DERLEME / REVIEW **Kulak Burun Boğaz** / Otorhinolaryngology

An Overview to Cytokeratin Pattern of Cholesteatoma

Ahmet Koç, Emrah Emre

Acibadem University School of Medicine, Department of Otorhinolaryngology, Istanbul, Turkey

ABSTRACT

Cholesteatoma is a destructive pathology characterized with progressive and chronic infiltration of keratinized squamous epithelium that can be seen the middle ear and mastoid regions of temporal bone. It has serious intra and extracranial complications which can be difficult to manage. Embryologic remnants of squamous epithelium, squamous metaplasia of middle ear epithelium, migration of epidermal cells from external ear canal and tympanic membrane and cell seeding because of middle ear surgery or trauma were all stated as causes of cholesteatoma. Cytokeratins are important markers of cell differentiation. The patterns of cytokeratin expression correlate well with the state of keratinocyte proliferation, migration and differentiation. These patterns are known to be affected during the formation of cholesteatoma. Increasing our knowledge about cytokeratin patterns may help us understand the unknown formation of cholesteatoma. In this review article, a literature survey was done about cytokeratin patterns of cholesteatoma.

Key words: cholesteatoma, cytokeratin

KOLESTEATOMADA SİTOKERATİN PATERNİNE GENEL BİR BAKIŞ

Kolesteatoma, temporal kemiğin özellikle orta kulak ve mastoid bölgelerinde görülen, keratinize yassı epitelin ilerleyici ve kronik infiltrasyonu ile karakterize tahrip edici bir patolojidir. Tedavisi güç olabilen ciddi kafa içi ve kafa dışı komplikasyonları vardır. Kolesteatoma oluşumunda; yassı epitelin embryolojik kalıntıları, orta kulak epitelinin yassı epitel metaplazisi, dış kulak yolu ve kulak zarından epidermal hücrelerin göç etmesi ve orta kulak cerrahisi yada travma nedeniyle hücre ekimi gibi nedenler suçlanmaktadır. Sitokeratinler, hücre farklılaşmasında önemli belirleyicilerdir. Sitokeratin oluşum paternleri; keratinosit proliferasyonu, göç ve farklılaşma ile yüksek oranda ilişkilidir. Bu paternlerin, kolesteatoma oluşumu sırasında etkili olduğu bilinmektedir. Sitokeratin paternleri hakkında bilgilerimiz arttıkça, kolesteatoma oluşumu hakkındaki bilgilerimiz de artacaktır. Bu makalede, kolesteatomada sitokeratin paternleri hakkında bir literatür taraması yapılmıştır.

Anahtar sözcükler: kolesteatoma, sitokeratin

Cholesteatoma is a pathologic process with progressive and continuous invasion of keratinizing squamous epithelium in middle ear and mastoid part of temporal bone. Cholesteatoma was defined as a tumoral process by a French pathologist, Cruveilhier (1829). Cruveilhier was the first pointed out the pathological characteristics of cholesteatoma. Johannes Mueller, a German physiologist, defined the process as "cholesteatoma" because of the cholesterol in the center of the tumour which was a wrong meaning (1830). Mueller described cholesteatoma as a fat tumor composed of layers, and different from other fatty tumors with presence of bile salts and fatty acids (cholesterin) dispersed between cells. Gray suggested the term "skin in the wrong place" (1). Today, we know that cholesteatoma is a tumour of keratin and cholesterin crystals. Because of this, the term of keratoma seems even better.

Treatment of this inflammatory condition which has serious intracranial and extracranial complications makes difficulties for ear surgeons since many years (2-4). Marsupialization of the middle ear was the best treatment modality before antibiotic era. In this period, all operations were radical mastoid surgeries and the only goal was to save life.

