Morphological Changes in the Temporal Bone and the Expression of Fas Ligand in Patients with Cholesteatoma

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Objectives. The unique feature of cholesteatoma is the hyperproliferation and accumulation of keratin debris within the middle ear and mastoid cavity, a process that leads to destruction of the surrounding structures in the temporal bone. The proliferation of keratin and the resulting bony destruction in the cholesteatoma matrix are associated with apoptosis. FasL, when conjugated with Fas, is known to trigger apoptosis. This study aimed to analyze the temporal bone patterns in patients with cholesteatoma and the expression of FasL in the cholesteatoma matrix.

Methods. From July 1999 to July 2001, all patients with cholesteatoma who received ear operations at the China Medical University Hospital were enrolled in this study. Surgical specimens from all patients were shown histopathologically to have cholesteatoma. The temporal bone patterns in these patients were reviewed by high resolution temporal bone computed tomography. Immunoperoxidase stain with a monoclonal antibody to FasL evaluated the expression of FasL in the cholesteatoma matrix. Postauricular skin, which was harvested during the same surgical procedure served as the control.

Results. The temporal bone patterns in the patients were classified as blunted scutum (58%), ossicular chain erosion (58%), erosion of tegmen mastoid-tympanicum (30%), erosion of the otic capsule (25%) and marked sclerosis (50%). Expression of FasL was not detected in the postauricular skin, cholesteatoma matrix or subepidermal granulation tissue.

Conclusions. The temporal bone patterns in the patients with cholesteatoma included bony destruction and new bone formation. No expression of FasL was detected in the cholesteatoma matrix or subepidermal granulation tissue. This indicates that apoptosis in the cholesteatoma matrix may not be through the FasL pathway. Therefore, the destruction of the temporal bone in patients with cholesteatoma may not be associated with FasL. **(Mid Taiwan J Med 2004;9:197-202)**

Key words

apoptosis, cholesteatoma, Fas ligand, high resolution temporal bone computed tomography

INTRODUCTION

The unique feature of cholesteatoma is the hyperproliferatiation of keratinizing squamous epithelium. Excess keratin debris accumulates in the middle ear and mastoid cavity, resulting in the destruction of surrounding structures in the temporal bone [1]. The bony changes associated with cholesteatoma are primarily caused by the

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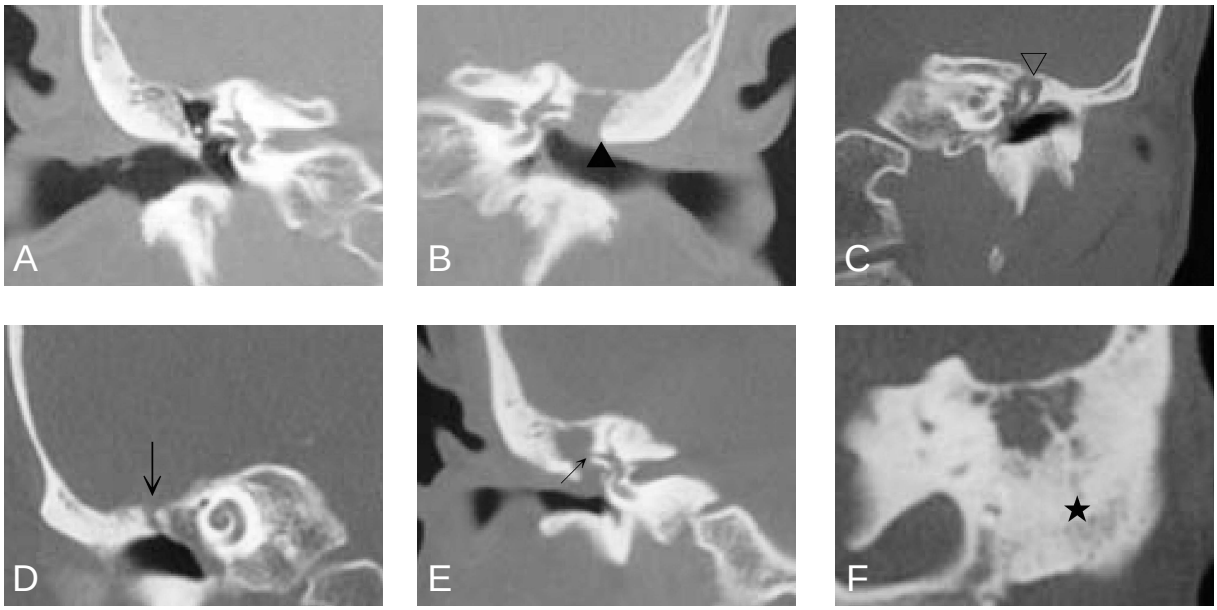

