ORIGINAL ARTICLE

Morphological considerations about middle ear cholesteatoma

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Abstract

The aim of this study is to present various morphologic aspects of the middle ear cholesteatoma, concerning both container (tympanic cavity) and content (cholesteatoma). There are two different aspects of the study: a mezoscopic study of the tympanic walls and the elements within the middle ear (ossicular chain, folds, ligaments, middle ear clefts) in order to evidentiate the critical areas (sinus tympani, facial sinus, anterior attic) and the pathways of spread for the cholesteatomas; a classical histological study of the middle ear cholesteatomas, in order to present the structural parts and the modifications in the neighboring structures.

***Keywords*:** anatomy, tympanic cavity, histology.

Introduction

A cholesteatoma consists of an accumulation of desquamated keratin epithelium in the middle ear cleft or any other pneumatized portion of the temporal bone. Cholesteatoma has a destructive character, by means of pressure erosion, as it destroys the ossicles, erodes the horizontal semicircular canal, uncovers the facial nerve, exposes dura of the temporal fossa or cerebellum, or causes other serious damage to important structures within or adjacent to the temporal bone. The envelope of a cholesteatoma is termed matrix, and desquamated keratin is shed continually by the matrix and forms the central mass of the cholesteatoma, similar to the layers of an onion. The term cholesteatoma is a misnomer, since the entity does not contain cholesterol. Cholesteatomas are classified as congenital or acquired; acquired cholesteatomas are subdivided into primary (attic retraction) or secondary [1].

In 1683, Duverney first described a temporal bone tumor probably corresponding to a cholesteatoma. Until 1838, as Müller coined the term cholesteatoma, nothing new appeared in medical publications [2].

Toynbee first mentioned the similarity between the squamae of what he called "*Molluscoum Contagiosum*" and the stratum corneum in 1850, not yet knowing how to explain the presence of epidermis in the middle ear [3, 4].

Von Troeltsch, while seeing cholesteatoma often desquamating into the external meatus, was the first to explain the possible epidermal origin of the disease. He supposed that epidermal debris, accumulated in the external meatus, was capable of inducing osteolysis of the bony wall of the meatus and thus of invading the mastoid and the middle ear [5–8].

Other suggested denominations were pearl tumor, by Cruveilhier, in 1829, epidermal cholesteatoma by Cushing, in 1922, and keratoma, by Shuknecht, in 1974.

Material and methods

Twenty temporal bones harvested from cadavers were dissected and cut in different planes, with an electric saw and diamond disc. The sections were analyzed on a stereomicroscope type Olympus SZ60 and photographed with a Camedia C5060/C7070 digital camera. The samples were illuminated with reflected and, in some cases, transmitted LED light. For macro pictures obtained using the macro function of the digital camera, halogen light illumination was used. The images were filtered and corrected with the image analysis software DP-Soft.

Tissue specimens of fifteen patients with cholesteatoma were obtained during middle-ear surgery. The tumor samples were fixed in buffered 8% formalin, paraffin embedded and sectioned at 4 μm on a Sakura SRM200 microtome, manually stained with Hematoxylin–Eosin and PAS. The microscope slide images were acquired with an Olympus Camedia C2000Z camera, mounted on a BX50 Olympus laboratory microscope, at a resolution of 1600×1200 pxl, under brightfield illumination, 10× and 20× objective magnification.

Results

The results of our study, following various morphologic aspects of the middle ear cholesteatoma, concerning both container (tympanic cavity) and content (cholesteatoma) are shown in Figures 1–11.

Discussions

Cholesteatomas are channeled along characteristic pathways by surrounding mucosal folds, the middle ear ossicles, and their suspensory ligaments. The middle ear can be divided into three compartments: the epitympanum, mesotympanum, and hypotympanum. The epitympanum lies above the level of the lateral process of the malleus; it contains the malleus head, incus body, and their associated ligaments and mucosal folds (Figures 1 and 2).

The annular ligament sends off fibrous bands from the anterior and posterior tympanic spines that meet at the neck of the malleus (tympano-mallear ligaments). Pars flaccida (Shrapnell's membrane) is exempt from the dense fibers that form the middle layer of the pars tensa. The lack of this structural support predisposes Shrapnell's membrane to retraction in case of negative pressure in the middle ear. The mesotympanum contains the stapes, long process of the incus, handle of the malleus and the oval and round windows. The eustachian tube exits from the anterior aspect of the mesotympanum (Figure 3).

