

중이진주종과 고막 경계부에서 Heat-Shock Protein 60 및 70의 발현

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Expression of Heat-Shock Protein 60 and 70 in the Junction between Cholesteatoma and Ear Drum

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-ABSTRACT-

Background and Objectives : Pathogenesis of cholesteatoma is characterized by cellular proliferation, differentiation, and programmed cell death of keratinizing epithelium in the middle ear cavity. This study was performed to investigate the role of Heat-shock protein 60 and 70 (HSP60 and 70) in the pathogenesis of cholesteatoma. **Materials and Methods** : Cholesteatoma samples were taken from 7 patients during operation. Immunohistochemical stain was used for HSP 60 and HSP 70. External ear canal skin and ear drum were used as a control group. **Results** : HSP60 is detected in all cases and HSP 70 is detected in 5 cases in all layers of cholesteatoma epithelium from the junction between drum and cholesteatoma. HSP60 and HSP70 are not detected in the skin of external ear canal and ear drum. **Conclusion** : HSP60 and HSP70 may have integral role in the pathogenesis of acquired cholesteatoma and we suggest abnormal hyperplasia of epithelial cells as a pathogenesis of acquired cholesteatoma. (J Clinical Otolaryngol 2002;13:188-198)

KEY WORDS : Cholesteatoma · Heat-shock proteins · Hyperplasia · Apoptosis.

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Heat - shock proteins(HSP)
(proteotoxic)

가

가 가

(prokaryote) (eukaryote)
 folding, assembly, translocation
 가
 가
 가
 HSP70
 Shinoda²⁾
 HSP
 HSP60 70

Table 1. The cases of the study

Cases (sex/age)	Extension of cholesteatoma
1 (M/36)	Antrum
2 (M/47)	Antrum
3 (F/46)	Attic
4 (M/43)	Antrum
5 (F/33)	Attic
6 (M/37)	Antrum
7 (M/36)	Antrum

HSP60
 가
¹⁾
²⁾
 10% formalin
 μm
 HSP60
 polyclonal
 rabbit anti - HSP60 antibody (SPA - 805, Stress Gen,
 Victoria, British Columbia, Canada)
 HSP70
 monoclonal mouse anti - HSP70
 antibody (SPA - 820, Stress Gen, Victoria, British
 Columbia, Canada)
 1 : 200

재료 및 방법

연구대상

가 7
 33~47 가 4 , 3
 가 5 ,
 2 (Table 1).

연구방법

1 × 0.5 cm
 gentian violet
 가
 10 mmol/L phosphate buffered saline(PBS)

polyclonal rabbit anti - HSP60 antibody
 anti - HSP70 antibody indirect immuno-
 peroxidase method
 1.0% hydrogen peroxidase 6
 , 3% rabbit serum 6 , rabbit anti -
 HSP60 antibody(10 μg/ml) monoclonal mouse
 anti - HSP70 protein antiserum 45 , peroxidase -
 conjugated goat antirabbit IgG 2.5 μg/ml 15 ,
 PBS 0.01% hydrogen peroxidase - 0.05% 3 -
 amino - 9 - ethylcarbazole 3 . count-
 erstain Mayer 's hematoxylin
 glycerol mount Olympus
 BH - 2(Olympus Optical Co. Tokyo, Japan)
 anti - HSP60 anti-
 body anti - HSP70 antibody rabbit nonimmune
 IgG 2

stain +/-, count-
 + ,
 +++ , ++

Table 2. Immunoreactivity of Heat-Shock Protein 60 in the cases

Cases	Cholesteatoma	Skin or ear drum
1	+	-
2	++	-
3	++	-
4	+	-
5	+	+/-
6	+	+/-
7	+	-

++ : reactive moderately, + : reactive slightly, +/- : trace, - : not reactive

Table 3. Immunoreactivity of Heat-Shock Protein 70 in the cases

Cases	Cholesteatoma	Skin or ear drum
1	+	-
2	+	-
3	-	-
4	+/-	-
5	+	-
6	+/-	-
7	+	-

+ : reactive slightly, +/- : trace, - : not reactive

결 과

7 HSP60
 2
 (Table 2). HSP70 3
 , 2 , 2
 (Table 3).

중이진주종에서의 HSP60 및 HSP70의 발현

HSP HSP
 P60 HS- (Fig. 1A)

HSP70
 HSP60
 (Fig. 1B).
 HSP60, HSP70 HSP

대조군으로 외이도 피부에서의 HSP의 발현

HSP60 2
 HSP70
 (Fig. 2).