Fig. 1. The temporal bone patterns in patients with cholesteatoma by high resolution temporal bone computed tomography. A: Normal middle ear and mastoid cavity, the sharp-angled scutum, intact tegmen mastoid-tympanicum and otic capsule. B: The blunted scutum (▲). C: Erosion of ossicular chain (▽). D: Erosion of tegmen mastoid-tympanicum (↓). E: Erosion of otic capsule (the lateral semicircular canal) (↗). F: Marked sclerotic change of mastoid (★).

overproduction of keratin and cellular death of the cholesteatoma epithelium [1,2]. The pattern of differentiation and proliferation in cholesteatoma epithelium is similar to the terminal differentiation of programmed cellular death in the squamous epithelium, and unlike the uncontrolled pattern of cellular proliferation in neoplasm [3,4]. Fas ligand (FasL) is an important modulator in the process of apoptosis. Conjugated with Fas, FasL is known to trigger apoptosis [5].

Few reports that describe the morphologic changes of the temporal bone in cholesteatoma have been published. High resolution temporal bone computed tomography (HRTCT) should be able to precisely demonstrate the extent of the bone erosion associated with cholesteatoma [6]. The purposes of this study were to analyze the temporal bone patterns in patients with cholesteatoma and the expression of FasL in the cholesteatoma matrix.

MATERIALS AND METHODS

We retrospectively reviewed all patients with cholesteatoma who received ear operations at the China Medical University Hospital from July 1999 to July 2001. Excluding the recidivistic cases, the temporal bone patterns of the patients with cholesteatoma were reviewed and analyzed by HRTCT. The specimens taken during surgery were confirmed to be cholesteatoma by traditional hematoxylin & eosin (H & E) staining.

The immunohistochemical stain with monoclonal antibody to FasL evaluated the expression of FasL in the cholesteatoma matrix. This immunohistochemical staining was performed by an immunoperoxidase method as previously described [7]. After deactivation with 3% H_2O_2, the slide preparations were infiltrated with FasL monoclonal antibodies (Transduction Laboratories, Lexington, KY). The chromogen was aminoethyl carbazole (AEC). The slide was counterstained with hematoxylin. The presence of crimson granules was proof of FasL monoclonal antibodies. Postauricular skin taken during surgery from the same postauricular wound served as the control.

RESULTS

A total of 56 patients with cholesteatoma received ear surgery from July 1999 to July 2001 at our institution. The recidivistic cases and the cases without preoperative HRTCT were

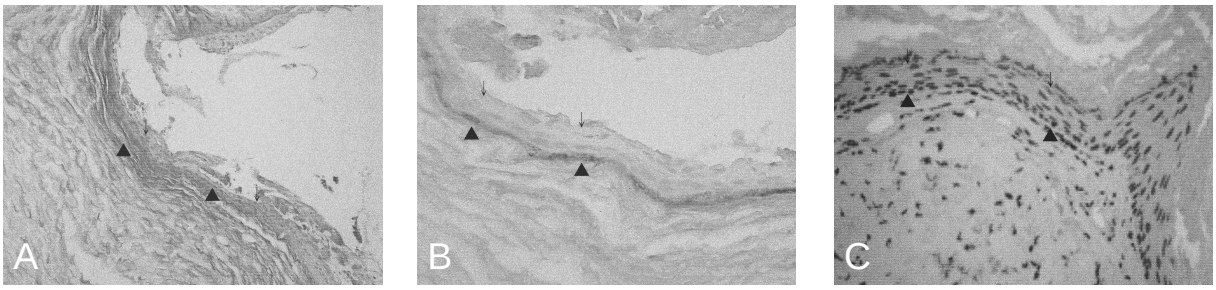

Fig. 2. Microscopic view of the epithelium of the cholesteatoma matrix and postauricular skin, from the basal layer (↓) to the granular layer (▲). A: The epithelium of the cholesteatoma matrix and the accumulation of keratin layer by layer (H&E stain; 100 ×). B: The negative expression of FasL in the epithelium of the cholesteatoma matrix (FasL; 100 ×). C: The negative expression of FasL in the control group, the postauricular skin (FasL; 100 ×).

