# Chitinase-like Protein YKL-40 Regulates Hyperoxia-induced Apoptosis in Human Airway Epithelial Cells.

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# Chitinase-like Protein YKL-40 Regulates Hyperoxia-induced Apoptosis in Human Airway Epithelial Cells

Directed by Professor Myung Hyun Sohn

The Master's Thesis submitted to the Department of Medical Science, the Graduated School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medical Science

Mi Na Kim

December 2011

This certifies that the Master's
Thesis of Mi Na Kim is approved

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_____

Thesis Committee Member # 2 : Se Hoon Kim

The Graduate School
Yonse University

December 2011

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그리고 이 모든 것을 허락하신 하나님께 감사드립니다.

2011년 12월
김미나

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## ABSTRACT

### Chitinase-like Protein YKL-40 Regulates Hyperoxia-induced Apoptosis in Human Airway Epithelial Cells.

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(Directed by Professor Myung Hyun Sohn)

YKL-40 is chitinase-like protein that lacks chitinase activity. Prolonged exposure to 100% oxygen causes hyperoxic acute lung injury characterized by alveolar epithelial cell injury and death. We investigated the role of YKL-40 regulating hyperoxia-induced apoptosis in human airway epithelial cells.

Human airway epithelial cell line, BEAS-2B, was exposed to > 93% oxygen for 24-72 hours. Hyperoxia induced apoptosis was confirmed by flow cytometry with Annexin-V and PI staining. The mRNA and protein expression of YKL-40, caspase 3 and caspase 7 was determined by real time PCR and Western blotting. YKL-40 short hairpin RNA (shRNA) and over-expression vectors were transfected to cells to examine the requirement of YKL-40.

Hyperoxia increased FITC-Annexin V positive cells compared with

room air. Caspase 3 and 7 , representative apoptosis regulators, were increased by hyperoxia on mRNA and protein level. YKL-40 expression was also increased. YKL-40 shRNA transfected cells expressed lower level of caspase 3 and 7 mRNA and protein than untransfected cells after exposing to hyperoxia, whereas YKL-40 over-expression vector transfected cells showed higher level of caspase 3 and 7 than untransfected cells.

We concluded that hyperoxia induced apoptosis associates with YKL-40 expression and that YKL-40 could be considered as one of apoptosis regulators in oxidative stressed human airway epithelial cells.

**Key words:** Hyperoxia, YKL-40, Chitinase-3 like 1, Apoptosis. Human airway epithelial cell, BEAS-2B.

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## Ⅰ. INTRODUCTION

Supplemental oxygen is often required to preterm babies and the adults suffering from respiratory failure to improve the delivery of oxygen to peripheral tissues. However, prolonged exposure to intensely high concentration of oxygen causes hyperoxic acute lung injury. The pathological changes in hyperoxia-injured lungs are characterized by injury and death of the alveolar endothelial and epithelial cells[1-5]. Apoptosis has been described as a major death mechanism in hyperoxia-induced lung injury in vivo and in vitro[6-12]. Apoptosis is a fundamental feature regulating cellular suicide that remove cells carrying abnormal genetic information to maintain the functional integrity of the cell population. Controlled by intercellular signaling, apoptosis also plays

a critical role in tissue remodeling and homeostasis[13,14]. Caspases, a family of cysteine proteases, are the central regulators of apoptosis. It is now well established that certain caspases, caspase-8, caspase-9 and caspase-10, play upstream initiator roles in apoptosis by coupling cell death stimuli to the downstream effector caspases, caspase-3, caspase-6 and caspase 7, which in turn execute apoptosis by cleaving cellular proteins following specific aspartate residues[15,16].