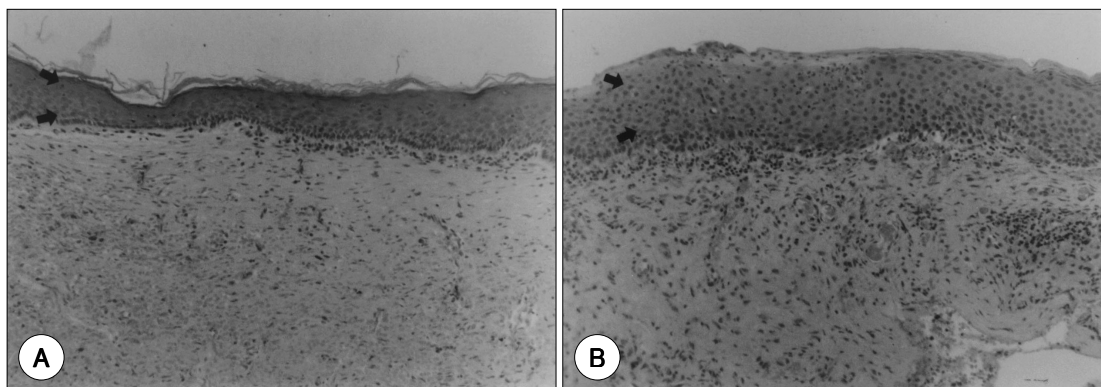


Fig. 1. Anti-HSP60 immunoreactivity (A) and Anti-HSP70 immunoreactivity (B) in cholesteatoma (x 100). Positive red-brown staining is seen in all layers of cholesteatoma epithelium (black arrows).

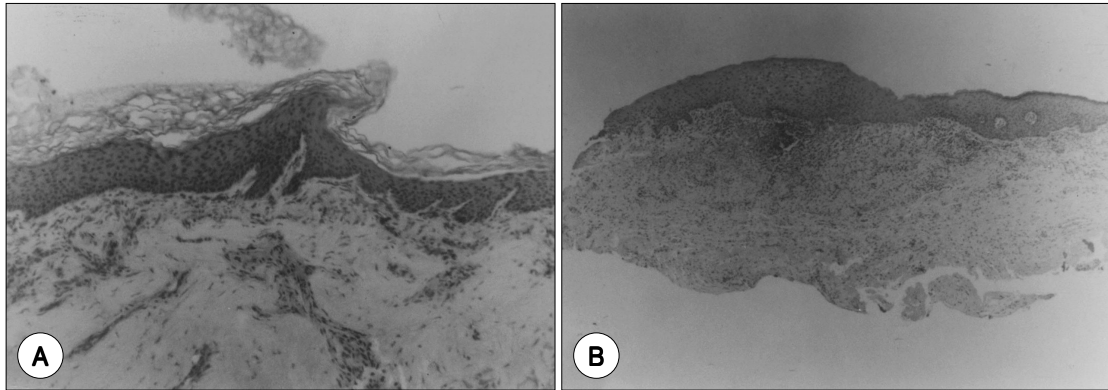


Fig. 2. Anti-HSP60 immunoreactivity (A) and anti-HSP70 immunoreactivity (B) in external ear canal skin ($\times 100$, A; $\times 40$, B). No positive staining is seen all layers of epithelium.

