

# 사람 정상 코점막 상피세포에서 겔 형성 점소(Gel-Forming Mucin) 분비에 대한 Prostaglandin E<sub>2</sub>의 효과

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## Effect of Prostaglandin E<sub>2</sub> on Gel-forming Mucin Secretion in Normal Human Nasal Epithelial Cells

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### ABSTRACT

**Background and Objectives** : Recently, Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) was found to induce MUC5AC production via an agonist of E-prostanoid (EP2/EP4), but not EP1/EP3, in normal human airway epithelium. However, the receptor that mediates MUC5AC has not been determined. This study was aimed to investigate the MUC5AC mucin gene and mucin secretion by PGE<sub>2</sub> and its receptors in cultured normal human nasal epithelial cells. **Materials and Method** : After treatment with PGE<sub>2</sub> and/or PGE<sub>2</sub> antagonist, gel-forming mucin mRNA expression was determined by reverse transcription-polymerase chain reaction. Total mucin and MUC5AC mucin levels were measured using an immuno-dot blotting assay. **Results** : PGE<sub>2</sub> increased the MUC5AC gene expressions and MUC5AC mucin, but not the expressions of other gel-forming mucin genes. An EP2 receptor antagonist (AH6809) did not suppress the PGE<sub>2</sub>-induced MUC5AC gene expression or MUC5AC mucin. However, an EP4 receptor antagonist (AH23848) significantly suppressed the level of PGE<sub>2</sub>-induced MUC5AC gene expression and MUC5AC mucin. **Conclusion** : These findings indicate that PGE<sub>2</sub> induces MUC5AC gene expression and mucin secretion via EP4 receptor in cultured normal human nasal epithelial cells. (Korean J Otolaryngol 2006;49:307-12)

**KEY WORDS** : Prostaglandin E<sub>2</sub> · Mucin · Secretion · Prostaglandin receptor.

가  
가  
(mucus) , , , ,  
(mucin) 20가  
(domain) (membrane -  
bound mucins) (gel - forming mucins)  
MUC1, MUC3, MUC4, MUC11, MUC12,  
MUC17, MUC18, MUC20 MUC2,  
MUC5AC, MUC5B, MUC6, MUC7, MUC9, MUC19  
MUC8<sup>2)</sup>

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가

PGE<sub>2</sub>로 유도된 점소 유전자 발현과 분비

가 medium(BEGM) Dulbecco 's modified Eagle 's me-  
 dium(DMEM) 1 : 1 <sup>7)</sup> 9

Prostaglandins(PGs) ALI PGE<sub>2</sub> 9

EP(E - prostanoid) PGE<sub>2</sub> 1 × 10<sup>-7</sup>M PGE<sub>2</sub> 24 PGE<sub>2</sub> 2

EP1 EP4 가가 AH 6809(EP2 recep-  
 tor antagonist, Sigma - Aldrich Inc., St. Louis., MO)

EP2 AH 23848(EP4 receptor antagonist, Sigma - Aldrich  
 Inc., St. Louis., MO)

EP4 TNF - , IL - 1 , lipopolysaccharide 1 × 10<sup>-5</sup>M AH 6809 AH  
 23848 PGE<sub>2</sub> 2 RNA 24

(arachidonic acid) PGE<sub>2</sub> (phase contrast microscopy)

EP2 EP4 2 , 7 , 14 , 21 RNA

PGE<sub>2</sub> PGE<sub>2</sub>가 EP2 EP4 Trisol<sup>®</sup>

MUC5AC, MUC5B, MUC6, MUC7, EP2 and EP4 mRNA  
 reverse transcription - polymerase chain reac-  
 tion(RT - PCR)

Air - liquid interface(ALI) Oligonucleotide primer Table 1

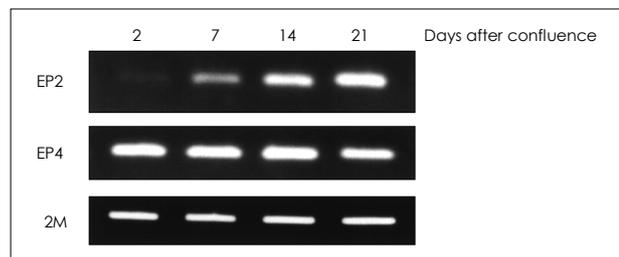
2 × 10<sup>5</sup> (passage - 2) microglobin oligonucleotide amplimer RT - PCR  
 (Transwell - clear, 24.5 mm, 0.45 μm pore size, Costar Co., Cambridge, MA) 0.5 ml , Clontech Laboratories(They generated  
 a 335 bp PCR fragment) . RT - PCR  
 basal epithelial growth Perkin Elmer Cetus DNA Thermal Cycler

