

인체 정상 코점막 상피세포에서 p38 MAP Kinase의 신호전달과정이 MUC8 유전자 발현 유도에 미치는 영향

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The Role of p38 MAP Kinase Signal Transduction in the Induction of MUC 8 Gene Expression in Normal Human Nasal Epithelial Cells

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ABSTRACT

Background and Objectives : In cystic fibrosis, chronic bronchitis, and asthma, the mucociliary mechanism is impaired when mucin is produced excessively. The mRNA encoding MUC8 has been shown to be the major up-regulated mucin under inflammatory condition and is likely to contribute to the airway mucus plugging characteristic of these diseases. The aim of this study is to determine the intracellular signaling pathway directly involved in the MUC8 regulation following inflammatory mediator treatments. **Materials and Method** : Passage-2 normal human airway epithelial cells were used in all experiments. Inflammatory signal-induced MAP kinase activity was measured by Western blot analysis using phosphospecific anti-active MAP kinase antibodies. Inflammatory signal-induced MUC8 expression was measured in the absence or presence of SB203580 by the semi-quantitative RT-PCR. **Results** : Inflammatory stimuli such as LPS, TNF- α , and IL-1 β activated the p38 MAP kinase and subsequently up-regulated the MUC8 expression. Interestingly, the TNF- α or IL-1 β - inducibility of the MUC8 gene expression was greatly enhanced by specific inhibition of the p38 MAP kinase by using SB 203580. **Conclusion** : These results suggest that the intracellular p38 MAP kinase activity is a negative regulator for the MUC8 up-regulation in human nasal epithelial cells following inflammatory stimuli. (**Korean J Otolaryngol 2000;43:719-23**)

KEY WORDS : Airway epithelial cells · p38 MAP kinase · MUC8 · Signal transduction.

(hyper - secretion) ⁶⁾⁷⁾ 가 ⁸⁾

mucociliary clearance ¹⁾ 가

가 가

²⁻⁵⁾ ⁹⁻¹⁷⁾ 12 가

mucociliary cle - arance 가

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(pathological mucin) 가

p38 MAP Kinase가 MUC 8

mucin gene 8(MUC8) 가 bronchial epithelial growth media(Clontics Corp. San Diego, CA, USA) Dulbecco's modified Eagle's medium(DMEM, Gibco - BRL, Gaithersburg, MD, USA) 가 가

¹⁸⁾¹⁹⁾ MUC8 cDNA , 1 : 1 가 가

가 MUC8 passage - 2
mitogen - activated MUC8 LPS(μ g/ml), IL - 1 (10 ng/ml), TNF - (10 ng/ml) 24
protein(MAP) kinase family , total RNA TRiZol agent
single stranded cDNA ¹⁸⁾

²⁰⁾ MAP kinase family extracellular signal regulated kinase(ERK), c - jun NH₂ terminal kinase (JNK)/stress - activated protein kinase(SAPK) cDNA MUC8 primer(sense : 5' - ACA GGG TTT CTC CTC ATT G - 3' ; antisense : 5' - CGT TTA TTC CAG CAC TGT TC - 3')
p38 MAP kinase 3가 subfamily가 cDNA 2
microglo - bulin primer(Sense : 5' - GCT TAC ATG TCT CGA TCC CAC TTA A - 3' ; antise - nse : 5' - CTC GCG CTA CTC TCT CTT TCT GG - 3') ¹⁸⁾ P38 MAP kinase
MUC8

²¹⁾ JNK/SAPK p38 MAP kinase , LPS, IL - 1, TNF - SB 203580(40 μ M) 4 RT - PCR
가 ²²⁾ MAP kinase SB 203580
MAP kinase oxidative p38 MAP kinase
stress epidermal growth factor receptor ²³⁾

ERK MAP kinase activity assay
MUC5AC 가 ¹⁰⁾ MAP kinase
Pseudomonas aeruginosa MAP kinase passage - 2
MUC2 ERK가 가 LPS , IL - 1 , TNF -
¹³⁾ 0, 15, 45 PBS
MUC8 SDS - PAGE sample buffer 5
MUC8 . MAP kinase family
가가 subfamily MAP kinase (ERK Thr²⁰²/Tyr²⁰⁴ , SAPK/
JNK Thr¹⁸³/Tyr¹⁸⁵ p38 Thr¹⁸⁰ /Tyr¹⁸²) (New
England Biolab, Inc., Beverly, MA, USA)
form specific
in vitro complex ki -
(Transwell - nase assay ²²⁾ sample
clear Costar Corp. Cambridge, MA, USA) air - liq -
uid interface(ALI) tubulin antibody We -
stern blot . Western blot

