Epidermal Differentiation in the Human External Auditory Meatus

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The differentiation of epidermis in the various parts of the human ear canal was documented on the basis of cytokeratin (Ck) expression patterns. Immunohistochemistry was performed on cryostat sections of normal meatal skin using a comprehensive panel of monospecific Ck antibodies representing the main lines of epithelial differentiation.

The epidermis of the cartilaginous part showed a Ck profile characteristic of normal skin type differentiation. The deep meatal skin, including the tympanic membrane, showed a peculiar type of differentiation: in addition to epidermal Cks, hyperproliferation-associated Cks 6, 16, and 17 were expressed in the suprabasal cells, while the simple epithelial cell marker Ck 19 was found in the basal cells.

The presence of hyperproliferative Cks in the deep meatal skin could only partly be related to areas of proliferative activity. Keratinocytes, which express markers of hyperproliferation, are migratory. Therefore, their presence in the meatal skin is likely to be related to the peculiar pattern of keratinocyte migration, the purpose of which is to keep the meatus free from desquamation products.

LARYNGOSCOPE, 106:470-475, 1996

INTRODUCTION

The epithelial lining of the external auditory meatus is an extension of the body skin. The epidermis of the lateral cartilaginous part is supplied with glands and hair follicles identical to those in other parts of the body.1-3 However, the epidermis covering the tympanic membrane and the adjacent part of the deep bony meatus differs from body skin in several respects. This deep meatal skin is devoid of adnexae, while the epidermis covering the tympanic membrane is extremely thin, which allows the transmission of sound impulses.4-5 In addition, it has been shown by ink dot marking that the skin surface in this part of the ear canal shows a peculiar pattern of keratinocyte migration.6,7

On the tympanic membrane, keratinocyte migration occurs in a radial and centrifugal direction, while the adjacent part of the meatal epidermis shows lateral migration that ceases at its junction with the cartilaginous part. This migration is important to prevent the accumulation of desquamated products, which can interfere with the vibration of the tympanic membrane. The exact nature of this migration is not yet clear, but the reduced intercellular adhesion and differentiation of keratinocytes, observed electronmicroscopically by Johnson and Hawke,4 might be related to their peculiar migratory activity.

The question arises as to whether these features are reflected in the composition of the cytoskeleton, notably the cytokeratin (Ck) polypeptides. The Cks, which constitute a group of at least 20 different proteins, are the major components of intermediate-sized filaments in epithelial cells.8-10 They are distributed in certain combinations, depending on the type of epithelium, the differentiation potential, the proliferative capacity, and the environmental conditions.9-11 This property of the Cks has proven very useful for studying both normal and abnormal differentiation in epithelial tissues.12

The present study was conducted to characterize the epidermis of the various parts of the external ear canal using Ck expression as a parameter for differentiation. For comparative purposes, skin from the retroauricular area was also examined. A comprehensive panel of mainly monospecific cytokeratin antibodies, representing all Ck subgroups, was applied. In addition, antibodies directed against vimentin, which occurs in mesenchymal cells, were included.

MATERIALS AND METHODS

All specimens were obtained from patients with a normal external ear canal who were operated on for an acoustic neuroma by the translabyrinthine route. The specimens included tympanic membranes with the adjacent part of the deep meatal skin (n=8) and skin biopsies from the cartilaginous meatus (n=8) and the retroauricular area (n=8).

Immediately after dissection, the specimens were...
frozen in liquid nitrogen and stored at −70°C. Cryostat sections 7 μm thick were placed on poly-L-lysine-coated slides, air dried for 30 minutes, and stored at −70°C until required. Immunostaining was performed according to a previously described indirect immunoperoxidase technique. 12 The specific properties of the antibodies that were applied are given in Table I. 10–14,25

RESULTS

The data obtained on Ck expression in the epidermis of the cartilaginous and bony meatus, the tympanic membrane, and the retroauricular skin are summarized in Table II and depicted in Figures 1, 2, and 3.

Cartilaginous Meatus and Retroauricular Area

The epidermis of the cartilaginous meatus and the retroauricular area showed homogeneous staining with the broadly reacting antibody RCK 103 (Fig. 1a). All the basal cells were stained with the antibodies for basal cell Cks, AE 14 (Ck5) (Fig. 1c) and RCK 107 (Ck 14) (Fig. 1d). A positive reaction was observed in all suprabasal cell layers with the keratinization marker RKSE 60 (Ck 10) (Fig. 1b).

There was no reaction in the skin with the hyperproliferation-associated cell markers KA 12 (Ck 6) (Fig. 1j) and LL025 (Ck 16) (Fig. 1k) and with the antibodies specific for simple and complex epithelia, including RCK 105 (Ck 7), M20 (Ck 8), Ck 18-2 (Ck 18), E3 (Ck 17) (Fig. 1l), and LP2K (Ck 19) (Fig. 1i). Only various cell subpopulations of the glands and hair follicles reacted with these antibodies. The epidermis and the skin adnexae failed to stain with Ck 7 (Ck 13) and EB10 (Ck 4), the stratification markers for noncornifying epithelia.

