Tendency towards Keratinization in the Stratified Squamous Epithelium Lining the Mouse Laryngopharynx near the Transitional Zone

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Summary: In the mouse laryngopharynx, the stratified squamous epithelium was studied by transmission electron microscope in order to determine its morphological characteristics. The superficial cells were filled with opaque filaments embedded in a less opaque interfilamentous substance. Further, they had thickened plasma membrane and contained a fairly large number of keratohyalin granules showing ultimate stages transforming into interfilamentous substance. It is suggested that the superficial cells appear to represent the transitional stage in the keratinizing process, and had morphologically close resemblance to both the granular cells and the cornified ones in keratinized epithelia.

Materials and Methods

Twenty SMA mice (10 male and 10 female) aged from 6 to 12 months were killed under chloroform anesthesia, and their pharyngeal regions were removed. They were fixed for 3 hr with 2.5% glutaraldehyde in phosphate buffer, sliced into blocks, and postfixed for 2 hr with 2% osmium tetroxide. They were dehydrated in ascending concentrations of ethanol, transferred to propylene...
oxide and embedded in Epon 812. Ultrathin sections were cut with a LKB 4801-A ultramicrotome, double-stained with uranyl acetate and lead citrate, and were examined with a Hitachi HU-12A transmission electron microscope.

Results

In the nasopharynx of the mouse, the "intermediate epithelium (Nakano, 1986)" occupies the transitional zone between the ciliated columnar epithelium, continuing from the nasal cavity, and the stratified squamous epithelium, lining the laryngopharynx. The stratified squamous epithelium near the transitional zone is investigated in this study. The cells in the deeper layer are polygonal or slightly elongated shape with many prominent cytoplasmic processes projecting into wide intercellular spaces. The cytoplasmic filaments are aggregated to form fibrils. The cytoplasm between the fibrils is packed with free ribosomes and also contains mitochondria and a few keratohyalin granules (Fig. 1). Keratohyalin granules have homogeneous contents of high electron density, and are surrounded by many ribosomes.

The cells undergo progressive flattening as they move upward. The cells in the middle layer contain many keratohyalin granules both in the cytoplasm and in the nucleus (Fig. 2). Many of the granules, including all those within the nucleus, are round masses of homogeneous content with high electron density (Figs. 2 and 3). Some granules consist of a central globular mass of somewhat lower electron density, exhibiting peripherally associated smaller round masses of high density (Fig. 3). All of the granules in the cytoplasm are surrounded by many ribosomes, but very few filaments are found in their immediate neighborhood (Figs. 2 and 3). Desmosomes persist in the granular layer and the cytoplasmic filaments form a network of fibrils. The intercellular cytoplasmic processes are approximately 0.1-0.2 \( \mu \text{m} \) wide (Figs. 2 and 7).

The superficial layer has several plates of flattened cells from which intact nuclei, mitochondria and other organelles have disappeared. The cytoplasm varies in electron density and consists mainly of fibrils (Fig. 4). Where the cytoplasm is brighter, the individual filaments are distinctly seen. The filaments show some tendency to form fibrils (Fig. 4). In cells where the cytoplasm is denser, opaque filaments are embedded in a less opaque interfilamentous substance. The individual filaments are not always clearly seen (Fig. 4). In the superficial layer, a fairly large number of keratohyalin granules are seen. Some keratohyalin granules consist of an amorphous component of high electron density, but the periphery of the granules is not so distinct (Fig. 4). In other granules, an amorphous component is condensed in a portion of the granules, either in the central portion (Fig. 5a) or in the periphery (Fig. 5b). They do not appear to be related to either ribosomes or filaments. The plasma membrane of the superficial layer consists of an asymmetrical trilaminar structure which is thicker and denser than that in the underlying layers. This thickening is made by an electron-dense inner leaflet of the membrane (Figs. 4 and 6). And in some areas, only the inner lamina is demonstrable and only fragments of the outer lamina may be distinguished. The dense intercellular plate of each desmosome becomes wider and more diffuse, and is not only present between the attachment plates embedded in the thick plasma membrane but also extended into the intercellular space (Fig. 6). The intercellular spaces vary in width and contain in part a rather opaque substance (Fig. 4).

As the cells move towards the superficial layer, the intercellular cytoplasmic processes become thicker and more closely applied to each other. In the superficial layer, the pro-
cesses and the microridges are approximately 0.3–0.4 μm in width, being twice the size of the processes in the underlying layers (Fig. 7).

**Discussion**

In general, it has been described that keratohyalin granules, nucleus and cytoplasmic organelles are disappeared in the transition from the granular layer to the cornified one, and that the cells in the cornified layer are entirely filled with a fibrous mass. In this study, however, the superficial cells contained a fairly large number of keratohyalin granules with various electron-density. Furthermore, the cells were filled with opaque filaments embedded in a less opaque interfilamentous substance to show the abnormal keratin pattern, and were characterized by an increased thickness and density of the plasma membrane as compared with the cells in the underlying layers. Considered from these findings, the superficial cells appear to represent the transitional stage in the keratinizing process, and have morphologically close resemblance to both the granular cells and the cornified ones in keratinized epithelia. In the epithelium lining the human cervix uteri, which did not normally keratinize but had ability of keratinization under certain pathological conditions, Hackemann et al. (1968) reported that the superficial cells contained keratohyalin granules and were characterized by a marked thickening of the inner leaflet of the plasma membrane. They also described that the majority of the tonofilaments were concentrated near the cell borders, but extremely small nucleus, degenerating mitochondria and lysosome-like bodies were remained (Hackemann et al., 1968). It is suggested that the superficial cells in the human cervix uteri appear to correspond to the superficial cells in this study if not exact structure, and that the structural differences between these 2 cells are attributed to the difference of the degree of keratinization.