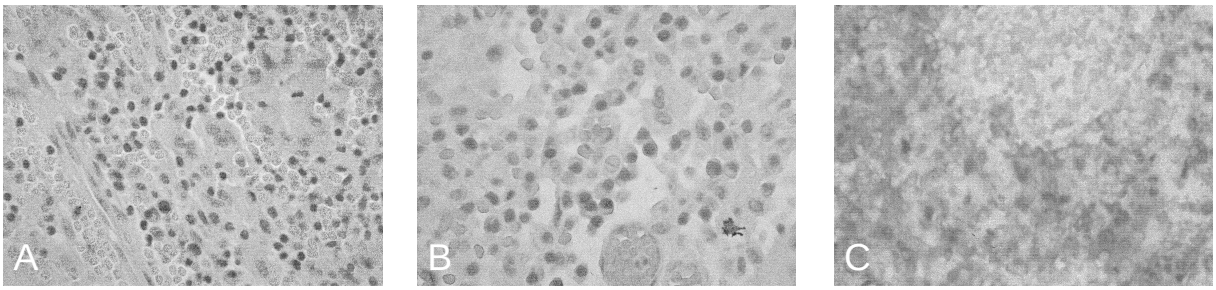

Fig. 3. FasL was not expressed in subepithelial cholesteatoma tissue. A: Inflammation is noted in the subepithelial granulation tissue, including lymphocyte infiltration and neovascularization (H & E stain; 400 ×). B: FasL is not expressed in the subepithelial granulation tissue of the cholesteatoma (FasL; 400 ×). C: Positive expression of FasL in the tumor nests from a nasopharyngeal cancer patient with skull base bony invasion (FasL; 200 ×).

Table. Temporal bone patterns in patients with cholesteatoma by high resolution temporal bone computed tomography (N = 40 ears)

Temporal bone patterns	n (%)
Blunted scutum	23 (58.0)
Erosion of ossicular chain	23 (58.0)
Erosion of tegmen mastoid-tympanicum	12 (30.0)
Erosion of otic capsule	10 (25.0)
Marked sclerosis of temporal bone	20 (50.0)

excluded; therefore, a total of 40 patients were enrolled in this study (18 males, 22 females; age range, 9 to 67 years; mean, 43 years). There were 21 right ears and 19 left ears.

The temporal bone patterns in these diseased ears were retrospectively analyzed (Table). Blunted scutum was found in 23 ears (58%), ossicular chain erosion was found in 23 ears (58%), erosion over tegmen mastoid-tympanicum was found in 12 ears (30%), erosion of otic capsule was found in 10 ears (25%), and sclerosis of temporal bone was found in 20 ears (50%) (Figs. 1A-1F).

Expression of FasL was evaluated by the immunoperoxidase method. No expression of FasL was detected in postauricular skin (control), epithelium of cholesteatoma matrix (Fig. 2) or subepithelial granulation tissue (Fig .3) from patients with cholesteatoma.

DISCUSSION

Cholesteatomas are generally classified into congenital and acquired types. The four basic theories for the pathogenesis of acquired cholesteatoma include invagination of pars flaccida, basal cell hyperplasia, migration through the perforated eardrum, and squamous metaplasia of middle ear epithelium [1].

Bony destruction of the middle ear and the otic capsule are common intraoperative findings in patients with cholesteatoma. Destruction of the ossicular chain, in particular erosion of the incus, has been found in 80% of patients while labyrinthine fistula, mostly in the lateral semicircular canal, has been found in 10% of patients with cholesteatoma [8]. HRTCT can

precisely evaluate the bony changes that occur in cholesteatoma [6]. In this study, we were able to classify the morphologic changes of the temporal bones as bony destruction and bony sclerosis. Bony destruction was most common in the scutum and the ossicular chains. One third of the patients had erosion of the tegmen and a quarter of them had erosion of the otic capsule. Marked bony sclerosis was found in half of the patients; this may have been caused by the formation of new bone following bony destruction.

Some researchers have proposed that bony destruction is caused by the mechanical factors of cholesteatoma. For example, the overproduction and accumulation of the keratin debris in the middle ear and mastoid cavity increase the pressure in the middle ear and mastoid cavity, which diminishes the blood supply to the middle ear and mastoid cavity and causes bone resorption [9]. Some humoral factors have been suggested to play an important role in the pathogenesis of bony destruction in cholesteatoma, such as endotoxins produced by bacteria [10], epithelial growth factors and their receptors [11,12] and tumor necrosis factor α [13] secreted by the epithelial cells in cholesteatoma. Subepithelial inflammation has frequently been found in patients with cholesteatoma. These inflammatory cells might produce protease, acid phosphatase, osteoclast activating factors and collagenase, which could resorb the surrounding bones [14].