YKL-40 is chitinase like protein which is involved in evolutionarily conserved 18 glycosyl hydrolase family. YKL-40 is produced by variety of cells including neutrophils, monocytes, macrophages, chondrocytes, synovial cells, smooth muscles, endothelial cells, tumor cells and epithelial cells[17-22]. Increased levels of YKL-40 have been noted to be associated with various pathological conditions including bacterial infections, rheumatoid arthritis, osteoarthritis, sarcoidosis, diabetes, allergic disease and varied malignancies[23-28]. High serum levels of YKL-40 are also associated with asthma, chronic obstructive pulmonary disease, and lung cancer[29-33]. Ober *et al*. reported that CHI3L1 is a susceptibility gene for asthma, bronchial hyper-responsiveness, and reduced lung function, and elevated circulating YKL-40 levels are a biomarker for asthma and decline in lung function[30]. Recent study demonstrated that YKL-40 and BRP-39, the mouse homologue for YKL-40, are critical regulators of oxidant injury, inflammation, and epithelial apoptosis in the murine and human lung[34]. The novel regulatory role of BRP-39/YKL-40 in cigarette

smoke induced inflammation and emphysematous destruction was also reported[35]. These studies indicate the pivotal role for YKL-40 in the lung with airway inflammation and oxidative stress.

Surprisingly, in spite of the importance of human airway epithelial cells as a essential alveolar capillary barrier and first line of defense, the role of YKL-40 in human airway epithelial cells undertaken oxidative stress have not been adequately defined. Therefore, in this study, we demonstrate the role of YKL-40 in hyperoxia exposed airway epithelial cells.

## Ⅱ. MATERIALS AND METHODS

### 1. Cell culture

BEAS-2B, human bronchial epithelial cell line, was purchased from American type Culture Collection (Manassas, NA, USA) and cultured in the defined medium, BEGE (bronchial epithelial growth medium; Lonza, East Rutherford, NJ, USA). Cells were grown in humidified incubator containing 95% air and 5% $CO_2$ at 37℃. For production of lentiviral particle, 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo scienctific Hyclone, USA) supplemented with 10% fetal bovine serum, 5% penicillin/streptomycin (Thermo scientific Hyclone, USA).

### 2. Hyperoxia exposure

For hyperoxia experiment, cells were plated in MIC-101 chamber (Modular Incubator, Billups- Rothenberg Inc., Germany) filled with 95% $O_2$ , 5% $CO_2$ up to 72 hours. The concentration of $O_2$ in the chamber was monitored by Max$O_2$ (Maxtec,Inc., USA). Room air cells were kept in air 21% $O_2$ , 5% $CO_2$ at 37℃. Gases were replaced everyday.

### 3. Annexin V/Propidium Iodide staining

Annexin V-FITC kit (BD Pharmingen™, USA) was used to determine apoptosis. We followed manufacturer's protocol. Briefly, after hyperoxia exposure for 24-72 h, cells were washed with PBS and $1\times10^5$ cells were transferred to a 5 ml tube. Then cells were resuspended with 1×binding buffer followed by staining with 5 μl of annexin V and 10 μl of

propidium iodide in the dark for 20min. Binding buffer (200 μl) was then added to each tube and analyzed by flow cytometry.

**4. Real-time polymerase chain reaction**

Total RNA was isolated from BEAS-2B cells using TRIzol reagent (Invitrogen, Charlsabad, CA, USA). The isolated RNA was dissolved in RNase-free water and quantified. 2 μg of total RNA was used to synthesize complemental DNA using 3 μg of Random Primer oligonucleotides, 1 μl of 10 mM dNTP Mix (2'-deoxynucleoside 5'-triphosphate) and 200 U of Superscript Ⅱ reverse transcriptase (Invitrogen, USA) in 20 μl volumes at 42℃ for 60 min. PCR amplification for detecting YKL-40, Caspase 3, Caspase 7 was carried out using the following specific primer pairs.

