.4) and dissected under a binocular using free forceps and scissors. Using this method, contamination of epidermal samples with remnants of small hair follicles could not be completely avoided. This was also true when epidermis was separated from dermis along the basal lamina using EDTA buffer (47). Alternatively, epidermal samples were obtained as slices using a cryocut knife (see b above) and then histologically appeared free from skin appendages, contaminated only with very little connective tissue adhering to the epidermis.

Outer root sheaths of anagen human hair follicles were prepared from plucked scalp and beard hairs. After cutting off the hair bulb, tissue consisting mainly of outer root-sheath could easily be stripped off from the hair shaft using forceps. The outer root-sheath cells were lyzed and extracted in buffer A (1.5 M KCl, 0.5% Triton X-100, 5 nM EDTA, 0.4 M phenylmethylsulfonyl fluoride [PMSF], 10 nM Tris-HCl, pH 7.2) for 20 min at 0°–4°C, centrifuged (1 min at 8,000 g), washed with buffer A for 20 min at 0°–4°C, centrifuged for 1 min at 8,000 g, washed with buffer B, and pelleted again.

For preparations of living foot-sole epidermis, foot-sole skin was vertically cut into 20-μm sections that were incubated with buffer A. Under the binocular (×50) the epidermal-layer could easily be distinguished: stratum corneum appeared opalescent, living epidermis was white. In the sections, living epidermal layers were separated from t. corneum and dermis with fine needles, following carefully the undulating profile of this type of epidermis.

Skin appendages were prepared by microdissection from 20-μm frozen sections of normal skin (Fig. 1) and extracted as described (42). Hair shafts were removed with a needle and discarded so that preparations of hair follicles consisted mainly of outer root sheath epithelium. Sweat-gland preparations contained acini as well as ducts, besides some connective tissue stroma. Pure eccrine sweat-gland ducts were prepared in the same way from foot-sole skin since in this tissue many sweat gland ducts are arranged perpendicularly to the epidermis. Routinely, for two-dimensional gel electrophoresis combined with silver staining, we applied the equivalents of 15 sections through hair follicles, 30 sections of sebaceous glands, and 30 sections through eccrine sweat glands (for sizes see Fig. 1).

For preparations enriched in basal cells of epidermis 12-μm cryostat sections of face skin were used. Regions of basal portions of interfollicular epithelium were dissected using a UV laser-beam dissection microscope (Fa. Biotechnik, Munich, Federal Republic of Germany; ×100) in such a way that only one or two cell layers of lower t. spinosum were included (Fig. 1). Thereafter the apical portion of dissected epithelium, including most of the t. spinosum, was scratched apart, using fine needles. Then a drop of cold buffer A was applied and the basal-cell layer-enriched tissue strips were transferred to a centrifuge tube and extracted as described (42).

Using similar techniques as described under (c), central portions of tumor nodules of basal cell epitheliomas were excised in order to separate the cytokeratin-positive tumor cells from associated, vimentin-positive connective tissue stroma (Fig. 2; for specific occurrence of cytokeratin in basal cell epitheliomas see refs. 27, 48).

Antibodies

The following guinea pig-antibody preparations against intermediate filament proteins were used: (a) antibodies against desmosome-attached tonofilaments of bovine-muzzle epidermis, recognizing epithelial as well as many nonepidermal cytokeratins (11, 13, 15, 21); (b) antibodies against total bovine-muzzle prekeratin polypeptides excised and eluted from preparative polyacrylamide gels and acetone-precipitated (13, 15); (c) antibodies raised against the electrophoretically purified bovine-muzzle prekeratin component I (mol wt 68,000; 15; cf. reference 20) which cross-reacts with several other “basic cytokeratins”, i.e. those focusing between pH 6.3 and 8.5 (13, 17); (d) antibodies raised against the electrophoretically purified bovine-muzzle prekeratin component VII which react with both components VII and VI (12); (e) antibodies to murine vimentin reacting also with human vimentin (16, 18, 19).

Immunofluorescence Microscopy

Indirect immunofluorescence microscopy using 4-μm thick, acetone-fixed cryostat sections was performed as described (15). Antibody staining was also used to distinguish tumor-stroma cells, identified by their reaction with vimentin antibodies, from epithelioma cells (Fig. 2).