From the mesotympanum, often impossible to visualize directly, two recesses extend posteriorly [9]. Sinus tympani lies between the medial wall of the mesotympanum and the facial nerve and is very difficult to be accessed surgically. The facial recess is bounded by the fossa incudis (superiorly) and the chordal eminence and chorda tympani nerve (laterally) (Figures 4 and 5).

Prussack's space is located between the tympanic membrane (pars flaccida) and neck of the malleus and the upper boundary of the lateral mallear fold. Posterior pouch of von Troeltsch lies between the tympanic membrane and the posterior mallear fold (Figure 6).

Epitympanic cholesteatomas start in Prussack's space. Cholesteatomas from Prussack's space spread via the posterior epitympanum, posterior mesotympanum and anterior epitympanum, in that order. The most common is the posterior epitympanic route where the cholesteatoma spreads to the superior incudal space lateral to the body of the incus potentially gaining access to the mastoid through the aditus ad antrum. The second most common is the inferior route, thought the posterior pouch of von Troeltsch (Figure 3).

This route allows cholesteatoma to gain access to the regions of the stapes, round window, sinus tympani and facial recess. Anterior epitympanic cholesteatomas form anterior to the malleus head. Facial nerve dysfunction may occur with these lesions, which can also gain access to the supratubal recess of the middle ear via the anterior pouch of von Troeltsch.

Microscopically the squamous epithelium of a cholesteatoma develops into a cyst of desquamating squames. This epithelium may rest on granulation tissue or fibrous tissue and sometimes (six in 15 cases) a part of the cholesteatoma overgrows normal mucosa.

Cholesteatoma has a keratinized stratified squamous epithelium named cholesteatoma matrix. It also presents a connective tissue, containing collagen fibers, fibrocytes and inflammatory cells, named perimatrix, which is in most of the cases in contact with squamous or ciliated cylindrical cells, remains from the original middle ear mucosa.

Some authors [10] describe the perimatrix as the most peripheral portion of the cholesteatoma, comprising granulation tissue or inflammatory subepithelial connective tissue, with lymphocytes, hystiocytes and neutrophils. The perimatrix appears as an inflammatory network that involves the cholesteatoma.

Sprekelsen BM *et al.* [11] stated that the matrix and perimatrix, in normal or pathological tissues, are formed by type IV collagen, tenascin, fibronectin, b-FGF and metalloproteinase (MMP).

In the study conducted by Paludetti G *et al.* [12], the perimatrix consisted of granulation tissue or inflamed subepithelial connective tissue.

The growth of cholesteatoma could require angiogenesis in the perimatrix connective tissue. Angiogenesis enables and supports the sustained migration of keratinocytes into the middle ear cavity [13].

Bony erosion occurs by two principal mechanisms [14]: *(a)* pressure effects produce bony remodeling, which occurs regularly throughout the normal skeleton; *(b)* enzymatic activity at the margin of the cholesteatoma enhances osteoclastic activity, which greatly increases the speed of bone erosion. These osteolytic enzymes appear to increase when a cholesteatoma becomes infected.

Dornhoffer JL *et al.* [15] studied the advisability of reusing the incus for ossicular reconstruction in cases involving cholesteatoma. Their examination showed that a number of specimens apparently free of cholesteatoma after macroscopic examination had microscopic evidence of cholesteatoma. Likewise, microscopic examination of an incus that appeared to be free of residual cholesteatoma revealed epithelial cells deeply invading the bone.

Macroscopic examination consistently under-estimated the amount of erosion that was clearly evident upon histologic examination. In light of these findings, gross examination of the incus after removal of cholesteatoma is not reliably predictive of invasive microscopic disease. Reusing the ossicles in this situation creates the potential of reimplanting the disease.

Cholesteatoma epithelium behaves more like a wound-healing process than a neoplasm. The available evidence to date does not indicate that cholesteatomas have inherent genetic instability, a critical feature of all malignant lesions. The induction of hyperproliferative cells in all layers of the cholesteatoma epidermis implicates a potential idiopathic response to both internal events as well as external stimuli in the form of cytokines released by infiltrating inflammatory cells. The presence of bacteria may provide a critical link between the cholesteatoma and the host, which prevents the cholesteatoma epithelium from continuing specific differentiation programs and returning to a quiescent state in which it becomes minimally proliferative, non-migratory, and noninvasive [16].

