# Regulation of MUC5AC expression by hypoxia in human airway epithelia

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# Regulation of MUC5AC expression by hypoxia in human airway epithelia

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Woo-Chul Shin is approved.

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ABSTRACT

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Hypoxia due to occlusion of sinus ostium is one of the major pathologic mechanisms of the sinusitis. In sinusitis, the expression of mucin genes such as MUC5AC, MUC5B is increased. Hypoxia-inducible factor 1 (HIF-1) is well known transcriptional factor, which induces cell response to the hypoxic condition and activates the transcriptions of several genes, such as erythropoietin and vascular endothelial growth factor. This transcriptional factor binds to the hypoxia-response element (HRE) in the promoter of certain target gene. A similar sequence to hypoxia-response element is also found in the promoter of MUC5AC, which is major mucin gene in the airway.

The aim of this study is to identify if hypoxia upregulates MUC5AC expression by binding of HIF-1α to putative HRE on the promoter of MUC5AC gene.

Here we show that the expression of mRNA and secreted protein of MUC5AC according to increased expression of HIF-1α protein as a fuction of time under hypoxia in normal human nasal epithelial cells.

Our results also show that the expression of MUC5AC mRNA was increased by the transfection of mammalian expression vector encoding HIF-1α under

normoxic condition in human lung mucoepidermoid carcinoma cell lines (NCI-H292 cells).

The luciferase assay for MUC5AC promoter demonstrated increased reporter activity under hypoxic condition, however, the mutation of the putative HRE in MUC5AC promoter attenuated the reporter activity.

In conclusion, hypoxia can upregulate MUC5AC expression by binding of HIF-1α to HRE in the promoter of MUC5AC. Taken together, hypoxia upregulates mucin gene expression and secretion by signaling using HIF-1α in the airway epithelia and hypoxia might be a pathophysiologic mechanism of hypersecretion in airway disease such as sinusitis.

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Key words : hypoxia, hypoxia-inducible factor, hypoxia-response element, MUC5AC, airway epithelium

# Regulation of MUC5AC expression by hypoxia in human airway epithelia

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## I. INTRODUCTION

In the pathophysiologic mechanism of sinusitis, hypoxia in the sinus cavities is considered as a major factor that causes progression of the disease. This hypoxia reflects occlusion of the sinus ostium which results in failure of transepithelial oxygenation, nonvascularized exudates, and the tendency of inflammatory hyperplasia to exceed neovascularization.[1] The severity of sinus mucosal disease which correlates with opacity on computed tomography scan is reported to be related to the level of oxygen in the sinuses of patients with acute sinusitis.[2] In chronic sinusitis, the tissue hyperplasia and polyp growth can overwhelm neovascularization, further impacting local tissue hypoxia.[1] When the bulk of tissue and secretion becomes great enough, the sinuses become occluded and oxygenation of the sinus cavity is decreased.

Mucus hypersecretion is also a major factor in the pathogenesis of the sinus disease. Kim *et al*.[3] and Ding *et al*.,[4] using reverse-transcription polymerase chain reaction, found that levels of MUC5AC and MUC5B mRNA in chronic rhinosinusitis were significantly increased compared with those in normal sinus mucosa. In addition, Ali *et al*.[5] showed that the main mucins secreted were

MUC5AC and MUC5B, and that the mean level of MUC5AC expression was higher compared to MUC5B in sinus mucus secretion. MUC2 was also detected but an inverse relationship between sinus MUC5AC and MUC2 was observed in majority of the cases.