Gel Electrophoresis

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) using 8.5% slab gels was performed according to Laemmli (35). For two-dimensional gel electrophoresis, neutral to acidic polypeptides were separated by isoelectric focusing (IEF; 44) whereas basic polypeptides could also be resolved when nonequilibrium pH gradient electrophoresis (NNEPHG; 45) was applied (ampholine range of pH 2–11, I lys buffer containing 0.25% SDS). Experiments using lys buffer without SDS did not show different results. Separation in the second dimension was performed using 12% polyacrylamide gels (35) with two modifications (60) implying a modified electrode buffer and an acrylamide, N,N'-...
FIGURE 1 Dissection of epidermis and separation of skin appendages from epidermis and dermis using frozen sections (methylene blue-stained in this case). (a) Survey micrograph of face skin showing epidermis (E), dermis (D) and a pilosebaceous unit consisting of hair follicle (HFu, upper portion; HF1, lower portion) and sebaceous gland (SG). The regions to be isolated (sebaceous gland and the upper portion of the hair follicle) are circumscribed and separated from surrounding tissue with a needle (42). After addition of a drop of buffer A, the structures are lifted with a needle and transferred, in this case to another microscope slide for demonstration (b and c). Only small residues of adjacent connective tissue (b and c) remain attached to the dissected epithelial tissues. (d and e) Dissection of a lower portion of hair follicle (below orifice of sebaceous gland duct). (f) Dissection of an eccrine sweat gland (ESG) containing secretory tubules as well as ducts. Separation gaps of the microdissection line are denoted by asterisks. (g) Phase-contrast micrograph showing UV laser-beam dissection, in a 12-μm thick section of face skin (arrows denote dissection line), of basal cell-enriched layer of interfollicular epidermis. Bracket demarcates epidermis. D, dermis. Bars, 100 μm.
methylene bis-acrylamide ratio of 30:0.15. Gels were stained either with Coomassie Brilliant Blue or, for detection of very small amounts of protein, by silver staining (58) modified as described (4; gels used for silver staining were made 0.75-1.0-mm thick instead of 1.5 mm).

Immunological Identification of Polypeptides

Polypeptides separated by SDS PAGE were characterized immunologically after blotting onto nitrocellulose paper (61; modified as described in reference 13). In most experiments, polypeptides were transferred electrophoretically in a buffer containing 192 mM glycine, 25 mM Tris-HCl (pH 8.3), and 20% (vol/vol) methanol. Immunoglobulins bound were detected by binding of 125I-labeled protein A from *Staphylococcus aureus* (New England Nuclear, Boston, MA).

Two-dimensional Tryptic Peptide Mapping of Radioiodinated Proteins

We used the procedure of Elder et al. (8), employing trypsin digestion of radioiodinated proteins of excised spots of Coomassie Blue-stained polypeptides separated by two-dimensional gel electrophoresis. The proteins were digested using 50 µg trypsin (Trypsin-TPCK; 217 U/mg, Millipore Corp., Bedford, MA) per gel piece, and peptides were lyophilized, analyzed by thin-layer electrophoresis and chromatography on cellulose-coated plates and detected by autoradiography.

RESULTS

Cytoskeletal Proteins of Normal Epidermis and Basal-Cell Epitheliomas

Cytoskeletal residues from epidermal tissues obtained after extraction in high- and low-salt buffer and Triton X-100 are enriched in intermediate-sized filaments of the keratin type. In several vertebrates, such epidermal tonofilaments display rather complex patterns of keratin polypeptides. For example, in tonofilaments from *S. spinosum*-rich epidermal tissue from bovine muzzle six polypeptide bands are resolved on normal SDS-polyacrylamide gel electrophoresis (7, 37, 51), and at least eight major keratin-polypeptide species can be distinguished by higher-resolution gel electrophoresis in one or two dimensions (11, 13, 15). A similarly great complexity of prekeratin composition is found in total human-epidermis from various locations (Fig. 3, slot 2, and Fig. 4a-d), which is essentially in agreement with findings of Fuchs and Green (25; for patterns of human-epidermal keratin polypeptides see also refs. 1, 3, 40, 49). The keratin nature has been shown for all these polypeptides by their inclusion in intermediate-sized filaments in vitro from proteins solubilized in urea (data not shown; cf. references 36, 46, 50, 52, 55, 59), by peptide maps (see below and references 23-25, 40) and by their binding of various antibodies specific for cytokeratins (Fig. 3, slot 2').