200 ng of cDNA was used for the real time PCR reaction with the primers in the AccuPower® Dualstar™ qPCR PreMix (Bioneer, Daejeon, Korea) using Exicycler (Bioneer, Daejeon, Korea). The sequences of the used primers were obtained from Primer Bank online (http://pga.mgh.harvard.edu/primerbank). Primer sequences were as follows : YKL-40: 5'-CCA AGG AGC CAA ACA TCC TA-3' (sense) and 5'-GAA GGG GAA GTA GGA TAG GGG-3' (antisense); Caspase 3: 5'-ATG GAA GCG AAT CAA TGG ACT C-3' (sense) and 5'-CTG TAC CAG ACC GAG ATG TCA-3' (antisense); Caspase 7: 5'-CGG TCC TCG TTT GTA CCG TC-3' (sense) and 5'-GGT GGT CTT GAT GGA TCG CA-3' (antisense); GAPDH: 5'-CCC CTT CAT TGA CCT CAA CT-3' (sense) and 5'-GAT GAC AAG CTT CCC GTT CT-3' (antisense).

Fold changes were calculated by the comparative ΔΔCt method.

**5. Western Blot analysis**

Cell protein levels of cleaved caspase 3, cleaved caspase 7 and YKL-40 were analyzed by Western blotting. Total proteins were extracted using a Mammalian Protein Extraction reagent (Pierce, Rockford, IL, USA). The protein concentration was determined using Bio-Rad Protein assay kit (Bio-Rad Laboratories, Hercules, CA). 5×SDS sample loading buffer was added to the protein extracts and boiled for 5 min. 10~20 μg of protein samples were then subjected to 10-13.5% sodium dodecyl sulfate polyacrylamide gels, seperated and transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, MA, USA). After blocking with 5% skim milk in 1×TBS containing 0.1% Tween 20 for 1 hour, membranes were incubated overnight at 4℃ with primary antibodies, 1:1000 diluted cleaved caspase 3, cleaved caspase 7 antibodies (Cell Signaling Technology, Beverly, MA, USA) and 1:500 diluted YKL-40 antibody (Santa Cruz Biotechnology, Delaware, CA), followed by horseradish peroxidase-conjugated anti-goat or anti-rabbit antibodies.(Cell Signaling Technology, Beverly, MA, USA). Protein bands were visualized by the ImageQuant™ LAS 4000 Mini Biomolecular Imager (GE healthcare, Sweden).

**6. Cell transfection**

To induce YKL-40 over-expression in BEAS-2B cells, over-expression clones were transfected with pcDNA3 (sigma) using Lipofectamine

(Invitrogen) according to the manufacturer's instructions. As a control, empty pcDNA3 vector was transfected. Transiently transfected cells were incubated for an additional 24 hours and exposed to either hyperoxia or room air. After 48 h, cell lysates were subjected to Western blot and real-time PCR. The efficiency of over expression vector was determined by Western blot analysis using the corresponding specific antibodies. YKL-40 over-expression vectors were kindly provided by Se-hoon Kim from Yonsei University College of Medicine.

**7. Lentiviral short hairpin RNA production and transduction**

Short hairpin RNA lentiviral transduction was conducted for YKL-40 gene knock down. YKL-40 lentiviral glycerol stock was purchased from sigmaldrich. Lentiviruses were prepared by transient transfection of 293T cells, using a liposomal cotransfection method. To summarize, the 293 T cells grown up to 50~70% confluency were transfected with YKL-40-pLKO.1-puro vector together with compatible packaging plasmids in DMEM without antibiotics. pLKO.1-puro control vector was used as a control. After 48 h, the supernatant was collected, centrifuged to remove the cellular debris, and filtered by 0.45 μm pore filteration.

Transduction of cells were performed in bronchial epithelial cell basal medium supplemented with 8 μg/mL polybrene (hexadimethrine -bromide, Sigma−Aldrich) to BEAS-2B cells by manufacturer's protocol. Before transduction, titer the concentration of puromycin lentivirus encoding cells was done.

In brief, $1\times10^6$ cells were plated in 60×15mm dishes. After incubation of the cells for 18-24 h, media was removed and lentiviaral constructs were added to cells with bronchial epithelial cell basal medium containing polybrene. Cells were incubated for 5 h and then media was changed to epithelial cell growth medium. Next day media was replaced with puromycin containing media and changed every 2 days until resistant colonies were identified. The efficiency of lentiviral transduction was determined by Western blot and real-time PCR.

