

Mongolian Gerbil에서 실험적으로 유도된 진주종에서의 신호전달체계에 관한 연구

이동훈¹ · 박기현² · 박홍준² · 전정민² · 황성철³

Signal Transduction System of Experimentally Induced Cholesteatoma in the Mongolian Gerbil

Dong-Hoon Lee, MD¹, Keehyun Park, MD², Hong-Joon Park, MD²,
Jeong-Min Chun MD² and Sung-Chul Hwang, MD³

¹Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, Hallym University, Seoul,

²Department of Otolaryngology, ³Pulmonary and Critical Medicine, Ajou University School of Medicine, Suwon, Korea

ABSTRACT

Background and Objectives : Hyperproliferative character of the cholesteatoma in the middle ear seems to be related to epithelial cell proliferation and differentiation. The proliferation of cells, their differentiation and organization in specialized tissues and the expression of their differentiated properties are under control of a large number of regulatory processes and complex interactions called signal transduction. PLC- 1 is a substrate of protein kinase located in EGFR, PDGFR- and - and signal transduction through PLC- 1 participates in the regulation of cell growth and differentiation. This study was undertaken to investigate the distribution of PLC- 1, EGFR and PDGFR in experimentally induced cholesteatoma, deep meatal skin and retroauricular skin of Mongolian gerbil. **Materials and Methods** : Using Western blotting and immunohistochemical techniques, we investigated the reaction patterns of antibody to PLC- 1, EGFR, PDGFR- and PDGFR- as a proliferation and differentiation marker in the experimentally induced cholesteatoma matrices of Mongolian gerbil. For the control, same study was performed with deep meatal skin and retrosuricular skin. **Results** : By Western blotting, considerably higher levels of PLC- 1, EGFR protein were detectable in cholesteatoma compared with control, however, PDGFR- and - were not detected in cholesteatoma. The immunostaining intensity of PLC- 1 and EGFR at suprabasal cell layer and basal cell layer were intense in cholesteatoma than in control. PDGFR- and - were not detected in both cholestatoma and control. **Conclusion** : Over-expression of PLC- 1 and EGFR in induced cholesteatomas may contribute abnormal proliferation and differentiation of their epithelial cell. Authors suggest that induced cholesteatoma in Mongolian gerbils can be a good model of signal transduction study for cholesteatoma. (**Korean J Otolaryngol 1998; 42:679-85**)

KEY WORDS : Induced cholesteatoma · Signal transduction · PLC- 1 · EGFR.

가 .
(hyperproliferation), (hyperdifferentiation),
(migration)

¹⁾²⁾

cytokeratin, involucrin, filaggrin

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: (0331) 219 - 5266, 5266 · : (0331) 219 - 5264
E - mail : parkkh@madang.ajou.ac.kr

가 . 가

transduction pathway) (signal 10% 24

4)5)

가 2)

6)7)8)

Western blotting

EBC (40 mM Tris/HCl, pH 8.0, 120 mM NaCl, 0.5% NP - 40, 2 μg/ml aprotinine, 2 μg/ml pepstatin, 2 μg/ml leupeptin, 100 μg/ml phenylmethylsulfonyl fluoride)

4 20 5 vortex

4 12,000 RPM 15

ELISA

Mongolian gerbil 2 mg/ml 2Xsodium dodecyl sulfate(SDS) sample (100 mM Tris/HCl, pH 6.8, 200mM dithiothreitol, 4% SDS, 0.2% bromophenol blue, 20% glycerol) 가 75 5 가

PDGFR - , PDGFR - Western blotting mini - gel kit(Bio - Rad)

가 100V 1.5 2

가 6.5% SDS/polyacrylamide nitrocellulose membrane

50V 1

Ponceaus nitrocellulose membrane nitrocellulose membrane 5% non - fat dry milk(Carnation)가 TNE (10 mM Tris/HCl, pH 7.5, 2.5 mM EDTA, 50 mM NaCl, 0.1% Tween 20) 1

25 60 75 g 90 120

Mongolian gerbil , ketamine hydrochloride(Ketar, 40 mg/kg) 10% sodium azide가

xylazine(Rompun, 8 mg/kg) 4 16 EGFR (Santa Cruz Biotec. Inc. Santa Cruz, CA, U.S.A.) 1 : 1,000 , PDGFR - (Santa Cruz Biotec. Inc. Santa Cruz, CA, U.S.A.) 1 : 1,000 , PD - GFR - (Santa cruz Biotec. Inc. Santa Cruz. CA, U.S.A.) 1 : 1,000 , PLC - 1 (Lab. of Cell Signaling at NIH, Bethesda, Mayland, U.S.A.) 1 : 4,000