In later years, some surgeons have tried to more conservative methods because of the morbidity of radical procedures (modified radical operation – Bryant 1906, radical conservative operation – Bondy 1910). After World War II, infections were taken to control with the introduction of antibiotics. With developing technological possibilities, tympanoplastic operations began to take place in surgical interventions. At these years again, self-containing mechanism of the outer ear canal and the mucous layer of middle ear were defined (5). In the next period, closed techniques (Jansen – posterior tympanotomy (6), Sheey et al – intact canal wall (7), Portmann – closed technique (8)) were popularized. Today, advantages and disadvantages of open and versus closed techniques are being discussed with notifying many surgical serials (9,10).

In the formation of cholesteatoma; embryological remnants of squamous epithelium (11), squamous metaplasia of middle ear epithelium (12), migration of epithelial cells from external auditory meatus or tympanic membrane through retraction, or through basal cell proliferation or through perforated tympanic membrane (13), sprinkled of epithelial cells by middle ear surgery or trauma (14) were all blamed.

Cholesteatoma can be studied under four headings:

1) external ear canal cholesteatoma,
2) iatrogenic cholesteatoma,
3) congenital cholesteatoma,
4) acquired cholesteatoma.

Acquired cholesteatoma is divided into two parts: primary acquired and secondary acquired. In primary acquired cholesteatoma, the Eustachian tube is open and the tympanic membrane is intact. Its formation is considered from expansion of external tympanic membrane epithelium to middle ear cavity, not remnants of embryological life. In secondary acquired cholesteatoma, cholesteatoma is formed by migration of epithelium to middle ear by way of marginal or attic perforation. Invagination, basal cell hyperplasia, otitis media with effusion and metaplasia are accused in primary acquired cholesteatoma, whereas implantation and epithelial invasion are accused in secondary acquired cholesteatoma and iatrogenic cholesteatoma. Congenital cholesteatoma can take origin from petrous apex, mastoid region, middle ear and external auditory canal (15).

The most appropriate description for cholesteatoma is, "skin in the wrong place with wrong healing process".

Cholesteatoma consist of keratinizing squamous epithelium with a sharp boundary across to middle ear mucosa. It has all the layers of skin with different thicknesses. Continuous renewal layer of the skin is the basal germinative layer. It produces new cells consistently. Skin cells are constantly renewed while dead cells are thrown out by desquamated lamella. Dead cells form the white lamella of cholesteatoma by accumulation. Basal germinative layer with surrounding connective tissue called *corion*, gets the name of "matrix" and creates the generative layer of cholesteatoma. Matrix is the part of cholesteatoma which in contact with middle ear walls. Continuous keratin production is the important characteristic of cholesteatoma. As a result; cholesteatoma is a pseudo-tumor with accumulation of desquamated epithelium and keratin comes from basal germinative epithelium and stratum (st) corneum (16,17).

Dead cells in the matrix are settled in layers on each other. This arrangement is lost away from matrix, because of this, the center of cholesteatoma has a amorphous character. As the infection progresses, heavy fat acids and cholesterin crystals which give the bad smell of cholesteatoma arise. In addition to this, granulation tissue and a papillary structure are observed around the matrix (16,17).

The epithelium of cholesteatoma is thinner than skin. It consists of four layers: st. germinativum, st.spinosum, st. granulosum and st. corneum. Keratin arises from the cells of st. corneum.

Why does the skin of external canal moving into middle ear and how do the epidermal cell hyperplasia and keratinization take place?. Answers of these questions are investigated with immunohistochemical studies. Skin of the external ear canal and cholesteatoma originate from the same cells. The differences are the presence of papillary proliferation reach out to the subepithelial layer and Langerhans cells. Their ratio is higher in cholesteatoma than canal skin. They are scattered at different levels in stratified squamous epithelium and infiltrated to the underlying subepithelial tissue. Langerhans cells which involved in st. spinosum are placed between keratinocytes. Smaller Merkel cells are present in st. germinativum. Bremond pointed out that these cells are not present in normal middle ear mucosa, he also added determining of these cells in the middle ear is the proof of the migration theory (16).