The polypeptide complexity of human-epidermal keratins is often greater than suggested from one-dimensional gel electrophoresis of the Laemmli type. SDS PAGE using some modifications (60) and two-dimensional gel electrophoresis can resolve more components (Fig. 4a-d). Human epidermis contains both slightly basic and acidic cytokeratin polypeptides (13) that can be separated and identified on nonequilibrium pH gradient electrophoresis (Fig. 4a). The following components are usually observed in weakly cornified human-epidermis (Fig. 4a, b): (a) a major component of mol wt 68,000 that is resolved into several (up to 4) isoelectric variants focusing between pH 7.0 and 7.8 (designated "K 68"); (b) a minor component of mol wt 65,500 focusing at about pH 7.8 ("K 65.5"); (c) a major component of mol wt 58,000 ("K 58") also displaying 3-4 isoelectric variant spots in the pH range from 6.8 to 7.4; (d) a prominent acidic component of mol wt 56,500 ("K 56.5") resolved into two major variants (pH 5.20-5.40); (e)
a similarly charged, slightly smaller acidic component (mol wt 56,000; "K 56a"); and (f) a major acidic component of mol wt 50,000 ("K 50"); at least two isoelectric variants. In addition, we have found in some epidermal samples (Fig. 4b, insert), but not in others, variable amounts of two relatively small and acidic cytoskeletal polypeptides of mol wts 48,000 ("K 48") and 46,000 ("K 46") which appear to be more frequent in preparations from skin containing a certain density of hair follicles. Although some keratin K 48 and K 46 has been noted, in variable amounts, in dissected epidermal samples containing some dermal material, including parts of hair follicles, they have not been seen in epidermal slices made with a cryocut knife. Also, interfollicular epidermis microdissected from frozen sections does not contain these two polypeptides (Fig. 4a). As the only exception, the acidic polypeptide K 48 is found in epidermis from foot soles, including microdissected living layers (Fig. 4c, d), and appears to correspond to the polypeptide band of foot-sole epidermis designated "mol wt 46,000" by Fuchs and Green (25; cf. reference 3). Because we have found polypeptide K 48 in different samples from foot soles but not in interfollicular epidermis from a broad range of different body locations, we conclude that it is expressed in living epidermal cells of a certain type, e.g. from foot sole, but not in epidermal cells from many other regions of skin.

Human basal-cell epitheliomas differ in their cytokeratin pattern from epidermis, most characteristically by the absence of the two relatively large and basic polypeptides of mol wts 62,000 and 65,500 and the presence of an additional component of an apparent mol wt 46,000 (K 46; Fig. 3, slot 3; Fig. 4 e and f). A similar pattern of prekeratin-polypeptide bands in basal cell epitheliomas has been reported by Kubilus et al. (34). Keratin polypeptide K 48 has not been detected in cytoskeletal preparations from seven out of eight different basal-cell epitheliomas (Fig. 4 e and f; in one preparation, trace amounts of K 48 have been found). By contrast, keratin K 46, which is more acidic than actin and keratin K 50 and usually appears...
as a series of three isoelectric variants (Fig. 4c and f), is the most prominent acidic keratin of this tumor; it has been identified as a member of the cytokeratin family of proteins by immunoblotting experiments and peptide maps (see below). The prominent acidic component K 50 of basal-cell epithelioma comigrates with epidermal K 50 (Fig. 4f, inset). A minor cytoskeletal component of mol wt 52,500 and almost isoelectric with BSA (Fig. 4f) has been shown, by coelectrophoresis and peptide map (not shown) to be identical to cytokeratin A originally detected in hepatocytes and intestinal cells of rodents (5, 11, 13, 21).