Mongolian gerbil 2 black silk

25 Mongolian gerbil 3

21

Ether Mongolian gerbil

TNE 10 3 nitrocellulose membrane 1

- 70 가 TNE chemi - luminescence reagent (NEN, Boston, MA)

가 - 70 X - ray film(Kodak) 3 5

10% Western blotting

24 10% EDTA(ethylenediamine tetraacetic acid) 2 3 band (+ +),

(±), (+), (-)

Paraffin 4 µm poly - slide

L - lysin slide

58 12 xylene 15

3 . 100%, 90%, 80%, 70%

ethanol 5

3%

methanol

1 : 9 15

30 40

EGFR (Santa Cruz Biotec. Inc. Santa Cruz, CA, U.S.A.) 1 : 100

PDGFR- (Santa Cruz Biotec. Inc. Santa Cruz, CA, U.S.A.) 1 : 30

PDGFR- (Santa Cruz Biotec. Inc. Santa Cruz, CA, U.S.A.) 1 : 30

PLC- 1 (Lab. of Cell Signaling at NIH, Bethesda, Mayland, U.S.A.) 1 : 150

4

16 18 . TBS

Avidin - biotin Complex 30

3 - amino - 9 - ethyl - carbizole (AEC) 4

Meyer 's hematozylin

16

5 slide

(basal layer) (suprabasal layer)

, 1 TBS

(focal staining, (±)), 400

(+), 100

, 400

10

Western Blotting

Phospholipase C-γ1(PLC-γ1)

8

, 5

30 µg protein/lane

145KD protein band

1

lane (Table 1, Fig. 1).

Epithelial growth factor receptor(EGFR)

5

3 60 µg protein/lane

170KD protein band

Table 1. Results of Western blotting (most commonly observed pattern of expression)

	C	D	S
PLC- 1	++	+	±
EGFR	++	±	±
PDGFR-	-	-	±
PDGFR-	-	-	±

C : cholesteatoma
 D : deep meatal skin
 S : retroauricular skin
 ++ : very strong positive
 + : positive
 ± : weak positive
 - : negative

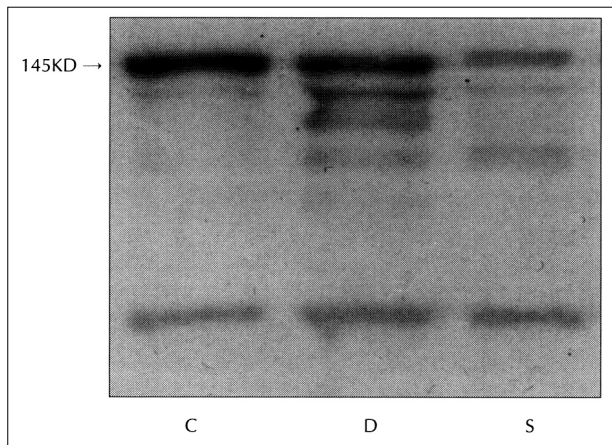


Fig. 1. Western blot analysis of PLC- 1 in cholesteatoma (C), deep meatal skin (D) and retroauricular skin (S). Strong band of PLC- 1 (145KD) is noted in cholesteatoma lane.

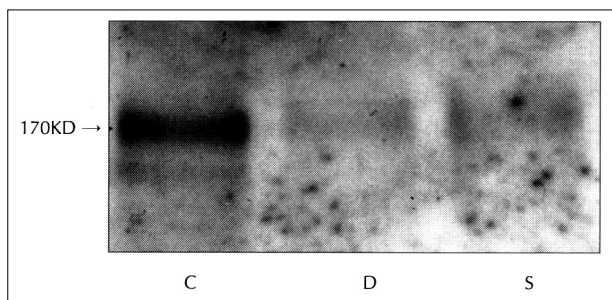


Fig. 2. Western blot analysis of EGFR in cholesteatoma (C), deep meatal skin (D) and retroauricular skin (S). Strong band of EGFR (170KD) is noted in cholesteatoma lane.

Table 2. Results of immunohistochemical stain : staining intensity (number of positive stained specimen/number of specimen)

	C		D		S	
	B	SB	B	SB	B	SB
PLC- γ 1	+	+	+	+	\pm	\pm
	(13/16)	(13/16)	(12/16)	(12/16)	(4/16)	(3/16)
EGFR	+	+	\pm	\pm	\pm	-
	(11/16)	(13/16)	(5/16)	(6/16)	(1/16)	(0/16)
PDGFR- α	\pm	\pm	\pm	-	\pm	-
	(1/16)	(1/16)	(2/16)	(0/16)	(2/16)	(0/16)
PDGFR- β	\pm	\pm	\pm	\pm	\pm	-
	(2/16)	(1/16)	(3/16)	(1/16)	(1/16)	(0/16)

C : cholesteatoma D : deep meatal skin S : retroauricular skin
 B : basal layer SB : suprabasal layer
 + : staining \pm : focal staining - : no staining

(Table 2, Fig. 4).

