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Immunohistochemical Staining Characteristics of Epidermal Appendages (Hair Follicles and Eccrine Sweat Glands) to Anti-epidermal Keratin Antisera

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The cellular characteristics of the epidermal appendages and their differentiations in relation to those of the epidermis have been chiefly studied from the morphological points in the past. We investigated them from the immunohistochemical characteristics of the constituent keratin protein to the antiserum against total keratin isolated from human plantar stratum corneum (TKA) which stains the whole epidermis, and to the antiserum against 64K keratin separated from total keratin (64KA) which stains the whole epidermis except the basal layer. In the hair follicles, medulla and cortex of the hair shaft and inner root sheath were positively stained with both types of the antisera only at the keratogenous zone. The staining pattern of the outer root sheath with 64KA were variable at different levels of the hair follicle. The secretory portion of the eccrine glands showed a heterogeneous staining pattern as compared with the ductal portions which were stained homogeneously by both antisera. In eccrine poroma, all of the tumor cells were stained positively with TKA, while negatively or positively stained cells were intermingled with 64KA, suggesting that only some tumor cells showed mature keratinization. *Key words: Epidermal appendage; Keratin; Anti-keratin antiserum.* (Received November 7, 1983.)

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Recent biochemical and immunohistochemical studies of epidermal keratin (water-insoluble fibrous protein) have shown close relationship between the kinds of keratin subunits and epidermal differentiation in normal epidermis (2, 4, 6, 16) and in skin disorders such as epidermal malignant tumors (7, 16–18) and warts (13). Immunohistochemical studies using specific antisera to keratin subunits separated from total keratin by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed that, relatively large M.W. keratins tended to be localized at the spinous and the horny layer (2, 6, 16) in contrast to small M.W. keratins which were noted at the whole epidermis (6, 16) or predominantly at the basal layer (2). With our own antiserum against total keratin extracted from human plantar stratum corneum (TKA), we have also demonstrated that the whole epidermis was stained positively, while the antiserum against 64K keratin, one of the largest M.W. subunits separated from total keratin by SDS-PAGE (64KA) stained the spinous and the horny layer but not the basal layer (6). We also found that the positively stained cells with 64KA in squamous cell carcinoma were well consistent with the cells histologically showing higher degree of differentiation and that basal cell epithelioma was stained negatively with 64KA except for a small number of cells which showed individual cell keratinization (7).

In this study, we investigated normal hair follicles and eccrine sweat glands immunohis-

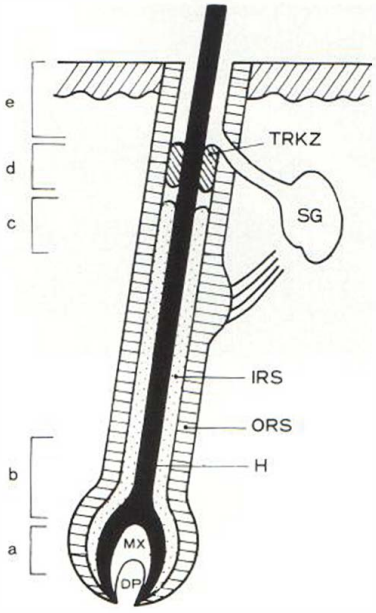
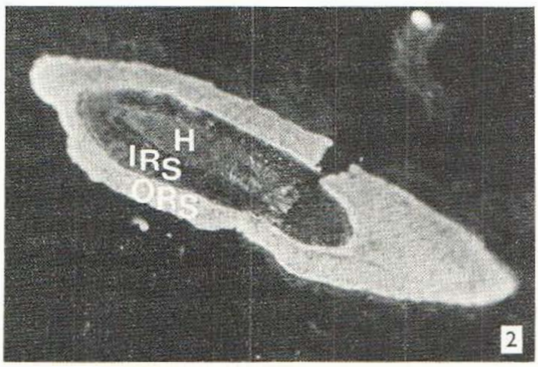


Fig. 1. A diagram of the normal hair follicle (anagen phase) which is devided into five portions. *a*, hair matrix; *b*, keratogenous zone; *c*, the lower part of the isthmus; *d*, the upper part of the isthmus; *e*, infundibulum; *TRKZ*, trichilemmal keratinization; *SG*, sebaceus gland; *IRS*, inner root sheath; *ORS*, outer root sheath; *H*, hair shaft; *MX*, hair matrix; *DP*, dermal papilla.

