

Classification of Epidermal Keratins according to Their Immunoreactivity, Isoelectric Point, and Mode of Expression

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ABSTRACT Human epidermal keratinocytes express under various growth conditions a total of at least nine keratins that can be divided into two subfamilies. Subfamily A comprises 40-, 46-, 48-, 50-/50'-, and 56.5-kilodalton (kd) keratins which are relatively acidic (pI <5.5) and, with the exception of 46-kd keratin, are recognized by AE1 monoclonal antibody. Subfamily B comprises 52-, 56-, 58-, and 65-67-kd keratins which are relatively basic (pI >6) and are recognized by AE3 monoclonal antibody. Within each keratin subfamily, there is a constant member (50-/50'- and 58-kd keratins of the subfamilies A and B, respectively) that is always expressed. The other seven keratins of both subfamilies are variable members whose expression depends upon the cellular differentiated state, which is in turn modulated by the growth environment. The 56.5-kd keratin (subfamily A) and the 65-67-kd keratins (subfamily B) are coordinately expressed during keratinization. In contrast, the 40-, 46-, and 48-kd keratins (subfamily A) and the 52- and 56-kd keratins (subfamily B) are characteristic of cultured epidermal cells forming nonkeratinized colonies. These results demonstrate that human epidermal keratins can be classified according to their reactivity with monoclonal antikeratin antibodies, isoelectric point, and mode of expression. The classification of keratins into various subgroups may have important implications for the mechanisms of epidermal differentiation, the evolution of keratin heterogeneity, and the use of keratin markers for tumor diagnosis.

The keratins represent a family of water-insoluble proteins (40-70-kilodalton [kd]¹) that form intermediate filaments (tonofilaments) in epidermal cells as well as almost all other epithelial cells (9, 12, 13, 55, 57, 58). Unlike other types of intermediate filaments (vimentin, desmin, glial filament, and neurofilament) which are relatively simple in subunit composition (for review, see 31), keratin composition is highly heterogeneous and varies depending on epithelial cell type, period of embryonic development, stage of epithelial differentiation, disease state, and cellular growth environment (for references, see 23, 34, 37, 61, 65).

To investigate the biological significance of keratin heterogeneity, we have produced three monoclonal antibodies (designated AE1, AE2, and AE3) against human epidermal keratins (67). Immunofluorescence data have established that

these antibodies are highly specific for keratin-type intermediate filaments. Immunoblot analysis further suggested that AE1 and AE3 antibodies react with two different groups of keratins and, in combination, recognize most keratin species (61, 67). Using these antibodies, we have shown that the expression of specific keratins can be correlated with different types of epithelial differentiation (simple vs. stratified, keratinized vs. nonkeratinized; 61; cf. 11, 34), and that individual epidermal keratins can be correlated with precise stages of epidermal keratinization (67; cf. 17, 47). These and other recent findings have led to the emerging concept that specific keratins may be regarded as molecular markers for different types and stages of epithelial differentiation (11, 52).

The epidermis is a keratinized epithelium in which the relatively undifferentiated basal cells give rise sequentially to spinous, granular, and finally cornified cells. This sequence of normal epidermal differentiation (keratinization) can be altered when keratinocytes are placed in culture. For example, cells grown in the presence of lethally irradiated 3T3 feeder

¹ Abbreviations used in this paper: EGF, epidermal growth factor; HC, hydrocortisone; kd, kilodalton; NEpHG, nonequilibrium-pH-gradient.

cells form nonkeratinized colonies that do not possess typical granular or cornified layers (29, 43) and lack the "large" 65–67-kd keratins (23, 56). However, these cells can rapidly reassume the keratinized morphology and reexpress the 65–67-kd keratins when they are injected subcutaneously into athymic mice (8, 29; cf. 3, 18, 19), suggesting that epidermal differentiation can be reversibly modulated by the external environment.

Although keratins of cultured epidermal cells appear to be similar to those of the "small-to-intermediate-sized" keratins of the epidermis with respect to their overall size distribution, immunological reactivity with polyclonal antikeratin antibodies, and peptide mapping patterns (16, 23, 28, 50, 56), the detailed structural and immunological relationship between the epidermal keratins expressed *in vivo* versus *in vitro* has not yet been determined. To further investigate the modulation of keratin expression within a single epithelial cell type and to better define the differentiated state of cultured keratinocytes, we have compared keratins of normal human epidermis with those of cultured human epidermal cells by one- and two-dimensional gel electrophoresis, and by the immunoblot technique using AE1 and AE3 monoclonal antikeratin antibodies. Our results indicated that under *in vivo* and *in vitro* conditions, postnatal human epidermal cells expressed a total of at least nine different keratins. All of these keratins could be divided into two subfamilies according to their immunoreactivities and charge properties. Keratins recognized by AE1 antibody were relatively acidic (subfamily A; A for *acidic*), whereas those recognized by AE3 antibody were relatively basic (subfamily B; B for *basic*). Within each keratin subfamily, there was a constant member that was always expressed, both *in vivo* and *in culture*; the expression of the remaining keratins was variable (variable members) and was apparently influenced by environmental factors.

MATERIALS AND METHODS

Monoclonal Antibodies: Mouse monoclonal antibodies AE1 and AE3 were prepared against SDS-denatured human epidermal (callus) keratins by the hybridoma technique (26), and were characterized by both immunofluorescence and immunoblot techniques (67). Culture media conditioned by repeatedly cloned hybridoma cell lines were used as the source of the antibodies (67).

Cell Culture: Human epidermal cells derived from newborn foreskin were used after two subcultures. Under "standard conditions", cells were grown in the presence of lethally irradiated 3T3 fibroblasts (4,000 Rads) in Dulbecco's modified Eagle's medium supplemented with 20% fetal calf serum, hydrocortisone (Calbiochem-Behring Corp., San Diego, CA; 0.4 μ g/ml) and epidermal growth factor (10 ng/ml; 43, 44). In vitamin A-deficiency experiments (18), cells were first grown in Dulbecco's modified Eagle's medium containing 20% delipidized fetal calf serum and hydrocortisone. When the cultures reached 20–30% confluence, the media were removed and replaced with Dulbecco's modified Eagle's medium supplemented with various combinations of epidermal growth factor, hydrocortisone, and vitamin A-containing fetal calf serum. Nine days later, water-insoluble cytoskeletal proteins were extracted from these cultures as described below.