2 ,
 (Table 1, Fig. 2).
 Platelet derived growth factor receptor- α (PDGFR- α)와
 platelet derived growth factor receptor- β (PDGFR- β)

16 PDGFR- PDGFR-

5

, PDGFR- (170KD) PDGFR- (180KD)

(Table 2).

protein band

(Table 1).

가

Phospholipase C- γ 1(PLC- γ 1)

16

12

cytokeratin 16, proliferation -
 associated nuclear antigen(Ki - 67),⁹⁾ filaggrin
 involucrin¹⁾³⁾

가

16

10

. 2

가

4

(Table

가

2, Fig. 3).

가

가

Epithelial growth factor receptor(EGFR)

16

13

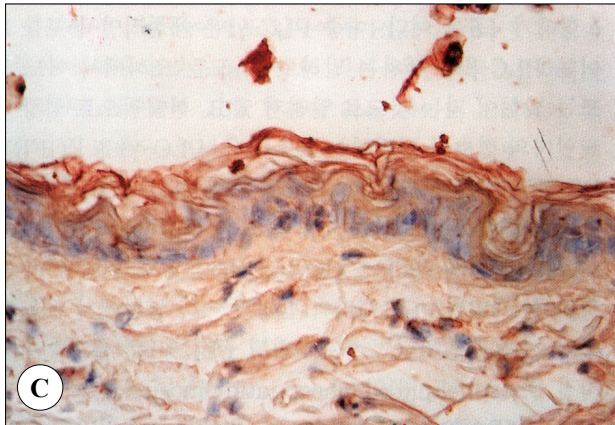
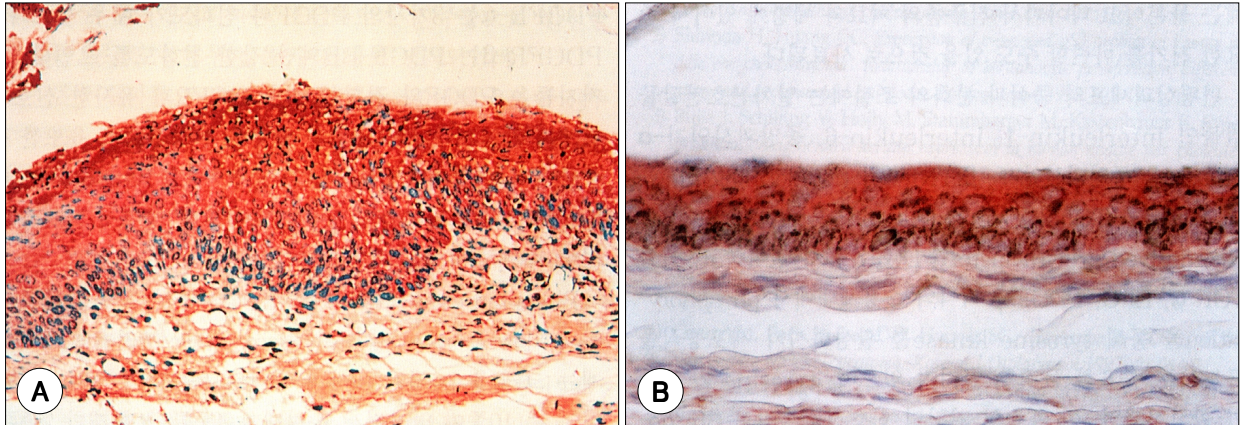


Fig. 3. PLC- 1 immunostaining. Cholesteatoma matrix (A) shows strong positive staining in the suprabasal layer and basal layer and deep meatal skin (B) also shows positive staining of suprabasal layer and basal layer, while retroauricular skin (C) shows only weak focal staining. (x 400)