Table I. *A summary of immunohistochemical stainings of the hair follicle and the eccrine sweat gland*

IF = immunofluorescence study, IP = immunoperoxidase study, TKA = anti-total keratin antiserum, 64KA = anti-64K keratin antiserum. *a–e* and other abbreviations are consistent with those in Fig. 1

	IF		IP	
	TKA	64KA	TKA	64KA
Hair follicle				
(*a*) MX	−	−	−	−
(*b*) H	+	+	+	+
IRS	+	+	+	+
ORS	++	+~++	++	++
(*c*) H	−	−	−	−
IRS	−	−	−	−
ORS	++	+~++	++	++
(*d*) H	−	−	−	−
TRKZ	++	++	++	++
(*e*) H	−	−	−	−
ORS				
Basal	++	−	++	−~±
Suprabasal	++	++	++	++
Eccrine sweat gland				
Secretory portion	+~++	+~++	+	+
Ductal portion				
Intradermal	++	++	++	++
Intraepidermal	(+)	(+)	(+)	(+)



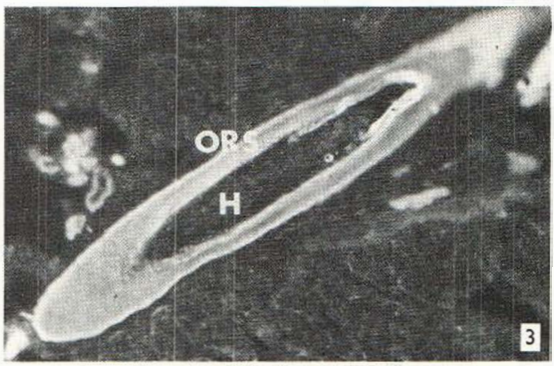


Fig. 2. IF staining pattern of the transverse section of the keratogenous zone of the hair follicle with 64KA (1 : 80 dilution). Medulla and cortex of the hair (*H*), and inner root sheath (*IRS*) are stained positively. In outer root sheath (*ORS*), stronger staining intensity is observed at the innermost and the outermost layers.

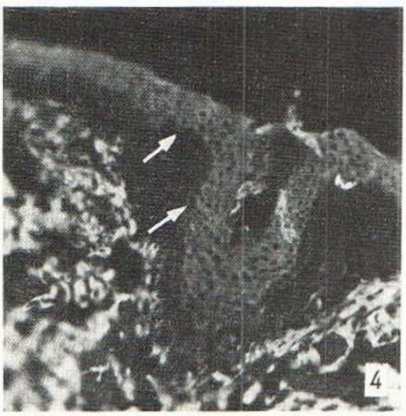
Fig. 3. IF staining pattern of the transverse section of the lower part of the isthmus with 64KA (1 : 160 dilution). The heterogeneity in staining pattern of outer root sheath (*ORS*) is distinct. Hair shaft (*H*) and inner root sheath are negatively stained.

tochemically with TKA and 64KA in detail, since only a brief mention was made about the epidermal appendages in the previous studies (3, 12, 15–16). In addition we studied a case of eccrine poroma in the same way.

MATERIALS AND METHODS

Keratin protein extraction and immune sera preparation

Total keratin was extracted from normal human plantar stratum corneum according to the method of Sun & Green (14) with 8 M urea and 0.1 M 2-mercaptoethanol, and 64K keratin was isolated by SDS-



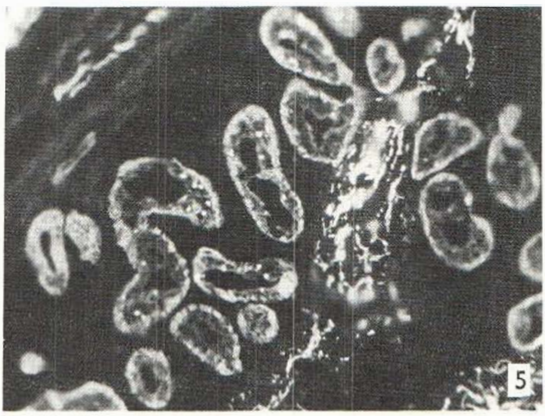


Fig. 4. IF staining pattern of infundibulum of the hair follicle with 64KA (1 : 80 dilution). The outermost basal layer of the outer root sheath showed negative staining, which is connected to the negatively stained neighboring epidermal basal layer (*arrows*).