Keratin Extraction: Fresh human epidermis was shaved from either newborn foreskin (exterior, nonmucosal portion) or adult abdominal skin obtained from autopsy, homogenized at 4°C in 25 mM Tris-HCl (pH 7.5) containing 1% Triton, 1 mM phenylmethylsulfonyl fluoride, pepstatin (Sigma Chemical Co., St. Louis, MO; 5 μ g/ml), antipain (Sigma Chemical Co.; 10 μ g/ml), 1 mM EDTA, and 1 mM EGTA. After centrifugation at 4°C at 10,000 *g* for 10 min, the water-insoluble pellet was further extracted with 25 mM Tris HCl containing 9.5 M urea. Since keratins of the cornified layers in human epidermis are cross-linked by intermolecular disulfide bonds while those of the living layers are not (2, 48, 56), this extraction condition (9.5 M urea in the absence of a reducing agent) selectively solubilizes keratins of the living layers

(67). These urea-soluble keratins are probably equivalent to the so-called prekeratins described by Matoltsy (33; prekeratin is operationally defined as citric-acid-extractable keratins and should not be construed as the precursor of keratins. For discussion, see 52). Cultured human epidermal cells were treated with EDTA to selectively remove 3T3 feeder cells and any contaminating human fibroblasts, rinsed with phosphate-buffered saline, scraped from the dishes with a rubber policeman, and extracted in two steps as described above for the *in vivo* tissues.

As an alternative procedure, the cytoskeletal proteins were extracted from the water-insoluble residues of the epidermis and cultured epidermal cells by heating at 95°C for 5 min in 1% SDS and 25 mM Tris HCl (pH 7.4). Keratins isolated by the two procedures yielded identical SDS gel patterns.

Gel Electrophoresis and Immunoblot Analysis: One-dimensional SDS polyacrylamide slab gel electrophoresis (SDS PAGE; Laemmli type, 12.5% acrylamide) was done as described earlier (56, 67). Two-dimensional gel electrophoresis in which the first dimensional separation was done either by nonequilibrium-pH-gradient (NEpHG) electrophoresis or by isoelectric focusing was performed as described by O'Farrell et al. (41, 42). For immunoblot analysis (60), proteins from unstained one- or two-dimensional gels were transferred electrophoretically to nitrocellulose sheets (Millipore type HA, 0.45- μ m pore size; Millipore Corp., Bedford, MA). The sheets were stained for 5–10 min with 0.1% Fast green in 50% methanol/10% acetic acid, destained in the same solvent or water, and subsequently reacted with mouse monoclonal antikeratin antibodies by the peroxidase-antiperoxidase technique (67). To record the peroxidase-antiperoxidase-reaction patterns, pictures were taken using Kodak CPP high-contrast film through a blue filter (Tiffen 47B) so that only the brown diaminobenzidine-reaction products were visible, but not the Fast-green-stained protein spots or bands on the same blot (67). This simple procedure allows the unambiguous identification of various AE1- and AE3-positive keratins.

RESULTS

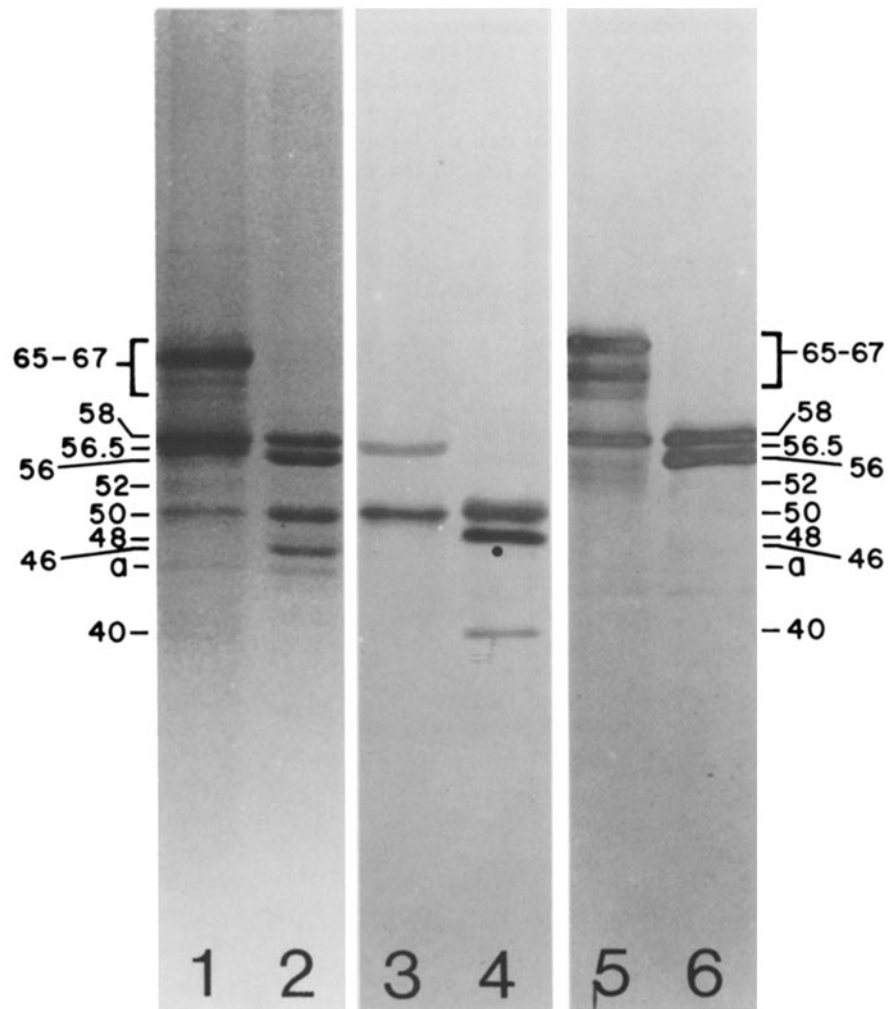
Two Subfamilies of Keratins as Defined by AE1 and AE3 Monoclonal Antibodies