Although hyperproliferation is the key characteristic of cholesteatoma, the proliferation of the cholesteatoma epithelium is less like the uncontrolled spread of cancer cells and more like the programmed cellular death and the terminal differentiation of epithelial cells [3,4]. Apoptosis is regulated by induction and inhibitory processes. FasL, Fas and TNFα induce the activation of apoptosis but the bcl-2 family will inhibit the process of apoptosis [15].

FasL is structurally like the type II transmembranous protein of TNFα with a molecular weight of about 42kDa. It produces lymphocyte-associated cytotoxicity by activating T lymphocytes, especially $CD8^+$ and Th1 $CD4^+$ T lymphocytes [5]. When FasL conjugates with Fas, the FasL-Fas complex activates the Fas associated death domain protein (FADD) and capsize. This activation induces sequential changes which leads to apoptosis [5,15,16]. Previous studies have shown that the c-jun [17], p53 protein [17,18], Bcl-xL protein [19] and Fas/APO-1 [20] are involved in the apoptotic process in cholesteatoma. In this study, no expression of FasL was found in the epithelial cells or the subepithelial inflammatory cells from cholesteatoma specimens.

A few studies have reported an association between FasL and bony destruction. Fas was expressed in human osteoblasts [21,22] and the interaction between Fas and FasL was shown to induce apoptosis in osteoblasts [5,16]. The expression of FasL in some tumors with bony destruction, such as multiple myeloma, correlated with the course of multiple myeloma [23]. FasL was found in patients with nasopharyngeal carcinoma and marked skull base invasion (Fig. 3C) [24] and in neoplastic cells in patients with breast cancer and bony metastasis [25]. Furthermore, FasL may induce apoptosis and inactivation of osteoblasts, resulting in bony resorption [21-23]. In this study, however, FasL was not expressed in cholesteatoma. Therefore, the regulation of apoptosis in cholesteatoma epithelium may not be regulated by the FasL/Fas pathway. Other apoptotic pathways such as the bcl-2 may be responsible [15].

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REFERENCES

1. Chole RA, Choo MJ. Chronic otitis media with cholesteatoma. In: Cummings CW, Harker LA, eds. Otolaryngology Head and Neck Surgery, 3rd ed. St Louis, Mosby, 1998:3026-34.
2. Canalis RF, Lambert PR. Chronic otitis media and cholesteatoma. In: Canalis RF, Lambert PR, eds. The Ear–Comprehensive Otology. Philadelphia: Lippincott Williams & Wilkins, 2000:409-31.
3. Kojima H, Tanaka Y, Tanaka T, et al. Cell proliferation and apoptosis in human middle ear cholesteatoma.