It has been reported that many infectious mediators and inflammatory/ immune response mediators activate the transcription of the mucin gene. MUC5AC and MUC2 genes have several putative NF-κB *cis*-elements in their promoters and *P. aeruginosa* upregulates MUC5AC expression following the activation of the p42/44 MAPK pathway via an EGFR signaling cascade.[6] Ligand-dependent activation of EGFR increases transcription of MUC2[7] and MUC5AC[8] genes. TNF-α also regulates expression of MUC5AC at the transcriptional level. IL-1β,[9] an inflammatory mediator expressed during early inflammation, induces MUC2 and MUC5AC expression in NCI-H292 or normal human nasal epithelial (NHNE) via MAPK activation of both ERK1/2 and p38 pathways.[10] Furthermore, IL-6 and IL-17 increase MUC5AC and MUC5B mRNA steady-state expression in NHBE cells[11] and IL-13 has been recently reported to increase the promoter activity of MUC5AC in clara cells,[12] while IL-9 increases MUC5AC expression in NCI-H292 cells.[13]

In contrast to the numerous studies performed on the relationship between mucin genes and inflammatory response, the mechanism for hypoxia-mediated regulation of transcription of mucin genes has not been investigated in the airway epithelia. It is believed that hypoxia-inducible factor 1 (HIF-1), a basic helix-loop-helix transcription factor of the PAS family[14] plays an essential role in the cellular response to hypoxic stress by transcriptional activation of various hypoxia-inducible genes, such as those encoding erythropoietin,[15, 16] vascular endothelial growth factor,[17] glycolytic enzymes,[18] glucouse transporters,[18] inducible nitric-oxide synthase,[19] heme oxygenase-1,[20] and transferrin,[21] in order to maintain oxygen homeostasis. HIF-1 is a heterodimer which is composed of an alpha and a beta subunit,[22] the latter being a constitutively-expressed aryl

hydrocarbon receptor (ARNT).[23] The activity of the HIF-1 is primarily determined by hypoxia-induced stabilization of HIF-1α which becomes rapidly degraded through the ubiquitin-proteasome pathway.[24] In hypoxia, stabilized HIF-1α dimerizes with HIF-1β and binds to the hypoxia-response element (HRE) in order to recruit the transcription coactivator p300/CBP onto the promoter of hypoxia-responsive genes for transcriptional activation.[24-26]

HRE is usually located at the proximal promoter (~100bp) which contains one or more of the HIF-1 binding sites (consensus sequence 5'-[A/G] CGTG-3').[27] Mutation of HRE results in the loss of transcriptional response to hypoxia.[28] The presence of an HIF-1 binding site is necessary but not sufficient for hypoxia-response-element function.[28, 29]

We have noticed that the promoter region of the MUC5AC gene, sequenced by Basbaum *et al.*,[30] contains a sequence very similar to the hypoxia response element within a 70bp sequence upstream of the transcriptional start site. Therefore, in this study, we aimed to first identify this sequence as a functionally active hypoxia response element and then prove the mechanism of hypoxia-induced MUC5AC gene regulation.

## II. MATERIALS AND METHODS

### 1. Cell culture

Passage-2 NHNE cells were prepared as described previously.[31] Passage-2 NHNE cells (1 x $10^5$ cells) were seeded in 0.5 ml of culture medium on 24.5 mm, 0.45 μm pore size, Transwell-clear (Costar Co., Cambrideg, MA, USA) culture inserts. Cells were cultured in a 1:1 mixture of bronchial epithelial growth medium (BEGM): Dulbeco's modified Eagle's medium (DMEM) containing all supplements.[32] The cultures were grown submerged for the first 9 days, during which time the culture medium was changed on Day 1 and every other day thereafter. An air-liquid interface (ALI) was created on Day 9 by removing the apical medium and restricting the culture feeding to the basal compartment. Following ALI creation the culture medium was changed daily. The cultured epithelial cells were treated with hypoxia on Day 16 of culture.

The human lung mucoepidermoid carcinoma cell lines (NCI-H292) were purchased from the American Type Culture Collection (CRL-1838: Manassas, VA ,USA) and cultured in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum in the presence of penicillin/ streptomycin at 37°C in a humidified chamber with 5% $CO_2$. For serum deprivation, confluent cells were washed twice with phosphate-buffered saline and recultured in RPMI with 0.2% fetal bovine serum. Hypoxic condition was produced by Forma 1029 anaerobic chamber (Thermo Fisher scientific, Waltham, MA, USA) with 95% $N_2$, 5% $CO_2$.