Water-insoluble "cytoskeletal" proteins from human epidermis and cultured human epidermal cells were compared by SDS PAGE (Fig. 1, lanes 1 and 2). Consistent with some earlier results, normal human epidermis (both abdomen and foreskin) expresses four major keratins (50-/50'-², 56.5-, 58-, and 65–67-kd; Fig. 1, lane 1; references 6, 17, 35, 67), whereas cultured epidermal cells synthesize four major (46-, 50-, 56- and 58-kd) and three minor (40-, 48-, and 52-kd) keratin-related proteins (Fig. 1, lane 2; references 17, 55, 56).

To characterize these proteins immunologically, we transferred them from an unstained SDS gel to nitrocellulose paper and stained them with AE1 and AE3 monoclonal antikeratin antibodies using the peroxidase-antiperoxidase technique (60, 67). In normal epidermis, AE1 antibody recognized the 50- and 56.5-kd keratins (Fig. 1, lane 3), whereas AE3 recognized the remaining 58- and 65–67-kd keratins (Fig. 1, lane 5; reference 67). On the other hand, in cultured cells, AE1 reacted with the 40-, 48-, and 50-kd keratins (Fig. 1, lane 4), whereas AE3 reacted with the 52-, 56-, and 58-kd components (Fig. 1, lane 6; also see Fig. 2). These results clearly demonstrated that AE1 and AE3 monoclonal antibodies, in combination, recognized almost all keratins expressed by human epidermal cells. Furthermore, the two antibodies defined two mutually exclusive subfamilies of epidermal keratins, both *in vivo* and *in vitro*. As a result, each epidermal keratin (with the exception of 46-kd keratin which was not recognized by either antibody) could be assigned to one of the two subfam-

² The 50-kd keratin can be resolved into two components by charge (50-kd, pI 5.3 and 50'-kd, pI 4.9). These two keratins are frequently co-expressed and are thus treated as a pair (50-kd/50'-kd) here for simplicity.

FIGURE 1 SDS polyacrylamide gel analysis of the cytoskeletal proteins from human epidermis and cultured human epidermal cells. Water-insoluble proteins were extracted from the living layers of human abdominal epidermis (lanes 1, 3, 5) and from cultured human newborn foreskin epidermal cells (lanes 2, 4, 6) and separated by SDS gel electrophoresis. The proteins were stained with Coomassie Blue (CB; lanes 1 and 2), or electrophoretically transferred to nitrocellulose paper and stained with monoclonal antikeratin antibody AE1 (lanes 3 and 4) or AE3 (lanes 5 and 6) using the peroxidase-antiperoxidase technique. AE1 and AE3 antibodies recognize two mutually exclusive subfamilies of keratins (AE3 reacts with 52-kd keratin only weakly; see Fig. 2, *b* and *f* for unambiguous staining of this keratin by AE3 antibody). Note that in lane 4, AE1 stains only the 50-, 48-, and 40-kd keratins, but not the 46-kd keratin (dot). Also note that AE1 stained the 48-kd keratin relatively much more intensely than the 50-kd keratin (see also Figs. 2 and 3). This could be due to a slight difference in the primary structure of the corresponding AE1 sites or, perhaps less likely, due to a difference in the number of antigenic sites per molecule. Values represent molecular weights $\times 10^{-3}$. a, residual actin.



ilies: 40-, 48-, 50-, and 56.5-kd keratins to the AE1 subfamily, and 52-, 56-, 58- and 65-67-kd keratins to the AE3 subfamily.

Keratins of the AE1 Subfamily Were Acidic Whereas Those of the AE3 Subfamily Were Basic

To further characterize the keratins expressed by epidermal cells under *in vivo* vs. *in vitro* conditions, we have analyzed these proteins by two-dimensional gel electrophoresis using either nonequilibrium-pH-gradient electrophoresis (NEpHG) or isoelectric focusing in the first dimension, followed by SDS PAGE in the second dimension (41, 42). Results from NEpHG-SDS gel electrophoresis showed that all keratins from both *in vivo* epidermis (Fig. 2*a*) and cultured cells (Fig. 2*b*) were resolved into well-defined spots, many with closely spaced isoelectric variants that presumably represented multiple phosphorylated isomers (5, 11, 21, 56). These keratins could be easily divided into two groups: those that were relatively acidic (40-, 46-, 48-, 50-/50', and 56.5-kd keratins; *pI* 5.0-5.5) and those that were relatively basic (52-, 56-, 58-, and 65-67-kd keratins; *pI* > 6; also see references 5, 46). Immunoblot analysis of these keratins showed that almost all acidic keratins, including the 40-, 48-, 50-/50', and 56.5-kd components, were AE1-reactive (Figs. 2, *c* and *d*); in contrast, all basic keratins (52-, 56-, 58-, and 65-67-kd) were AE3-positive (Figs. 2, *e* and *f*). These results, schematically sum-

marized in Fig. 3, demonstrated that keratins recognized by AE1 antibody were relatively acidic (thus referred to as subfamily A; A for *acidic*), whereas those recognized by AE3 antibody were more basic (subfamily B; B for *basic*).

