



가  
 1)2). PLC- 1  
 epidermal growth factor receptor tyrosine  
 kinase 가  
 (EGF) PLC- 1  
 가 3).  
 cytokine

가  
 PLC- 1 4).  
 PLC- 1  
 가  
 PLC- 1

재료 및 방법

1. 조직표본

8  
 10% 48  
 5mm western blotting  
 -70

2. Western blot분석

8  
 EBC  
 (40mM Tris/HCl, pH 8.0, 120mM NaCl, 0.5% NP-  
 40, 2 µg/ml aprotinine, 2 µg/ml pepstatin, 2 µg/ml  
 leupeptin, 100mg/ml phenylmethylsulfonyl flu-

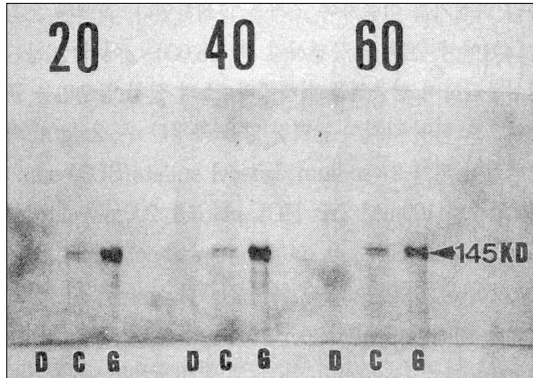
oride) 가 ,  
 4 20  
 15,000 xg 20  
 , 2mg/ml 2 x  
 sodium dodecyl sulfate(SDS) sample (100  
 mM Tris/HCl, pH 6.8, 200mM dithiothreitol, 4%  
 SDS, 0.2% bromophenol blue, 20% glycerol)  
 가 5  
 mini - gel kit(Bio - Rad) , buffer sy -  
 stem Laemmli<sup>13)</sup> , 6%  
 SDS/polyacrylamide  
 nitrocellulose membrane  
 . Nitrocellulose membrane 5%  
 non - fat dry milk(Carnation)가 TNE  
 (10mM Tris/HCl, pH 7.5, 2.5mM EDTA, 50mM  
 NaCl, 0.1% Tween 20) 1 PLC -  
 1 1 2  
 , 0.05% Tween 20 TNE  
 3 chemilumin - nescence reagent(NEN,  
 Boston, MA) X - ray film(Kodak) 3  
 1

3. 면역조직화학적염색

3%  
 H<sub>2</sub>O<sub>2</sub>가 methanol 30  
 peroxidase . LSAB  
 Kit(Dako, Carpinteria, CA, USA) ,  
 (Lab. of Cell Signaling at NIH, Bethesda,  
 Maryland, USA) 10mg/ml blocking  
 1 ,  
 AEC . Harris  
 hematoxylin .

결 과

PLC- 1  
 western blot  
 20, 40, 60 µg protein/lane 가

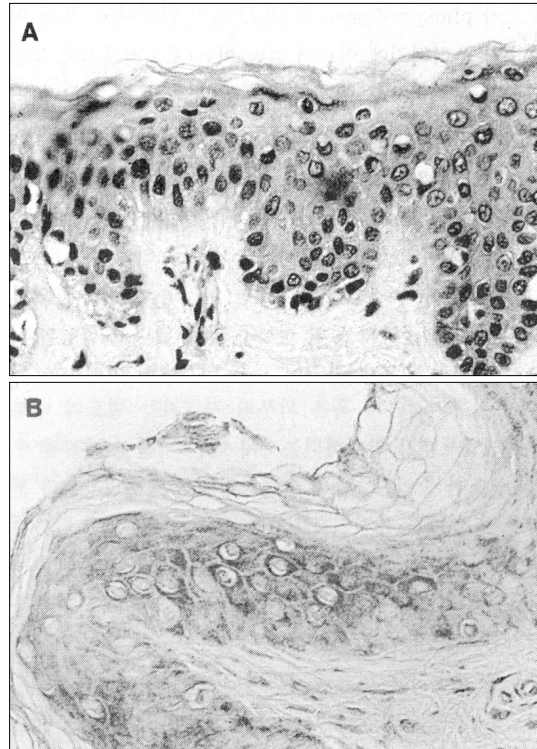


**Fig. 1.** Immunoblotting deep meatal skin and cholesteatoma with monoclonal anti-PLC-1 antibody. Total extract (20, 40, 60mg protein/lane) from both deep meatal skin and cholesteatoma tissues were separated on 6% SDS-PAGE and transferred to nitrocellulose membrane and blotted with monoclonal anti-PLC-1 antibody. The bands were visualized with HRP conjugated goat antimouse antibody using electrochemiluminescence. D : deep meatal skin, C : cholesteatoma, G : purified PLC-1 standard. Numbers indicate total extract (mg protein/lane) and arrow indicates the molecular weight.