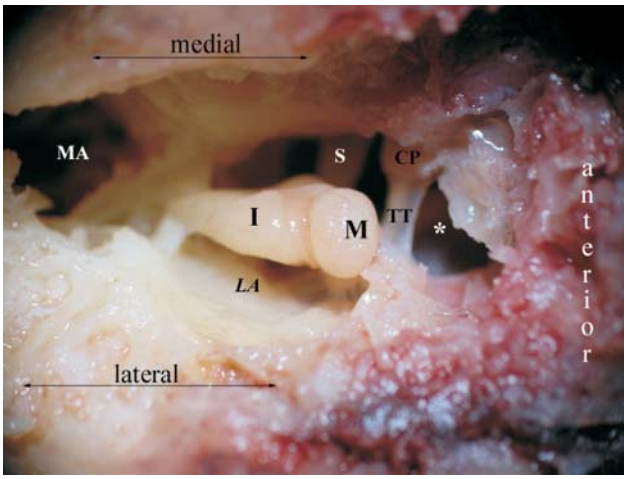


Figure 1 – *Left temporal bone (fresh specimen). Superior view of the attic (epitympanum) and mastoid antrum (MA): LA – lateral attic; M – malleus head; I – incus body; S – stapes; CP – cochleariform process; TT – tensor tendon; * – tensor fold is absent*

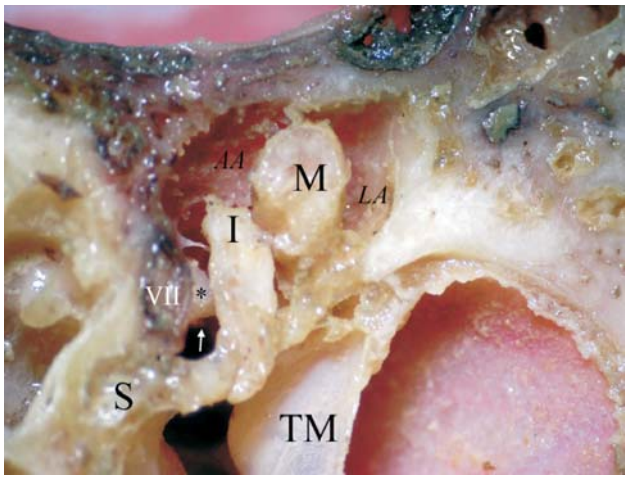


Figure 2 – *Right temporal bone (fresh specimen). Frontal section, anterior view: M – tympanic membrane; M – malleus; I – incus, S – stapes; VII – facial nerve; * – cochleariform process; white arrow – protympanum; LA – lateral attic; AA – anterior attic*

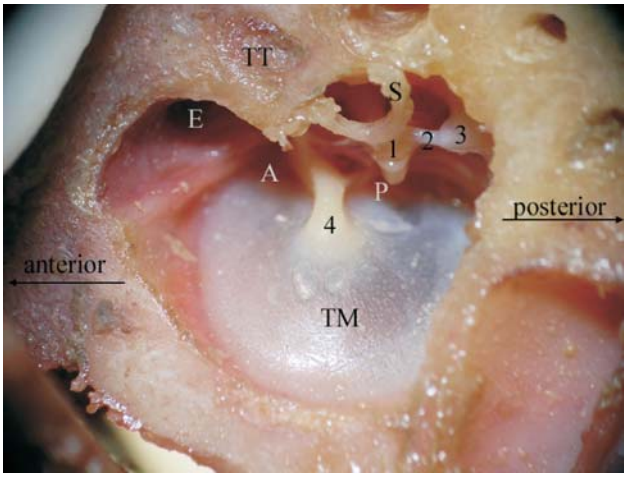


Figure 3 – *Right temporal bone (fresh specimen). Antero-posterior section, medial view of the tympanic membrane and mesotympanum: TM – tympanic membrane; E – Eustachian tube opening; TT – tensor tympani muscle; S – stapes; A – anterior pouch von Troeltsch; P – posterior pouch von Troeltsch; 1 – incudo-stapedial joint; 2 – pyramidal muscle; 3 – pyramidal eminence*