A comparison of the keratins expressed by *in vivo* epidermis vs. cultured keratinocytes revealed that only the 50-/50'-kd (subfamily A) and 58-kd (subfamily B) components were present in both samples (Figs. 1 and 2). Other keratins were found either *in vivo* or *in vitro*, but not under both conditions. Thus the 56.5-kd keratin (subfamily A) and the 65-67-kd keratin (subfamily B) were detected only in normal epidermis, whereas the 40-, 46-, and 48-kd keratins (subfamily A), and the 52- and 56-kd keratins (subfamily B) were characteristic of cultured keratinocytes. These results showed that the 50-/50', and 58-kd components were unique among epidermal keratins in that they were always expressed (constant members), whereas the expression of other keratins was variable and was apparently influenced by the cellular growth environment (variable members).

Environmental Modulation of Variable Keratins

To investigate the significance of the variable epidermal keratins, we grew human keratinocytes under various conditions known to affect epidermal differentiation. As noted earlier, epidermal cells grown by the feeder technique form nonkeratinized colonies (Fig. 4*a*). Recent results by Fuchs

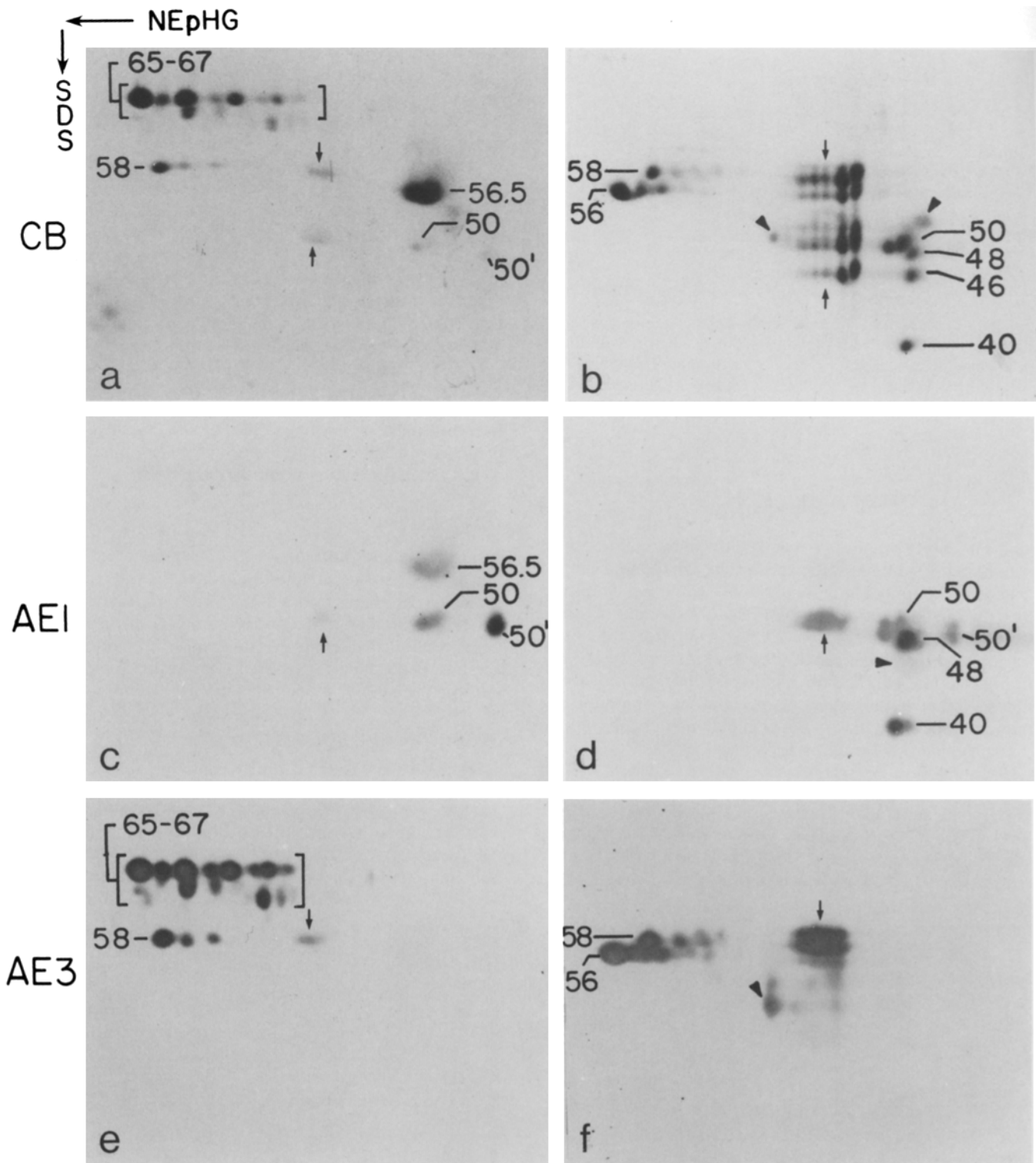


FIGURE 2 Two-dimensional polyacrylamide gel electrophoresis of keratins from normal human epidermis (*left*) and cultured human epidermal cells (*right*). The first dimensional separation was done by nonequilibrium pH gradient electrophoresis (*NEpHG*); basic polypeptides are to the left, and the second dimensional separation by SDS PAGE. Proteins were either stained with Coomassie Blue (*a*, foreskin epidermis; *b*, cultured human foreskin epidermal cells), or reacted with AE1 antibody (*c*, foreskin epidermis; *d*, cultured cells) or AE3 antibody (*e*, foreskin epidermis; *f*, cultured cells) by the immunoblot technique. Keratins are identified by molecular weights ($\times 10^{-3}$) (Fig. 1). Arrows indicate keratin complexes incompletely separated during *NEpHG* electrophoresis but dissociated during SDS PAGE. The arrowheads in Fig. 2, *b* (*right*) and *d* mark the positions of a 54- and a 46-kd keratin, respectively, which were not recognized by AE1 or AE3. The arrowheads on left side of gels *b* and *f* denote the AE3-positive 52-kd keratin (no. 8 of Moll et al. [34]). Note the relatively strong staining of the 50'-kd keratin (as compared with the 50-kd keratin) by AE1 antibody.