Kaneko et al (18) demonstrated keratin directly penetrated into destructed bone tissue because of fragmentation of matrix. According to Abramson et al (19), frequent occurrence of bone resorption is related to excessive production of keratin and epithelial debris. Creating foreign body reaction by keratin and epithelial debris was demonstrated in animal studies by Kaneko et al and Abramson et al. Keratin also makes an ideal environment for microorganisms. It provides high concentrations of microorganisms by blocking of drainage of inflammatory flow and also makes a low oxygenated ambient for evolving of anaerobic bacteria.

The basic proteins of the cytoskeleton are; microtubules, microfilaments and intermediate filaments. Intermediate filaments are divided into five groups according to specific appearances: vimentin, desmin, glial fibrin protein, neurofilaman proteins and cyrokeratins (20). Cytokeratins (Ck) are the most complex and diverse polypeptide groups between the intermediate filaments of human epithelial cells and are important determinants of all differentiation (21-23). Cytokeratins have been introduced as markers of cellular proliferation. They are insoluble, low-molecular – weight proteins. Special forms are found according to the type of epithelium (e.g. simple, stratified, transitional), the location of cell in the epithelium, stage of development of the epithelium and the relative stage of keratinocyte differentiation and tissue development (23-28). Biochemical studies and studies with monoclonal antibodies demonstrated 21 soft and 8 hard cytokeratins (20,21,29). Soft cytokeratins are divided into two main groups; group 1) relatively bigger and basic polypeptides, numbered from 1 to 8, group 2) relatively smaller and acidic polypeptides numbered from 9 to 21. Epithelium is defined as its cytokeratin pattern. Differences of acidic and basic cytokeratins are correlated with the diversity of epithelium. Studies have revealed that some of the general rules:

- filament formation needs polymerization of one acidic and one basic cytokeratin,
- cytokeratin 7,8,18 and 19 are located usually in simple epithelium,
- cytokeratin 5 and 14 are located in basal layers of stratified and complex epithelium,
- cytokeratin 1,2,10 and 11 are located in suprabasal layers of cornified epithelium,
- cytokeratin 4 and 13 are located in non-cornified epithelium,
- cytokeratin 6 and 16 are located in hyperproliferated epithelium,

- cytokeratin pattern of stratified epithelium may vary locally, for example differentiated cells of epidermis have cytokeratin 1 and 10,
- whereas non-keratinized oral mucosa has cytokeratin 4 and 13.

The pattern may change in malign transformation, in the cell cultures, in the wound healing and in the case of inflammation. In the pathogenesis of inflammatory diseases, cytokeratins express their role and act as messengers between cells. Cytokeratins are released by macrophages, lymphocytes and other cells at the site of infection and inflammation. Cytokeratin polypeptides can be demonstrated by immunohistochemical methods using with monoclonal antibodies.

The mechanism of region-specific occurrence of cytokeratins is not fully understood. During development, epithelial – mesenchymal relationships due to regional differences may be responsible for this.

In order to elucidate the pathogenesis of cholesteatoma, so many studies have been performed related to distribution pattern of cytokeratins (28,30-42). Cytokeratins can be used as proliferation, migration and differentiation markers. Studies on the appearance of cytokeratins in cholesteatoma revealed that cytokeratin pattern of cholesteatoma looks like external canal skin and points the hyperproliferative stage (31,35,36). Lee et al (43) used monoclonal antibodies for revealing and comparing the cytokeratin structure of normal canal skin and cholesteatoma. He determined that there was not any difference in staining patterns. Kakoi et al (20) examined cytokeratins of cholesteatoma and epithelial differentiations. They used cytokeratins of pharyngeal mucosa as control group and determined cytokeratin 1,5,10 and 14 in cholesteatoma. Cytokeratin 5 and 14 were synthesized in basal lamina cells and are used as squamous epithelial marker. Otherwise cytokeratin 1-10 synthesized in suprabasal layer cells and they were the markers for keratinization in squamous epithelium or differentiation (44). Sasaki et al (45) studied cytokeratin 6 which was observed in epidermal hyperproliferative stage in epidermis of cholesteatoma and cytokeratin 13 which was observed in active cell proliferation in epidermal cells. In this immunohistochemical study, cytokeratin 16 was showed in suprabasal layers of cholesteatoma, canal skin and tympanic membrane. These results are similar to the results in active psoriatic lesions (46). According to Sasaki et al (45), these results suggest that cholesteatoma, canal skin and tympanic membrane are all in hyperproliferative stage. Ergun