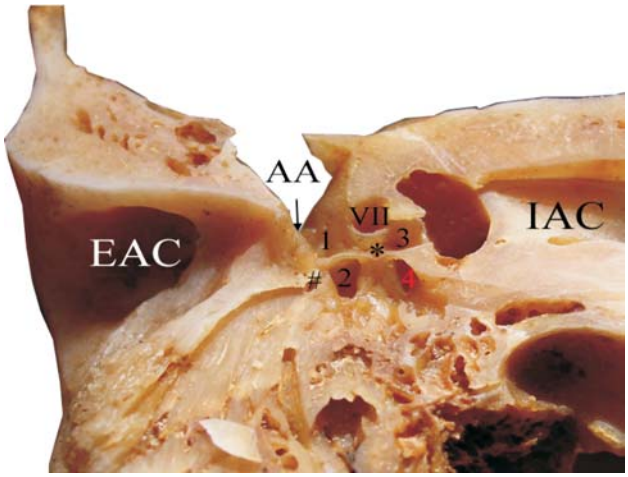


Figure 4 – *Right temporal bone-frontal section: EAC – external auditory canal; IAC – internal auditory canal. Posterior wall of the tympanic cavity: VII – facial nerve canal; # – chorda tympani canal; * – pyramidal eminence; black arrow – fossa incudis; 1 – facial sinus; 2 – lateral facial sinus; 3 – posterior lateral sinus; 4 – sinus tympani; AA – aditus ad antrum*

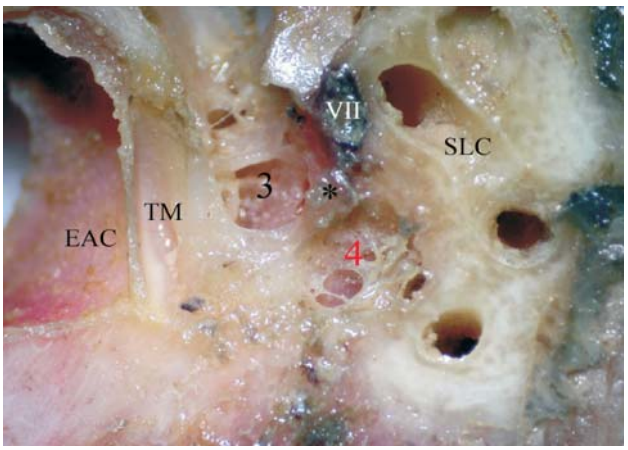


Figure 5 – *Right temporal bone (fresh specimen). Frontal section, posterior view: EAC – external auditory canal; TM – tympanic membrane; VII – facial nerve; SLC – semicircular lateral canal; * – pyramidal eminence; 4 – sinus tympani lined by mucosa; 3 – lateral facial sinus lined by mucosa*

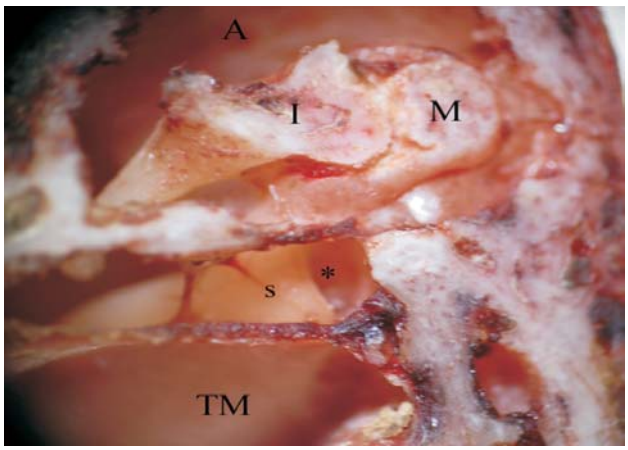


Figure 6 – *Right temporal bone (fresh specimen). Antero-posterior section, lateral view of the tympanic membrane and epitympanum: TM – lateral surface of the tympanic membrane; A – attic; M – malleus head; I – incus body; s – short (lateral) process of the malleus; * – Prussack's space*