Bony Meatus

In comparison with the cartilaginous part, the skin of the bony part was thinner and lacked adnexae. The whole epidermis showed a positive reaction with the broadly reacting antibody RCK 103 (Fig. 1e). Antibodies to Ck 5 (Fig. 1g) and Ck 14 (Fig. 1h), specific for basal cells, stained the basal cell layer and, in most of the specimens, also the parabasal cell layer.

Staining with the keratinization marker Ck 10 (Fig. 1f) was present in all suprabasal cell layers. The antibody directed against the hyperproliferation-associated Ck 6 (Fig. 1n) showed staining of two or more suprabasal cell layers, and the basal cells were also involved at some sites. The hyperproliferation-associated marker Ck 16 (Fig. 1o) revealed a heterogeneous-to-homogeneous reaction in one or two suprabasal cell layers. The same pattern was seen for Ck 17 (Fig. 1p).

No reaction was observed with the antibodies directed against stratification markers for nonkeratinizing epithelium or the markers for simple and complex epithelia, except for Ck 19 (Fig. 1m), which was present in scattered and large clusters of basal cells. Ck 19 was absent at the sites where Ck 6 reacted with the basal cells.

Tympanic Membrane

The Ck profile of the tympanic membrane did not differ fundamentally from that of the bony meatus. The broadly reacting antibody RCK 103 (Fig. 2a)

<table>
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<tr>
<th>Specimen</th>
<th>Broadly Reacting</th>
<th>Simple Epithelia</th>
<th>Basal Cell</th>
<th>Stratified Epithelia</th>
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</thead>
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<tr>
<td>Retroauricular</td>
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<td>−</td>
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<td>Cartilaginous</td>
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<td>−</td>
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<tr>
<td>Tympanic Membrane*</td>
<td>+</td>
<td>−</td>
<td>−</td>
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</table>

*For the expression in different parts of the tympanic membrane, see text.

Ck = cytokeratin; − = no staining; + = homogeneous staining; b = staining of basal cells; b1 = staining of basal cells and parabasal cell layer; b2 = patchy staining of basal cells; sb1 = staining of all suprabasal cell layers; sb2 = heterogeneous to homogeneous staining of one or two suprabasal cell layers; sb3 = varying number of suprabasal cell layers and Ck 19-negative basal cells.

Laryngoscope 106: April 1996
Vennix et al.: Cytokeratin Expression
Fig. 1. Immunohistochemical staining of the epidermis of the cartilaginous parts of the external meatus (photographs a through d and i through l) and the bony parts of the external meatus (photographs e through h and m through p) with antibodies directed against various cytokeratins (Cks). Photographs a and e. Broadly reacting antibody RCK 103. Photographs b and f. Keratinization marker Ck 10 (PKSE 80). Photographs c and g. Basal cell marker Ck 5 (AE 14). Photographs d and h. Basal cell marker Ck 14 (RCK 107). Photographs i and m. Simple epithelial cell marker Ck 19 (LP2k). Photographs j and n. Hyperproliferation-associated marker Ck 6 (KA 12). Photographs k and o. Hyperproliferation-associated marker Ck 16 (LL025). Photographs l and p. Ck 17 (ES). Note the lack of expression of Cks 6, 16, 17, and 19 (photographs i through l) in the skin of the cartilaginous meatus (original magnification ×380).
showed homogeneous staining in all the cell layers, while the keratinization marker Ck 10 (Fig. 2b) was found in all the suprabasal cells.

The reactions with the other antibodies differed locally. All the basal cells were positive with the basal cell markers Ck 5 (Fig. 2c) and Ck 14 (Fig. 2d), but in the annular region, Shrapnell's membrane, and the region of the umbo, the parabasal cells also reacted. Hyperproliferation-associated Ck 6 (Figs. 2f and 3b) revealed a fairly homogeneous expression pattern in one or more suprabasal cell layers. In the annular region, nearly the whole suprabasal compartment contained cells that were positive for Ck 6 (Fig. 3b).

In all specimens, Ck 16 (Figs. 2g and 3c) and Ck 17 (Figs. 2h and 3d) showed a nearly homogeneous expression in one or two parabasal cell layers of the annulus, the peripheral part of the pars tensa, the pars flaccida, and the area of the umbo. The staining patterns of these Cks in the central area of the pars tensa in the different specimens varied from focal to homogeneous.

The Ck 19 (Figs. 2e and 3a) revealed a patchy expression pattern in the basal cell layers in the area of the annulus (Fig. 3a), the pars flaccida, the region of the umbo, and the peripheral part of the pars tensa. In the central part of the pars tensa (Fig. 2e), the variation in expression was similar to that observed for Ck 16 (Fig. 2g) and Ck 17 (Fig. 2h).

No reaction was observed with the other markers for simple and complex epithelia or with the stratification markers for noncornifying epithelium. Staining with the vimentin antibodies was limited to the Langhans' cells, which were present in varying numbers in the different parts of the meatal epidermis.