### 2. RNA analysis

Total RNA was isolated from NHNE cells and NCI-H292 lung cancer cell line using TRIzol (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized with random hexamer (PerkinElmer Life Sciences, Waltham, MA, USA) using Moloney murine leukemia virus-reverse transcriptase (PerkinElmer Life

Sciences, Waltham, MA, USA). Oligonucleotide primers for PCR were designed based on the GenBank sequence of MUC5AC (GenBank accession number AJ001402, 337 bp, sense 5'-CGACA ACTAC TTCTG CGGTG C-3'and antisense 5'-GCACT CATCC TTCCT GTCGT-3'). The following PCR conditions used involved 35 cycles : denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and polymerization at 72°C for 30 s. The oligonucleotide primers for HIF-1α were designed based on the GenBank sequence (GenBank accession number NM181054, 320 bp, sense 5'-GCAGC CAGAT CTCGG CGAAG-3' and antisense 5'-CTGTG TCCAG TTAGT TCAAA CTG-3'). PCR parameters used involved 24 cycles as follows : denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and polymerization at 72°C for 30 s. The oligonucleotide primers for $\beta_2$-microglobulin (used as a control for the RT-PCR) were designed based on the GenBank sequence (GenBank accession number NM004048, 334 bp, sense 5'-TCGCG CTACT CTCTC TTTCT-3' and antisense 5'-GCTTA CATGT CTCGA TCCCA CTTAA-3'). PCR parameters used involved 24 cycles as follows : denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and polymerization at 72°C for 30 s. The PCR products were run in a 2% agarose gel and visualized with ethidium bromide under a transilluminator.

3. Western blot analysis

After hypoxia treatment, the NHNE cells and NCI-H292 cells were lysed with 2x lysis buffer (250 mM Tris-C, pH 6.5, 2% SDS, 4%-mercaptoethanol, 0.02% bromphenol blue, 10% glycerol). Equal amounts of whole cell lysates were resolved by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). Membranes were blocked with 5% skim milk in Tris-buffered saline (50 mM Tris-Cl, pH 7.5, 150 mM NaCl) for 2 h at room temperature. This blot was then incubated overnight with primary antibody (BD bioscience, San Jose, CA, USA) in TTBS (0.5%

Tween 20 in Tris-buffered saline). After washing with TTBS, the blot was further incubated for 45 min at room temperature with anti-mouse antibody (Cell Signaling, Danvers, MA, USA) in TTBS and then visualized by using the ECL system (Amersham, Little Chalfont, Buckinghamshire, United Kingdom).

4. Quantification of secreted MUC5AC

To analyze the production of MUC5AC, accumulated apical secretion fluid was collected and analyzed by dot blot analysis. Briefly, diluted apical secretion fluid was applied to nitrocellulose membrane, which were incubated with human MUC5AC antibody, followed by a reaction with horseradish peroxidase-conjugated goat anti-mouse IgG. The signal was detected by chemiluminescene using the ECL kit (Amersham, Little Chalfont, Buckinghamshire, United Kingdom).

5. Transient transfection and luciferase assay

NCI-H292 cells were transiently transfected with the plasmids : 1) for gain-of-function study of HIF-1α, pCMV-basic and pCMV-HIF-1α 2) for MUC5AC promoter luciferase assay, pGL3-basic, pGL3-MUC5AC promoter (-1376/+4), pGL3-mutated MUC5AC promoter (-1376/+4) using FuGENE 6 transfection reagent (Roche Applied Science, Bagel, Switzerland) according to the manufacturer's instructions. After transfection of reporter, the cells were incubated under the defined conditions for 6 h and assayed for luciferase activity, using a luciferase assay system (Promega, Madison, Wisconsin, USA), according to the manufacturer's instructions. β-galactosidase activity was also assayed to standardize the transfection efficiency of each sample. Mutation of the putative regulatory elements that start from -65 bp upstream of MUC5AC gene was generated by mutagenic PCR (5'-ACGTG-3' 5'-AAATG-3', -65/-61).