et al (47) also observed the hyperproliferative nature of cholesteatoma. This study indicated the expression pattern of epithelial markers in cholesteatoma corresponds to that in normal epidermis. Some think that cytokeratin distribution of cholesteatoma changes in different stages of the disease (17,40), as well as others who claim the opposite (42).

It is thought that epidermal cytokeratin pattern of cholesteatoma points the epithelial migration, in addition non-epidermal cytokeratin points the hyperproliferative or ectopic metaplasia (35). There are various ultrastructural (28,36,39), immunhistochemical (23,31,33,35-38,40,42,48,49) and clinic studies support that epidermal migration is the most common cause of cholesteatoma. When st. corneum layer of cholesteatoma is examined with electron microscopy, the findings are similar to the findings of deep part of external ear canal and pars flaccida part of tympanic membrane. This finding supports the hypothesis that cholesteatoma is formed from epithelial migration of tympanic membrane and external ear canal (28). Immunohistochemical studies of cytokeratins indicate that cholesteatoma arises from keratinized epithelium of tympanic membrane and canal (32,33,35,42). In some studies, it was shown that, in the region where normal epithelium meets cholesteatoma, there were active keratinocytes indicating proliferation and migration (40,41). Nevertheless, normal squamous epithelium has not any migration capability to middle ear (30). For the migration of squamous epithelium, a formation like inflammation, granulation tissue or organized effusion is required acting as a bridge (41). Olszewska et al (50) studied with Ck 10-14-18-19-34 in congenital cholesteatoma epithelium, pediatric acquired cholesteatoma epithelium, middle ear mucosa, glandular epithelium and external ear canal (skin) epithelium. Olszewska et al demonstrated an identical expression pattern of Ck 10-14-18-19 and 34 antigens in the matrix of congenital and acquired pediatric cholesteatoma. According to Olszewska et al, this finding supports the hypothesis that both types of cholesteatoma have an epidermal origin. In addition to this, in this study, the matrix of acquired cholesteatoma shows the same characteristics as the external matrix suggesting the theory that cholesteatoma has an epidermal origin. Lepercque et al (48) observed a similar cytokeratin pattern in middle ear cholesteatoma, annular region of the external meatus and the lateral side of eardrum. With these findings Lepercque et al stated that cholesteatoma originates from external ear canal or the lateral side of the tympanic membrane.

Epithelial cell proliferation is another mechanism proposed in the pathogenesis of cholesteatoma. Some studies have defined the hyperproliferated behavior of cholesteatoma epithelium according to the expression of Ck 4,10,13,14,16 and 19 (37,51-53). This theory was supported by experimental animal (23,36) and human (35,38,49) studies. In their experimental study which was conducted with cytokeratins, Vennix et al (40) argued that cholesteatoma has an epidermal origin. They advocated that non-epidermal cytokeratins like Ck 4, Ck 13 and Ck 16 indicate hyperproliferation rather than metaplasia. They also added that infection cause proliferation of canal epidermis which transplanted in middle ear, and this finding supported the progression of cholesteatoma was connected to inflammatory process (23). Increased appearance of Ck 13 and 16 which are the markers of hyperproliferative keratinocytes has been shown in cholesteatoma (38,46,49,54). In immunohistochemical studies, there are remarkable differences between the intensity and localization of certain cytokeratins. These changes may be related to an altered process of differentiation. Olszewska et al observed Ck 10 in all suprabasal cell layers of the meatal skin and congenital cholesteatoma. Ck 10 is known as a keratinization marker and corresponds the extent of differentiation within keratinocytes. While Olszewska et al (50) considered that Ck 10 expression was affected by the extent of cholesteatoma like Kim et al (53), others demonstrated a decrease of Ck 10 expression in cholesteatoma (37,55,59). Liang et al (52) observed the expression of Ck 10 in the granular and spinous layers of the external ear canal epidermis and superficial layer of epidermoid formation. While Liang et al didn't observe positive immunohistochemical reaction with staining for Ck 19 within basal and suprabasal keratinocytes in the epidermis or in the cholesteatoma epithelium, Olszewska et al observed definitely positive reactions with Ck 19 antibodies within the middle ear mucosa around the glands. Results of immunohistochemical staining reactions for cytokeratins at various epithelial cites and cholesteatoma in different studies were summarized in Table 1. Stainig epithelial sites are meatal skin, cartilage and bone of external ear canal, medial and lateral sides of tympanic membrane, middle ear mucosa and glandular epithelium. Cytokeratin 4,5,7,8,10,13,14,16,18,19 and 34bE12 were studied. In cholesteatoma, all of these were observed in almost all studies. In epithelial sites, a dispersion was observed.

