

사람 정상 코점막 및 비용 상피세포에서 점액과 리소자임의 발현양상

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Mucin and Lysozyme Expression in the Epithelium of Normal Human Nasal Inferior Turbinate and Polyps

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ABSTRACT

Background and Objectives : In chronic bronchitis, rhinitis or cystic fibrosis, the number of goblet cells increases along with hypertrophy of mucous cells in submucosal gland, resulting ineffective mucociliary clearance. But, it is still not fully understood what role each gene plays in producing airway secretions. This study aimed to figure out which mucin gene is expressed in the epithelium of normal human nasal mucosa and nasal polyps, and to verify whether the epithelium of nasal polyp itself contributes to the increased nasal secretion as in chronic sinusitis with nasal polyp. **Materials and Methods** : Normal nasal epithelial cells were obtained from the inferior turbinates of ten normal healthy volunteers, and nasal polyps from six patients who underwent sinus surgery. The epithelial cells were enzymatically isolated, and intracellular levels of mucin and lysozyme were measured from cell lysates using immunoblot assay. And, RT-PCR was used for the detection of mucin mRNA and lysozyme mRNA. **Results** : The level of intracellular mucin was 2.9 times higher in the epithelium of nasal polyp, and this was statistically significant. Among seven mucin genes (MUC1, 2, 4, 5AC, 5B, 7, 8) expressed in the epithelium of normal inferior turbinate and polyps, MUC2 and MUC8 were more strongly expressed in the epithelium of nasal polyp than those of normal inferior turbinate. **Conclusion** : This results suggest that the polyp epithelium itself is contributing to increased secretion in chronic sinusitis, and MUC2 and MUC8 are thought to be responsible for this change. However, further study is required to uncover the full sequence of MUC8 mRNA and its exact function. (**Korean J Otolaryngol 2000;43:377-82**)

KEY WORDS : Mucin Lysozyme · Mucin gene · Nasal epithelium · Polyp.

Clara
 가
 가 (transitional phenotype)
 가¹⁾
 가 가 ,
 가²⁾³⁾
 가
 가
 가⁴⁻⁵⁾ 10 30 MDa
 가

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serine, threonine¹⁻³⁾ proline⁶⁾ 1% pronase가 가 (DME : F12=1 : 1) . 16 18

9가 (MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC8)⁴⁻⁵⁾⁷⁻¹²⁾ 37 1

MUC1, MUC2 MUC7 6가 가

가 가¹³⁾ 가

1% Tween - 20(Bio - Rad, Hercules, CA, USA), 1 mM EDTA protease inhibitors(Complete, Berhringer Mannheim, Indianapolis, IN, USA)가 가 50 mM Tris buffered saline Gray immunoblot assay¹⁴⁾ (generous gift from Dr. Davis CW, University of North Carolina, NC, USA) (Sigma, St. Louis, MO, USA) H6C5(a gift from Dr. C.W. Davis) , (DAKO, Carpintera, CA, USA)

가 가 mRNA horseradish peroxidase conjugated goat antimouse IgG antirabbit IgG , chemilumi - nesc - ence(ECL kit ; Amersham, Bucking - hamshire, UK)

10 Western blot ± student's t - test

6 mRNA reverse transc - ription polymerase chain reaction Northern blot mRNA 가가 가 E PCR Guzman RT - ¹⁶⁾ Oligonucleotide primers MUC1