The main basic cytokeratin of the epitheliomas comigrates with epidermal keratin K 58, showing also the same distribution of isoelectric variants as in epidermis (not shown). These tumor cells also contain a minor component slightly more basic than K 58 of normal epidermis, which has a mol wt of approximately 56,000 and seems to correspond to keratin K 56b observed in some preparations of epidermis (e.g., Fig. 4c).

**Cytoskeletal Proteins of Epithelial Cells of the Pilosebaceous Tract**

The finding of cytokeratin K 46 in basal-cell epitheliomas and in certain follicle-containing preparations of epidermis has pointed out the possibility that this cytokeratin polypeptide is a constituent of normal skin but is restricted to follicle epithelium. We have therefore examined in detail the outer root-sheath epithelium of hair follicles and have compared its cytoskeletal proteins with those of sebaceous glands and of interfollicular epidermis. The cytokeratin pattern of hair-follicle epithelium (Fig. 3, slots 4, 4' and 4''); Fig. 4g and h) is different from that of normal interfollicular epidermis of adjacent body-skin but is strikingly reminiscent of the cytokeratin-polypeptide pattern of basal-cell epitheliomas. Outer root-sheath epithelium does not contain any detectable amounts of cytokeratins larger than mol wt 58,000 but is rich in two prominent basic cytokeratins, K 58 and K 56b, and three acidic ones, K 50, K 48, and K 46. When follicle epithelial cytokeratins are coelectrophoresed with those from basal-cell epitheliomas, the two components designated K 46 in each tissue comigrate and are clearly separated from polypeptide K 48 present in both hair follicles and foot-sole epidermis (Fig. 4h, inset).

This cytokeratin pattern is characteristic only for the external root-sheath epithelium of the lower part of the follicle, i.e., below the entry of the sebaceous duct, as it is enriched in plucked-hair follicles (for hair-follicle anatomy see 43). We have also microdissected pilosebaceous units into external root-sheaths of lower portions of hair follicle, upper portions of hair follicle (pilosebaceous ducts), and sebaceous glands, and analyzed the cytokeratin proteins (Fig. 3, slots 7 and 8, and Fig. 5a–c). Epithelial material from hair follicles, which includes pilosebaceous ducts, shows the presence of the large basic keratin K 68 typical of interfollicular epidermis, in addition to the keratin polypeptides of plucked-hair follicles (Fig. 3, slot 8). Pure microdissected-pilosebaceous ducts reveal a keratin pattern almost identical to that of interfollicular epidermis (Fig. 5a), showing the presence of keratin K 68 and the absence of keratins K 48 and K 46 (see also reference 3). By contrast, dissected lower portions of hair follicles show a pattern identical to that of the root sheaths associated with plucked-hair follicles (Fig. 5b).

Cytoskeletons of dissected sebaceous glands as obtained after extraction with high-salt buffers and detergent display essentially the same pattern of keratin polypeptides as external root-sheaths of hair follicle (Fig. 3, slot 7, and Fig. 5c). In addition, cytoskeletons of sebaceous glands display a special basic polypeptide (K 59 in Fig. 5c) besides some minor spots in the region of acidic keratins K 56.5 and K 56a, and polypeptide K 48 is found only in trace amounts.

**Cytoskeletal Proteins of Eccrine Sweat-Glands**

Eccrine sweat-gland epithelium is in direct continuity, via the ductal epithelium, with the epidermis but it is constituted by morphologically and functionally grossly different types of cells, including “dark” and “light” secretory cells and myoepithelial cells (9, 43). We have compared the keratin pattern of excised total sweat-glands as well as of separated glandular and ductal portions with those of the pilosebaceous tract and of epidermis (Fig. 3, slots 9 and 11 and Fig. 5d). Whereas the ductal epithelium contains major cytokeratin-polypeptides similar to those described for the pilosebaceous tract, but without K 46 (Fig. 3, slot 11), preparations including secretory portions of sweat glands are characterized by the presence of an additional cytokeratin smaller (mol wt 40,000; "K 40") and more acidic than actin (Fig. 5d). This small cytokeratin comi-
brates with the cytokeratin of mol wt 40,000 recently described in small intestine, colon, and rectum (21, 42; not shown) and seems to be similar to keratin "40 K" observed in certain cultures of human squamous-cell carcinomas (65) and conjunctival keratinocytes (26). Glandular portions of sweat glands (Fig. 5d) also consistently show two polypeptides of mol wts 54,000 ("K 54") and 52,500 ("K 52.5"), the smaller of which appears to be identical with the human cytokeratin-component A as described in human liver and hepatocellular carcinomas (5), in colorectal epithelia and a related cloacogenic carcinoma (42) and, as a minor component, in basal-cell epitheliomas (Fig. 4f of this study).

