Squamous Metaplasia of the Middle Ear Epithelium

W. KUIJPERS,1 P. P. C. A. VENNIX,1,2 T. A. PETERS1 and F. C. S. RAMAEKERS3

From the Departments of Otorhinolaryngology,1 University of Nijmegen and 2University of Leiden, and 3Department of Molecular Cell Biology and Genetics, University of Limburg, Maastricht, The Netherlands


This study deals with the expression of cytokeratins (Cks) in squamous cell metaplastic lesions in rat and human middle ear. In rats, squamous metaplastic lesions could be induced during chronic otitis media. The histological features of these lesions were similar to those observed in the human middle ear. Immunohistochemistry revealed that squamous cell metaplasia in both rat and human middle ear is characterised by a loss of simple epithelial cell related Cks and the appearance of Cks characteristic of stratified and cornifying epithelia. This indicates a true change in the differentiation of the middle ear epithelium. It is concluded that the Ck profile of the cholesteatoma matrix cannot be used as a variable to decide whether the origin of cholesteatomas is epidermal or metaplastic. This rat model is suitable for studying squamous cell metaplasia in relation to cholesteatoma genesis. Key words: rats, humans, cytokeratin expression.

INTRODUCTION

Squamous metaplasia is assumed to be a possible source of cholesteatoma and has been suggested to account for about 4% of the total number encountered (1). However, this contribution is difficult to prove because these lesions will usually go unnoticed until the tympanic membrane perforates. This makes them indistinguishable from cholesteatoma genesis from ingrowing epidermis.

The metaplastic theory is mainly based on the presence of stratified epithelium in the middle ear during chronic otitis media (OM). However, these studies chiefly showed the presence of areas of non-cornifying epithelium, while cornifying epithelium has only been observed incidentally (1–6). Also, in experimental animals, squamous metaplasia has been observed after the application of a large variety of irritating chemicals (7–9), but metaplasia as a consequence of middle ear infections seems to be very rare.

This investigation is a continuation of a previous experimental study in which we succeeded in inducing an infection-related squamous cell metaplasia by Eustachian tube obstruction in rats with an upper respiratory tract infection (10). In the present study we were interested in the relation between cytokeratin expression and histological changes during squamous cell metaplasia. Cytokeratins (Cks) are intermediate filament proteins which are exclusively present in epithelial cells. The expression of the different types of Cks (numbered 1–20) depends on the type of epithelium and stage of differentiation (11–13). Because of these properties Cks seem very suitable for a better understanding of squamous cell metaplasia in the middle ear. The metaplastic lesions in the rat were studied with a broad panel of monospecific antibodies directed against various cytokeratins. In addition, squamous metaplastic lesions in biopsies taken from human cases with chronic OM were included in this study.

MATERIAL AND METHODS

For the induction of purulent OM, 100 adult Wistar rats that displayed clinical symptoms of an upper respiratory airway disease were used. The animals were checked otoscopically for the absence of otitis and the eustachian tube was obstructed on both sides as described previously (14). All the animals developed a purulent middle ear disease within one week after operation. After survival times varying from 4 months up to 6 months the animals were killed by an intracardiac injection of pentobarbital sodium and decapitated. The specimens were either processed for embedding in glycol methacrylate (GMA) for histological studies or for preparing cryosections for immunohistochemistry.

For histology the whole middle ear was dissected from the skull and fixed in phosphate buffered (0.1 mol/L; pH 7.4) 2.5% glutaraldehyde and subsequently decalcified in a solution containing 10% EDTA (pH 7.4). After dehydration the specimens were embedded in glycol methacrylate (GMA). Sections (2 μm) were stained with toluidine blue.

For immunohistochemistry, the middle ears were immediately after dissection from the skull stored in a decalcification solution containing 10% EDTA and 7.5% polyvinylpyrrolidone in 0.1 mol/L of TRIS-HCl buffer (pH 7.2) at 4 ºC for a period ranging from 4–6 days. After rinsing in the same solution without EDTA, the specimens were frozen in liquid nitrogen. Cryosections were placed on poly-L-lysine coated-slides, air dried and fixed in acetone. Immunohisto-
chemical staining of these sections was performed as described previously (10). The following monoclonal cytokeratin antibodies were used: RCK 103 (broadly reacting); RCK 105 (Ck 7); CK 18-2 (Ck 18); M20 (Ck 8); 6B10 (Ck 4); 1C7 (Ck 13); RCK 107 (Ck 14); RKSE 60 (Ck 10). For specifications of these antibodies and references, see (15). All these antibodies with known specificities for human Cks cross-react with the rat cytokeratins except for M20 (Ck 8).