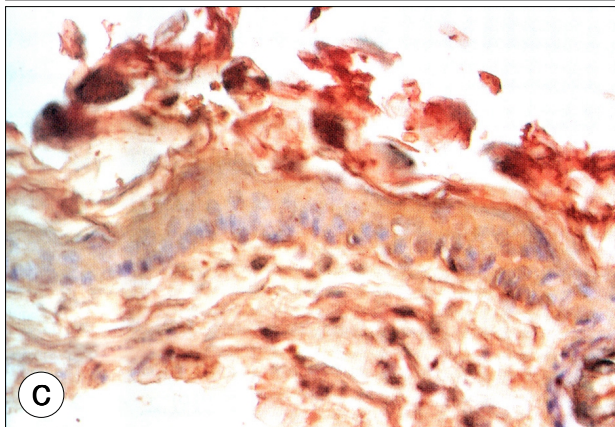
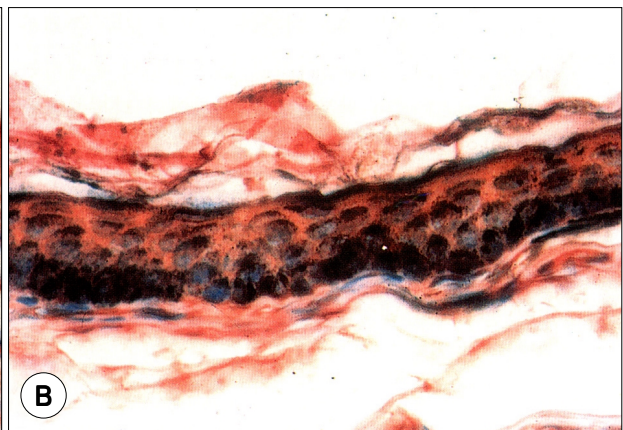
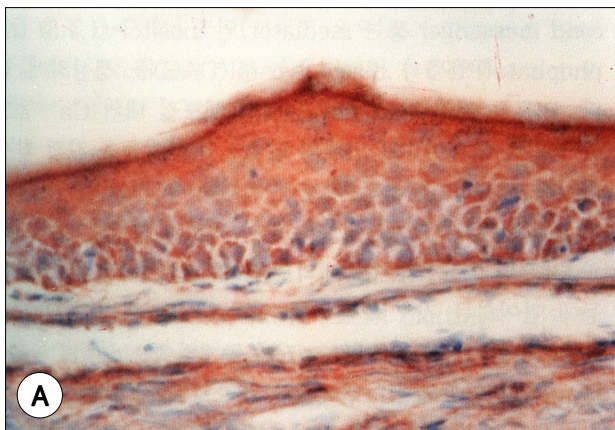


Fig. 4. EGFR immunostaining. Cholesteatoma matrix (A) shows positive staining in the suprabasal layer and basal layer, while deep meatal skin (B) and retroauricular skin (C) show focal or negative staining. (x 400)

가 interleukin - 1, interleukin - 6, (TNF -), (TNF -), transforming growth factor - (TGF -), prostaglandin, leukotrien, platelet derived growth factor(PDGF), TGF - , EFG, PDGF phosphorylation tyrosine kinase, tyrosine kinase, Src homology 2(SH2) domain, phosphatidylinositol 3' kinase(PI₃-kinase), Phospholipase C - (PLC -), Grb2/Sos1, Src 10

EGFR 170KD (intracellular tyrosine kinase domain), (extracellular ligand-binding domain), 3 EGF가 tyrosine kinase, c - jun, c - fos transcription factor DNA, EF - GR

8)9)14)15) EGFR EGFR 14) 9) Mongolian gerbil

EGFR

가 가

9)14) PDGFR PDGF - (170 KD) PDGF - (180KD) 2 PDGF tyrosine kinase . PDGF dimerized A chain B chain disulfide bond , PDGF - AA, AB BB 3가 isoform

PDGFR - 3가 , PDGFR - PDGF - BB 5)16) PDGF PDGFR PDGFR - Western blotting PDGF Phospholipase C(PLC) 1987 Ryu isozyme 17) 가 , 가 10 가 4 , 가 2 , 가 4 PLC - 1 PLC - 1 EGFR PDGFR EGF PDGF 가 tyrosine kinase 1989 Meisenhelder 가 11)18) PLC - 1 (Phospholipid) phosphatidylinositol 4,5 - biphosphate(PIP2) 가 (second messenger mediator) inositol - (1,4,5) triphosphate(PIP3) diacylglycerol (DAG) PIP 3 Ca⁺⁺ Ca⁺⁺ 가 , DAG protein kinase C , 4)5)19) PLC - 1 Ca⁺⁺ protein kinase C Na⁺/H⁺ exchanger . PIP 3 DAG , PLC - 1 20) , PLC - 1

가
 , Mongolian gerbil
 PLC -
 가
 PLC - 1 가
 EGFR
 Western blotting
 EGFR PDGFR
 PLC - 1 가
 EGFR
 ,
 tyrosine kinase
 PLC - 1
 antiphosphotyrosine
 orylation
 ,
 가가
 ,
 Mongolian gerbil
 Western blotting
 EGFR PLC - 1
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 가
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 EGFR.
 , PLC - 1

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