

사람 중이진주종에서 간극결합단백 Connexin 26, 43의 발현양상

정연훈 · 박기현 · 강성욱 · 신유리 · 조민정 · 윤용로

Expression Pattern of Gap Junction Protein, Connexin 26 and 43 in Human Middle Ear Cholesteatomas

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ABSTRACT

Background and Objectives : A human cholesteatoma in the middle ear is characterized by the presence of a keratinizing epithelium from hyperproliferative properties. It needs intercellular signal exchange through gap junctions as well as intracellular signal pathway for hyperproliferation. Connexin (Cx) is a gap junction protein for intercellular communication, and especially Cx26 and Cx43 are plenty in human epithelial cells. The objective of this study was to analyze the expression pattern of Cx43 and Cx26 in human middle ear cholesteatomas against normal epitheliums. **Subjects and Method** : Ten retroauricular skins (RAS), ear canal skins (ECS), and cholesteatomas were taken during middle ear operations at the Department of Otolaryngology. Immunohistochemical staining, reverse transcription-polymerase chain reaction (RT-PCR), and Western blotting were used to detect Cx43 and Cx26. **Results** : In human cholesteatomas, Cx43 were expressed in the whole suprabasal layers, especially in the middle portion, except in the basal layer, and Cx26 were usually expressed in the supra layer and in the basal layers. But normal RASs showed weak expression of Cx43 in the upper spinosal and granular layers, but not in the basal layers, and the restricted localization of Cx26 in the basal layer. The expression of Cx43 and Cx26 in EASs was weak but showed similar patterns to that of cholesteatomas. In RT-PCR and Western blot, the expression of Cx43 and Cx26 were increased in cholesteatomas than in RASs. **Conclusion** : Human middle ear cholesteatomas showed upregulated expression and different localization of Cx43 and Cx26, gap junction proteins for intercellular communication, compared with normal RASs, suggesting that perturbations of intercellular communication through gap junctions may be associated with the pathology of human middle ear cholesteatomas. (Korean J Otolaryngol 2006;49:29-34)

KEY WORDS : Cholesteatoma · Gap junction · Connexin 26 · Connexin 43.

factor receptor,¹⁾ platelet derived growth factor receptor,¹⁾ c - jun,²⁾ p53,²⁾ phospholipase C - ¹⁾³⁾ cytokeratin13/16³⁾
(intracellular communication) 가 (intercellular communication)
가
epidermal growth 가
(extracellular communication)
(proliferation),
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중이진주종에서 Connexin의 발현

(differentiation), (apoptosis) . , .

가 (gap junction) 3 . , (immunohistochemistry) Cx26, Cx43 (Reverse transcription - polymerase chain reaction) Cx26, Cx43 mRNA .⁴⁾⁵⁾ Cx Western blot Cx26, Cx43 (endoplasmic reticulum) (golgi apparatus) 6 가 connexon (hemichannel) (traf- Western blot ficking) .⁶⁾⁷⁾ Cx multigenic family 20 isoform Cx26 , 4% paraformaldehyde (paraffin) , Cx43 , , -70 Cx RT - PCR Western blot Cx Cx43, 26, 30, 31, 37, 45 .⁸⁾ Cx26 Cx43 Cx 가 6 um , xylene .⁸⁾ 가 Cx26 3 7 100%, 90%, 80%, 70% citric acid K⁺ ,⁹⁾¹⁰⁾ buffer 10 가 Cx 가 Cx 가 .¹¹⁾ Cx 20 normal horse serum blocking 1 rabbit polyclonal anti - connexin 26 antibody (Zymed, CA, U.S.A.) mouse monoclonal anti - connexin 43 antibody (Zymed, CA, U.S.A.) 1 : 100 1 , 2 30 . Vectastatin Universal Elite ABC kit (Vector Laboratories, Burlington, Ontario, Canada) 30 DAB(Vector Laboratories) 5 Mayer 's hematoxylin(Vector Hematoxylin QS, Vector Laboratories) 70%, 80%, 90%, 100% 2 , xylene 3 3 Canadian balsam . 10 (basal layer) (suprabasal layer) , 200 .

(-),
 (focal, ±),
 ++),
 Cx43
 Cx26
 Cx43, Cx26 1
 buffered saline(PBS)
 1 ml TRizol®(GIBCOBRL,
 Grand Island, NY, U.S.A.)
 RNA
 RNA 2 µg 10x Buffer RT 2.0 µ , dNTP Mix
 (5 mM each dNTP) 2.0 µ , Oligo - dT primer(10 µM)
 2.0 µ , RNase inhibitor(10 units/µ) 1.0 µ , Omniscript
 Reverse Transcriptase 2 Unit, RNase - free water가
 20 µ 37 60 , 94 5
 cDNA . PCR Minicycler™
 (MJ research, Waltham, MA, U.S.A.)
 cDNA Taq DNA polymerase 1 Unit(Roche Diag-
 nostics Co., Indianapolis, IA, U.S.A.) primer
 primer
 Cx 26 ; Up 5 ' - TCT - TTT - CCA - GAG - CAA -
 ACC - GC - 3 '
 Down 5 ' - CTG - GGC - AAT - GAG - TTA - AAC -
 TGG - 3 '
 Cx 43 ; Up 5 ' - TAC - CAT - GCG - ACC - AGT -
 GGT - GCG - CT - 3 '
 Down 5 ' - GAA - TTC - TGG - TTA - TCA - TCG -
 GGG - AA - 3 '
 PCR 96 3
 96 30 , 55 30 , 72 30
 30 cycle (extension) 72 5
 mRNA Cx26 725
 bp, Cx43 294 bp