In addition to these rat ears, 50 biopsy specimens of human middle ear epithelium from cases of chronic OM were included in this immunohistochemical study. The biopsies were processed in the same way as the rat specimens, but the decalcification step was omitted.

RESULTS

Rats

All the rat ears were filled with a purulent mass. The middle ear mucosa was largely thickened and contained numerous inflammatory cells. The hypertrophic epithelial lining was very irregular with cyst-like formations. It often showed local discontinuities and contained numerous secretory cells. Two ears which were embedded in GMA revealed areas of squamous cell metaplasia. These areas consisted of stratified non-cornifying epithelium with a regularly arranged layer of columnar basal cells and flattened suprabasal cells. There was an abrupt transition between the hyperplastic pseudostratified epithelium and the squamous epithelium (Fig. 1).

From the ears which were processed for immunohistochemistry two specimens revealed areas of cornifying squamous metaplasia. Immunohistochemical staining showed a homogeneous expression of the simple epithelial cell Cks 18 and 19 in the middle ear epithelium whereas Ck 7 was expressed heterogeneously. From the stratification markers, Ck 4 was expressed sparse to heterogeneously and Ck 13 was absent. Expression of the basal cell Ck 14 was limited to the basal cells. These data do not fundamentally

<table>
<thead>
<tr>
<th>Cytokeratin expression in middle ear epithelium of rats and humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Homogeneous positive reaction, – Negative reaction, ± Sparse to heterogeneous reaction, B Staining of basal cells,</td>
</tr>
<tr>
<td>1Data from references 10 and 15, 2Antibody M20 does not cross-react with rat Ck 8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ck</th>
<th>Rats Normal1</th>
<th>Metaplasia</th>
<th>Humans Normal1</th>
<th>Metaplasia non-cornifying</th>
<th>Metaplasia cornifying</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>+ B</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>±</td>
<td>–</td>
<td>±</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>18</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>19</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>–</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>+ B</td>
<td>–</td>
<td>+ B</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>+ B</td>
<td>+</td>
<td>+ B</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>
Fig. 2. Cytokeratin expression in metaplastic cornifying squamous epithelium in the rat middle ear, 4 months after the induction of chronic otitis media. (A) Survey showing expression of Ck 4 in metaplastic epithelium (m), but not in the middle ear epithelium (e). (B) Survey showing expression of Ck 14 in both metaplastic epithelium (m) and middle ear epithelium (e). (C) Detail of metaplastic epithelium in (A) showing expression of Ck 4 in all suprabasal cell layers. (D) Metaplastic epithelium showing homogeneous expression of Ck 14 in all cell layers. (E) Metaplastic epithelium showing scattered suprabasal expression of Ck 10. Bars represent 500 μm (A, B), 100 μm (C–E).

differ from those observed in the normal middle ear epithelium (Table I, (10, 15)). The immunohistochemical data of the metaplastic epithelium are summarised in Table I and depicted in Fig. 2. This epithelium showed homogeneous expression of Cks 4 and 13 in all suprabasal cell layers (Fig. 2A, C), while the basal cell marker Ck 14 was expressed in all cell layers (Fig. 2B, D). The cornification marker Ck 10 showed a sparse to heterogeneous expression in the suprabasal cells (Fig. 2E). No expression of the markers for simple and complex epithelia (Cks 7, 18, 19) was observed.