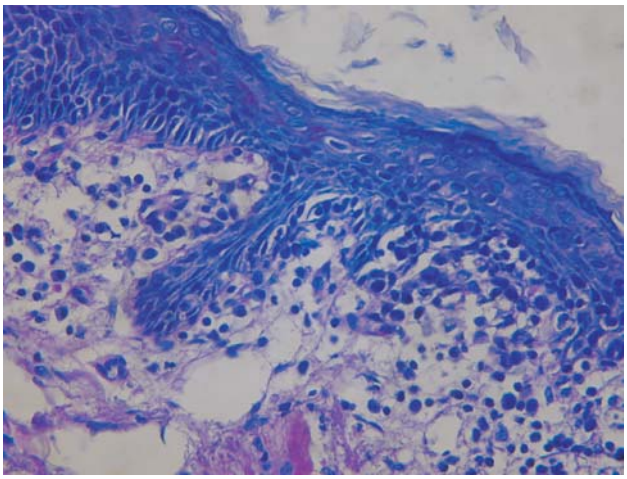
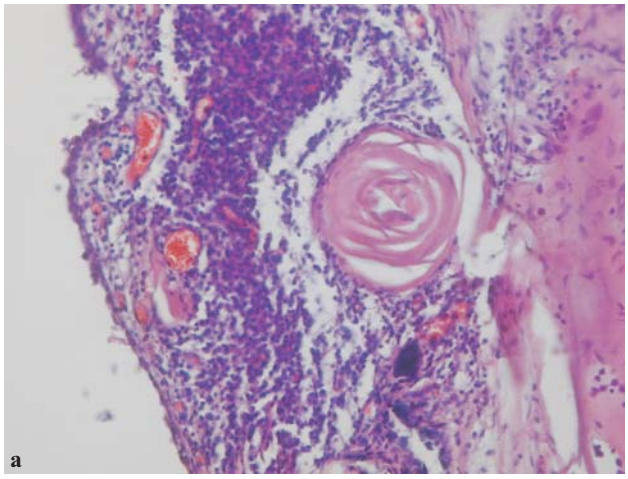


Figure 7 – ***Cholesteatoma. Keratinized squamos epithelium limited by a loose fibro–connective tissue infiltrated with many lymphocytes, monocites and macrophages (HE staining, ×20)***



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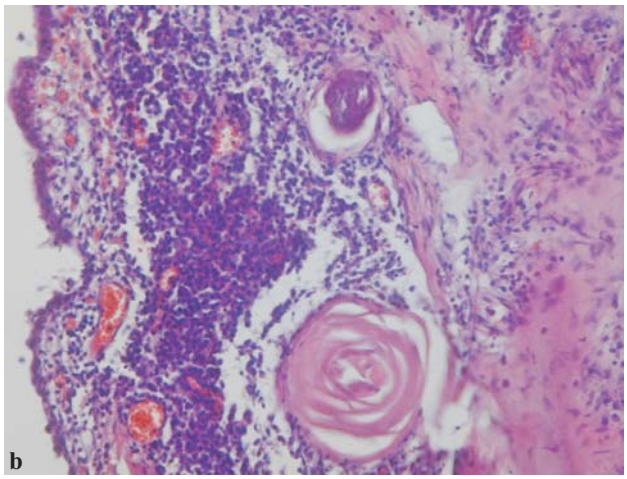

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Figure 8 a, b – ***Internal surface mucosa of the tympanic membrane. Cholesteatoma foci of different ages near manubrium mallei. Dense inflammatory infiltrate and hyperemic vessels (HE staining, ×10)***

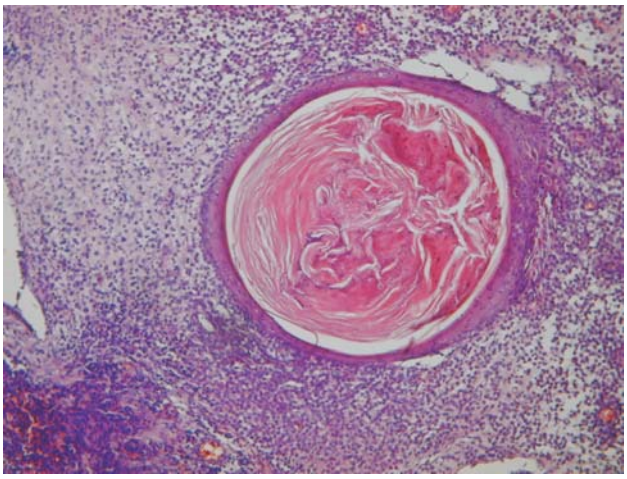

Figure 9 – ***Details of previous image. Perimatrix consisting of loose connective tissue with a rich diffuse chronic inflammatory infiltrate (HE staining, ×20)***

