Differential immunohistochemical detection of cytokeratins and vimentin in the surgically removed human endolymphatic duct and sac

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Summary. Immunohistochemical detection of intermediate filament proteins and different subgroups of cytokeratins (Cks) was used to characterize the epithelium of the surgically removed adult human endolymphatic duct (ED) and sac (ES). The epithelium of the ED and ES demonstrated immunostaining for Cks 7, 8, 14, 17, 18 and 19, a pattern typical of so-called “complex” or “mixed” epithelia. This is a remarkable finding, since this pattern differs strikingly from previously reported data on the adult human cochlea and vestibular labyrinth that demonstrated a Ck pattern typical of “simple” (or single-layered) epithelia. Furthermore, the epithelium of the ED and ES exhibited co-expression of Cks 7 and 19. The present data indicate that the epithelium of the ED and ES exhibits another type of epithelial differentiation and demonstrates a higher degree of complexity than the other epithelia in the adult human inner ear.

Key words: Human endolymphatic duct and sac – Intermediate filaments – Cytokeratins – Immunohistochemistry

Introduction

Cytokeratins (Cks) are a family of polypeptides which belong to the class of intermediate filament proteins (IFPs) and are part of the cytoskeleton of epithelial cells. Vimentin is also an IFP and in general is synthesized by mesenchymally derived cells. Apart from this tissue-specific distribution, the co-expression of Cks and vimentin has been described in several epithelia. Desmin, glial fibrillary acidic protein (GFAP) and neurofilament pro-

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nomenclature for the subdivision of the human duct and sac as proposed by Bast and Anson [7] seems most appropriate. This latter system divides the ED into the sinus and isthmus portion while the ES is divided into the rugose and smooth portion (i.e. endolymphatic sac proper). Since the rugose portion in man is restricted to the part of the sac within the vestibular aquaduct (i.e. the intraosseous part), the smooth portion of the human ES may also be termed the extraosseous part.

Mainly based on animal experiments, various functions have been attributed to the different epithelial regions of the ED and ES. These include an active role in the turnover of inner ear fluids [17], a possible role in a local immunological defense system [28], and a possible role in statoconial formation and degradation [16].

Several reports have described the ultrastructure of the different epithelial cell types in both the human ED and ES [6, 14, 26]. However, apart from previous immunohistochemical studies on various animal species [2, 3, 12] and human embryos [4], the first report on the immunohistochemical characterization of the epithelium of the adult human ES has only recently been published [1]. In this study by Altermatt’s group, several Cks and vimentin were demonstrated in the ES epithelium [1]. However, this study was restricted to the extraosseous (smooth) portion of the human ES, thus excluding the highly differentiated epithelial cells of the intraosseous rugose portion and the ED. Moreover, only three monoclonal antibodies to different Cks were used in this study, thus leaving the possible presence of many Ck subunits undetermined.

Recently, we have described the immunohistochemical detection of Cks in the epithelium of the adult human cochlea, demonstrating a Ck pattern typical of simple epithelia [9]. A similar Ck pattern was also found in the adult human vestibular labyrinth [10]. The aim of the present study was to characterize the epithelium of the adult human ED and ES by investigating its immunohistochemical expression of Cks and other IFPs in the various regions of the duct and sac.

Materials and methods

Tissue preparation. The ED and ES of five patients (ages 21–69 years) were dissected and removed during surgery for acoustic neuroma through a translabyrinthine approach. Subsequently, the tissues were embedded in Tissue-Tek II and cryosectioned.

Antiserum. Tissue sections were incubated with polyclonal rabbit and monoclonal mouse antibodies to five classes of IFPs: i.e., vimentin, neurofilament proteins, desmin, GFAP, and different Ck subunits. Unless indicated otherwise, antibodies were obtained from Euro-Diagnostics BV, Apeldoorn, The Netherlands. The Ck subunit classification described in this paper is that described by Moll et al. [18].