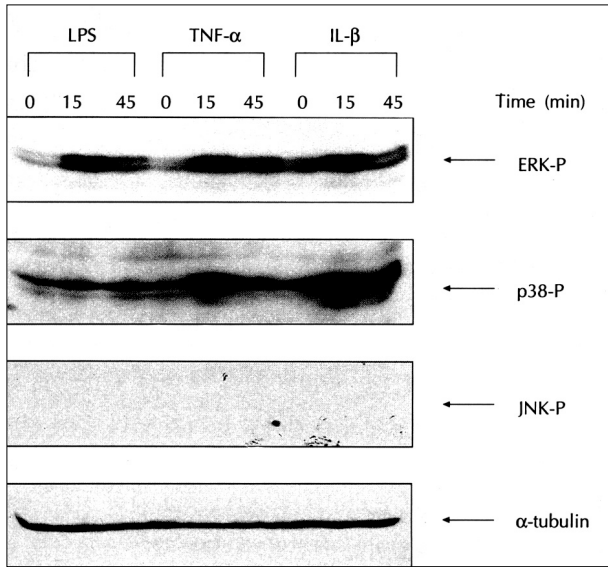


Fig. 1. Activation of MAP kinase family by various inflammatory stimuli. Following treatment of LPS (5 μ g/ml), TNF- α (10 ng/ml), and IL-1 β (10 ng/ml), cells were harvested, and soluble fraction (100 μ g/lane) was analyzed by Western blot analysis using phosphospecific anti-active form antibodies as described in "Materials and methods". The data were representative of three separate experiments. Anti- α -tubulin antibody was used as a loading control.

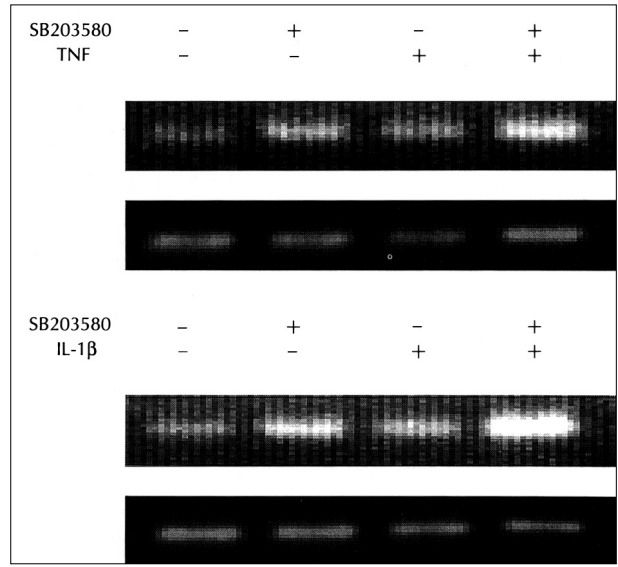


Fig. 2. Effects of SB203580 on TNF- α and IL-1 β -induced MUC8 gene expression. TNF- α and IL-1 β -induced MUC8 gene expression was enhanced by specific inhibition of p38 MAP kinase. Following treatment of TNF- α and IL-1 β , cells were subjected to total RNA extraction. In case of p38 MAP kinase inhibition, cells were pretreated for 4 hours in the presence of SB203580 (40 μ M). The cDNA was synthesized and an aliquot was subsequently amplified using specific primer pairs for MUC8 gene. Constitutively expressed β -2 microglobulin was also amplified as a control. The PCR products were separated on ethidium bromide-agarose gel. The amounts of PCR products were quantified by CSC-camera based densitometry and are prepared as the MUC8 expression (first and third panels) relative to constitutive β -2 microglobulin gene expression (second and fourth panels).

21)22)

MAP kinase MUC8

MAP kinase family LPS, IL-1, TNF- α , IL-1 β

0, 15, 45

(Fig. 1).

MAP kinase family ERK, p38, JNK

15

p38 MAP kinase

LPS

p38

15

TNF- α

IL-1 β

45

SAPK/JNK

MAP kinase

MAP kinase

MAP kinase subgroup

p38 MAP kinase kinase

가

가

p38 MAP kinase가 MUC8

MUC8 가

IL-1 TNF- α 가 p38 MAP kinase

IL-1 TNF- α

p38 MAP kinase MUC8

TNF- α MUC8 mRNA 가 IL-1 p38

MAP kinase RT-PCR

MAP kinase family p38

MAP kinase SB203580

23)

SB203580

DMSO

(DMSO)

p38 MAP Kinase가 MUC 8

IL - 1 TNF - MUC8
 가 , SB203580
 IL - 1 TNF - MUC8
 가 (Fig. 2).
 SB203580 MUC8
 가 basal level p38 MAP
 kinase MUC8
 MUC8 IL - 1 TNF -
 negative regulator 가 p38 MAP kinase

가

2-5) 가

가 MUC8
 19) 가

18) MUC8

MUC8

MUC8
 가
 MAP kinase family
 p38 MAP kinase가
 가

SB203580 IL - 1 TNF -
 MUC8 p38 MAP kinase
 negative regulator
 MUC8 p38 MAP
 kinase가 MUC5AC
 epidermal growth factor receptor 가
 TGF - MUC5AC
 가 11)

TNF -
 가 Epidermal growth factor rec-
 eptor ERK
 MUC5AC ERK
 가 oxidative stress ligand - indepe -
 ndent epidermal growth factor receptor MU -
 C5AC 가 ERK up - stream kinase MEK
 PD98059
 10) MUC2 ERK
 13) MUC2 MUC5AC

ERK
 MUC8

가

ERK MUC8
 MEK PD98059
 MUC8

MUC5AC
 p38 MAP kinase
 p38 MAP kinase MUC2 MUC5AC
 ERK 가

가
 MUC8 promoter region
 reporter construct MAP kinase do -
 minant - negative construct cotransfection
 reporter assay p38 MAP kinase
 MUC8

MUC8

p38 MAP kinase MUC8

1996

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