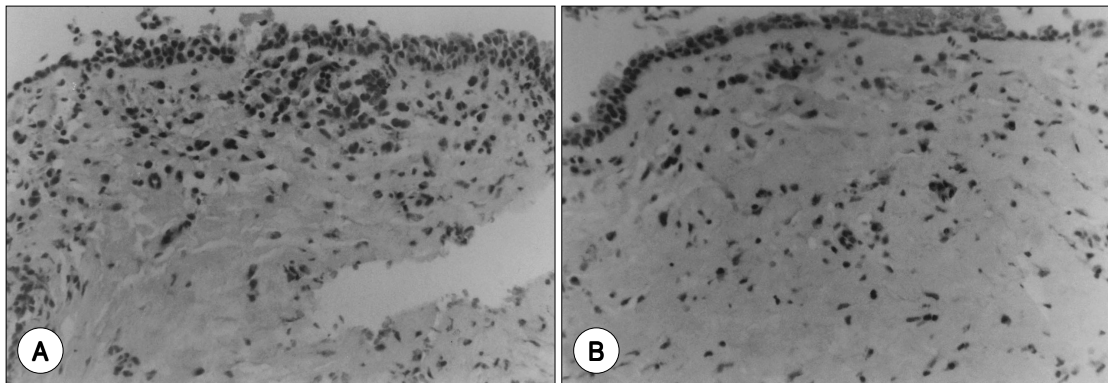


Fig. 3. Anti-HSP60 immunoreactivity (A) and anti-HSP70 immunoreactivity (B) in mucosa of mastoid antrum ($\times 200$). No positive staining is seen in mucosa.

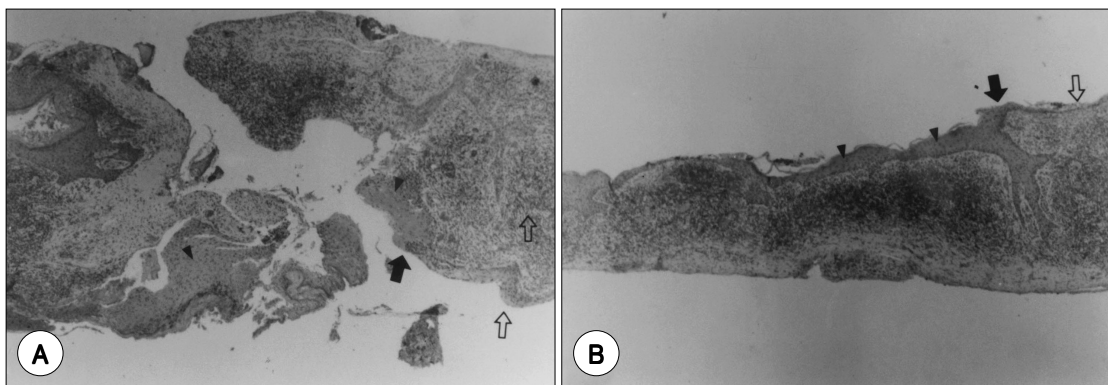


Fig. 4. Anti-HSP60 immunoreactivity (A) and anti-HSP70 immunoreactivity (B) in the junction between cholesteatoma and the ear drum ($\times 40$). Reddish brown staining is seen at the junction (black arrow) and in all layers of cholesteatoma (arrowheads). Subepithelial connective tissue of both cholesteatoma and the drum shows diffuse inflammatory reaction but no positive staining is seen in all the tissue of the drum (white arrows).

대조군으로 유양동 점막에서의 HSP의 발현

HSP60 HSP70
(Fig. 3).

중이진주종과 고막 경계부에서의 HSP의 발현

HSP60 HSP70
HSP60
5 , HSP70 3
가 (Figs. 4 and 5).