Fig. 5. IF staining of the eccrine sweat glands with TKA (1 : 40 dilution). In the secretory portions, strongly positive cells are observed discontinuously along the basement membrane, while weakly positive cells are aligning the lumens. Homogeneously stained ductal portions are seen at the left.

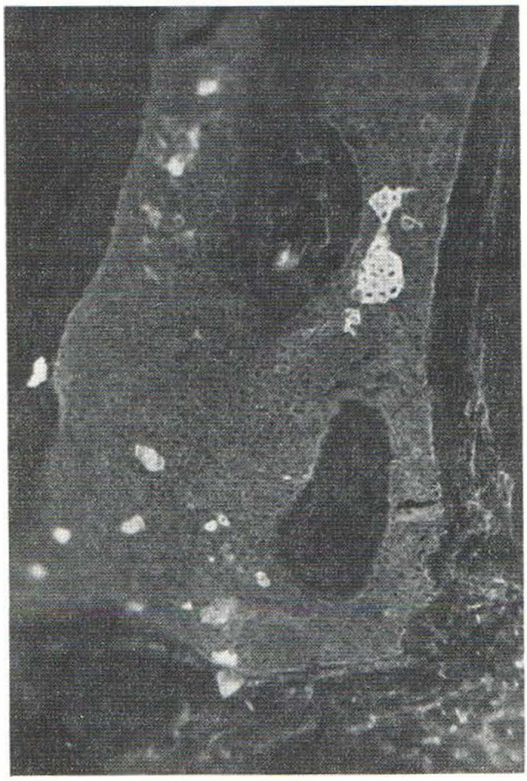


Fig. 6. IF staining pattern of a part of eccrine poroma with 64KA (1 : 160 dilution). Most of the tumor cells are stained negatively, with some of the positively stained cells in clusters.

PAGE as described before (6). Immune sera against both keratins were raised in rabbits respectively (6).

Tissue specimens

Tissue sections from normal skin of various sites of the body including sole and scalp were used as a material. Eccrine poroma was obtained from the palm of a 25-year-old male.

Immunohistochemical techniques

(*a*) *Immunofluorescense* (*IF*) *technique*. The indirect IF technique was described previously (6–7). Frozen sections were cut into 6 μm in a cryostat, incubated with keratin antisera (1 : 40 to 1 : 160 dilution) and further incubated with fluorescein isothiocyanate-antirabbit IgG antiserum (1 : 32 dilution, Miles Laboratories, USA). Samples were examined under Nikon fluorescence microscope.

(*b*) *Immunoperoxidase* (*IP*) *technique*. This was performed according to the method of Schlegel et al. (12). Deparaffinized tissue sections were sequentially incubated for 30 min at room temperature with each of the following reagents. (1) methanolic hydrogen peroxide, (2) normal swine serum (1 : 10 dilution), (3) anti-keratin antisera (1 : 40 to 1 : 640 dilution), (4) swine antirabbit serum IgG (1 : 20 dilution), (5) horseraddish peroxidase–rabbit anti-horseraddish peroxidase soluble complex (1 : 100 dilution). (2), (4) and (5) were purchased from Dakopatts, a/s Denmark. Antibody localization was determined by detection of peroxidase activity by 2 min incubation with 3,3′-diamino benzidine tetrahydrochloride (Sigma Chemical Company, USA) plus 3% hydrogen peroxide.

RESULTS

Hair follicles

Normal hair follicles in anagen phase were arbitrarily devided into five portions according to their difference in staining pattern (Fig. 1); *i*, *e*, *a*, hair matrix, *b*, keratogenous zone, *c*,

the lower part of the isthmus, *d*, the upper part of the isthmus (a part of trichilemmal keratinization, TRKZ) and *e*, infundibulum. The immunohistochemical staining pattern of each portion is summarized in Table I.