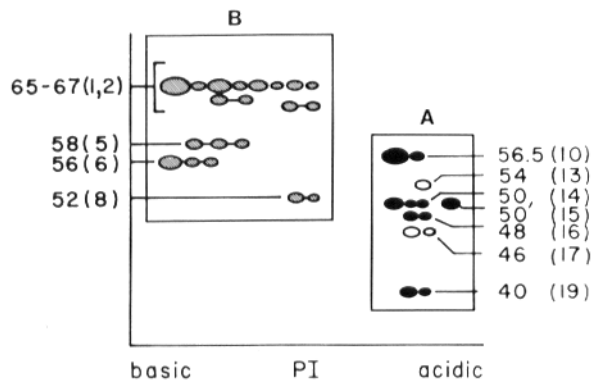


FIGURE 3 A schematic drawing showing the two subfamilies of human epidermal keratins. Keratins expressed by normal human epidermal cells under *in vivo* and *in vitro* conditions, as defined by two-dimensional gel electrophoresis (Fig. 2), are represented by circles. The numbers are relative molecular weights $\times 10^{-3}$ dalton; numbers in parenthesis are keratin nomenclature according to Moll, Franke, and co-workers (34). Solid and hatched circles represent AE1- and AE3-positive keratins, respectively. The acidic, AE1-negative 54-kd protein was found in some but not all samples of cultured human epidermal cells.

and Green (18) indicated that keratinocytes cultured with delipidized (vitamin-A-deficient) serum assume to a limited extent a keratinized morphology (23). We have confirmed this finding. In addition, we found that the deletion of hydrocortisone and epidermal growth factor (EGF) from the culture medium further enhanced the degree of morphological keratinization (Fig. 4*b*). This observation is consistent with earlier findings that both hydrocortisone and EGF promote the growth but inhibit the terminal differentiation of cultured human epidermal keratinocytes (43, 54).

Keratins were extracted from epidermal cells cultured in the presence or absence of vitamin A, hydrocortisone, and EGF, and were analyzed by SDS PAGE. Consistent with earlier results (18), Coomassie Blue-staining showed that epidermal cells grown under conditions conducive to keratinization expressed an increased, albeit small, amount of the

(variable) 65–67-kd keratin, as well as decreased amounts of the 40- and 52-kd keratins (Fig. 5*a*).

Immunoblot analysis of the keratins expressed under various growth conditions using AE1 antibody enabled us to demonstrate that the 56.5-kd keratin, previously detected only in keratinized tissues, was also synthesized by cultured keratinocytes concomitant with the appearance of a keratinized morphology (Fig. 5*b*). Moreover, a comparison of AE1 (Fig. 5*b*) and AE3 (Fig. 5*c*) staining results showed that in various samples the expression of 56.5- and 65–67-kd keratins was coordinated, and that the levels of these two keratins could be correlated with the degrees of morphological keratinization. These results lend additional support to our previous suggestion that the 56.5- and 65–67-kd keratins represent molecular markers for phenotypic keratinization (61, 67).

Our results also indicated that there existed a continuous spectrum of keratin expression. The 50- and 58-kd keratins (constant members) were expressed throughout this spectrum. In contrast, the expression of other keratins varied, depending on the growth/differentiation state of the keratinocytes. These results are schematically summarized in Fig. 6.

DISCUSSION

Although we and others have previously presented evidence that there exist two subfamilies of keratins (see below), the present work established for the first time that these two keratin subfamilies, as defined immunologically and electrophoretically, can account for all known human epidermal keratins. Our data also provided clear evidence for the constant vs. the variable mode of keratin expression.

Keratin Subfamilies A and B

Our results indicate that human epidermal cells can express a total of at least nine different keratin species with molecular weights of 40-, 46-, 48-, 50-/50'-, 52-, 56.5-, 58-, and 65–67-kd. These keratins can be divided into two subfamilies (Fig. 3). Subfamily A is comprised of the 40-, 46-, 48-, 50-/50'-, and 56.5-kd keratins which are all relatively acidic, and, with

