

# 진주종상피에서 Apoptosis를 통한 세포의 사멸기전

1, 2, 3

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## Mechanism of Apoptotic Cell Death in Cholesteatoma Epithelium

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### ABSTRACT

**Background and Objectives :** Accumulation of keratin debris in the middle ear is one of the characteristics of the cholesteatoma. It is related to increased rate of cell death and differentiation of keratinocytes compare to normal skin. This kind of cell death is known as apoptosis. In this study, we plan to investigate the apoptotic cell death and expression of Fas in both normal and cholesteatoma epithelia. **Materials and Methods :** Seven cholesteatomas and retroauricular skins were obtained from patients undergoing middle ear operations. Detection of the fragmented DNA in apoptosis was done by in situ TUNEL methods and agarose gel electrophoresis. For the morphologic confirmation of apoptosis, transmission electron microscopy (TEM) was done. Immunohistochemistry was also performed for detection of Fas expression on the tissue. **Results :** In TUNEL staining, many positive staining nuclei were observed in upper layers of cholesteatoma epithelium whereas a few positive cells were found on the granular layer of retroauricular skin. Typical "ladder pattern" was seen on the gel electrophoresis of the genomic DNA of cholesteatoma. On TEM study, we observed condensation of chromatin in the keratinocytes of the cholesteatoma epithelium. Immunohistochemical studies revealed that Fas protein was expressed in all layers of cholesteatoma epithelium, while retroauricular skin showed weak reactions only in the granular layer. **Conclusion :** We confirmed that increased apoptosis and up-regulated expression of Fas in cholesteatoma epithelium. Since Fas is known as apoptosis triggering protein, the authors suggest that accumulation of keratin debris is due to increased apoptotic cell death and further investigation should be needed about the mechanism of cell death in cholesteatoma. (**Korean J Otolaryngol 1998;41(4):425-429**)

**KEY WORDS :** Cholesteatoma epithelium · Cell death · Apoptosis · Fas.

keratin debri 가  
 tokeratin 가 1-3) cy - apoptosis 6)7)  
 4)5)가 keratin debri  
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Fas Apoptosis

가 .<sup>8)</sup>

apoptosis

Te -

terminal deoxynucleotidyl transferase(TdT) - mediated dUTP Nick End - Labeling(TUNEL) ,

, DNA Fas

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7

TUNEL 분석

10%

poly - L lysine 4 μm

xylene

proteinase K(20 μg/ml,

, 30 ) PBS

(10 , 2 ) endogenous peroxidase

H<sub>2</sub>O<sub>2</sub>(3%, 5 ) PBS

terminal deoxynucleotidyl transferase (TdT) labeling 37 , 90

PBS

(10 , 3 ) anti - FITC HRP conjugate 37 , 30

In situ Apoptosis De -

tection Kit(Takara Shuzo Co., Japan)

AEC Substrate - Chromogen System(DAKO, CA, USA) 2 5

투과전자현미경관찰

Karnovsky fixative solution(1% paraformaldehyde, 2% glutaraldehyde, 0.002M calcium chloride, 0.1M cacodylate buffer, pH 7.4) 2