Arch Otolaryngol Head Neck Surg 1998;124:261-4.
4. Ergun S, Carlsoo B, Zheng X. Apoptosis in meatal skin, cholesteatoma and squamous cell carcinoma of the ear. *Clin Otolaryngol* 1999;24:280-5.
5. Nagata S. Apoptosis by death factor. [Review] *Cell* 1997;88:355-65.
6. Maroldi R, Farina D, Palvarini L, et al. Computed tomography and magnetic resonance imaging of pathologic conditions of the middle ear. [Review] *Eur J Radiol* 2001;40:78-93.
7. Chiu CF, Chow KC, Lin FM, et al. Expression of DNA topoisomerase II alpha and multidrug resistance p-glycoprotein in acute leukemia. *Zhonghua Yi Xue Za Zhi (Taipei*) 1997;60:184-90.
8. Wang JC, Hung CM. The ossicalar destruction in chronic otitis media. *J Taiwan Otolaryngol Head Neck Surg* 1994;29:344-9. (In Chinese; English abstract)
9. Chole RA, McGinn MD, Tinling SP. Pressure-induced bone resorption in the middle ear. *Ann Otol Rhinol Laryngol* 1985;94:165-70.
10. Macri JR, Chole RA. Bone erosion in experimental cholesteatoma--the effects of implanted barriers. *Otolaryngol Head Neck Surg* 1985;93:3-17.
11. Kojima H, Matsuhisa A, Shiwa M, et al. Expression of messenger RNA for keratinocyte growth factor in human cholesteatoma. *Arch Otolaryngol Head Neck Surg* 1996;122:157-60.
12. Bujia H, Holly A, Schilling V, et al. Aberrant expression of epidermal growth factor receptor in aural cholesteatoma. *Laryngoscope* 1993;103:326-9.
13. Yan SD, Huang CC. The role of tumor necrosis factor-alpha in bone resorption of cholesteatoma. *Am J Otolaryngol* 1991;12:83-9.
14. Huang CC, Yi ZX, Chao WY. Effects of granulation tissue conditioned medium on the in vitro differentiation of keratinocytes. *Arch Otorhinolaryngol* 1988;245:325-9.
15. Mostafapour SP, Hockenbery DM, Rubel EW. Life and death in otolaryngology: mechanisms of apoptosis and its role in the pathology and treatment of disease. [Review] *Arch Otolaryngol Head Neck Surg* 1999; 125:729-37.
16. Strasser A, Newton K. FADD/MORT1, a signal transducer that can promote cell death or cell growth. [Review] *Int J Biochem Cell Biol* 1999:31:533-7.
17. Shinoda H, Huang CC. Expressions of c-jun and p53 proteins in human middle ear cholesteatoma: relationship to keratinocyte proliferation, differentiation, and programmed cell death. *Laryngoscope* 1995;105:1232-7.
18. Albino AP, Reed JA, Bogdany JK, et al. Expression of p53 protein in human middle ear cholesteatomas: pathogenetic implications. *Am J Otol* 1998;19:30-6.
19. Kojima H, Miyazaki H, Tanaka Y, et al. Role of Bcl-xL protein in differentiation and apoptosis of human middle ear cholesteatoma epithelium. *Arch Otolaryngol Head Neck Surg* 1999;125:738-42.
20. Park HJ, Park K. Expression of Fas/APO-1 and apoptosis of keratinocytes in human cholesteatoma. *Laryngoscope* 1999;109:613-6.
21. Kawakami A, Eguchi K, Matsuoka N, et al. Fas and Fas ligand interaction is necessary for human osteoblast apoptosis. *J Bone Miner Res* 1997;12:1637-46.
22. Jilka RL, Weinstein RS, Bellido T, et al. Osteoblast programmed cell death (apoptosis): modulation by growth factors and cytokines. *J Bone Miner Res* 1998;13:793-802.
23. Silvestris F, Tucci M, Cafforio P, et al. FasL up-regulation by highly malignant myeloma plasma cells: role in the pathogenesis of anemia and disease progression. *Blood* 2001;97:1155-64.
24. Tsai MH, Chow KC, Lin TY, et al. Expression of Fas ligand in patients with evident skull base involvement of nasopharyngeal carcinoma. *Oncol Rep* 2002;9:247-51.
25. Fromigue O, Kheddoumi N, Lomri A, et al. Breast cancer cells release factors that induced apoptosis in human bone marrow stromal cells. *J Bone Miner Res* 2001;16:1600-10.

中耳膽脂瘤的顳骨骨質變化與Fas ligand的表現

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背景 中耳膽脂瘤的特徵在於上皮細胞過度增生與角質碎屑堆積在中耳乳突腔中，引起顳骨骨質的破壞，這種細胞過度增生堆積的現象與膽脂瘤上皮細胞的凋亡作用有關。Fas ligand (FasL)爲上皮細胞凋亡作用的重要調控因子之一，並與骨細胞的活性有關。本研究除了藉高解析度顳骨電腦斷層掃描分析膽脂瘤患者的顳骨表現型態以外，分析膽脂瘤中FasL的表現情況，並討論FasL是否與骨質破壞有關。

方法 蒐集過去兩年間中耳膽脂瘤接受手術患者，回溯分析顳骨電腦斷層掃描的顳骨表現型態，手術時取出之膽脂瘤標本以傳統染色切片確定爲膽脂瘤，並利用免疫化學組織的方法檢測膽脂瘤中FasL之表現情況。

結果 針對過去未曾接受過耳部手術且有術前顳骨電腦斷層掃描的膽脂瘤患者共40名；男性18名，女性22名，平均年齡43歲。以電腦斷層掃描分析其顳骨表現型態，可以發現58%鼓室頂板或聽骨鏈磨損，乳突鼓室蓋磨損30%，耳囊磨損25%，骨質明顯硬化50%。免疫組織化學檢測則顯示耳後皮膚、膽脂瘤基質上皮細胞層以及基質表皮下發炎肉芽組織FasL的表現均成陰性反應。

結論 膽脂瘤的顳骨表現型態包括骨質破壞與骨質硬化或增生，前者的影響範圍以鼓室頂板及聽骨鏈最多；膽脂瘤上皮細胞及表皮下肉芽組織的FasL均呈陰性反應，由於FasL可以誘發導致細胞死亡的凋亡作用，因此膽脂瘤過度增生分化與死亡終極分化的凋亡作用不是經由FasL此一凋亡路徑，FasL與膽脂瘤常伴隨的骨質破壞可能無關。（**中台灣醫誌 2004;9:197-202**）

關鍵詞

凋亡作用，膽脂瘤， Fas配體，高解析度顳骨電腦斷層掃描

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