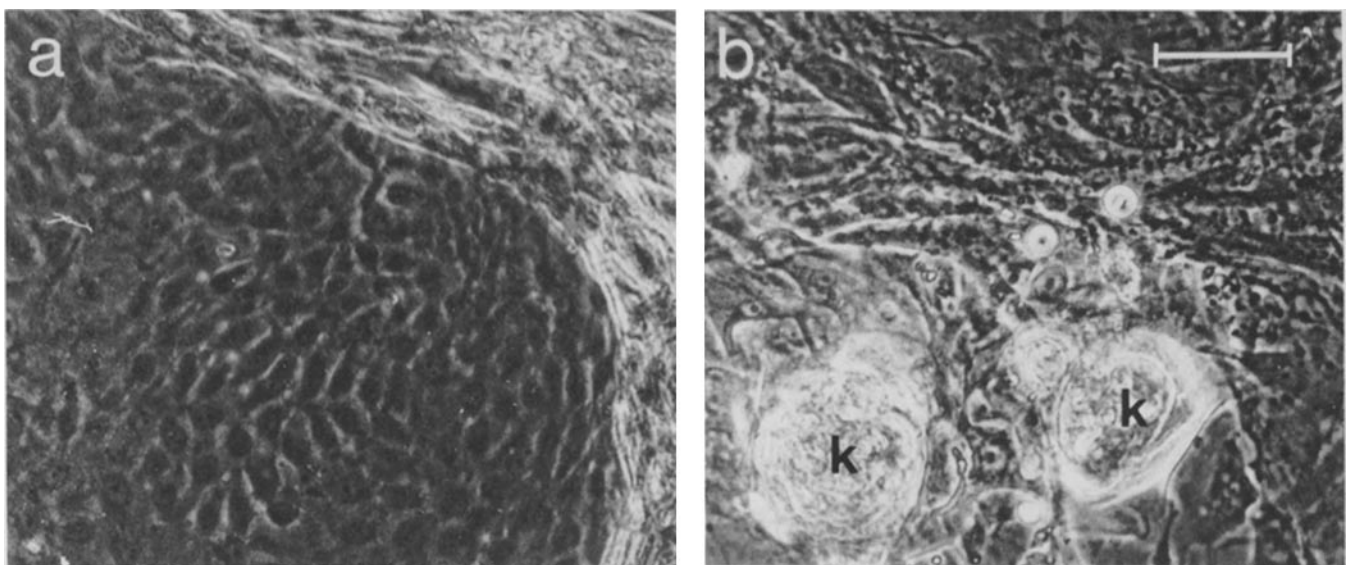
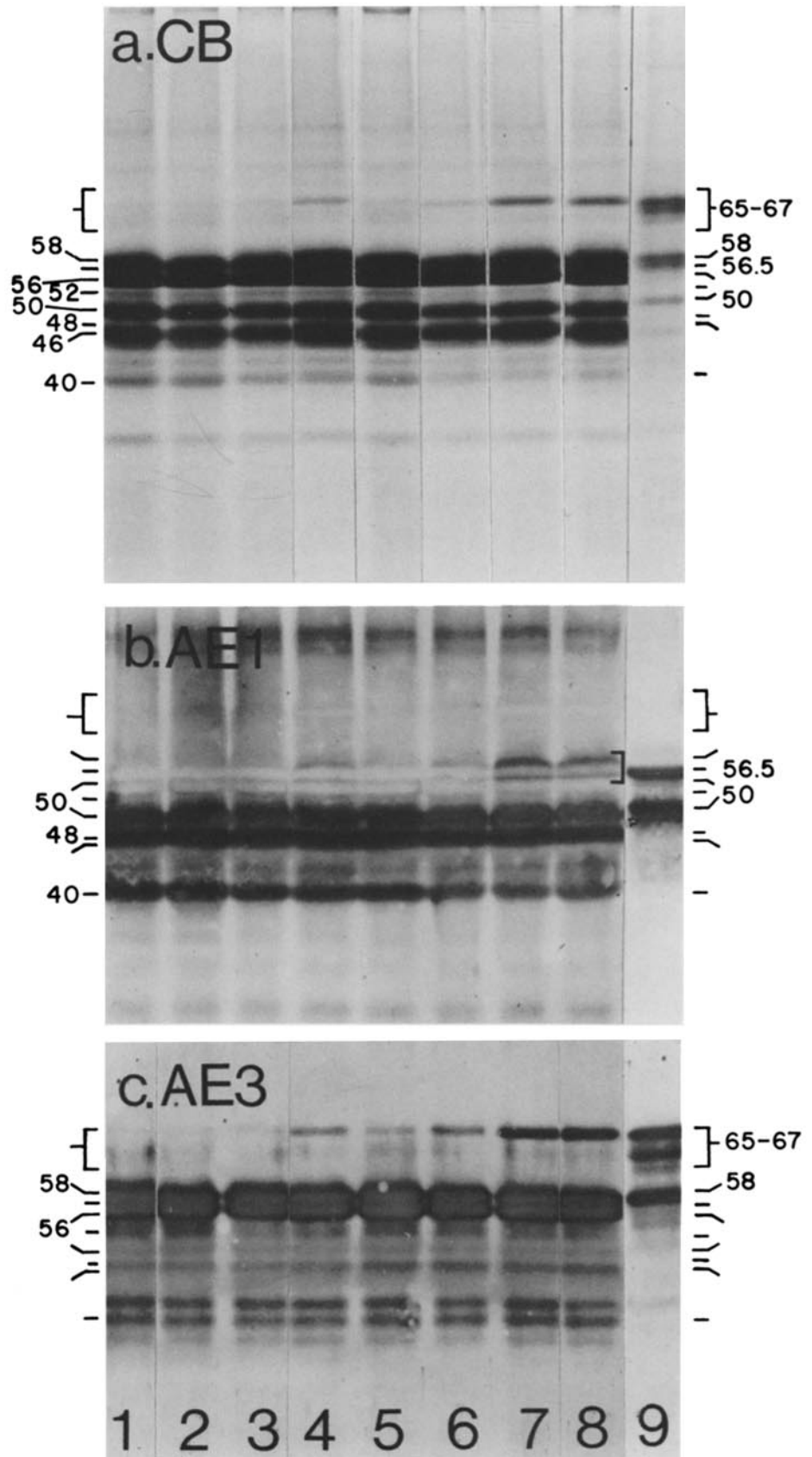


FIGURE 4 Phase contrast microscopy of cultured human epidermal cells grown in the presence (a) or absence (b) of hydrocortisone, vitamin A, and epidermal growth factor. Note the formation of large, refractile "keratinized" colonies (K) when the three factors were omitted from the culture medium. Bar, 100 μ m.

FIGURE 5 Immunoblot analysis of keratins from cultured human epidermal cells grown in the presence (+) or absence (-) of hydrocortisone (HC), vitamin A, and epidermal growth factor. (a) Coomassie Blue staining (CB); (b) immunoblot staining using AE1 antibody; and (c) immunoblot staining using AE3 antibody. The gels were intentionally overloaded to clearly demonstrate the synthesis of the 56.5- (AE1-positive) and 65-67-kd (AE3-positive) keratins. In b, lanes 4-8, the 56.5-kd keratin appeared as a doublet (bracket) after AE1 staining; this is probably a staining artifact due to the overlapping AE1-negative 56-kd band (no. 6 of Moll et al. [34]) which is present in a large quantity in cultured cells. A small amount of high molecular weight, AE1-positive bands (100-kd) are found in all specimens, probably representing some keratins cross-linked through γ -glutamyl-lysine linkage. In c, AE3 also showed reproducible staining of several minor, low molecular weight keratins which are not well characterized (40). Lane 1, + HC, + vitamin A, + EGF (standard conditions); lane 2, - HC, + vitamin A, + EGF; lane 3, + HC, + vitamin A, - EGF; lane 4, + HC, - vitamin A, + EGF; lane 5, - HC, + vitamin A, - EGF; lane 6, - HC, - vitamin A, + EGF; lane 7, + HC, - vitamin A, - EGF; lane 8, - HC, - vitamin A, - EGF; lane 9, human *in vivo* abdominal epidermis.