Studies suggest that increasing number of researches about cytokeratins will also increase our understanding of pathogenesis of cholesteatoma. Progress about this subject will support surgical and medical treatments.

Table 1. Results of immunohistochemical staining reactions for cytokeratins at various epithelial cites and cholesteatoma in different studies.

	Ck 4	Ck 5	Ck 7	Ck 8	Ck 10	Ck 13	Ck 14	Ck 16	Ck 18	Ck 19	Ck34bE12
Meatal skin	+ (53,58) -(35,59)	+ (35,59)	- (35,59)	- (35,59)	+ (35,47,50,53,58,59)	+ (53) -(35,59)	+ (35,47,59) -(50,58)	± (35,59)	- (35,50,58,59)	- (35,50,58,59)	+ (50,58)
External ear canal (cartilage)	-(48)	+(48)	-(48)	- (48)	+(48)	-(48)	+(48)	-(48)	-(48)	-(48)	
External ear canal (bone)	-(48)	+(48)	-(48)	-(48)	+(48)	-(48)	+(48)	+(48)	-(48)	+(48)	
Tympanic membrane	+(53)	+(53)			+(47,53)	+(53)	+(47)				
Tympanic membrane (lateral side)	-(48)	+(48)	-(48)	-(48)	+(48)	-(48)	+(48)	+(48)	-(48)	+(48)	
Tympanic membrane (medial side)	+(48)	-(48)	+(48)	+(48)	-(48)	+(48)	+(48)	-(48)	+(48)	+(48)	
Middle ear mucosa	+ (58) - (35,53,59)	+ (35,59) - (53)	- (35,59)	- (35,59)	+ (47,53,58) - (35,50,59)	- (35,53,59)	+ (35,47,59) -(50) ±(58)	+ (35,59)	- (35,50,58,59)	+ (50,58) - (35,59)	- (50,58)
Cholesteatoma	+ (58) ± (35,59)	+ (35,59)	± (35,59)	± (35,59)	+ (35,47,50,58,59)	± (35,59)	+ (35,47,50,59) ± (58)	+ (35,59)	± (35,59) - (50,58)	± (35,59) - (50,58)	+ (50,58)
Middle ear glandular epithelium	-(58)				-(50,58)		-(50,58)		-(50,58)	+(50,58)	-(50,58)

References

1. Gray D. The treatment of cholesteatoma in children. Proceedings of the Royal Society of Medicine, London, 1964;57: 769-771

2. Osma C, Cureoglu S, Hosoglu S. The complications of chronic otitis media: report of 93 cases. J Laryngol Otol 2000;114:97-100