(Genbank accession #J05582, 388 bp, 5' primer : TCTCACCTCCTCCAATCAC ; 3' primer : GAAATG-GCACATCACTCAC) ; MUC2(Genbank accession #L21998, 440 bp, 5' primer : TGCCTGGCCCTGTC-TTTG ; 3' primer : CAGCTCCAGCATGAGTGC) ; MUC4(Genbank accession #AJ000281, 466 bp, 5' primer : TTCTAAGAACCACCAGACTCAGAGC ; 3' primer : GAGACACACCTGGAGAGAATGAGC), MUC5AC(Genbank accession #U06711, 680 bp, 5' primer : TCCGGCTCATCTTCTTCC ; 3' primer : ACTTGGGC-ACTGGTGCTG) ; MUC5B(Genbank accession #Z72496, 338 bp, 5' primer : ACTCCAGAGACTGTCCACAC ; 3' primer : TACCACTGGTCTGTGTGCTA) ; MUC7(Genbank accession #L132283, 209 bp, 5' primer : CCACACCTAATTCTTCCC ; 3' primer : CTATTGCTCCACCATGTC) ; MUC8(Genbank accession #U14383, 239 bp, 5' primer : ACAGGGTTTCTCCTCATTG ; 3' primer : CGTTTATTCCAGCACTGTTC) ; (Genbank accession #J03801, 350 bp, 5' primer : CTCTCATTGTTCTGGGGC ; 3' primer : ACGGAC-AACCCTCTTTGC)

primer (Table 1). RT-PCR
335 bp 2 microglobulin(2M) oligonucleotide amplimers(Clontech Laboratory, CA, USA)
. RT-PCR Perkin Elmer Cetus DNA Thermal Cycler

Table 1. Primers used for polymerase chain reaction amplification

Gene	Genbank no.	Product (bp)	Primer s equence
MUC1	J05582	368	F : TCTCACCTCCTCCAATCAC R : GAAATGGCACATCACTCAC
MUC2	L21998	440	F : TGCCTGGCCCTGTCITTTG R : CAGCTCCAGCATGAGTGC
MUC4	AJ000281	466	F : TTCTAAGAACCACCAGACTCAGAGC R : GAGACACACCTGGAGAGAATGAGC
MUC5AC	U06711	680	F : TCCGGCTCCATCTTCTCC R : ACTTGGGC ACTGGTGCTG
MUC5B	Z72496	338	F : ACTCCAGAGACATGTCCACAC R : TACCACTGGTCTGTGTGCTA
MUC7	L132283	209	F : CCACACCTAATTCTTCCC R : CTATTGCTCCACCATGTC
MUC8	U14383	239	F : ACAGGGTTTCTCCTCATTG R : CGTTTATTCCAGCACTGTTC
Lysozyme	J03801	350	F : CTCTCATTGTTCTGGGGC R : ACGGACAACCCTCTTTGC

total RNA(1 µg/20 µl reaction volume)
random hexanucleotide primers Moloney murine leukemia virus reverse transcriptase cDNA
40%, 2M 4% 0.2 mM
. PCR
95 1 , MUC4,
MUC7, MUC8 55 , MUC2, MUC5AC
2M 60 1 , 72 1
. PCR 50 ng/ml ethidium bromide
2% Seakem agarose gel(FMC, Rockland, ME, USA)
55
mRNA
. PCR
PCR . MUC2
27 35 , MUC4 27 40 , MUC5AC
32 40 , MUC5B 23 29 , MUC7 MUC8 32
40 , 25 30 , 2M 26 30
mRNA genomic DNA
RT rev-
erse transcriptase
, RT-PCR PCR fragment sequenc-
ing(dsDNA Cycle Sequencing System, GIBCO-BRL, Gaithersburg, MD, USA)

10⁶ 2918.0
±1931.9 µg
10⁶ 1001.5 ± 263.8 µg
2.9 가
(p<0.05)(Fig. 1).
10⁶ 56.6 ± 28.1 µg
34.4 ± 20.3 µg
(Fig. 2).
mRNA
MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC7,
MUC8 mRNA가
MUC2 mRNA가
6 3 , MUC8 mRNA가 6 5