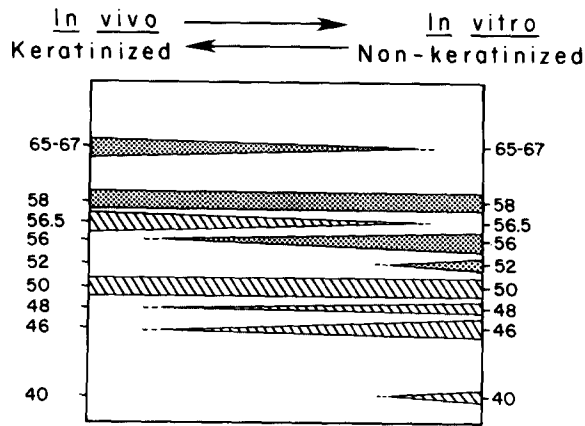


FIGURE 6 Schematic diagram illustrating the spectrum of keratin expression by human epidermal cells. Bars with various width denote the relative expression of keratins by epidermal cells under different conditions. The left side shows keratins of the in vivo, keratinized epidermis, whereas the right side shows those of the nonkeratinized epidermal colonies cultured under the regular "3T3 feeder" conditions in the presence of hydrocortisone, vitamin A, and epidermal growth factor (43). The patterns of keratin expression can be modulated by the culture environment (Fig. 5). The hatched and stippled bars represent keratins of subfamilies A (acidic) and B (basic), respectively. The acidic, AE1-negative 54-kd component present in some but not all samples of cultured keratinocytes (Fig. 2b) was not included in this diagram (see text and [34]). Values represent keratin molecular weight $\times 10^{-3}$.

the exception of the 46-kd component, share an AE1 determinant; whereas subfamily B is comprised of the 40-, 46-, 50-, 52-, 56-, 58-, and 65-67-kd keratins which are all relatively basic and share an AE3 antigenic site.

This classification of epidermal keratins into a relatively acidic (A) and a relatively basic (B) subfamily is quite consistent with existing data. For example, Fuchs et al. have shown by hybrid-selection that the 40-, 46-, and 50-kd keratins of the A subfamily are related to Type I wool keratins, whereas the 56- and 58-kd keratins of the B subfamily are related to Type II wool keratins (15, 24, 25). In a separate series of experiments, Franke and his collaborators have demonstrated by two-dimensional peptide mapping that the 56-, 58-, 65-67-kd, and several other keratins (of the B subfamily) are closely related to one another and are structurally distinct from the 46-, 48-, and 50-kd keratins (of the A subfamily; references 35, 46). Moreover, they have recently described a monoclonal antibody K_C8.13 that showed strong reactivities with the 52-, 56-, 58-, and 67-kd keratins (all of the B subfamily; see Table I), but weak or no reactivity with any keratins of the A subfamily (20). Finally, Bladon et al. have also shown that, of the four major keratins expressed by in vivo epidermis, two are basic and the other two are acidic (5). These results are therefore in excellent agreement with ours (present study and references 40, 53, 61, 67), and lend strong, independent support to the concept of the existence of two keratin subfamilies.

Functional Significance of Constant Members

The 50-/50'-kd keratin (subfamily A) and 58-kd keratin (subfamily B) are always expressed by epidermal keratinocytes and therefore belong to the category of constant members. Tissue-distribution data showed that these keratins are present in various quantities only in stratified epithelia, suggesting

that they may be regarded as markers for this cell type (34, 61). In basal cells of the human epidermis, the 50- and 58-kd keratins are the only two major keratins detected (47, 67). Since at least two keratins are thought to be required for filament reconstitution (32, 49), this pair of keratins may represent the minimum requirement for epidermal tonofilament formation (67).

Functional Significance of Variable Members

The smallest keratins of the A (40- and 46-kd) and B (52-kd) subfamilies are present not only in cultured keratinocytes, but also in many simple epithelia as the major tonofilament subunits (Table I; 34, 61). The 48-kd (subfamily A) and 56-kd (B) keratins are another two variable members made by cultured keratinocytes (55, 56). Based on an analysis of keratin expression in various epidermal diseases and cultured keratinocytes, we have recently suggested that the 48- and 56-kd keratins represent molecular markers for hyperproliferative keratinocytes (Table I; see reference 64).

Recent data suggest that the 56.5- and 65-67-kd keratins of subfamilies A and B, respectively, may be regarded as molecular markers for keratinization (Table I). Tissue-distribution studies showed that these two keratins are normally limited to keratinized epidermis (34, 61). Immunolocalization data have established that in normal human epidermis these keratins are expressed only by the terminally differentiated, suprabasally located cells (51, 67; cf. 17, 47, 62, 63). The present work further demonstrated that cultured keratinocytes could be induced to synthesize coordinately the 56.5- and 65-67-kd keratins under conditions permissive for keratinization (Fig. 5).

Concluding Remarks

As shown in Table I, there exist in both keratin subfamilies markers for simple epithelia or cultured keratinocytes (40-, 46-, and 52-kd keratins which represent the smallest members of both subfamilies); markers for hyperproliferative keratinocytes (48- and 56-kd keratins); permanent markers for keratinization (50- and 58-kd keratins); and markers for keratinization (56.5-kd and 65-67-kd keratins which represent the largest members of both subfamilies). The striking similarity between the A and B subfamilies with regard to the "relative size vs. function" relationship strongly suggest that the two keratin subfamilies may have evolved toward a greater complexity in a parallel or coordinated fashion (61), and that corresponding members from subfamilies A and B ("keratin-pairs"; reference 53) may play complementary roles in tonofilament assembly and function.