3. Sennaroglu L, Sozeri B. Otogenic brain abscess: review of 41 cases. Otolaryngol Head Neck Surg 2000;123:751-755

4. Yetiser S, Tosun F, Kazkayasi M. Facial nevre paralysis due to chronic otitis media. Otol Neurotol 2002;23:580-588

5. Sade J. Middle ear mucosa. Arch Otolaryngol 1966;84:137

6. Jansen C. Posterior tympanotomy: access to the middle ear with preservation of the external ear canal. Arch Klin Exp Ohren Kehlkopfheilkd 1967;188:558-9

7. Sheehy JL, Patterson MR. Intact canal wall tympanoplasty with mastoidectomy. A review of eight years' experience. Laryngoscope 1967;77:1502-1542

8. Portmann M. Open or closed technique in surgery of the middle ear. Ann Otol Rhinol Laryngol 1968;77:927-937

9. Inanlı S, Ozturk O, Batman C, Tutkun A, Uneri C, Sehitoglu MA. Clinical importance of supratubal recess in cholesteatoma surgery. Otol Neurotol 2001;22:754-760

10. Gocmen H, Kılıc R, Ozdek A, Kızılkaya Z, Safak MA; Samim E. Surgical treatment of cholesteatoma in children. Int J Pediatr Otorhinolaryngol 2003;67:867-872

11. Derlacki E, Clemis JD. Congenital cholesteatoma of the middle ear and mastoid. Ann Otol Rhinol Laryngol 1965;74:706-725

12. Sade J, Babiacki A, Pinkus G. The metaplastic and congenital origin of cholesteatoma. Acta Otolaryngol 1983;96:119-129

13. Wullstein HL, Wullstein S. Cholesteatoma, etiology, nosology and tympanoplasty. ORL J Otorhinolaryngol Relat Spec 1980;42:313-335

14. Von Haacke NP, Wilson JA, Murray JA, Duvall E. Cholesteatoma following stapedectomy. J Laryngol Otol 1987;101:708-710

15. Cureoglu A, Osma U, Oktay MF, Nazaroglu H, Meric F, Topcu I. Congenital cholesteatoma of the mastoid region. J Laryngol Otol 2000;114:779-780

16. Bremond G, Magnan J, Acquaviva F. Cholesteatome et metaplasie epidermoide. Acta Otorhinolaryngol Belg 1980;34:34-42

17. Anniko M, Mendel L. Cholesteatoma: a clinical and morphological analysis. Acta Otolaryngol 1981;91:275-283

18. Kaneko Y, Yuasa R, Ise I, Iino Y, Shinkawa H, Rokugo M, Tomioka S, Shibahara Y. Bone destruction due to the rupture of a cholesteatoma sac: a pathogenesis of bone destruction in aural cholesteatoma. Laryngoscope 1980;90:1865-1871

19. Abramson M, Asarch RG, Litton WB. Experimental cholesteatoma causing bone resorption. Ann Otol Rhinol Laryngol 1975;84:425-432

20. Kakoi H, Kitamura K, Ishida T, Kitajima Y, Hiraide F. Characterization of cytokeratins in cholesteatoma. In: Cholesteatoma and Mastoid Surgery, Proceedings of the Fourth International Conference, Nakano Y (ed), Kugler Publications, Amsterdam / New York, 1993:pp 151-54

21. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalogue of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 1982;31:11-24

22. Sun TT, Tseng SCG, Huang AHW, Cooper D, Schermer A, Lynch MH, Weiss R, Eichner R. Monoclonal antibody studies of mammalian epithelial keratins: a review. Ann NY Acad Sci 1985;455:307-329

23. Vennix PP, Kuijpers W, Peters TA, Tonnaer E, Ramaekers F. Growth and differentiation of meatal skin grafts in the middle ear of the rat. Arch Otolaryngol Head Neck Surg 1994;120:1102-1111

24. Ruedi L. Pathogenesis and surgical treatment of the middle ear cholesteatoma. Acta Otolaryngol (Stockh) Suppl 1979;361:1-45

25. Tseng SCG, Jarvinen MG, Nelson WG, Huang JW, Woodcock-Mitchell J, Sun TT. Correlation of spesific keratins with different types of epithelial differentiation: monoclonal antibody studies. Cell 1982;30:361-372