III. RESULTS

1. mRNA expression of MUC5AC according to hypoxic stimulation in NHNE cells

To determine whether hypoxic stimulation induces the expression of MUC5AC gene in NHNE cells, mRNA expression of MUC5AC in NHNE cell was analysed by RT-PCR after incubating the NHNE cells in the hypoxic chamber with 95% $N_2$, 5% $CO_2$. The mRNA levels of MUC5AC were measured with increasing time at 0, 2, 6, 12, 24 h after hypoxic treatment. Compared with non-treated cells, the mRNA levels of MUC5AC were increased as a function of time in the cells under hypoxic treatment. These results indicate that MUC5AC expression in NHNE cells is induced by hypoxia (Fig. 1).

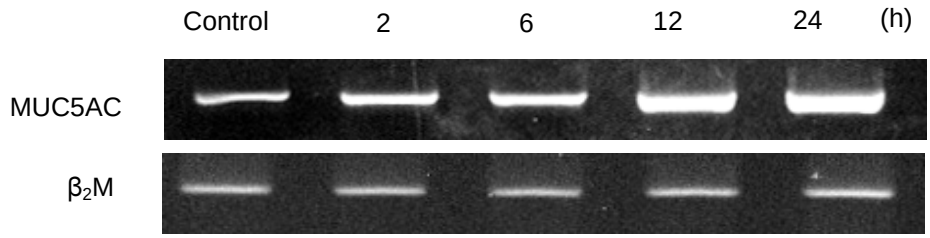



Figure 1. mRNA expression of MUC5AC according to hypoxic stimulation in NHNE cells. NHNE cells were exposed to 95% $N_2$, 5% $CO_2$ in the hypoxic chamber for 0-24 h. Total RNA of the NHNE cells was isolated and the mRNA of MUC5AC was analyzed by RT-PCR. The expression of MUC5AC mRNA was increased under the hypoxic condition with time compared to the control group. Control : NHNE cells without hypoxic stimulation.

2. HIF-1α mRNA and protein expression according to hypoxic stimulation in NHNE cells

To test whether hypoxic stimulation induces the expression of HIF-1α in NHNE cells, HIF-1α mRNA levels were measured using RT-PCR in the cells incubated for 24 h under hypoxic condition of 95% $N_2$, 5% $CO_2$. However, no change was detected in mRNA levels of HIF-1α in the cells with hypoxic stimulation for 0-24 h (Fig. 2). Therefore, the protein levels of HIF-1α in NHNE cells under the control and hypoxic condition were assayed by western blot analysis. Compared with NHNE cells without hypoxic stimulation, the protein levels of HIF-1α were increased in NHNE cells under hypoxic stimulation with 95% $N_2$, 5% $CO_2$ for 24 h (Fig. 3).

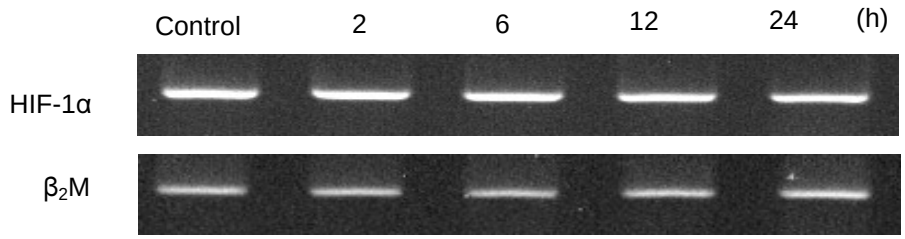



Figure 2. mRNA expression of HIF-1α according to hypoxic stimulation in NHNE cells. NHNE cells were exposed to 95% $N_2$, 5% $CO_2$ in the hypoxic chamber for 0-24 h. Total RNA of the NHNE cells was isolated and the mRNA of HIF-1α was analyzed by RT-PCR. The transcription of HIF-1α was not increased in the NHNE cells under the hypoxic condition compared to the control group. Control : NHNE cells without hypoxic stimulation.