Western blot
 150 mM NaCl, 1% NP40, 0.5%
 DOC(deoxycholic acid), 0.1% sodium dodesyl sulfate
 (SDS), 50 mM Tris - Cl(pH 7.5) RIPA buffer
 1.0 ml , 0 가 가
 , 4 2 . 14,000
 rpm 15

Bio - Rad protein assay(Bio - Rad, Hercules,
 CA, USA)
 sodium dodesyl sulfate(SDS) - polyacrylamide gel
 electrophoresis(PAGE) polyvinylidene
 difluoride(PVDF) membrane(Amersham, Arlington Hei-
 ghts, IL. USA) 4 polyclonal rab-
 bit anti - Cx26 antibody(Zymed Laboratories Inc., CA,
 U.S.A.), monoclonal mouse anti - Cx43 antibody(Zymed
 Laboratories Inc., CA, U.S.A.)
 membrane 0.2% Tween - 20 PBS
 peroxidase - conjugated donkey anti - rabbit
 antibody(Santa Cruz biotechnology, Inc. CA, USA)
 anti - mouse IgG, horseradish peroxidase linked whole
 antibody(Amersham, UK) . 1
 enhanced chemiluminescence detection
 system(ECL, Amersham, UK) X - ray film

Cx43 (Fig. 1, Table 1)
 Cx43

Cx43

가 가

Cx 26 (Fig. 2, Table 2)
 Cx26

Table 1. Expression patterns of Cx43 in human middle ear cholesteatomas (Chole), retroauricular skins (RAS), and ear canal skins (ECS) in immunohistochemical staining (n=10)

	RAS		ECS		Chole	
	B	SB	B	SB	B	SB
-	8	0	7	0	9	0
±	2	2	3	2	1	1
+	0	7	0	8	0	4
++	0	1	0	0	0	5

B : basal cell layer, SB : suprabasal cell layer, - : negative, ± : focal, + : positive, ++ : strong positive

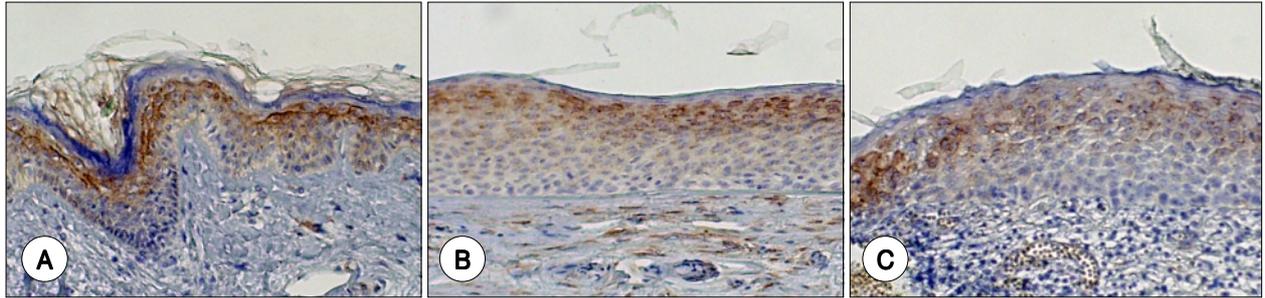


Fig. 1. Immunohistochemistry using primary anti-Cx43 antibody (× 200). A : Retroauricular skin showed positive staining in the spinous and granular layers. B : Ear canal skin showed positive staining in the granular layer. C : Middle ear cholesteatoma showed positive staining (especially strong in the middle portion) in the suprabasal layer, while none was found in the basal layer.