26. Sun TT, Eichner R, Nelson WG, Tsengf SCG, Weiss RA, Jarvinen M, Woodcock-Mitchell J. Keratin classes: molecular markers for different types of epithelial differentiation. J Invest Dermatol 1983;81:109-115

27. Eichner R, Sun TT, Aebi U. The role of keratin subfamilies and keratin pairs in the formation of human epidermal intermediate filaments. J Cell Biol 1986;102:1767-1777

28. Young R, Rowles P. The spatial organization of keratinocytes in acquired middle ear cholesteatoma resembles that of external auditory canal skin and pars flaccida. Acta Otolaryngol 1990;110:115-119

29. Moll R, Schiller DL, Franke WW. Identification of protein IT of the intestinal cytoskeleton as a novel type I cytokeratin with unusual properties and expression patterns. J Cell Biol 1990;111:567-580

30. O'Donoghue GM. The migration theory of cholesteatoma formation: some experimental observations. J Laryngol Otol 1986;100:395-398

31. Broekaert D, Cornille A, Eto E, Leigh I, Ramaekers F, Van Muijen G, Coucke P, De Bersaques J, Kluyskens P, Gillis E. A comparative immunohistochemical analysis of the cytokeratin and vimentin expression in middle ear mucosa and cholesteatoma and in epidermis. Virchows Arch Pathol Anat 1988;413:39-51

32. Huang CC, Shi GS, Yi ZX. Experimental induction of middle ear cholesteatoma in rats. Am J Otolaryngol 1988;9:165-172

33. Chao WY, Huang CC. An immunocytochemical study of cytokeratin expression in human middle ear cholesteatoma. Arch Otorhinolaryngol 1989;246:37-42

34. Masaki M, Wright CG, Meyerhoff WL. Experimental cholesteatoma: epidermal ingrowth through tympanic membrane following middle ear application of propylene glycol. Acta Otolaryngol 1989;108:113-121

35. Van Blitterswijk CA, Grote JJ, Lutgert RW, Hesseling SC, Out CJ, Van Muijen GN, Fransen JA. Cytokeratin patterns of tissues related to cholesteatoma pathogenesis. Ann Otol Rhinol Laryngol 1989;98:635-640

36. Vennix PP, Kuijpers W, Tonnaer E, Peters TA, Ramaekers FC. Cytokeratins in induced epidermoid formation and cholesteatoma lesions. Arch Otolaryngol Head Neck Surg 1990;116:560-565

37. Broekaert D, Coucke P, Leperque S, Ramaekers F, Van Muijen G, Boedts D, Leigh I, Lane B. Immunohistochemical analysis of the cytokeratin expression in middle ear cholesteatoma and related epithelial tissues. Ann Otol Rhinol Laryngol 1992;101:931-938

38. Buija J, schilling V, Holly A, Stammberger M, Kastanbauer E. Hyperproliferation associated keratin expression in human middle ear cholesteatoma. Acta Otolaryngol 1993;113:364-368

39. Sudhoff H, Bujia J, Borkowshi G, Koc C, Holly A, Hildmann H, Fisseler-Eckhoff A. Basement membrane in middle ear cholesteatoma: immunohistochemical and ultrastructural observations. Ann Otol Rhinol Laryngol 1996;105:804-810

40. Vennix PP, Kuijpers W, Peters TA, Tonnaer E, Ramaekers F. Keratinocyte differentiation in acquired cholesteatoma and perforated tympanic membranes. Arch Otolaryngol Head Neck Surg 1996;122:825-832

41. Goycoolea MV, Hueb MM, Muchow D, Paparella MM. The theory of the trigger, the bridge and the transmigration in the pathogenesis of acquired cholesteatoma. Acta Otolaryngol 1999;119:244-248

42. Kim CS, Chung JW. Morphologic and biologic changes of experimentally induced cholesteatoma in Mongolian gerbils with anticytokeratin and lectin study. Am J Otol 1999;20:13-18