**Table 1.** Designed primers used for PCR amplification

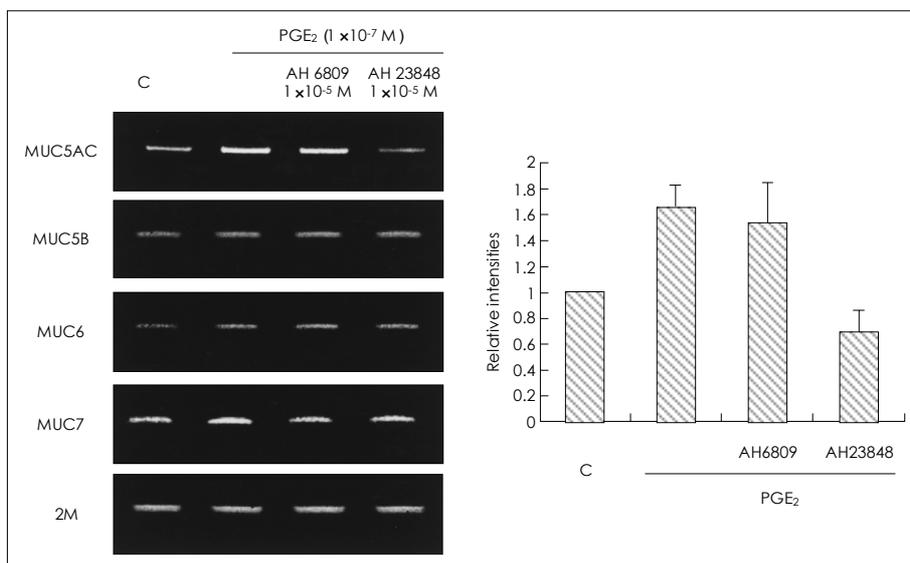
Name	Sequence	Annealing temperature	Cycle	Size
MUC5AC	F- CGA CAA CTA CTT CTG CGG TGC R- GCA CTC ATC CTT CCT GTC GTT	60	33	360 bp
MUC5B	F- ACT CCA GAG ACT GTC CAC AC R- TAC CAC TGG TCT GTG TGC TA	55	30	338 bp
MUC6	F- TCA CCT ATC ACC ACA CAA C R- GGA GAA GAA GGA AAA AGA G	55	30	293 bp
MUC7	F- CCA CAC CTA ATT CTT CCC R- CTA TTG CTC CAC CAT GTC	55	30	209 bp
EP2	F- CGA GAC GCG ACA GTG GCT TCC R- CGA GAC GCG GCG CTG GTA GA	60	30	409 bp
EP4	F-TCG CGC AAG GAG CAG AAG GAG AC R-GAC GGT GGC GAG AAT GAG GAA GGA	60	30	468 bp
2M	F- TCG CGC TAC TCT CTC TTT CTG G R- GCT TAC ATG TCT CGA TCC CAC TTA A	55	28	360 bp

mRNA  
(Comparative kinetic analysis)<sup>7)</sup>  
 PCR 50 ng/ml ethidium bromide 2%  
 Seakem agarose gel(FMC, Rockland, ME)  
 55  
 (negatives) Molecular Dynamics Densitometer  
 (Sunnyvale, CA) (signal) ImageQuant  
 software (amplified products)  
 mRNA (reverse transcriptase)  
 PCR  
 (negative control)  
 PCR sequencing(ABI  
 3100 Genetic Analyzer, Applied Biosystem, Foster City,  
 CA)  
 MUC5AC Immunodetection  
 Immuno - dot blotting  
<sup>8)</sup> 17Q2(1 :  
 2000 ; Covance Inc., Berkely, CA) MUC5AC  
 anti - human MUC5AC antibody(1 : 1000 ; NeoMarkers  
 Inc., Fremont, CA)  
 (horseradish pe-  
 roxidase - conjugated goat anti - mouse anti - rabbit  
 IgG) chemiluminescence(ECL kit, Amer-  
 sham, Little Chalfont, UK)

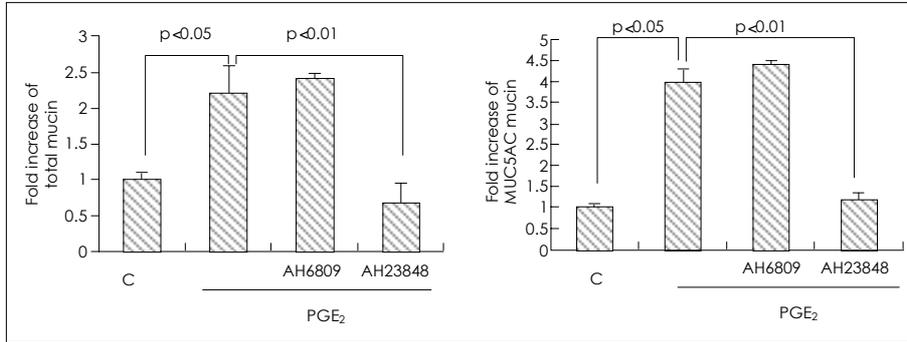
Student *t* -  
 test(SPSS ver. 11.5 Chicago, IL) (p<  
 0.05).  
 ±  
 mRNA  
 EP2 EP4  
 mRNA  
 EP2 EP4  
 가  
 EP2  
 가  
 (Fig. 1).  
 EP4 mRNA  
 EP2 mRNA  
 RT - PCR



**Fig. 1.** EP2 gene expression increased as a function of mucociliary differentiation. However, EP4 gene expression was not altered as a function of differentiation.