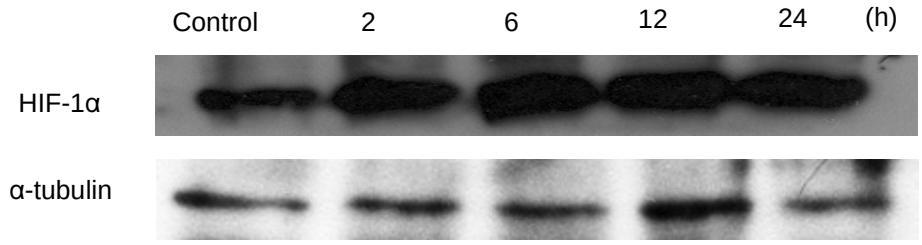



Figure 3. HIF-1α protein expression according to hypoxic stimulation in NHNE cells. NHNE cells were incubated in the hypoxic chamber with 95% $N_2$, 5% $CO_2$ for 0-24 h. The protein of HIF-1α was measured by western blot analysis. The protein expression of HIF-1α was increased in NHNE cells under the hypoxic condition compared to the control group. Control : NHNE cells without hypoxic stimulation.

3. MUC5AC secretion according to hypoxic stimulation in NHNE cells

To figure out whether the secretion of MUC5AC is increased by hypoxic stimulation in NHNE cells, NHNE cells were incubated in the hypoxic chamber with 95% $N_2$, 5% $CO_2$ for 24 h. The secretion of NHNE cells was collected at 2, 6, 12, 24 h after incubation and analyzed by dot blot. MUC5AC secretion was increased under hypoxic stress compared to the normoxic condition. The secretion was maximally increased at 12 h then decreased at 24 h (Fig. 4).

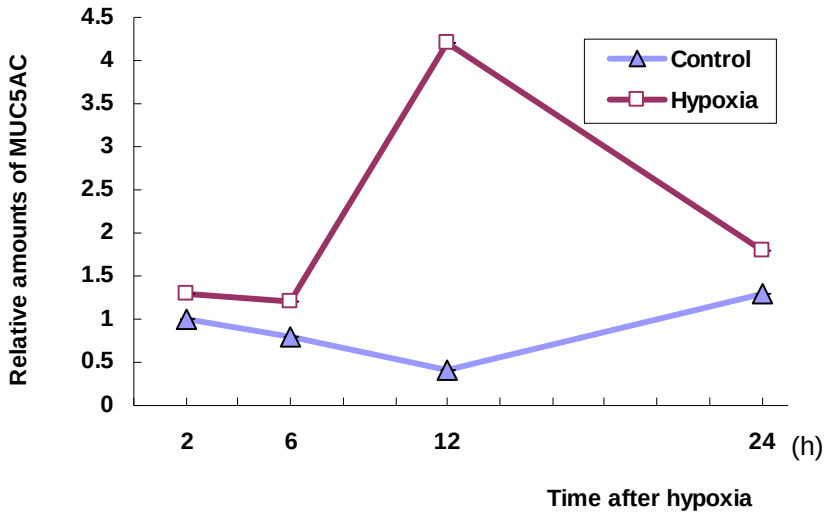


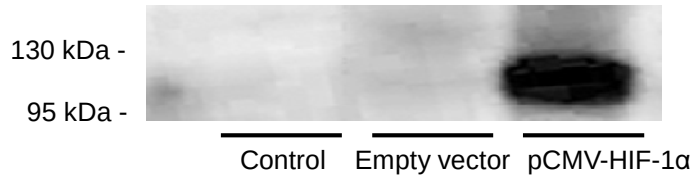

Figure 4. MUC5AC secretion according to hypoxic stimulation in NHNE cells. NHNE cells were incubated in the hypoxic chamber with 95% $N_2$, 5% $CO_2$ for 24 h. The secretion of NHNE cells was collected at 2, 6, 12, 24 h after incubating in the hypoxic condition and MUC5AC was measured by dot-blot. The amount of secreted MUC5AC was increased in NHNE cells under the hypoxic condition compared to the control group. Control : NHNE cells without hypoxic stimulation.