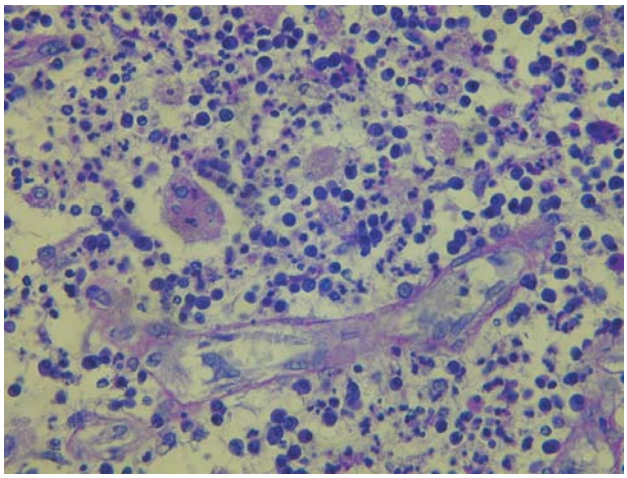

Figure 10 – ***Perimatrix: developing granulation tissue with neoformation vessels and an inflammatory infiltrate with many lymphocytes, plasmocytes, monocytes and macrophages (PAS staining, ×10)***

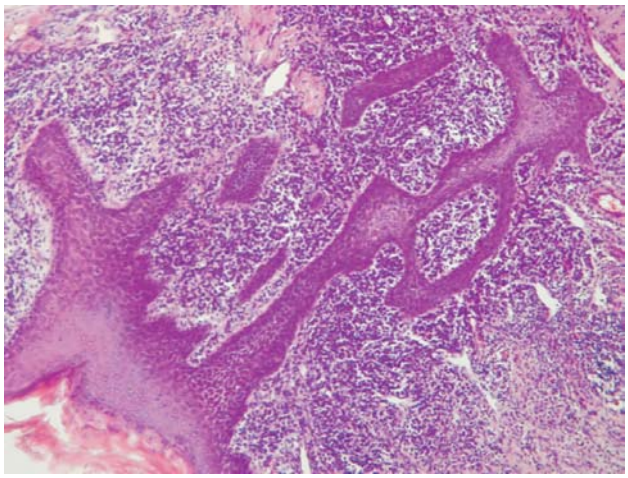

Figure 11 – ***Proliferated cholesteatoma. Islands and strips of squamos epithelium (matrix) penetrate deeply the perimatrix which consists of diffuse chronic inflammatory infiltrate during fibroblastic–fibrocytic remodelation. Small fragments of bone tissue in osteolysis (HE staining, ×10)***

Figures 7–11 present structural histological components of a cholesteatoma and its related structures. No data suggest that there are any obvious molecular or cellular differences among the various types of cholesteatomas (e.g., primary and secondary acquired, recidivistic, and congenital).

Conclusions

The exact mechanism or pathogenesis of cholesteatoma seems not to be identified; however, neither the aggressiveness of the disease nor the description of its key elements is yet clear.

Cholesteatoma appears as a benign lesion, having a dynamic proliferative pattern, with an associated locally distructive potential and entertaining an inflammatory reaction in the perimatrix; during its evolution, it will induce a diffuse fibrosis, affecting both the function of the tympanic membrane, and the ossicular chain within the middle ear.

In some cases, the size and the aggressive character of the lesion can mimic a malignant process. More than that, the pathological examination of tissue fragments could not always certify the diagnostic elements (the matrix/perimatrix interface could not be evaluated), thus leading to a false positive diagnosis of malignant tumor.

We consider that the first part of the study needs a further immunohistochemical assessment of the squamous differentiation pattern of the matrix (heavy cytokeratin vs. light cytokeratin ratio), and also of some epidermal growth factors and nuclear proliferation factors.

A further study concerning the assessment of the matrix/perimatrix interface will be made, by evaluating the expression of certain extracellular matrix elements (type IV collagen, laminin, fibronectin), and the phenotype of certain fibroblastic cells (miofibroblastic differentiation aspects).

A basic knowledge of the anatomy of the middle ear provides the fundament for understanding the disease progression and concepts for surgical management. Anterior epitympanic cholesteatoma may be easily overlooked during tympanomastoidectomy if not explored. The most common location for cholesteatoma persistence after chronic ear surgery is sinus tympani and the facial recess. Sinus tympani may be directly accessed via a posterior approach, through the mastoid (posterior tympanotomy or facial recess approach).

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