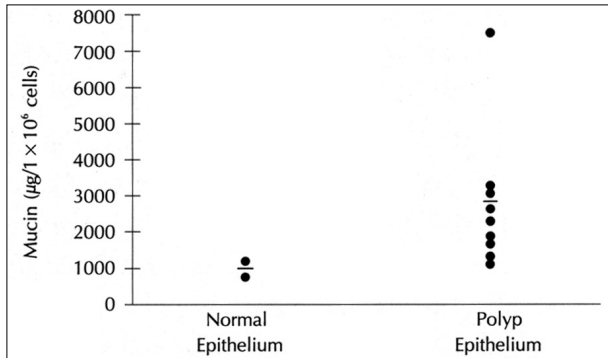


Fig. 1. Level of intracellular mucin in the epithelial cell of normal human inferior turbinate mucosa and nasal polyp measured by immunoblot. Mean value of intracellular mucin in the epithelial cells of nasal polyp was $2918.0 \pm 1931.9 \mu\text{g}/10^6$ cells, which is significantly higher than $2918.0 \pm 1931.9 \mu\text{g}/10^6$ cells of inferior turbinate mucosa ($p < 0.05$). (- : mean value)

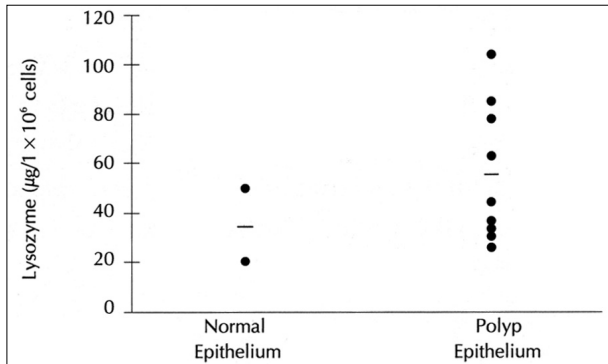


Fig. 2. Level of intracellular lysozyme in the epithelial cell of normal human inferior turbinate and nasal polyp measured by immunoblot. Mean value of intracellular mucin in the epithelial cells of nasal polyp was $56.6 \pm 28.1 \mu\text{g}/10^6$ cells, which is slightly higher than $34.4 \pm 20.3 \mu\text{g}/10^6$ cells of inferior turbinate, which was not statistically significant. (- : mean value)

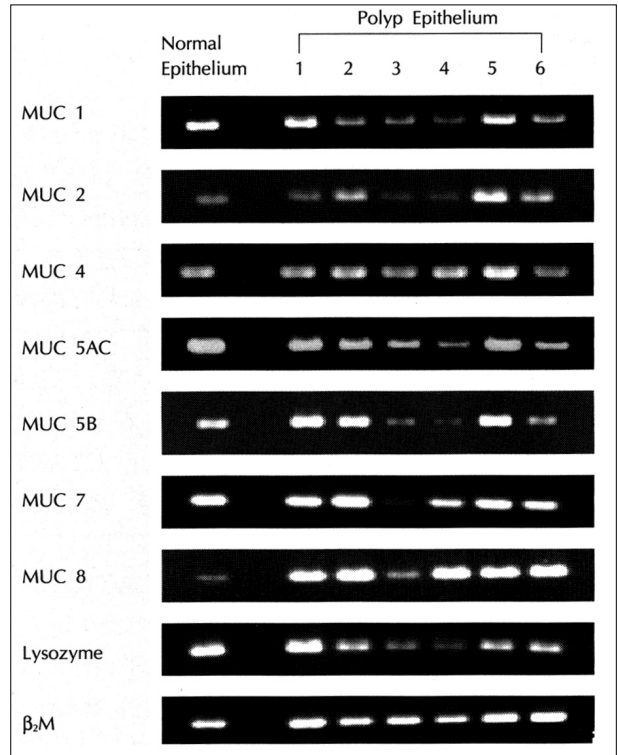


Fig. 3. Expression of mRNA of mucin and lysozyme in the epithelium of normal human inferior turbinate and nasal polyp. The mRNAs of MUC2 and MUC8 are more strongly expressed in the epithelium of nasal polyp compared to that of inferior turbinate, among seven expressed mucin genes (MUC1, 2, 4, 5AC, 5B, 7, 8).

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(Fig. 3).

가

Periodic Acid - Schiff(PAS)

가

1)

가

가

가

가

20)

가

가

200 1000 nm

가

21)

가

가

22)

MUC8 mRNA 가 MUC8 mRNA가
 가
 MUC8- 가
 MUC8 mRNA
 MUC8 mRNA MUC8 cDNA

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