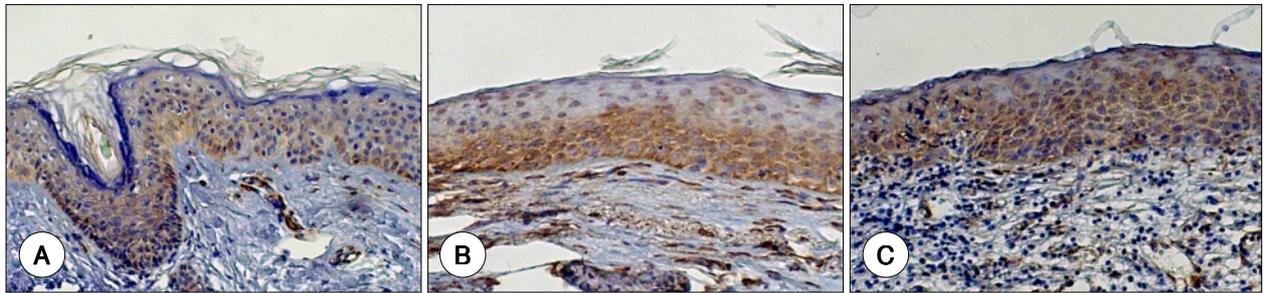


Fig. 2. Immunohistochemistry using primary anti-Cx26 antibody (× 200). A : Retroauricular skin showed focal positive staining in the basal layer. B : Ear canal skin showed positive staining in the spinous and basal layers. C : Middle ear cholesteatoma showed strong positive staining in the suprabasal and basal layers.

Table 2. Expression patterns of Cx26 in human middle ear cholesteatomas (Chole), retroauricular skins (RAS), and ear canal skins (ECS) in immunohistochemical staining (n=10)

	RAS		ECS		Chole	
	B	SB	B	SB	B	SB
-	4	9	3	3	2	3
±	6	1	5	6	2	2
+	0	0	2	1	5	4
++	0	0	0	0	1	1

B : basal cell layer, SB : suprabasal cell layer, - : negative, ± : focal, + : positive, ++ : strong positive

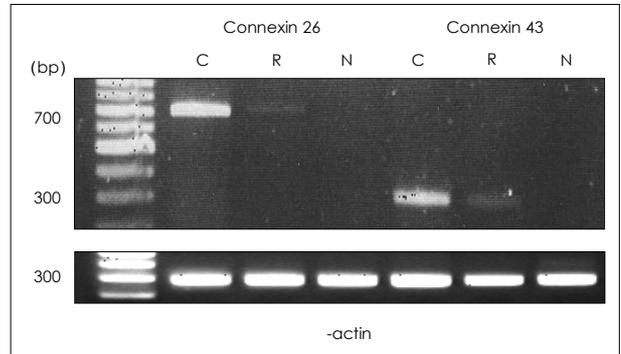


Fig. 3. RT-PCR of human middle ear cholesteatoma and retroauricular skin using Cx26 and Cx43 primers. Cx26 and Cx43 mRNAs were expressed to a greater degree in the cholesteatoma than in the retroauricular skin. C : cholesteatoma, R : retroauricular skin, N : negative control.

가
가
가
mRNA (Fig. 3)
Cx43 Cx26
mRNA 5
Cx43 5 5
mRNA
, Cx26 1
, 4 mRNA

Western blot Cx (Fig. 4)
Cx43 Cx26
5 Cx43
Cx43
, Cx26 3
, 2

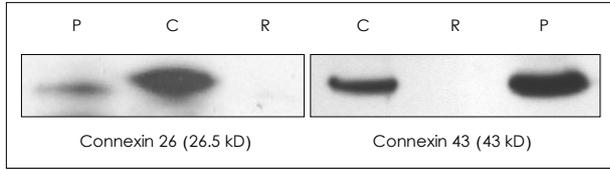


Fig. 4. Western blotting of human middle ear cholesteatoma and retroauricular skin. Cx26 and Cx43 proteins were expressed to a greater degree in the cholesteatoma than in the retroauricular skin. C : cholesteatoma, R : retroauricular skin, P : positive control.

, cAMP, inosi-
tol 1, 4, 5 - triphosphate(IP3) 1000 Da
Na⁺, K⁺, Ca²⁺
second messengers

Cx 30, 31, 37, 45가
Cx 43 (epidermal adnxae)
Cx 43, 26, Cx43, Cx26 (interfollicular epi-dermis)

Cx26¹⁶⁾

Cx26

¹²⁻¹⁴⁾

가

connexin

Cx26 (tape - stripped epi-dermis)¹¹⁾¹²⁾¹⁷⁾

Cx26

가

Cx26

Cx43

Cx43¹⁷⁾

가

Cx43¹⁸⁾

가

Cx26 retinoic acid

Cx43

Cx43

Cx43

Gibson¹⁹⁾

Cx43

Cx43 Cx26

가

가

Cx

Cx26,¹¹⁾

Cx43

Lee²⁰⁾ HeLa

Cx26

Cx26

, Cx

Cx26, Cx43

가

cription)

Cx26, Cx43 mRNA, (trans-lation)

(trans-lation)

Cx43

가

가

Cx26

Cx26

Cx26

Cx43

Cx26

Cx43

mRNA
 가 5
 Western blot
 Cx26,
 Cx43
 mRNA
 Cx26 mRNA 5 1 , 5 2
 Cx26 Cx43 Cx26 가
 가
 Cx26 , Cx26
 Cx43 Cx26
 가 Cx
 : Connexin 26 Connexin 43.

2004

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