Cytokeratins of Microdissected Basal Strata from Interfollicular Epidermis

The finding of small and acidic cytokeratins in pilosebaceous epithelium (K 46) and sweat glands (K 40) but not in interfollicular epidermis has prompted us to examine the possibility that only a small proportion of epidermal cells, e.g. basal cells, may contain these polypeptides. Therefore, we have micro dissected basal-cell-enriched epidermal tissue and compared its cytoskeletal polypeptides with those of total interfollicular epidermis and of basal-cell epithelioma. As is shown in Fig. 3 (slots 3 and 6), no polypeptides migrating faster than keratin K 50 are found in such epidermal-cell layers; specifically, components K 46 and K 40 are absent. When high loads of cytoskeletal material from interfollicular epidermis are applied to gel electrophoresis no components similar to K 48 and K 46 are seen in Coomassie Blue staining (e.g., Fig. 4a). Trace amounts of polypeptides similar to K 48 and K 46 are only detected after silver-staining, and amounts of K 46 appear to be <1% of K 50 (not shown; for sensitivity of silver staining see also 58). Thus, in interfollicular epidermis keratins K 46 and K 48 are unlikely to make a significant contribution to the structure of the tonofilaments of the basal cell layer as a whole. Rather, the minute amounts of K 48 and K 46 observed in such preparations may reflect contaminations by pilosebaceous material or the occurrence of a very small proportion of special epidermal cells containing these two small cytokeratins.

Comparison of Keratin Polypeptides in Two-dimensional Tryptic Peptide Maps

The molecular relationship of the individual keratinlike polypeptides has also been examined by peptide mapping of polypeptide spots excised after two-dimensional gel electrophoresis. The neutral-to-basic cytokeratin polypeptides K 68, K 58, and K 56b as well as those of various other stratified squamous epithelia (cf. reference 13) show conspicuous similarities in such tryptic-peptide maps (not shown), indicating that they represent a defined subfamily of keratin polypeptides (for related data see 22). In the present study we have made a detailed comparison of the various small acidic cytokeratins K 46, K 48, and K 50. For each of these polypeptides the different isoelectric variants are practically identical in their peptide maps (not shown), in agreement with the notion that these variants represent different degrees of phosphorylation of the same polypeptide. This is also indicated by studies of 32P-phosphate incorporation into keratins of epidermis (28, 53) including K 46, K 48, and K 50 of hair follicles and basal-cell epitheliomas (not shown; for related data in mouse liver and several cultured cells see 13, 53, 55). Comparison of the peptide maps of polypeptide K 50 from different sources has confirmed that this keratin is identical in hair-follicle epithelium, interfollicular epidermis and basal-cell epithelioma (not shown). Since we have previously observed that human keratin K 50 coelectrophoreses, on two-dimensional gel electrophoresis, and immunologically cross-reacts with prekeratin VII from bovine-muzzle epidermis (13) we have also compared these two pro-
teins (Fig. 6a and b) and similar tryptic-cleavage patterns have been obtained although some differences in the two species are also apparent.

Human cytokeratin K 48 is also identical in hair follicles and in foot-sole epidermis (not shown). When one compares the tryptic map of radioiodinated K 48 with that of K 50 (Fig. 6a and c) it is obvious that these two polypeptides are related but not identical. Detailed comparison reveals a number of different peptides, indicating that K 48 is not derived from K 50 by proteolytic processing.