43. Lee RJ, Sidey CM, Narula AA, McKenzie IC, James RFL. Keratin patterns in aural epithelia and cholesteatoma cell culture. In Cholesteatoma and Mastoid Surgery, Proceedings of the Fourth International Conference, Nakano Y (ed), Kugler Publications, Amsterdam / New York, 1993;pp 167-171

44. Woodcock-Mitchell J, Eichner R, Nelson WG, Sun TT. Immunolocalization of keratin polypeptides in human epidermis using monoclonal antibodies. J Cell Biol 1982;95:580-588

45. Sasaki H, Huang CC. Cytokeratin expression in cholesteatoma, meatal skin and tympanic membrane. In: Cholesteatoma and Mastoid Surgery, Proceedings of the Fourth International Conference, Nakano Y (ed), Kugler Publications, Amsterdam / New York, 1993;pp 143-150

46. Mare DS, Jong DE, Van Erp PEJ, Van de Kerkhof PCM. Markers for proliferation and keratinization in the magrin of the active psoriatic lesion. Br J Dermatol 1990;122:469-475

47. Ergun S, Zheng X, Carlsöö B. Antigen expression of epithelial markers, collagen IV and Ki67 in middle ear cholesteatoma. An immunohistochemical study. Acta Otolaryngol 1994;114,295-302

48. Lepercque S, Broekaert D, Van Cauwenberge P. Cytokeratin expression patterns in the human tympanic membrane and external ear canal. Eur Arch Otorhinolaryngol 1993;250,78-81

49. Sasaki H, Huang CC. Expression of cytokeratins 13 and 16 in middle ear cholesteatoma. Otolaryngol Head Neck Surg 1994;110:310-317

50. Olszewska E, Lautermann J, Koc C, Schwaab M, Dazert S, Hildmann H, Sudhoff H. Cytokeratin expression pattern in congenital and acquired pediatric cholesteatoma. Eur Arch Otorhinolaryngol 2005;262:731-736

51. Rosenfeld RM, Moura RL, Bluestone CD. Predictors of residual – recurrent cholesteatoma in children. Arch Otolaryngol Head Neck Surg 1992;118:384-439

52. Liang JN, Chrobok V, Michaels L, Wright A. Incidence and cytokeratin analysis of the epidermal formation. Registry 2000;8:1-4

53. Kim HJ, Tinling SP, Chole RA. Expression patterns of cytokeratins in cholesteatomas: evidence of increased migration and proliferation. J Korean Med Sci 2002;17:381-388

54. Weiss RA, Eichner R, Sun T. Monoclonal antibody analysis of keratin expression in epidermal disease: a 48- and 56-k Dalton keratin as molecular markers for hyperproliferative keratinocytes. J Cell Biol 1984;98:1397-1406

55. Weiss RA, Guillet GYA, Freedberg IM, Farmer ER, Small EA, Weiss MM, Sun TT. The use of monoclonal antibody to keratin in human epidermal disease: alterations in immunohistochemical staining pattern. J Invest Dermatol 1983;81:224-230

56. Shinkawa H, Hozawa J, Usami S. Immunohistochemical study on CK and vimentin expression in congenital cholesteatoma. In: Nakano Y (ed). Cholesteatoma and Mastoid Surgery. Kugler, Amsterdam, 1993;pp 155-166

57. Kim HJ, Tinling SP, Chole RA. Expression patterns of cytokeratins in retraction pocket cholesteatoma. Laryngoscope 2001;111:1032-1036

58. Olsewska E, Sudhoff H. Comparative cytokeratin distribution patterns in cholesteatoma epithelium. Histol Histopathol 2007;22:37-42

59. Lifton WB. Epidermal migration in the ear: the location and characteristics of the generation center revealed by utilizing a radio-active desoxyribose nucleic acid precursor. Acta Otolaryngol (Stockh) (Suppl) 1968;240:-39