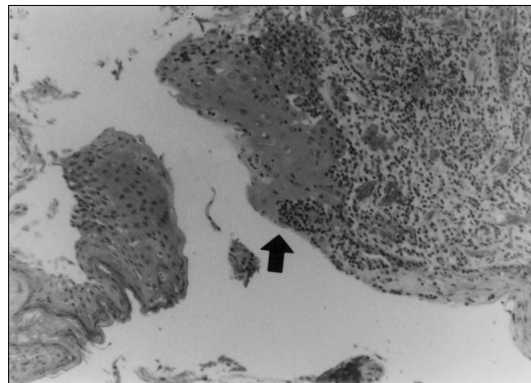


Fig. 5. Anti-HSP60 immunoreactivity at the junction between cholesteatoma and ear drum (x 100). Beginning of positive staining is noted (arrow).

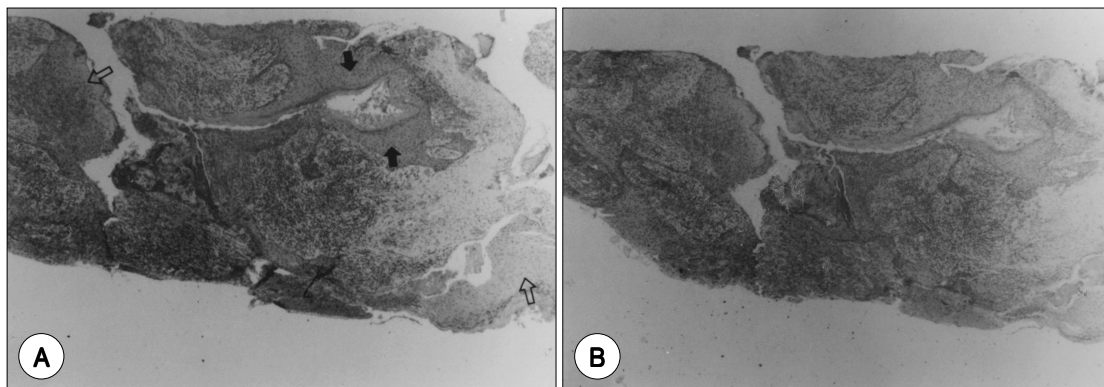


Fig. 6. Anti-HSP60 immunoreactivity (A) and anti-HSP70 immunoreactivity (B) in cholesteatoma near the junction between cholesteatoma and drum (x 40). It is noted that ingrown epithelium in cholesteatoma shows stronger immunoreactivity (black arrows) than that of surface epithelium (white arrows), especially for HSP60.

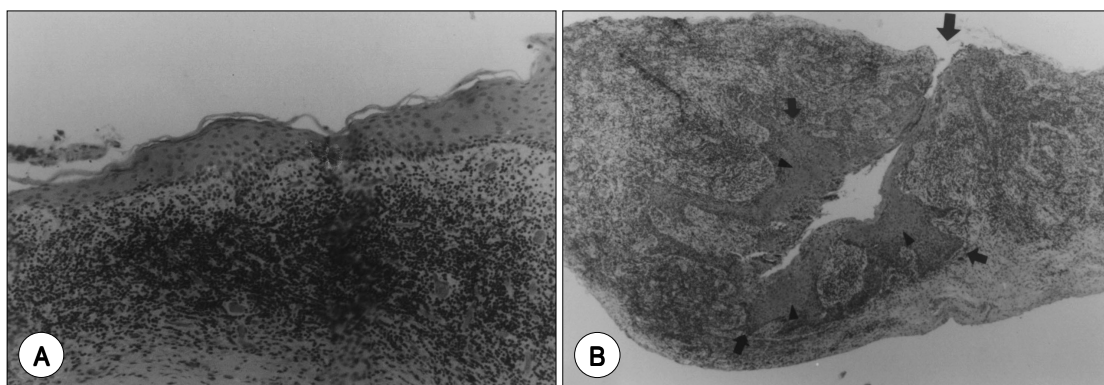


Fig. 7. Comparison of morphology and Anti-HSP60 immunoreactivity between cholesteatoma far from the drum (A) and cholesteatoma near the drum (B) (x 100). Cholesteatoma near the drum shows ingrowing pattern (black arrows) and stronger immunoreactivity in the upper part of epithelial cell layers (arrowheads).