4. Gain-of-function study with mamalian expression vector for HIF-1α in NCI-H292 cells

To determine whether HIF-1α is involved in the regulation of MUC5AC, mammalian expression vector containing HIF-1α (pCMV-HIF-1α : generous gift from Dr. Koh EM, SMC, Seoul, Korea) was transiently transfected into the human mucoepidermoid carcinomal cell lines (NCI-H292 cells). Initially, western blot analysis was carried out to confirm the expression of HIF-1α. The expression was increased in the cells tranfected with HIF-1α expression

plasmids compared to the cells with empty vectors (Fig. 5A). mRNA of MUC5AC was measured by RT-PCR which showed that transfection of plasmids encoding HIF-1α increased the expression of MUC5AC mRNA in the NCI-H292 cells (Fig. 5B).

A




B

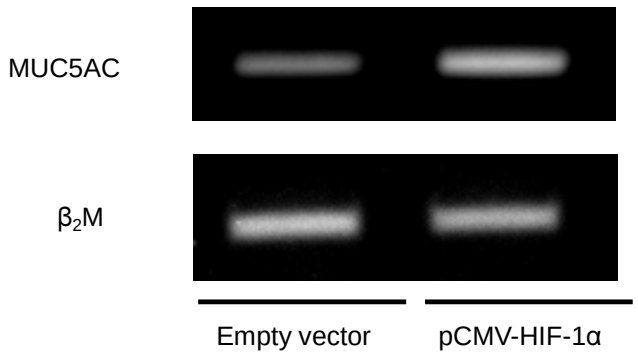



Figure 5. Gain-of-function study with mammalian expression vector of HIF-1α in NCI-H292 cells : A, Protein expression of HIF-1α. pCMV vector encoding HIF-1α and pCMV vector without HIF-1α were transiently transfected into NCI-292 cells. The expression of HIF-1α was increased in the cells transfected with pCMV-HIF-1α vector compared to the cells with empty vectors in the normoxic condition. B, mRNA expression of MUC5AC. The increase of MUC5AC mRNA was noted in the cells with pCMV vectors encoding HIF-1α compared to the cells with empty vectors. Control : NCI-292 cells without

mammalian vector, Empty vector : NCI-292 cells with pCMV-basic vector, pCMV-HIF-1α : NCI-292 cells with pCMV vector encoding HIF-1α, HIF-1α size : 120 kDa, MUC5AC size : 337 bp.

5. Luciferase assay of MUC5AC promotor in hypoxia

To figure out whether putative HRE of MUC5AC promoter plays a role in hypoxia-induced MUC5AC expression, luciferase assay with mutagenesis of putative HRE was carried out. The putative HRE is located 61~65 upstream of the MUC5AC gene, of which sequence is 5'-ACGTG-3'. By mutagenic PCR, putative HRE sequence was mutated to 5'-AAATG-3'. These wild-type or HRE-mutated MUC5AC promoters were inserted into pGL3 plasmids, which have the luciferase reporter gene. NCI-H292 cells were transfected with pGL3 vectors containing wild-type MUC5AC promoter, pGL3 vectors with HRE-mutated MUC5AC promoter, and pGL3-basic vectors, respectively. After treatment of hypoxia with 95% $N_2$, 5% $CO_2$ for 6 h, the relative luciferase activity in the cells with pGL3-wild-type MUC5AC promoter was increased by 8.3-fold compared to the HRE-mutated group and by 11.2-fold compared to the pGL3-basic vector group. In normoxia, the luciferase activity in the cells with plasmids encoding wild-type MUC5AC promoter was increased compared to the groups with either HRE-mutated promoter or pGL3-basic vector. Compared with the normoxic condition, the luciferase activity was increased by 1.7-fold in the cells with wild-type MUC5AC promoter in the hypoxic condition. Hypoxic stimulation did not increase the activity of luciferase reporter when the MUC5AC promoter HRE was mutated (Fig. 6).