In portion *a*, hair matrix was not stained with any antisera. Only in keratogenous zone (*b*), medulla and cortex of the hair shaft and inner root sheath (IRS) showed positive staining with both types of the antisera (Fig. 2), while they were no longer stained positively above this zone (Fig. 3). In contrast, outer root sheath (ORS) showed positive staining throughout the hair follicle, whose transverse sections revealed much stronger staining at the innermost and outermost layers especially with 64KA by IF technique (Figs. 2–3). Such a heterogeneous staining pattern was not distinct by IP technique. Portion *d*, where ORS shows TRKZ, revealed equally positive staining with both types of the antisera. In the infundibulum (*e*), the outermost basal cell layer of ORS was no more stained with 64KA, from where the negative staining continued to the basal cell layer of the epidermis (Fig. 4, arrows). This feature was not observed with TKA.

Eccrine sweat glands

The staining pattern of normal eccrine sweat glands is also summarized in Table I. There was no difference in staining pattern between the two antisera. By IF study, strongly stained cells were observed discontinuously at the outermost layers of the secretory portion, while the cells aligning the lumen showed only weak staining (Fig. 5). It was difficult to identify the further characteristics of the positively stained cells at the outermost layers. By IP technique, the secretory portion was stained in equal intensity. The ductal portions were stained homogeneously by both techniques, although the intraepidermal ducts were difficult to define because of the strong staining of the surrounding epidermal keratinocytes.

Eccrine poroma

In eccrine poroma, all of the tumor cells showed uniform positive staining with TKA, whereas negatively or positively stained cells were observed with 64KA in various ratios at various parts of the tumor (Fig. 6).

DISCUSSION

Although there have been several studies reporting positive immunohistochemical stainings of the hair follicles with anti-epidermal keratin antisera (12, 15–16), details have not been described; only Bertolino et al. (3) reported that the most intense reaction in the mouse was noted in the constituents of the hair cortex. Our present study using antisera against human epidermal keratins disclosed that the staining pattern of the human hair follicles changes at different levels of the development, which might reflect the various differentiation states in the hair follicle.

It is interesting that hair with medulla and cortex and IRS were stained positively with both antisera only in the keratogenous zone. In the hair follicle, the matrix cells differentiate into medulla, cortex, cuticle and IRS, which are different morphologically and biochemically (8–9). Baden et al. (1) found that keratins derived from hair showed different urea and SDS-PAGE patterns as well as amino acid compositions from those of stratum corneum. Furthermore, they noted that antibody to stratum corneum keratin did not react with hair keratin and vice versa. Our finding suggests that proteins which could react with anti-epidermal keratin antisera are only transiently formed in the keratogenous zone. Another possibility is that, though these tissues contain immuno-reactive proteins to anti-

epidermal keratin antisera, their antigenicities have been masked when these tissues reach full development.

At present, we cannot explain the reason of the heterogeneous staining pattern of the transverse sections of ORS below isthmus by IF technique. Further ultrastructural studies are required to clarify this phenomenon. The upper part of the isthmus (a part of TRKZ) was stained uniformly with both antisera. Benign neoplasms showing TRKZ such as trichilemmoma or trichilemmal cyst were also stained uniformly with these antisera (unpublished data). The cells composing infundibulum which are not able to be distinguished morphologically from the adjacent epidermal cells (11), also could not be distinguished even immunohistochemically.

We confirmed that eccrine sweat glands are immuno-reactive to anti-epidermal keratin antisera (12, 15–16). In our study, myoepithelial-like cells were observed to be discontinuously stained along the basement membrane of the secretory portion as reported by Sun et al. (15). However, it is difficult to identify at this level of magnification whether they are really myoepithelial cells, which awaits further immunoelectronmicroscopic study. Furthermore we found that secretory cells surrounding the lumens also showed weakly positive reaction.

The origin of eccrine poroma has been considered to be intraepidermal eccrine duct cells on the basis of the findings of histological, histochemical and ultrastructural studies (5, 10). Hashimoto and Lever demonstrated that the tumor cells showed ability to differentiate either into ductal cells or into keratinizing cells (5). Although it is suggested that the positively stained cells with 64KA in the tumor consist of the cells with mature keratinization, we are not sure if the negatively stained cells with 64KA belong to the cells differentiating to the ducts, because normal intraepidermal eccrine ducts showed positive reaction with 64KA in the present study. Some of the tumor cells might lose their maturely keratinizing ability in transformation as seen in epidermal malignant tumors.

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