**DISCUSSION**

This immunohistochemical study demonstrated clearly that there is considerable difference in the Ck profile of the skin in the different parts of the external ear canal. In the skin of the lateral cartilaginous part, the basal cells showed the expression of Cks 5 and 14, while the suprabasal cells showed the expression of the marker of skin type differentiation Ck 10, which was similar to that observed in other parts of the skin. However, the deep meatal skin showed the expression of Ck 19 in the basal cells and the expres-
sion of Cks 6, 16, and 17 in the parabasal cell layer(s), in addition to skin type differentiation.

The Cks 6 and 16 are considered to be hyperproliferation-associated proteins. They are only present in skin areas with a high cell turnover, such as the sole of the foot and the palm of the hand and under conditions of hyperproliferation, such as in wound healing, cell culture, hyperproliferative skin diseases, and malignant transformation. Despite the close relationship of Cks 6 and 16 with hyperproliferative activity, there is no unanimity on their function in this process. They are not usually expressed in the basal cell layer (i.e., the site of proliferation), but they are found in the suprabasal nonproliferative compartment.

Kopan and Fuchs suggested that the expression of Cks 6 and 16 is more likely to be the consequence than the cause of increased proliferation, while de Mare, et al. proposed that Cks 6 and 16 are a trigger induced by disturbed integrity, precluding hyperproliferation. In normal skin type differentiation, Maisbridge and Knapp regarded the appearance of Cks 6 and 16 as evidence for an alternative pathway of differentiation, the so-called "regenerative maturation type." According to this reasoning, the phenotype of the deep mental skin can be considered to combine two separate differentiation phenotypes: the normal epidermal type and the regenerative maturation phenotype.

In the deep mental skin, Ck 17 showed an expression pattern similar to that of the hyperproliferation-associated Cks 6 and 16. The Ck 17 is normally only expressed in the basal cells of a group of complex epithelia. In normal epidermis, Ck 17 is absent, except in a special sensory structure, the so-called "haar-scheiben." However, its expression has been observed in keratinocyte cultures. Psoriasis, and carcinomas. In these cases, Ck 17 can be coexpressed with Cks 6 and 16. The present observations in the mental epidermis are in line with the assumption that in stratified epithelia, Ck 17 can also be considered to be a hyperproliferation-associated cyto keratin.

The presence of Ck 19 in the basal cells of the deep mental skin, also observed by Leperoque, et al., was very exceptional. Normally, Ck 19 is only found in simple and complex epithelia and in the basal cell layer of stratified epithelia, where two types of differentiation coexist, as, for example, in the mouth and the cervix. However, according to Stasiak, et al. and Bosch, et al., the synthesis of Ck 19 cannot be considered a general criterion for epithelial stem cell differentiation, because it is absent in the basal cells of the epidermis. Stasiak, et al. suggested that Ck 19 expression in these areas of variable differentiation could indicate a transformation-sensitive population of cells. The deep mental skin showed the coexistence of two types of differentiation, which might explain the presence of Ck 19.

What is the significance of this special pathway of differentiation in the deep mental skin? Studies on the proliferative activity of the deep mental skin have been performed on the guinea pig and the mouse. In the guinea pig, Litton observed that mitotic activity was limited to the epidermis just distal to the annulus, except in the superior part. This mitotic activity was reported to be much higher than in the epidermis elsewhere. Boedt and Broeckaert reported the presence of Ck 16 exclusively in the inferior annular region and concluded that this expression reflected the proliferative nature of this area.

These data are at variance with those reported in other studies on mitotic activity and also with the present observations on the expression of hyperproliferation-associated Cks. In mice, mitoses were observed not only in this area but also in the pars flaccida and the region of the handle of the malleus. However, the number of mitoses was very low and did not exceed that of normal skin. Therefore, whether these areas can be considered hyperproliferative remains questionable. Furthermore, the present immunohistochemical observations demonstrated that hyperproliferation-associated Cks are also expressed outside these areas, including in the pars tensa, where no mitotic activity has been observed. Thus, their presence cannot be related exclusively to proliferation or hyperproliferation.

Keratinocytes that express hyperproliferation-associated Cks exhibit different behavior from normal keratinocytes. One of their main characteristics is the ability to migrate. As mentioned previously, the epithelium of the deep meatus, including the tympanic membrane, shows a peculiar migration pattern in a lateral direction. Therefore, the presence of hyperproliferation-associated Cks in the keratinocytes in the deep part of the meatus is more likely to be related to their ability to migrate than to proliferation or hyperproliferation. The presence of these Cks in the parabasal cell layers seems to support the suggestion of Johnson and Hawke that migration occurs in the deeper cell layers of the stratum spinosum.

CONCLUSION

We conclude that the keratinocytes in the deep external ear canal and on the tympanic membrane express a set of Cks that are only encountered in other epidermal sites of the body during wound healing or in pathologic conditions. The expression of this special set of cytoskeletal proteins is probably related to the migratory characteristics of these keratinocytes. Whether these properties of deep mental skin keratinocytes contribute to the invasion of epidermal cells into the middle ear in chronic otitis media remains to be proven.
BIBLIOGRAPHY