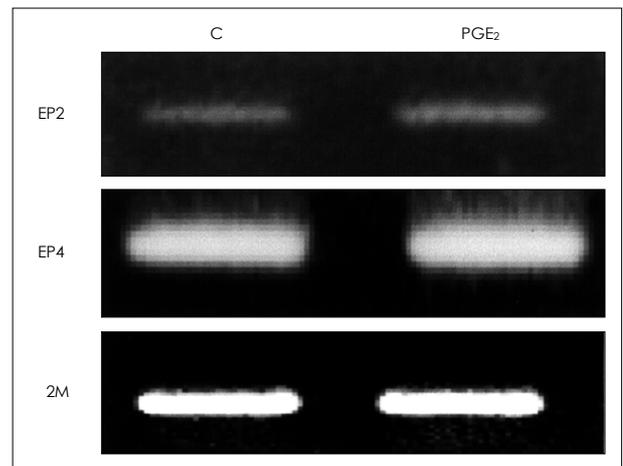


**Fig. 2.** Effects of prostaglandin E<sub>2</sub> on gel-forming mucin gene expressions and inhibitory effects of PGE<sub>2</sub> receptor antagonists. PGE<sub>2</sub> increased only MUC5AC gene expression, but not the expression other gel-forming mucin genes. An EP2 receptor antagonist (AH6809) did not suppress PGE<sub>2</sub>-induced MUC5AC gene expression. However, an EP4 receptor (AH23848) antagonist significantly suppressed MUC5AC gene expression.



**Fig. 3.** Effect of PGE<sub>2</sub> on total mucin and MUC5AC mucinsecretion. PGE<sub>2</sub> increased total mucin secretion and promoted a 4-fold increase of MUC5AC mucin secretion. An EP4 antagonist (AH23848) significantly suppressed both total mucin and MUC5AC mucin secretion.

EP2 mRNA 가  
2  
PGE<sub>2</sub> PGE<sub>2</sub>  
, PGE<sub>2</sub>  
MUC5AC 1.68 가  
EP2 (AH6809) PGE<sub>2</sub>  
MUC5AC EP4  
(AH23848) MUC5AC  
(Fig. 2).  
PGE<sub>2</sub>가 EP4 MUC5AC  
가



**Fig. 4.** Detection of EP (E prostanoïd) mRNA expression in cultured NHNE cells. PGE<sub>2</sub> did not affect EP2 or EP4 gene expression.

MUC5AC PGE<sub>2</sub> PG  
PGE<sub>2</sub> 가 MUC5AC  
4 가 EP4 MUC5AC (Fig. 4).  
MUC5AC  
(Fig. 3). MUC5AC 가 PGE<sub>2</sub>  
가 EP4  
MUC5AC 가  
(arachidonic acid)  
cyclooxygenase PGs thromboxanes  
(prostanoids)  
PGE<sub>2</sub>  
10) IL - 1 TNF -  
PGE<sub>2</sub> 11)

autocrine paracrine  
MUC5AC MUC8  
가 5)12 - 15)  
, PGE<sub>2</sub>가 16)  
17) EP2 EP4 가

EP4, PGE<sub>2</sub>가 MUC2

EP, EP가

EP2가

EP4

EP2

EP4

Gray<sup>4)</sup> (misoprostol) EP2 EP4

EP2 EP4가 PGE<sub>2</sub> MUC5AC

PGE<sub>2</sub> MUC5AC EP2

level) PGE<sub>2</sub>가

PGE<sub>2</sub> EP4

MUC5AC PGE<sub>2</sub>가 EP4

Takahashi<sup>16)</sup> PGE<sub>2</sub>가 EP4

Belly Chadee<sup>17)</sup> PGE<sub>2</sub>가 EP4 (exocytosis)

MUC5AC 1.68가 MUC5AC (transcriptional level)

MUC5AC (post-transcriptional level)

가

가 EP2 EP4

EP2 EP4 PGE<sub>2</sub> MUC5AC (activating downstream signaling molecules)

PGE<sub>2</sub> (transcriptional level) MUC5AC

EP4

EP4

<sup>18)</sup> EP4

가

EP2 EP4가 PGE<sub>2</sub>, PGE<sub>2</sub>

MUC5AC

가

EP4

: PGE<sub>2</sub> . . . PG

Korean Research Foundation(KRF - 2004 - 015 - E00074)

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