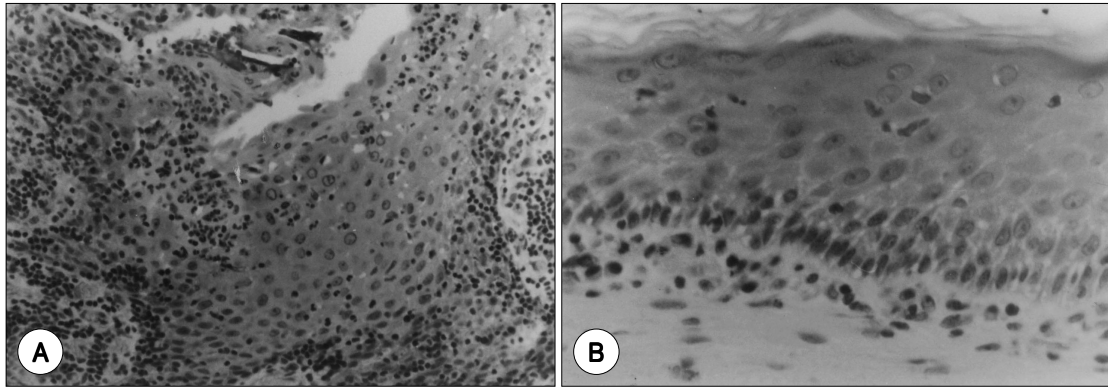
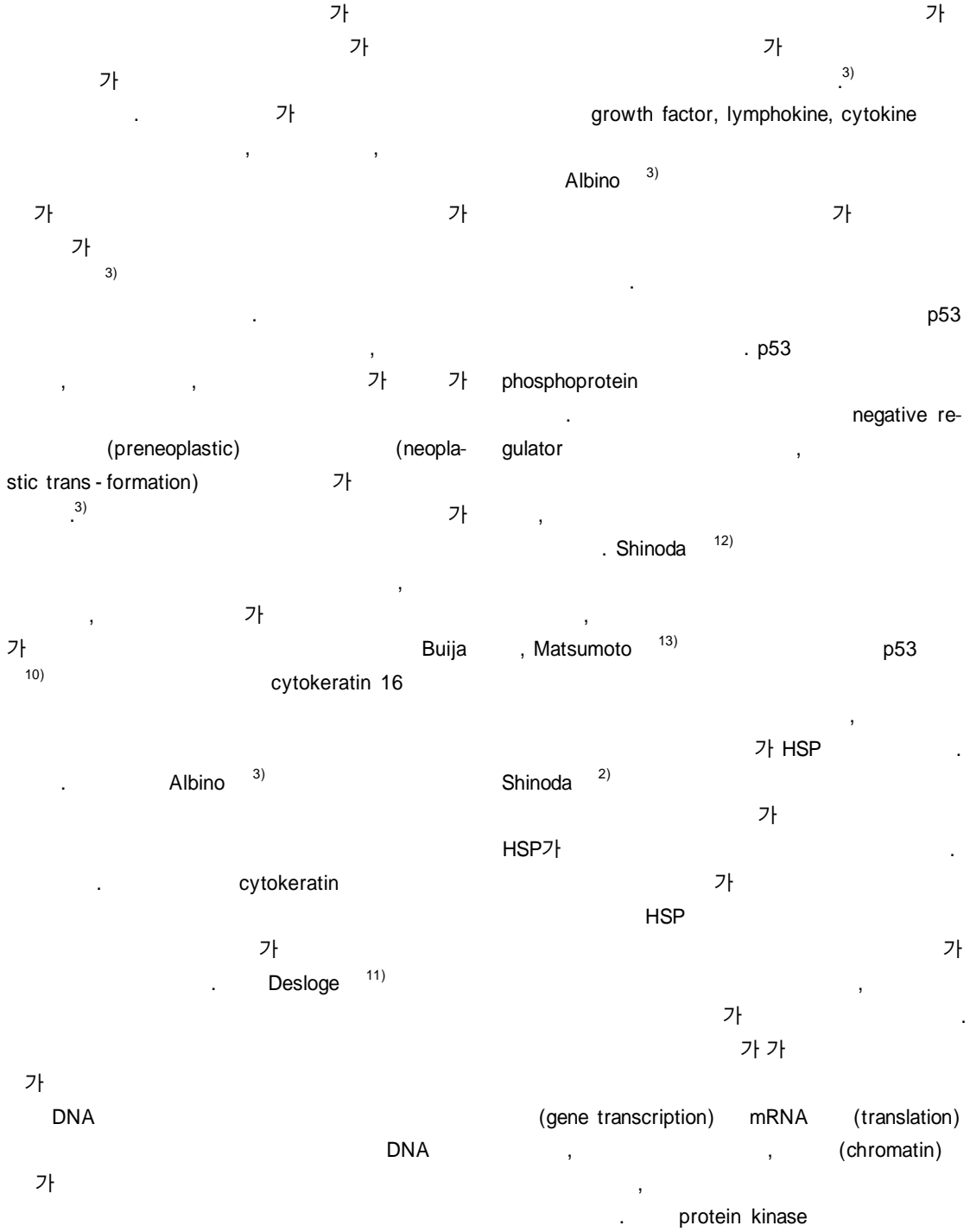