The tryptic-peptide map of keratin K 46 from hair follicle is identical to that of the main acidic cytokeratin K 46 from basal-cell epithelioma (Fig. 6d and e). As K 46 from basal-cell epithelioma comigrates, on two-dimensional gel electrophoresis, with cytokeratin component 3 from HeLa cells (13) we have also compared these two polypeptides by tryptic-peptide mapping. The results (Fig. 6e and f) indicate that K 46 is a common cytokeratin of hair follicles, basal-cell epithelioma cells and HeLa cells, i.e., cells derived from an adenocarcinoma of the cervix uteri. All other HeLa cytokeratins are different, by two-dimensional gel electrophoresis and in peptide maps, from any of the keratins of epidermis and pilosebaceous tract (not shown; cf. reference 13). Peptide maps of keratins K 46 and K 48 from hair follicle are rather similar but also show a number of different peptide spots (Fig. 6c, d). An even greater similarity is observed for tryptic-peptide maps of keratins K 46 and K 50 (Fig. 6a and d), but detailed examination also reveals some peptide spots unique to each of the two polypeptides.

Occurrence of Cytokeratin K 46 in Other Human Tumors

A cytokeratin polypeptide comigrating with K 46 of hair follicles and basal-cell epitheliomas has also been found in some other human epithelial-tumors such as adamantinomas, i.e. tumors derived from cells related to, or derived from, the epithelial portion of tooth germs in the “enamel organ” (for review see 62; for keratinlike proteins in tooth germs see reference 38). An example of this tumor, displaying a typical form of growth in clumps or columnar aggregates of cells resembling basal-cell epithelioma, is shown in Fig. 7a and b. Cytoskeletons from this tumor (Fig. 7c and d) reveal the presence of cytokeratin K 46, together with two basic keratins and K 50 and K 48. Furthermore, this tumor produces cytokeratin polypeptides K 52.5 and K 40 which have both been described for the eccrine sweat glands (this study) and other epithelia (5, 11, 13, 21). Tryptic peptides from K 46 of adamantinoma are identical to those of K 46 from hair follicle and basal-cell epithelioma (not shown).

DISCUSSION

The epithelial tissue covering the body is a continuous closed system but it is not uniform and homogeneous as far as cell types and functions are concerned. Rather, the epithelial moiety of the skin represents a mosaic of different functional domains formed by different types of epithelial cells that are all derived from cells of the embryonal epidermis (for reviews see references 9, 43). The following types of cutaneous epithelial-complexes can be distinguished: (a) Interfollicular epidermis sensu stricto; (b) epithelia of the pilosebaceous unit, i.e. the hair proper, outer root sheath, sebaceous gland, sebaceous-duct epithelium, and the common pilosebaceous duct; (c) epithelia of alveoli and ducts of eccrine sweat-glands; (d) epithelia of apocrine glands and their ducts; (e) epithelia lining the ducts and alveoli of mammary gland.

In the present study we demonstrate that the epithelia of cutaneous appendages, albeit derived from basal-cell layers of embryonal epidermis, differ in their patterns of cytoskeletal proteins from each other as well as from epidermis, including cells of the basal layer. For example, the eccrine sweat-glands do not contain large neutral-to-basic cytokeratins (K 64–K 68) as they are typical of epidermal differentiation. They do express, however, polypeptides K 40 and K 52.5, which are also

FIGURE 6 Autoradiographs of tryptic-peptide maps of keratin polypeptides separated by two-dimensional gel electrophoresis, excised and radio-iodinated. E, direction of electrophoresis; C, chromatography. Maps of the most basic isoelectric variant spot of each polypeptide are shown. (a) K 50 from human interfollicular epidermis; (b) prekeratin VII from bovine-muzzle epidermis; (c) K 48 from human-hair follicle; (d) K 46 from human-hair follicle (outer root-sheath); (e) K 46 from basal-cell epithelioma; (f) HeLa cytokeratin component 3. Arrows in (a) and (b) denote similar tryptic-cleavage products of human K 50 and bovine VII, which comigrate in mixtures of peptides from both polypeptides. Bars in (a) and (e) designate peptide spots facilitating distinction of K 48 or K 46. Note identity, in all detectable spots, of K 46 from hair follicle, basal-cell epithelioma and HeLa cells. All details were also confirmed by mapping of mixtures of peptides from different polypeptides (not shown).
found in various simple epithelia, including intestinal cells (e.g. 5, 11, 13, 17, 21, 42; for K 40 see also reference 65). As K 40 is detected in microdissected glandular portions but not in ducts, we conclude that it is specifically expressed in the secretory cells.