It is reasonable to assume that the cells representing the transitional stage in the keratinizing process may exist in keratinized epithelia. It has been pointed out that the transition between the granular layer and the cornified one is very abrupt and there exist no transitional stages. However, Brody (1959, 1960) reported in human and guinea pig epidermis that there existed the “transitional cells” in between the granular layer and the cornified one. He described that the transitional cells showed morphological similarities with both the granular cells and the cells in the basal part of the cornified layer; e.g., a flattened nucleus was sometimes visible and the cytoplasm was filled with keratohyalin-like large masses with high electron-density. The cells very similar to the transitional cells were also observed in the anal canal epithelium of man (Baba, 1968; Ogawa, 1971) and the lingual dorsal epithelium of chicken (Iwasaki and Kobayashi, 1986), both of which were keratinized. According to Lavker and Matoltsy (1970), in the ovine rumen the epithelial cells transforming the granular layer to the cornified one were more numerous. Further, Muto (1971) observed the “intermediate layer” between the granular layer and the cornified one in the epidermis of naked mouse. In the filiform papilla of the hamster tongue, Fernandez et al. (1978) observed the “transitional layer” just beneath the cornified layer. The cells in the transitional layer did not present a nucleus and any cytoplasmic organelles and contained a large number of microfilaments, although they showed lower electron-density as compared with the cornified cells and contained keratohyalin granules in different stages of disintegration (Fernandez et al., 1978). It is considered that these cells in keratinized epithelia represent the transitional stage in the keratinizing process, and correspond to the superficial cells in this study.

The relationship between the morpho-
logical type of keratohyalin granules and the degree of keratinization has been the matter of discussion. In general, the epithelium which shows the keratin pattern contains irregularly shaped keratohyalin granules associated with filaments, while in the epithelium showing the abnormal keratin pattern these granules are regularly shaped and are not associated with filaments (Gibbins, 1962; Yukino, 1967; Hayward et al., 1973; McMillan, 1979). Muto (1970a, b) regarded the irregularly shaped keratohyalin granules as the fully matured granules. It is suggested that the mature keratohyalin granules contribute to production of the opaque interfilamentous substance and that the keratin pattern is thus formed. On the other hand, in the epithelium which shows the abnormal keratin pattern the development of keratohyalin granules is inhibited and the immature granules can not produce the opaque interfilamentous substance. Considered from their morphological features, the keratohyalin granules in this study appear to correspond to the immature granules. That is, the development of the keratohyalin granules is inhibited to form the abnormal keratin pattern and, thus, the keratinizing process does not increase beyond a certain degree.

It has been suggested that keratohyalin granules contribute to the formation of the interfilamentous substance. In this study, the superficial layer contained a fairly large number of keratohyalin granules. The periphery of some granules is not so distinct, giving an impression that the component is infiltrating the filamentous network and undergoing changes into interfilamentous substance. In other granules, an amorphous component is condensed in a portion of the granules. The similar granules were observed in the interpapillary epithelium of rat tongue (Farbman, 1966) and in mouse tongue (Matolsy and Parakkal, 1967). It is suggested that the electron translucent area represents an area which have undergone changes into interfilamentous substance. That is, these granules appear to show ultimate stages transforming into interfilamentous substance.

In this study, the intercellular cytoplasmic processes became thicker and more closely applied to each other, as the cells move towards the superficial layer. In the superficial layer, the processes are approximately 0.3-0.4 μm in width, being twice the size of those in the underlying layer. It is suggested that the intercellular processes increase in size, as a result of cell membrane distortion associated with keratinization, to form the tightly applied intercellular interdigitations as the cells move towards the superficial layer.

References


Explanation of Figures

Plate I

Fig. 1. The deeper layer. The cells are polyhedral or slightly elongated shape with many cytoplasmic processes. arrow = keratohyalin granule. arrowheads = basement membrane. f = fibril. × 10,000.

Fig. 2. The middle layer. The cells contain many keratohyalin granules both in the cytoplasm (K) and in the nucleus (Kn). arrows = desmosome. N = nucleus. × 20,000.
Plate II

Fig. 3. Higher magnification of the keratohyalin granules in the middle layer. The granule consists of a central mass of somewhat lower density (c) exhibiting peripherally associated smaller round masses of high density (arrowheads). × 50,000.

Fig. 4. The superficial layer. The cytoplasm of the cells varies in density and contains some keratohyalin granules (K). The cytoplasmic filaments shows tendency to form fibrils (A) or are embedded in a less opaque interfilamentous substance (B). The keratohyalin granules (K) consist of an amorphous component of high density. × 50,000.
Plate III

Fig. 5. Keratohyalin granules in the superficial layer. The component of the granules is condensed in the central portion (arrow; fig. 5a) or in the periphery (arrow; fig. 5b) of the granules. × 50,000.

Fig. 6. The cells in the superficial layer. The plasma membrane consists of a thick and dense asymmetrical trilaminar structure. In some areas, only the inner lamina is demonstrable (arrowheads). arrow = desmosome. × 50,000.

Fig. 7. Electron micrograph to show the middle (M) and the superficial layers (S). The cytoplasmic processes in the superficial layer (arrowheads) are thicker and about double size of those in the middle layer (arrows). × 20,000.