Fig. 8. Anti-HSP60 immunoreactivity in cholesteatoma near the drum (A) and cholesteatoma far from the drum (B) (x 200,A ; x 400,B). Positive staining is seen in the cytoplasm and nucleus of the epithelial cell of cholesteatoma.

HSP60, HSP70 (5 ory) . Ars⁵⁾ 가
 가
 가 Sade⁶⁾
 , 가 ann⁷⁾ . Wolfm-
 HSP60, HSP70 Michaels⁹⁾
 (Figs. 6 and 7). HSP 12
 60 HSP70 (Fig. 8).

고 찰

(apoptosis)²⁾ 가
 가³⁾ 가
 가 4-8)
 가 가 . Huang⁸⁾ chinchilla
 propylene glycol
 가
 ttmack⁴⁾ . Wi- (invagination the-



transcriptional activation
HSP가

가 . 가
heat - shock gene . gradative pathway
Ciechanover¹⁶⁾
ubiquitin pathway

가 . 가 HSP HSP90
HSP
가 . 가
HSP가 HSP가
HSP 가 HSP
4가 . 110 kDa
(HSP110), 90 kDa(HSP90), 70 kDa(HSP70), 60
kDa(HSP60)가 HSP90 HSP86 HSP84 .¹⁷⁾
가 HSP70 HSP68 HSP70
가 . HSP
HSP60 HSP70 chaperone
HSP70 HSP70¹⁷⁾ Polla¹⁷⁾
가 HSP ther Platelet activating factor(PAF) ace-
HSP60 HSP70 HSP HSP 70 family
heat - shock cognate(HSC) . Shinoda²⁾ HSP60 HSP70
HSP 가 . Polla¹⁷⁾
HSP chaperone . Chaperone 2
polypeptide
가 . HSP Polla¹⁷⁾
HSP HSP HSP가
HSP70
HSP¹⁴⁾ HSP HSP가
ubiquitin pathway 가 HSP HSP
Rechsteiner¹⁵⁾가 , HSP70 lysosome de-

HSP ger²⁰⁾ HSP 70 가

DeNagel¹⁸⁾ 가 , HSP premature folding HSP60 HSP70 . Shin-

가 oda²⁾ HSP60 HSP70

HSP60 HSP70 T Langerhan , HSP60 HSP70 . DeNagel¹⁸⁾ Shinoda²⁾

HSP , major , HSP70 histocompatibility complex(MHC) T HSP60 antigen processing MHC HSP60 HSP70 HSP70 , 가 가 HSP 가 HSP60 HSP 가 가 HSP HSP70 가 가 HSP가 가 HSP HSP HSP 가 antigen processing , , HSP 가 HSP