cacodylate buffer 1% osmium tetroxide 1.5% potassium ferrocyanide 1

. 50 100% alcohol Poly/Bed 812 resin(Pelco) 37 6 , 48

12 , 60 24

uranyl acetate lead citrate

Zeiss EM 902A

Fragmented Genomic DNA의 전기영동분석

fragmented DNA

0.5 ml lysis buffer(10 mM Tris, 1 mM EDTA, 0.2% Triton X - 100) 13,000 × g 5

fragmented DNA가

5M NaCl(0.1 ml, ice - cold) 2 - propanol(0.7 ml, ice - cold) 20

70% alcohol

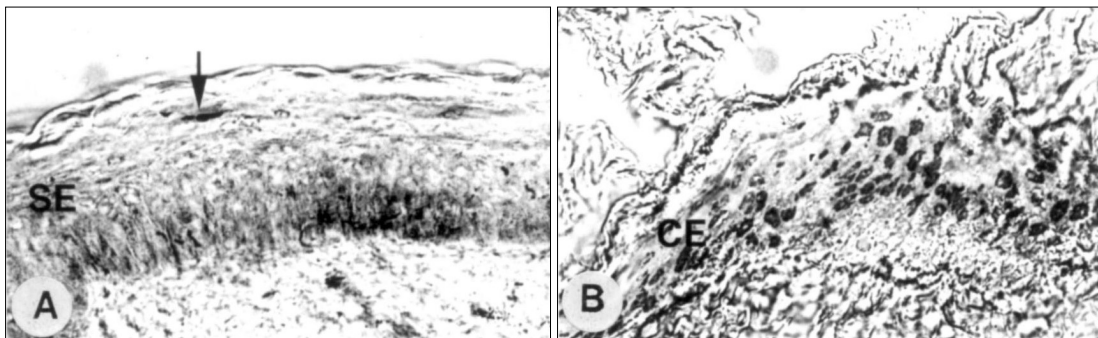
. DNA pellet 1.5%

agarose 50V, 2 ethidium bromide

UV lamp

면역조직화학염색

TUNEL



**Fig. 1.** TUNEL staining of retroauricular skin and cholesteatoma epithelium. A : Nuclei of fragmented chromatin are presented in the granular layer of retroauricular skin ( × 400) (arrows-keratinocytes in apoptosis) B : Increased number of TUNEL positive nuclei are observed in upper layers of the human cholesteatoma epithelium ( × 400). SE-epithelium of retroauricular skin, CE-epithelium of cholesteatoma.

H<sub>2</sub>O<sub>2</sub>(3%, 5 ) Fas pri-  
 mary antibody(Santa Cruz, CA, USA) 4  
 Vectastain ABC reagent(Vector,  
 CA, USA) AEC

ethidium bromide apoptosis  
 genomic DNA nucleosomal  
 (" ladder ")  
 (Fig. 3).

Fas

Fas

TUNEL

TUNEL  
 TUNEL  
 (Fig. 1A).  
 TUNEL  
 (Fig. 1B).

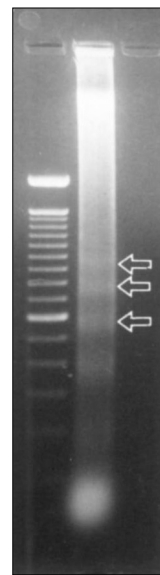
7  
 가 가

TUNEL

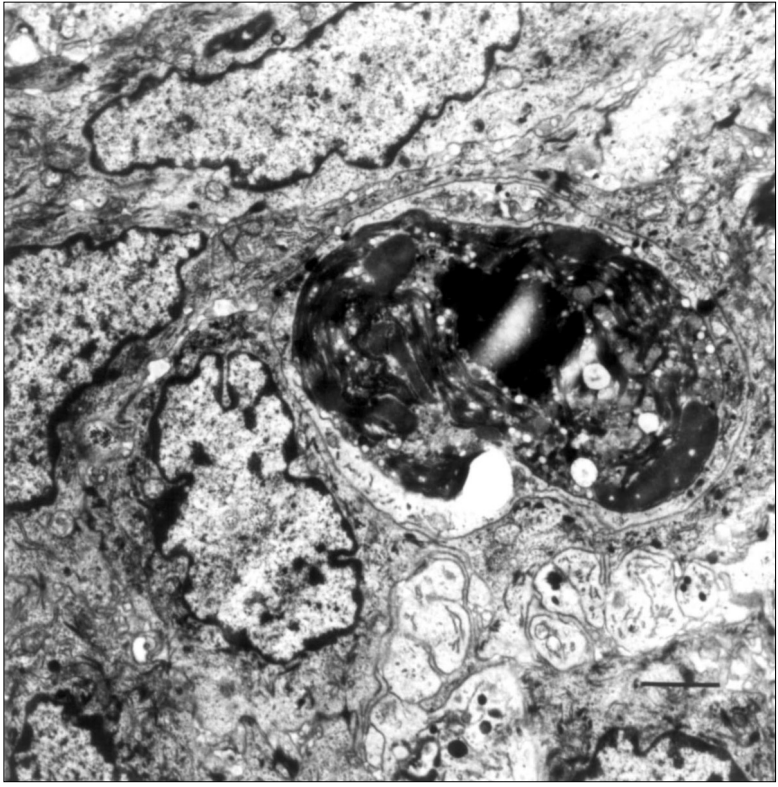
apoptosis  
 condensation  
 (Fig. 2).

