Architectural Organization of Filiform Papillae in Normal and Black Hairy Tongue Epithelium

Dissection of Differentiation Pathways in a Complex Human Epithelium According to Their Patterns of Keratin Expression

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Background: An inadequate understanding of the complex morphologic characteristics of human filiform papillae has hampered the histopathological characterization of disorders affecting tongue keratinization. To better define the 3-dimensional cytoarchitecture of tongue epithelium, we performed detailed immunohistochemical analyses of normal and black hairy tongue tissues using a panel of antikeratin antibodies.

Observations: The dome-shaped base of the human filiform papilla (primary papilla) is surmounted by 3 to 8 elongated structures (secondary papillae). These secondary papillae are composed of a central column of epithelial cells expressing hair-type keratins and an outer rim of cells expressing skin-type keratins. The epithelium overlying the primary papillae and between the individual primary papillae express esophageal-type keratins. In black hairy tongue disease, there is a marked retention of secondary papillary cells expressing hair-type keratins.

Conclusions: Using a panel of antikeratin probes, we define the precise topographical localization of cell populations undergoing 3 distinct differentiation programs in dorsal tongue epithelium. Comparative analyses of black hairy tongue specimens indicate that defective desquamation of the cells in the central column of filiform papillae results in the formation of highly elongated, cornified spines or “hairs”—the hallmark of this disease.

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HUMAN TISSUE

Normal human tongues were obtained from autopsy within 48 hours of death. The 3 biopsy specimens of BHT were obtained from former patients of Manhattan Veterans Affairs Medical Center in New York as well as Medical University of Lübeck in Germany.

ANTIKERATIN ANTIBODIES

Detailed characterizations of the monoclonal antibody AE 8, which reacts specifically with the esophageal-type keratin K13, and the antibody AE 13, which reacts with 44- to 46-kd acidic hair keratins, are described elsewhere. The AE 20 antibody was generated against trypsinized human skin cells according to the methods described previously. The antibody to K6 was generously provided by Dennis Roop, PhD, Baylor University, Houston, Tex.

KERATIN EXTRACTION, GEL ELECTROPHORESIS, AND IMMUNOBLOTTING

Normal human epidermis and cultured epithelial cells from various tissues were first extracted with 25-mmol/L Tris-hydrochloride, 0.6-mol/L potassium chloride, 1% Triton X-100 supplemented with 5 protease inhibitors. The residual aqueous-insoluble cytoskeletal preparation, containing mainly keratin proteins, was then solubilized with 2% sodium dodecyl sulfate in 25-mmol/L Tris-hydrochloride (pH 7.4) as described. One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subsequent immunoblotting were performed according to the methods described previously.

IMMUNOHISTOCHEMICAL STAINING

The biopsy specimens were embedded in OCT compound (Ty Miles Inc, Westchester, Ill), snap frozen in liquid nitrogen, and cut into 6-µm cryostat sections. Sections were stained by the indirect immunofluorescent and immunoperoxidase technique.

We present the characterization of a new antikeratin K1 monoclonal antibody and its use to definitively localize the skin-type compartment in human tongue epithelium. In addition, we provide a more detailed description of the 3-dimensional organization of the separate domains within human filiform papillae. Finally, we demonstrate that the hairlike projections observed in black hairy tongue disease (BHT) are due primarily to the formation of abnormally long extensions of the “hair compartment” of the filiform papillae.

SPECIFICITY OF THE AE 20 ANTIBODY FOR HUMAN EPIDERMAL KERATIN

In our prior study of human tongue epithelium, indirect immunofluorescent staining was performed using the monoclonal antibody AE 2, which recognizes markers of skin-type differentiation, K1 and K10. Unfortunately, AE 2 cross-reacts with filaggrin, a major component of keratohyalin granules (data not shown), making it difficult to localize the skin-type keratins. To obtain more definitive immunolocalization data, we generated a monoclonal antibody, AE 20, that reacts specifically with the basic 67-kd human epidermal keratin, K1, as assessed by immunoblotting (data not shown). No AE 20 immunoreactivity was detected in keratin extracts from a variety of other tissues and cultured cells tested (data not shown).

SEVERAL DISTINCT DIFFERENTIATION DOMAINS OF CROWN-SHAPED HUMAN FILIFORM PAPILLA

The human filiform papilla consists of an elevated connective tissue core covered by a partially keratinized stratified epithelium. Small connective tissue protrusions emanate from the top of this central core and are surmounted by a column of epithelial cells undergoing cornification. These elongated cornified spines are referred to as secondary papillae and characteristically tilt toward the pharynx. In contrast to the papillary epithelium, the interpapillary surface is nonkeratinized.

To facilitate the topological analysis of this complex structure, we used a panel of monoclonal antibodies recognizing keratins that are characteristic of different types of stratified epithelia. The AE 8 monoclonal antibody specifically reacts with the K4 keratin, which is expressed in nonkeratinized, stratified epithelia such as in the esophagus. The AE 13 antibody recognizes acidic hair keratins, which are expressed in hair cortex, nail plate, and mouse tongue filiform papillae. The AE 20 antibody, described above, recognizes K1, which is characteristic of keratinized epithelia such as skin. The antibody to K6 recognizes a keratin that is normally expressed at a few restricted sites of the body, including volar skin, foreskin, oral mucosa, and outer root sheath cells of the hair follicle, as well as by all stratified epithelial cells undergoing hyperproliferation.