PLC-1 가 (Fig. 1). Western blot PLC-1

(-), (+), (++) (F) (Fig. 2, 3). 8 1 8 1 (Table 1, 2).

고찰



**Fig. 2.** Immunohistochemistry of deep meatal skin with monoclonal anti-PLC-1 antibody shows negative staining(A) and positive staining(B) (ABC method, x 400).

retraction theory papillary proliferation theory 가 가 (TNF-), transforming growth factor - cytokine co-upling tyrosine kinase c-jun c-fos transcription factor DNA 4)5) 가 가 6)7) terminal differentiation

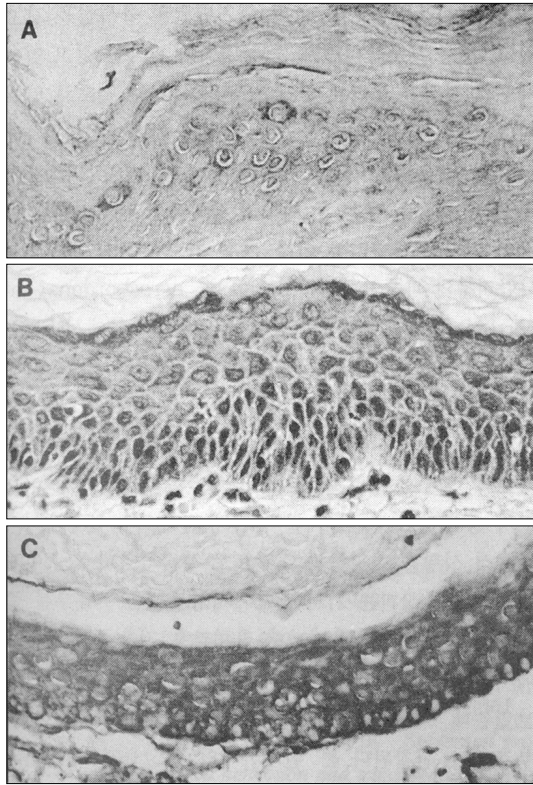


Fig. 3. Immunohistochemistry of cholesteatoma with monoclonal anti-PLC-1 antibody shows focal staining(A), positive staining(B), and strong positive staining(C) (ABC methods,  $\times 400$ ).

가 terminal differentiation 가 soft keratinization apoptosis가 4)8)9) 가 cytokine 가

가 PLC-1 가 phospholipids, phospholipase, protein phosphorylation Inositol 가 , 1975 Mitchell Inositol 1, 4, 5-triphosphate(IP3)가 calcium 가 10) 2 가 phosphoinositide-specific phospholipase C(PLC) 가 phosphatidyl inositol 4, 5-biphosphate (PIP<sub>2</sub>)가 PLC inositol 4, 5-biphosphate (PIP<sub>3</sub>) diacylglycerol 3)11)12) tyrosine kinase G-protein 가 IP3 , dia-cylglycerol protein kinase C stimulator , protein kinase C 2) Phospholipase C  $\alpha$ , 가 PLC- G-protein action site

**Table 1.** Localization of PLC- 1 in human cholesteatoma & deep meatal skin

Specimen No.	Epithelial layers containing protein		
	Basal layer	Spinous layer	Granular layer
1. Cholesteatoma	F	+	++
Deep meatal skin	-	-	-
2. Cholesteatoma	+	+	+
Deep meatal skin	-	-	-
3. Cholesteatoma	+	+	+
Deep meatal skin	F	F	F
4. Cholesteatoma	F	+	++
Deep meatal skin	-	-	F
5. Cholesteatoma	+	+	+
Deep meatal skin	F	F	F
6. Cholesteatoma	-	+	+
Deep meatal skin	-	-	-
7. Cholesteatoma	++	++	++
Deep meatal skin	+	+	+
8. Cholesteatoma	++	++	++
Deep meatal skin	F	F	F

F : Focal staining, - : Negative staining, + : Positive staining, ++ : Strong positive staining

**Table 2.** Localization of PLC- 1 in human cholesteatoma & deep meatal skin

Specimens		Basal layer	Spinous layer	Granular layer
Cholesteatoma	-	1	0	0
	(8)	+	3	6
		++	2	2
		F	2	0
Deep meatal skin	-	4	4	3
	(8)	+	1	1
		++	0	0
		F	3	3

Staining/F : focal, - : negative, ++ : positive, ++ : strong positive

psoriasis, acrochordons, seborrheic keratoses

PLC- 1

13)

14)15)

16)17)18)

, PLC- src

PLC- 1

1 EGF - R tyrosine kinase  
가 EGF

PLC-

가 activity

PLC- 1

tyrosine kinase

tyrosine kinase

가 key hole

kinase  
 PLC- 1 가  
 가  
 가  
 가  
 결론  
 PLC- 1 가  
 western blot  
 가

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