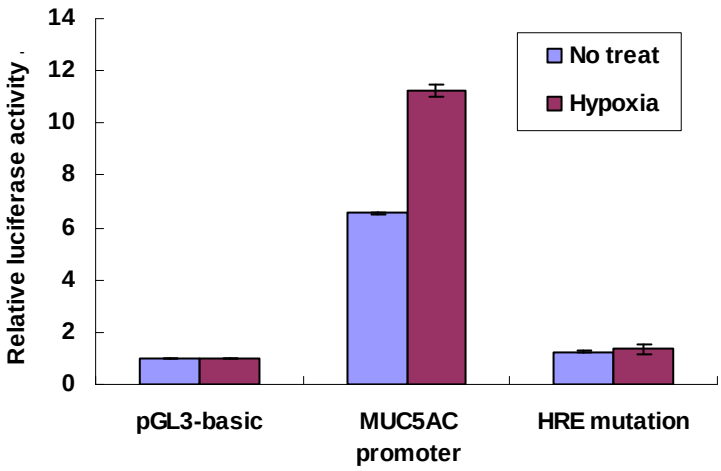



Figure 6. Luciferase assay of MUC5AC promoter. NCI-H292 cells were transfected with pGL3-basic vectors, pGL3 vectors encoding wild-type MUC5AC promoter or pGL3 vectors encoding HRE-mutated MUC5AC promoter. After cells were incubated in the hypoxic chamber with 95% $N_2$, 5% $CO_2$ for 6 h, luciferase activities were measured. In the hypoxic condition, the luciferase activity of the wild-type MUC5AC promoter was increased by 8.3-fold compared to the cells with HRE- mutated MUC5AC promoter and by 11.2-fold compared to the cells with pGL3-basic vectors. pGL-basic : NCI-H292 cells with pGL3-basic vector, MUC5AC promoter : NCI-H292 cells with pGL3 vector encoding wild-type MUC5AC promoter, HRE mutation : NCI-H292 cells with pGL3 vector encoding HRE-mutated MUC5AC promoter.

## IV. DISCUSSION

This study was performed based on the presumption that the hypoxic condition within the sinuses can directly induce the hypersecretin of mucin which is one of the major pathophysiologic mechanisms of sinusitis. Although hypoxia is known as a potent stimulant for inflammation[1] and tissue remodeling[33] that can contribute to sinus diseases, no studies have been performed to investigate the effect of hypoxia on mucin secretion in airway epithelia system. Only MUC3 gene in intestinal epithelium is known to be regulated by hypoxic stimulation.[34]

This study demonstrated that the MUC5AC gene is induced by hypoxia in normal human nasal epithelial cells (Fig. 1,4). The transcription of MUC5AC occurred after the cells were exposed to hypoxic conditions. One possible mechanism by which hypoxia can regulate MUC5AC gene expression is through the transcription factor HIF-1. HIF-1 is heterodimeric proteins composed of an oxygen regulated α subunit and an oxygen independent β subunit.[35] The α subunits are continuously transcribed and translated. Under normoxic conditions, two prolines in the α subunit are hydroxylated, which enables the protein to be ubiquinated by the von Hippel-Lindau tumor suppressor and degraded in the 26S proteosome.[36]

When oxygen levels decrease, the α and β subunits heterodimerize and bind to specific elements in promoters of many genes, termed hypoxia response elements to induce transcription. In this study, although the mRNA expression of HIF-1α was not influenced by hypoxic stimulations (Fig. 2), the protein level of HIF-1α was increased with time under hypoxic conditions (Fig. 3), which suggests post-translational regulation of HIF-1α gene.

We found that the MUC5AC promoter region contains a putative HRE in MUC5AC promoter. The HRE of the MUC5AC promoter exhibits core

sequence ACGTG that are similar to the sequences found in the promoters of transferrin and lactate dehydrogenase. To evaluate the role of HIF-1 as assessed by its overexpression, HIF-1α expression vectors were transfected in human lung mucoepidermoid carcinoma cell lines. The MUC5AC promoter activity was enhanced by increased expression of HIF1a. However, compared to the increased amount of HIF-1α protein (Fig. 5A), the increased MUC5AC mRNA levels in the cells with overexpression of HIF-1α did not appear to be sufficient (Fig. 5B). It may be suggested that additional overexpression of HIF-1β could contribute to higher activity of MUC5AC promoter as observed in other studies in which co-transfection of HIF-1α and HIF-1β resulted in higher activity of transferrin receptor promoter than HIF-1α alone.[37] These results suggest that the MUC5AC gene contains an enhancer regulated by HIF-1. Moreover, our studies demonstrated that the wild-type but not HRE-mutated MUC5AC promoter conferred inducibility to the luciferase reporter gene under the hypoxic condition in NCI-H292 cells (Fig. 6). However, the difference of relative activity between the cells incubated in the hypoxic and normoxic conditions was not very significant, meaning that there may be another mechanism involved in activating the MUC5AC gene mediated by the putative HRE.