Particularly interesting is the finding that both outer root sheath cells of hair follicles and sebaceous-gland epithelium differ in their keratin proteins from interfollicular epidermis in that they do not contain neutral-to-basic keratin polypeptides larger than mol wt 59,000, but express large proportions of a specific acidic cytokeratin of mol wt 46,000 (K 46). This polypeptide K 46 is related to K 50 expressed in all cutaneously derived epithelia, including mammary gland (not shown), and to K 48 which is also produced in foot-sole epidermis devoid of pilosebaceous units. Polypeptide K 46 is also expressed in certain tumor cells such as in basal-cell epitheliomas, adamantinomas, various squamous-cell carcinomas (unpublished results), and in cultured HeLa cells. Whether it is identical or related to the keratin polypeptide designated "46 K" in cultures of keratinocytes (26, 65) remains to be examined. The specific expression of such a relatively small, acidic cytokeratin of approximate mol wt 46,000 in hair-follicle epithelium but not in interfollicular epidermis is also seen in other species such as cow (these authors, unpublished data). We propose that synthesis of K 46 defines a certain cell type which is frequent in pilosebaceous epithelium but can also occur elsewhere in the body and can be maintained in certain tumors. Our finding that polypeptide K 46 is expressed in human basal-cell epitheliomas but not in several carcinomas (5, 13) indicates that the occurrence of this protein in tumors is not due to the formation of an abnormal keratin during tumorigenesis (63) but rather is the result of the selection of a cell type related to the epithelium of the pilosebaceous tract. A relationship of basal-cell epitheliomas to pilosebaceous cells is in harmony with current views of the nature and possible origin of this type of tumor (31, 39).

Our data do not allow us to decide whether these tumors are derived from cells already committed to pilosebaceous differentiation or whether they are produced from a small subpopulation of basal-epidermal cells developing some features of differentiation reminiscent to those of the pilosebaceous tract (the latter mode of origin of tumor is suggested by findings summarized in references 29, 39, 66). Unexpected is the finding of polypeptide K 46 in HeLa cells, a cell line derived from an "adenocarcinoma" of the cervix uteri (33), i.e. a tissue that can produce sebaceous metaplasias (43). Further systematic studies of the cytokeratin patterns of different epithelial tissues and cells will have to show the restriction and cell type-specificity of this protein.

Our finding of different cytokeratin patterns in the different cutaneous epithelia suggests that in embryonic development epidermal cells laterally differentiate into cells forming glands and hair follicles and that during these differentiation processes changes of expression of cytoskeletal proteins take place. These observations of differences of expression of cytokeratins in the various epidermal derivatives also add to the increasing number of cases of cell type-specific expression of keratinlike proteins in epidermis as well as in various nonepidermal organs (6, 11, 13, 17, 21-26, 41, 64, 65). The functional importance of these differences in the cytokeratin patterns is not clear. The expression of different but related polypeptides included in the same structure in the various epithelia may reflect the expression of higher-order sets of differentiation programs rather than the synthesis of specific proteins serving distinct cell type-specific functions.

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FIGURE 7 Human adamantinoma. (a and b) Immunofluorescence microscopy on frozen sections, showing similar growth patterns of a stroma-rich basal-cell epithelioma (a) and adamantinoma (b), stained by antibodies to cytokeratins. Tumor cells are strongly stained, stromal tissue is negative. Bars, 100 µm. (c and d) Two-dimensional gel electrophoresis of cytoskeletal proteins of adamantinomas, using NEPHG electrophoresis (c) and IEF (d) in the first dimension. The two components designated K 58 and K 56b comigrate, in coelectrophoresis experiments, with polypeptides K 59 and K 58 of sebaceous glands (see Fig. 5 c).
Note Added in Proof: We have recently shown that acinar cells of apocrine glands from human axillary skin contain the same cytokeratin polypeptides (K39, K58, K54, K52.5, K50, K46, K45 and K40) as cytoplasmic intermediate filaments that are present only in K45, K52.5, K46 and K45 are identical to cytokeratin polypeptides originally designated cytokeratins 1, 2, 3, and 4 of HeLa cells (see reference 13).

REFERENCES