Using this panel of antikeratin antibodies, we confirmed that the epithelium covering filiform papillae is subdivided into at least 3 distinct cell domains (data not shown). As reported previously, the antiesophageal keratin antibody, AE 8, reacted with the nonkeratinizing epithelium between the filiform papillae as well as between the cornified hairlike projections. The AE 20 immunoreactivity was confined to cells at the bases of the cornified spines, indicating that the skin-type keratin K1 is restricted to this cell population. The AE 13 antihair keratin antibody reacted only with those cells directly beneath the cornified spines, adjacent to the AE 20–positive cells. Immunostaining with the antibody to K6 was uni-
formally positive in the suprabasal cells of the dorsal tongue, similar to other oral epithelium.13

Detailed analysis of horizontal sections of human filiform papillae allowed us to further refine our understanding of the 3-dimensional architecture of the filiform papillae and the topological locales of different epithelial domains (data not shown). Within the large central area surrounded by a ring of 5 to 12 cornified spines are AE 8–positive cells, which synthesize esophageal-type keratins. The interpapillary epithelial cells likewise produce esophageal-type keratins, but they appear to constitute an esophageal-type compartment distinct from the AE 8–positive papillary epithelium. A thin band of AE 20–positive cells that undergo skin-type differentiation are distributed at the base of each of the cornified spines, encircling the cluster of AE 13–positive cells. Clusters of AE 13–positive cells, as well as their overlying cornified spines, are distributed in a ring at the periphery of the filiform papillae. These cells synthesize a “hair” keratin and undergo hair-type differentiation. Thus, the human filiform papilla is essentially crown shaped, with the outer rim being formed by the ring of hairlike spines. A scheme of the histological architecture of human filiform papillae is shown in Figure 1.

ABERRANT ELONGATION OF THE HAIRLIKE DOMAIN IN BHT TONGUE EPITHELIUM

Since the histological architecture of normal filiform papillae has never been described precisely, many common denominators make it difficult to differentiate between normal and BHT epithelium. Furthermore, the most striking finding on routine histological examination of BHT is the presence of numerous small fragments of cornified cells, which results from tangential sectioning. To avoid this problem, we made an effort to prepare well oriented longitudinal sections of the BHT biopsy specimen. Immunofluorescent staining using our panel of monoclonal antibodies demonstrated that the overall pattern of keratin expression in BHT is similar to that of normal tongue epithelium. The cells in the center of the filiform papillae as well as those in the interpapillary region are AE 8–positive, indicating the presence of esophageal-type keratins (Figure 2, A). The column of cells directly beneath the cornified spines are AE 20–positive, reflecting the presence of skin-type keratins (Figure 2, B). The cells adjacent to the AE 20–positive cells are stained with AE 13 and thus undergo hair- and nail-type differentiation (Figure 2, C and D). Our studies could not exclude, however, the possibility that the entire filiform papilla is involved, resulting in mild hyperkeratosis in both the skin-type and central intraspinous esophageal-type domains. Interestingly, the interpapillary esophageal domain (the dorsal tongue epithelium between the individual primary filiform papillae) shows no alterations in its differentiation, based on both morphological and immunolocalization criteria.

COMMENT

The tongue is covered by a complex epithelium composed of several functionally distinct cell populations. Our data have enabled us to definitively localize at least 3 unique domains within the tongue epithelium: (1) cells in the secondary filiform papillae that express acidic hair-type keratins, (2) a ring of cells surrounding this hair compartment that express skin-type keratins, and (3) cells overlying the central mound of the primary papillae as
frequently in chronic smokers. Clinically, the mid-dor-sal tongue is covered with long hairlike structures, hence the name. Although BHT has been histopathologically characterized extensively by Winzer et al, most of their descriptions also apply to normal histological characteristics of the tongue. For example, they noted that pathological changes associated with BHT include a markedly digitated surface, pointed rete ridges, focal parakeratosis, focal preservation of the granular zone, balloon cells with pale cytoplasm in the spinous zone, and the presence of both keratohyalin- and trichohyalin-like granules. However, since normal tongue epithelium consists of distinct but closely juxtaposed differentiation domains, the above “pathological” findings are also observed in normal tongue. Without a clear understanding of the precise histological topography of the tongue and its specialized epithelial domains, one cannot meaningfully analyze the pathological processes in tongue diseases. Our present study shows that the designation of black hairy tongue is by chance consistent with the specific keratin expression in cells that cause the characteristic morphologic features. Cells underneath the strikingly elongated cornified spines are indeed derived from the hair domain of filiform papillae. However, it remains unclear why the hair-type compartment shows a prominent retention of cornified spines, and how the pathogenic factors implicated in promoting BHT, such as smoking, oxidizing agents, and antibiotics, inhibit hair compartment desquamation. Further studies are necessary to elucidate the relevant mechanisms. In this regard, it might be interesting to investigate whether medicines that influence hair growth may also cause morphologic alterations of the filiform papillae.

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