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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THE DORSAL surface of mammalian tongue is covered by densely packed filiform papillae. The shape and size of these papillae vary markedly from species to species. In humans, the papillary architecture is more complex, consisting of a central body surrounded by several threadlike cornified projections, often referred to as secondary papillae. Morphologically, human tongue epithelium seems to be divided into discrete domains that undergo distinct pathways of terminal differentiation, similar to what occurs in rodent and cow tongue epithelium. However, the morphologic compartments of the human tongue have not been as well characterized as those of the rodent because of their greater complexity.

Keratins are a heterogeneous family of polypeptides that form a subclass of intermediate filaments. They serve as excellent markers for various pathways of epithelial differentiation and have been used to define discrete populations of keratinocytes within the tongue epithelium. The cumulative data indicate that, in addition to the ubiquitous expression of the stratified epithelial-type keratins (K5 and K14), different regions of the tongue epithelium display distinct patterns of keratin expression. The nonkeratinized epithelium of the lateral and ventral surfaces of the tongue, as well as the epithelium covering the lateral aspects of filiform and fungiform papillae and the interpapillary mucosa of the dorsal tongue, express esophageal-type keratins (K4 and K13). In contrast, the orthokeratinized epithelium overlying the tips of filiform papillae makes skin-type keratins (K1 and K10), and the epithelium covering taste buds synthesizes simple epithelial-type keratins (K8, K18, and K19). Finally, we and others have shown that tongue epithelium also produces an acidic hard-type keratin, characteristic of hair- or nail-type differentiation.

In this article, we extend our immunolocalization studies on the structure and compartmentalization of the human filiform papilla and begin to explore the role of such compartments in human disease.
MATERIALS AND METHODS

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.
formly 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 specimens. 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.

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
well as between the papillae that express esophageal-type keratins. It is fascinating that filiform papillae express both soft (epithelial) keratins and hard (trichocyte) keratins. It has been proposed that the coexistence of these different programs of keratin expression reflects the dual requirements of tongue epithelium to be both rigid and flexible to resist friction and expansion accompanying tongue movements during food handling and grooming.4

While the importance of the above observation remains to be determined, the existence of 3 distinct domains in filiform papillae raises an interesting question with respect to their cell origin. Do cells in the hair-, skin-, and esophageal-type domains originate from a common pluripotent stem cell, or does each arise from unique stem cells located at the base of its domain? Recent results26-30 suggest that epithelial stem cells, as defined by their kinetic properties, reside in specific sites within each epithelial tissue. Since stem cells are normally slow cycling, they can be identified as label-retaining cells after the labeling of all cycling cells by a continuous administration of isotope for a prolonged period. Using this approach, the stem cells of epidermis, intestinal epithelium, corneal epithelium, and hair follicles were shown to be located at the bottom of deep rete ridges, the crypts, the limbal region, and the bulge area, respectively.26,28-30 Interestingly, these findings were further supported by the studies of graft-vs-host disease, in which the sites of involvement were thought to correlate precisely in the localization of the stem cells. In graft-vs-host disease, it was shown that cytotoxic lymphocytes infiltrate preferentially around the bottom of the epidermal rete ridges, the follicular bulge cells, and the anterior and posterior shoulders of filiform papillae.31-33 In this regard, our finding that filiform papillae can be divided into several differentiation compartments is noteworthy. It may be important to distinguish the stem cells of these domains because different stem cells may give rise to different neoplasms of the tongue epithelium.

The distinctive spatial arrangement of the hair domains of filiform papillae is of particular interest, given that hairs in skin also develop with a patterned distribution rather than at random.34-37 For example, a common grouping of follicles is the “trio” in which the primary hair follicle or tylotrich first erupts from the germ, and then 2 secondary hair follicles develop on both sides of the primary one, resulting in a central large tylotrich hair follicle flanked by 2 smaller hair follicles. Alternatively, the primary tylotrich follicle can be surrounded by a cluster of up to a dozen smaller follicles. If one assumes that the topological arrangement of the hair-type compartment (secondary papillae) at the periphery of the primary papillae in the tongue is analogous to the distribution of secondary hairs around a central tylotrich follicle in the skin, then the molecular mechanisms responsible for regulating these embryonic patterning events may be similar.

Black hairy tongue is an acquired disorder seen most frequently in chronic smokers.38 Clinically, the middorsal tongue is covered with long hairlike structures, hence the name. Although BHT has been histopathologically characterized extensively by Winzer et al,39 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, balloononed 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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