Because putative HRE-mutated MUC5AC promoter attenuated the reporter activity, putative HRE in the MUC5AC promoter can be a strong candidate for a functional HRE. To confirm that HIF-1α can bind to the putative HRE in the MUC5AC promoter, RNA interference for HIF-1α and EMSA with supershift may be performed in the future.

Our study reveals that hypoxia can also contribute to the sinus disease by directly regulating MUC5AC secretion via the activation of HIF-1.

MUC5AC is the one of the major secreted mucin in sinusitis. Considering that a marked increase of MUC5AC and MUC5B secretion was reported in sinus diseases and the mean level of MUC5AC expression was higher compared to MUC5B in sinus mucus secretion,[5] the role of hypoxic stress in the

pathophysiology of sinusitis could be more accentuated. Therefore, in the management of upper airway diseases like sinusitis, the mechanical or pharmacological restoration of ventilation would be cardinal.

## V. CONCLUSION

Our study revealed that the secretion of MUC5AC which is the major component of mucin in sinusitis can be increased with hypoxic stimulation in NHNE cells. HIF-1 is the well-known transcription factor which activates several genes responding to hypoxic stimulation. The alteration of the activity of HIF-1 with overexpression of HIF-1α affects the secretion of MUC5AC. Mutation of the putative HRE in the MUC5AC promoter attenuated the reporter activity in promoter luciferase assay.

In conclusion, HRE in the MUC5AC promoter plays an important role for HIF-1 mediated activation of MUC5AC gene.

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ABSTRACT (IN KOREAN)

# 사람 기도점액상피세포에서 저산소 환경에 의한 MUC5AC 유전자의 발현 조절

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신 우 철

저산소 환경은 부비동 질환의 주요한 병리 기전의 하나이며 부비동 질환이 발생하면 MUC5AC, MUC5B 등의 점액 발현이 증가 되는 것으로 알려져 있다.

HIF-1 (hypoxia-inducible factor-1) 은 잘 알려진 전사인자로서 저산소 환경에서 erythropoietin이나 endothelial growth factor 등 유전자의 전사를 활성화 시킨다. 이 전사인자는 특정 유전자의 promotor에 있는 HRE (hypoxia-response element) 에 결합하는데 MUC5AC promoter에는 HRE와 유사한 부위가 존재한다. 이 연구의 목적은 저산소 환경에서 MUC5AC promotor에 존재하는 HRE 유사부위가 실제로 HIF-1에 반응하는 enhancer로서 역할을 하여 MUC5AC 유전자의 활성을 증가시키는지 알아보는 것이다.

본 연구에서 사람 코점막 상피세포를 저산소 환경에 두었을 때 MUC5AC의 mRNA 및 protein 발현이 증가하였고 HIF-1α의 protein 발현이 증가되었다.

사람 폐점액상피암주 (NCI-H292) 에 HIF-1α를 가진 mammalian vector를 삽입할 때 정상 산소 환경에서도 MUC5AC의 mRNA가 증가되었고 MUC5AC promotor에 대한 luciferase assay에서 저 산소 환경에서 luciferase 활성도가 증가하였으며 MUC5AC내의 HRE

유사부위가 mutation이 되었을 때는 luciferase 활성도가 증가하지 않았다.

결론적으로 MUC5AC내의 HRE 유사부위는 HIF-1α가 결합하는 enhancer로서 저산소 환경시 MUC5AC 유전자의 발현을 증가시키는 작용을 할 수 있다. 이는 저산소 환경이 사람 기도상피에서 MUC5AC 점액분비를 조절하여 부비동 질환등 상기도 질환의 병리기전이 될 수 있음을 시사한다.

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핵심되는 말 : 저산소 환경, hypoxia-inducible factor, hypoxia-response element, MUC5AC, 기도상피