Cytokeratins. Monoclonal antibodies 6B10 (dilution 1:1 directed to Ck 4), RCK 102 (dilutions 1:2.5 to 1:5 directed to Ck 5 and 8), RCK 105 (dilutions 1:1 to 1:5 directed to Ck 7), M20 (dilutions 1:5 to 1:20 directed to Ck 8), RKSE 60 (dilution 1:1 directed to Ck 10) and IC7 (dilution 1:1 directed to Ck 13) were used. RPN 162 (dilutions 1:1 to 1:2.5 directed to Ck 7) and RPN 1665 (dilutions 1:5 to 1:20 directed to Ck 19) were obtained from Amer-
Fig. 1 A–F. Immunostaining of the endolymphatic sac and duct. A Intraosseous rugose portion of the endolymphatic sac immunostained with monoclonal antibody RPN 1162 to cytokeratin 7. Intense immunostaining of the epithelium of the endolymphatic sac is present but with differences in the degree of staining between individual cells. × 120. B Intraosseous rugose portion of the endolymphatic sac immunostained with monoclonal antibody RCK 107 to cytokeratin 14. Although the majority of epithelial cells lack immunoreactivity, other cells demonstrate clear immunostaining. × 100. C Endolymphatic duct immunostained with monoclonal antibody RCK 102 to cytokeratins 5 and 8, showing intense immunostaining of the epithelial lining of the duct. × 60. D Intraosseous part of the endolymphatic sac immunostained with monoclonal antibody RPN 1165 to cytokeratin 19. All cells are stained in the epithelial lining of the endolymphatic sac. × 180. E Intraosseous rugose portion of the endolymphatic sac immunostained with monoclonal antibody RV 202 to vimentin. Immunoreactivity is seen in the epithelial lining of the endolymphatic sac (arrow) as well as in the subepithelial fibroblasts. × 130. F Endolymphatic duct immunostained with monoclonal antibody RV 202 to vimentin. There is immunostaining of both the epithelial lining of the endolymphatic duct (arrows) and of the underlying fibroblasts. × 70.
positive to negative) between morphologically otherwise similar individual cells. This especially occurred with antibodies RCK 107 (Ck 14), LI.002 (Ck 14) and E 3 (Ck 17), which demonstrated variable immunostaining in approximately only 40% of the epithelial cells throughout the ED and ES (Fig.1B). The cells which stained positively for antibodies detecting Cks 14 and 17 were morphologically not distinguishable at the light microscopical level from negatively staining adjacent epithelial cells. The other immunoreactive Ck antibodies, i.e., RCK 102 (Cks 5 and 8), RCK 105 (Ck 7), RPN 1162 (Ck 7), M20 (Ck 8), RCK 106 (Ck 18) and RPN 1165 (Ck 19), stained the majority of the epithelial cells throughout the ED and ES (Figs. 1A, C, D, 2–4).

No immunostaining was observed with antibodies 6B10 (Ck 4), RKSE 60 (Ck 10) and IC7 (Ck 13).

Vimentin

Positive immunostaining occurred in the epithelium of the ED and ES, as well as in the subepithelial connective-tissue fibroblasts (Fig. 1E, F).

Desmin, GFAP and neurofilament proteins

All antibodies to these IFPs demonstrated absence of immunostaining.
Discussion

The procedure for surgical removal of the adult human ED and ES allows optimal preservation of immunoreactivity, since chemical fixation and decalcification are avoided. Thus, detection of antigens is performed on cryosections of freshly frozen tissue, offering the most reliable immunohistochemical results [8]. A disadvantage of the removal of the ED and ES during surgery, however, is that a substantial portion of the smooth or extraocular part of the ES cannot be adequately dissected because of its close relation to the dura mater. Another possible disadvantage of our specimens is the presence of an acoustic neuroma adjacent to the ES. However, we have found no reason to assume any untoward influence on our immunohistochemical results.

The monoclonal antibodies to cytokeratins (Cks) 4, 7, 8, 10, 13, 14, 17, 18 and 19 used in this study included markers for the single-layered, stratified keratinizing and non-keratinizing and complex or mixed epithelia. We have demonstrated immunoreactivity for Cks 7, 8, 14, 17, 18 and 19 in the epithelium of the human ED and ES, while reactivity for Cks 4, 10 and 13 was absent. In addition, the epithelium of the adult human ED and ES demonstrated co-expression for Cks 10 and 13. The remaining IFPs tested for, i.e. desmin, GFAP and neurofilament proteins, could not be detected immunohistochemically. Still, the absence of immunostaining for these IFPs and for certain CK subunits, without support of biochemical analysis, does not definitely exclude the presence of the antigens concerned and therefore must be interpreted with caution.