It has been suggested that cultured keratinocytes are equivalent to the basal or basal plus spinous layers of the in vivo epidermis (e.g., 17, 56, 59). This suggestion was based in part on the assumption that cultured keratinocytes synthesize the same 46-58-kd keratins as the in vivo epidermis. Our present findings, which agree with previously published data (16, 17, 56) but are more refined, emphasize that significant differences exist between the 46-58-kd keratins that are expressed by in vivo epidermis and cultured cells (also see reference 45). Moreover, since cultured keratinocytes do not express the 65-67-kd keratins that are characteristic of the spinous (and upper) cells of the in vivo epidermis, cultured cells do not form a typical spinous layer. Whether the basal cells of the cultured epidermal colonies are equivalent to those of the in

TABLE I
Classification of Human Epidermal Keratins

Subfamily A			Subfamily B			Mode of expression	Markers for
mol wt × 10 ⁻³	pI	Monoclonal antibody	mol wt × 10 ⁻³	pI	Monoclonal antibody		
56.5 (10)*	5.3	AE1	67** (1)	7.8	AE3	Variable	Keratinization
			66*	7.0	AE3		
			65* (2)	6.1	AE3		
50/50 [†] (14/15)	5.3/4.9	AE1	58 (5)	7.4	AE3	Constant	Keratinocytes
48 (16)	5.1	AE1	56 (6)	7.8	AE3	Variable	Hyperproliferative keratinocytes [‡] Cultured keratinocytes and simple epithelia
46 (17)	5.1	—	52 (8)	6.1	AE3	Variable	
40 (19)	5.2	AE1					

Classification of human epidermal keratins according to their immunoreactivity, relative charge, and mode of expression. Keratin subfamilies (A and B) were classified according to charges and immunoreactivities with AE1 and AE3 monoclonal antikeratin antibodies. Although not listed here, a 55-kd corneal-specific keratin (no. 12 of Moll et al. [34]) and a 54'-kd keratin (no. 13) characteristic of internal, nonkeratinized stratified epithelia also belong to subfamily A (53). Schiller, Franke, and Geiger (46) have previously shown by peptide mapping that keratins of subfamily B are structurally related and have called these and several other proteins "a subfamily of relatively large and basic cytokeratin polypeptides." This subfamily includes a 64-kd corneal-specific keratin (no. 3), and a 59-kd keratin (no. 4) which are AE3-positive and are relatively basic in charge (53, 61). Molecular weights were determined by SDS-gel electrophoresis (17, 56). These values are in excellent agreement with those used by Moll et al. (34). Numbers in parentheses represent the nomenclatures of Moll et al. (34). Isoelectric points were estimated by isoelectric focusing and by comparing the mobility of protein spots with standard proteins with known pI during nonequilibrium pH gradient electrophoresis. These values are also in close agreement with those reported by Moll et al. (34). Reactivities with monoclonal antikeratin antibodies were determined by one- and two-dimensional immunoblot experiments (Figs. 1 and 2; [67]). Keratins that were expressed in significant quantities irrespective of the growth/differentiation state of epidermal cells are classified here as constant members, although minor modulations cannot be ruled out. In contrast, keratins that can undergo clear-cut, "on-off" type of expression are classified here as variable members.

* As shown previously (67), AE2 antibody reacts with the largest members (the 56.5- and 65-67-kd keratins) of the A and B subfamilies.

† The 65-, 66-, and 67-kd keratins share AE2 and AE3 antigenic determinants and are frequently co-expressed in keratinized tissues. Therefore, for simplicity, these three closely related components are treated here as a group (also see reference 67).

‡ The 50'-kd keratin which is the most acidic one among all epidermal keratins has been described previously by Wu et al. (68) and Moll et al. (35). Although the precise relationship between the 50- and 50'-kd keratins is not yet clear, they are frequently co-expressed and are therefore treated as a group here.

§ See the accompanying paper by Weiss et al. (64).

vivo epidermis is not yet clear (for a discussion of the tremendous size difference between the basal cells of the in vivo epidermis and cultured keratinocytes, see reference 54).

There are three possible explanations for the altered keratin expression in cultured keratinocytes (8, 38). First, a minor subpopulation of atypical epidermal stem cells (30) may have been selected in culture. Second, cultured keratinocytes may undergo irreversible changes, thereby losing their ability to keratinize. Third, the expression of specific keratins may be related to particular states of epidermal differentiation, which may in turn be determined by the external environment. Results from this study (Fig. 5) and several earlier reports (1, 8, 18, 19) have shown, however, that cultured epidermal cells retain their ability to express keratins characteristic of the in vivo tissue. These observations strongly favor the third possibility and suggest that environmental modulation is primarily responsible for the changes in keratin expression in cultured epidermal cells (for a general discussion of the environmental effects on cellular differentiation, see references 4 and 66).

The concept of "constant vs. variable keratins" may have important implications for tumor diagnosis. Franke and his co-workers have demonstrated previously that tumors derived from hepatocytes, intestinal epithelium, and some other simple epithelia retain the keratin patterns of the original normal tissues (7, 10, 14, 34, 36). The situation appears to be much more complicated, however, for tumors derived from stratified squamous epithelia. For example, the keratin patterns of certain epidermal neoplasms are so different from that of normal epidermis that they may appear unrelated (27, 34-36, 64). Results from the present study established, however, that many keratins are variable members whose expression can be readily affected by the growth and/or differentiation state of

the keratinocytes. Such findings suggest that the presence or absence of these variable keratins may not be a reliable criterion for determining the tissue-origin of stratified carcinomas. In contrast, constant members (i.e., the 50-/50'- and 58-kd keratins), whose expression appears to be relatively independent of the differentiated state of epidermal cells, may provide more reliable markers for identifying neoplasms or cultured cells of epidermal and other stratified epithelial origins (39, 40; cf. 34, 36).

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