Fragmented Genomic DNA

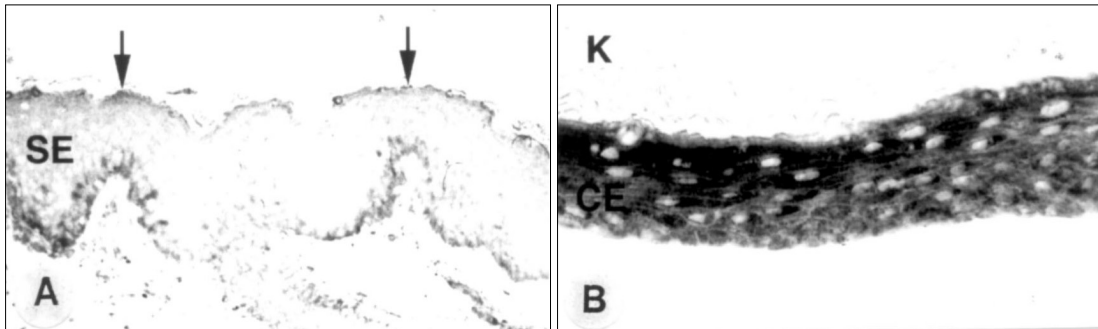
DNA 1.5% agarose gel



**Fig. 3.** Agarose gel electrophoresis of genomic DNA from cholesteatoma epithelium. Distinct pattern of fragmented genomic DNA is observed in 1.5% agarose gel electrophoresis with ethidiumbromide staining (Left lane, 100 bp DNA marker ; Right lane, Genomic DNA of human cholesteatoma epithelium) (arrows-typical ladder patterns of fragmented DNA).



**Fig. 2.** Photography of transmission electron microscope of the keratinocyte under apoptotic process in cholesteatoma epithelium. Condensation of the chromatin can be observed in the keratinocyte (Scale bar, 1.7 μm).



**Fig. 4.** Immunohistochemical staining of normal retroauricular skin and cholesteatoma epithelium using polyclonal anti-human Fas protein antibody. The reaction was done with avidine-biotin-peroxidase complex and AEC. A : Weak positive staining can be seen only in the keratin layer of normal retroauricular skin (arrows). B : Positive reactions can be found in entire cholesteatoma epithelium ( × 400) K-Keratin debris of cholesteatoma, CE-cholesteatoma epithelium.

(Fig. 4).

가 . TUNEL apoptosis  
in situ <sup>12)</sup> necrosis가

가  
가  
가 . Apoptosis DNA fragmentation  
internucleosomal sites  
double - stranded DNA 200 bp  
band (ladder pattern).<sup>13)</sup>  
DNA ladder pattern  
(Fig. 3).  
DNA fragmentation apoptosis  
apoptosis  
shrinkage condensation <sup>14)15)</sup>  
apoptosis 가

apoptosis가  
apoptosis 가 <sup>11)</sup>  
TUNEL TUNEL  
condensation  
(Fig. 2).  
keratinocyte Apoptosis Fas, p53, bcl - 2  
epi- Shinoda Huang (1995)  
p53 apoptosis  
dermal growth factor receptor가 <sup>8)</sup> Tumor necrosis  
cytokeratin 13, 16 Fas  
<sup>1-3)</sup> <sup>4)5)</sup>  
debris 가  
apoptosis 가 apoptosis p53  
TUNEL apoptosis가 ( )

19) Fas p53 apoptosis  
In vitro  
p53 Fas 가 apoptosis  
20) Fas  
Fas apoptosis  
Fas  
Fas apoptosis  
Fas 가 apoptosis  
Fas in vivo  
가 가  
Fas Apoptosis  
가 가  
keratin  
debris 가  
: Apoptosis Fas.

1997

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