Our results on the expression pattern of Cks in the adult human ED and ES are generally in agreement with earlier, albeit limited, data on mice [2, 3, 12]. These studies demonstrated the presence of Cks 7, 8, 18, and 19 in the ED and ES but without testing for Cks 14 and 17, two subunits which we also found to be present. However, when compared to a study by Anniko et al. [4] on human fetuses, the results of which may differ considerably from those obtained in the human adult due to the transient expression of IFPs during embryonic development, there are notable differences. In Anniko’s study, probes were used for Cks 7, 8, 16 and 18, and indicated the presence of Ck 10, apart from Cks 7, 8 and 18 [4]. We were not able to detect Ck 10 in our material. This latter Ck is a polypeptide typical of keratinized epidermis whose presence in the inner ear seems very unlikely on theoretical grounds. Also, we were not able to confirm recently published data by Altermatt et al. [1] on the presence of Ck 10, besides Cks 18 and 19, in the extracerebral smooth portion of the adult human ES, a subunit typical of non-keratinizing stratified epithelia (as present in esophageal, tongue and cervical epithelia).

The Ck pattern in the epithelium of the adult human ED and ES found in our study may be characterized as typical of a complex or mixed epithelium, since it constitutes a mixture of Cks typical of simple epithelia (Cks 7, 8, 18 and 19), keratinocyte-specific Ck (Ck 14), and Ck 17 which is a Ck subunit that is frequently co-expressed with Ck 14 [15]. In addition, markers for both keratinizing (Ck 10) and non-keratinizing (Cks 4 and 13) stratified epithelia were absent.

As recently demonstrated by Parkins et al. [21], the 5/14 Ck pair is expressed by all squamous basal cells (cells in contact with the basal lamina but out of contact with the epithelial surface) of each multilayered or mixed epithelium, whether it concerns glandular, ductal, secretory or stratified squamous epithelia. Furthermore, the 5/14 Ck pair has been shown to be absent in all homogeneous simple or single-layered epithelia, of which the cells border both the basal lamina and the epithelial surface [21]. Thus, our finding of Ck 14 expression in a subpopulation of cells throughout the epithelial lining of the ED and ES with two different antibodies likely to recognize different epitopes indicates the presence of basal cells, thus supporting the classification of this epithelium as a mixed epithelium.
Morphological data presented in two recent studies on the ultrastructure of the human ES [6, 26] and ED [14] demonstrated a highly differentiated epithelium in which an enormous variability exists in the size and shape of the epithelial cells (either flattened, cuboidal or cylindrical). However, while Schindler [26] reported an occasionally pseudostratified appearance of the epithelium in the rugose folds and villi of the intraseptal portion of the sac, Bagger-Sjöbäck et al. [6] did not report such findings and described the epithelium to be purely single-layered. Our immunohistochemical data present new evidence for pseudopatification, since the expression of Ck 14 has, until now, never been found in homogeneous single-layered epithelia [21]. The immunohistochemical classification of the epithelium of the human ED and ES as a complex or mixed epithelium differs strikingly from results by our group on the epithelia of the coehlea [9] and vestibular labyrinth [10], in which Cks were demonstrated typical of simple epithelia (i.e. Cks 7, 8, 18 and 19). The epithelium of the ED and ES apparently has a higher degree of complexity and differs considerably in its immunohistochemical phenotype from the other epithelia in the adult human inner ear.

Although differences in the ultrastructure of epithelial cells have been described between different regions of the ED and ES (e.g. differences in junctional characteristics between the duct and proximal part of the sac, and the rugose and more distal part of the sac [3]), we were not able to detect clear regional differences in immunostaining for either cytokeratins or vimentin.

As with certain other epithelial structures in both the coehlea and vestibular labyrinth [10, 11], we found that the entire epithelium of the ED and ES demonstrated co-expression of Cks and vimentin. Similar findings have been described by Altermatt et al. [1] for the extravasal or smooth portion of the adult human sac. However, the meaning of co-expression of Cks and vimentin is not yet understood. Although it has been demonstrated in several neuroepithelial tissues, it has also been found in other unrelated epithelia and mesothelial cells [19].

Our immunohistochemical results indicate a high degree of cellular and functional differentiation of the ED and ES epithelium and give support to the view that the ED and ES are a functionally active part of the inner ear. Future studies will have to elucidate how certain functions of this organ correlate with the type of epithelial differentiation seen.

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