Takahashi¹⁹⁾ S - 100 protein HSP Langerhan 가 HSP Langerhan 가 HSP가 가 , HSP

HSP Langerhan 가 Matsumoto¹³⁾ HSP가 p53 HSP가 Lan- . HSP

가 , p53

HSP60 HSP70

가 가 , 가

HSP 가 가

13) 가 p53 가 가

HSP HSP

중심 단어 : Heat - shock proteins

Shinoda 2)

HSP60 HSP70 HSP60

HSP70

3 2

HSP

가 , 가 가

HSP가 HSP가 가

결 론

HSP60 HSP70 7 5

HSP60 HSP60, HSP70

가 HSP60 HSP70

REFERENCES

- Schlesinger MJ. *Heat shock proteins: the search for functions. J Cell Biol* 1986;103:321-5.
- Shinoda H, Huang C. *Heat shock protein in middle ear cholesteatoma. Otolaryngol Head Neck Surg* 1996;114:77-83.
- Albino AP, Kimmelman CP, Parisier SC. *Cholesteatoma: a molecular and cellular puzzle. Am J Otol* 1998;19:7-19.
- Wittmack K. *Wie Entsteht ein Genuines Cholesteatom? Arch Otorhinolaryngol* 1933;137:306-12.
- Ars B, Craemer W. *Tympanic membrane lamina propria and middle ear cholesteatoma. In: Tos M, editor. Cholesteatoma and mastoid surgery. Amsterdam; Kugler and Ghedini publications;1989. p.429-31.*
- Sade J, Berco E. *Atelectasis and secretory otitis media. Ann Otol Rhinol Laryngol* 1976;85:66-72.
- Wolfmann DE, Chole RA. *Osteoclast stimulation by positive middle ear pressure. Arch Otolaryngol Head Neck Surg* 1986;112:1037-42.
- Huang CC, Shi GS, Yi ZX. *Experimental induction of middle ear cholesteatoma in rats. Am J Otolaryngol* 1988;9:165-72.
- Michaels L. *Biology of cholesteatoma. Otolaryngol Clin North Am* 1989;22:869-81.
- Buija J, Schilling V, Holly A, Stammberger M, Kastenbauer E. *Hyperproliferation-associated keratin expression in human middle ear cholesteatoma. Acta Otolaryngol* 1993;113:364-8.
- Desloge RB, Carew JF, Fistad CL, Steiner MG, Sassoon J, Levenson MJ, et al. *DNA analysis of human cholesteatomas. Am J Otol* 1997;18:155-9.
- Shinoda H, Huang CC. *Expression 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.
- Matsumoto H, Shimura M, Omatsu T, Okaichi K, Majima H, Ohnishi T. *p53 proteins accumulated by heat stress associate with heat shock proteins HSP72/HSC73 in human glioblastoma cell lines. Cancer lett* 1994;87:39-46.
- Pelham HRB. *Speculation of the function of the major heat shock and glucose regulated proteins. Cell* 1986;46:959-61.
- Rechsteiner M. *Natural substrates of the ubiquitin proteolytic*

- pathway. Cell 1991;66:615-8.*
- 16) Ciechanover A, Finley D, Varshavsky A. *Ubiquitin dependence of selective protein degradation demonstrated in the mammalian cell cycle mutant ts85. Cell 1984;246:382-5.*
 - 17) Polla BS, Kantengwa S. *Heat shock proteins and inflammation. Curr Top Microbiol Immunol 1991;167:93-105.*
 - 18) DeNagel DC, Pierce SK. *Heat shock proteins in immune responses. Crit Rev Immunol 1993;13:71-81.*
 - 19) Takahashi S, Nakano Y. *Immunohistochemical demonstration of Langerhan's cell in cholesteatoma using an antiserum against S-100 protein. Arch Otolaryngol 1989;246:48-52.*
 - 20) Langer T, Neupert W. *Heat shock proteins hsp60 and 70. Curr Top Microbiol Immunol 1991;167:3-30.*