Human specimens
The mucosa of the biopsy specimens from chronic OM invariably showed the presence of numerous
inflammatory cells. The epithelium was hypertrophic. It contained many secretory cells and crypts lined with a varying number of secretory and non-secretory cells. In three biopts squamous metaplasia was observed. It consisted of non-cornifying squamous epithelium in two specimens. The third specimen showed a gradual change from hyperplastic pseud stratified epithelium into non-cornifying stratified epithelium, which gradually passed into cornifying epithelium with keratohyalin granules (Fig. 3). There was an abrupt transition between the cornifying epithelium and the middle ear epithelium (Fig. 3A). The Ck profile of the middle ear epithelium in all the biopts was similar to that observed in the normal epithelial lining (Table I, (10)). It showed homogeneous expression of the simple epithelial cell Cks 7, 8, 18, 19 and a scarce to heterogeneous expression of the stratification markers Cks 4 and 13. Expression of the basal cell marker Ck 14 was limited to the basal cells. The same expression profile was observed in the hyperplastic pseudostratified epithelium in the metaplastic area (Fig. 3) although the expression of Cks 4...
and 13 was more marked. In the non-cornifying metaplastic squamous epithelium Cks 4 and 13 were homogeneously expressed in all suprabasal cell layers (Fig. 3C), whereas Ck 14 was expressed in all cell layers (Fig. 3D). No expression was observed for the cornification marker Ck 10 (Fig. 3E) and the markers of simple epithelia (Fig. 3A), except for Ck 19 which was expressed in all cell layers (Fig. 3B). The cornifying epithelium showed suprabasal expression of the cornification marker Ck 10 (Fig. 3E). The basal cell marker Ck 14 extended into all suprabasal cell layers (Fig. 3D). No expression of Cks 4, 13, 7, 8, 18 and 19 was observed in this area (Fig. 3A–C). In the area of non-cornifying epithelium adjacent to the cornifying epithelium Cks 10, 4 and 13 were co-expressed.

**DISCUSSION**

This study demonstrates that the middle ear epithelium of the rat can undergo squamous cell metaplasia during chronic OM. This finding is in line with comparable observations in the human middle ear (1) and in other areas of the respiratory tract (16, 17), which shows the susceptibility of this epithelium for squamous cell metaplasia.

The present immunohistochemical observations demonstrate that squamous metaplasia of the middle ear epithelium of the rat is associated with a loss of simple epithelial cell related Cks. This is paralleled by the appearance of the stratification markers of non-cornifying epithelia Cks 4 and 13 and to a lesser extent of the cornification marker Ck 10. In addition, the basal cell marker Ck 14 extends into all cell layers. This altered Ck profile must be due to a modification of the differentiation character of the progenitor cells of the middle ear epithelium.

A similar change of the Ck profile is observed in the human specimens. However, in these specimens the simple epithelial cell marker Ck 19 is retained in the non-cornifying lesion, whereas in one specimen squamous cell metaplasia had developed further into a lesion which phenotypically resembled epidermis. In this lesion Ck 10 expression substituted completely for Cks 4 and 13, which makes the epithelium indistinguishable from normal epidermis. Remarkably, also in the human lesions expression of the basal cell Ck 14 extended into the suprabasal cells. This upregulated Ck 14 expression indicates a state of hyperproliferation.

These immunohistochemical findings are very similar to those observed in squamous cell metaplasia of the lung (16) and the nose (17), except for the expression of the basal cell Cks, which remain limited to the basal cells. Extension of the basal cell Cks into the suprabasal cell layers has only been reported in squamous cell metaplasia induced by vitamin A depletion (18).

It has been suggested that the presence of non-epidermal Cks in cholesteatoma matrix refers to a metaplastic origin (19). However, the similarity of the Ck profile in the epidermal type of differentiation of a metaplastic lesion (exclusive expression of Ck 10) with that of the cholesteatoma matrix originating from ingrowing epidermis (10) is in conflict with this suggestion. In addition, expression of non-epidermal Cks has also been observed in dysplastic areas of the advancing front of cholesteatomas originating from ingrowing epidermis (10, Vennix, unpubl. data).

Summarizing, this study shows that infection-induced squamous cell metaplasia in the rat middle ear appears to be an appropriate model for studying metaplasia in relation to cholesteatoma genesis.

**REFERENCES**


Address for correspondence:
Dr. W. Kuijpers
Dept. of Otorhinolaryngology
University of Nijmegen
Ph. van Leydenlaan 15
NL-6500 HB Nijmegen
The Netherlands
Tel: +31 24-3615144
Fax: +31 24-3540251