**8. Statistical Analysis**

Data were expressed as mean ± SEM of at least three individual experiments. Statical analysis comparing between treatment and control groups was assessed by student *t* test. ($p<0.05$ was considered significant)

# Ⅲ. RESULT

## 1. Hyperoxia induces apoptosis in human airway epithelial cells.

To investigate whether hyperoxia induces apoptosis in airway epithelial cells, BEAS-2B cells were exposed to hyperoxia (95% $O_2$ , 5% $CO_2$ ) for 24, 48 and 72 h, respectively and analyzed by flow cytometry after staining with Annexin-V FITC. As shown in Fig. 1, apoptotic cell death rate gradually increased up to 48 h and then has significantly grown at 72 h.

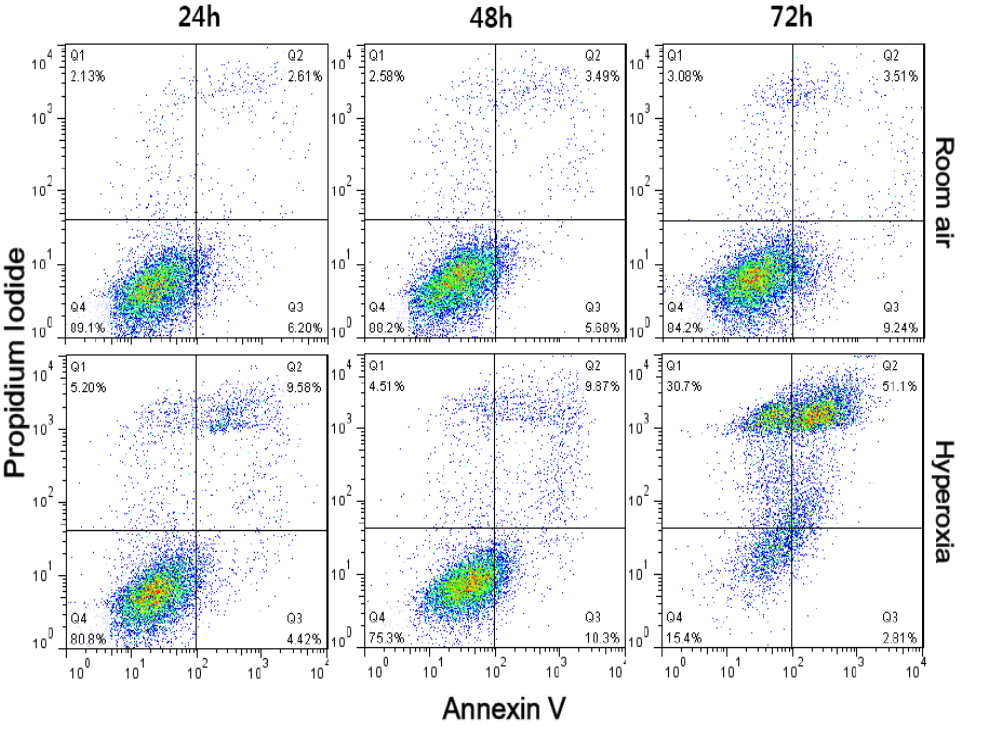
It is well established that caspase 3 and 7 execute apoptosis by cleaving cellular proteins following specific aspartate residues. To support apoptosis caused by hyperoxia, we determined the caspase 3 and 7 expression by real time PCR and Western blot analysis. It was resulted that mRNAs and activated proteins of caspase 3 and 7 were all up-regulated in a time dependent manner and showed the most distinct increase at 72 h. These result suggest that hyper oxygen stimulation exposure induces apoptosis in human airway epithelial cells.

## 2. Effect of hyperoxia on YKL-40 expression

To examine whether hyperoxia affects YKL-40 expression in airway epithelial cells, the level of YKL-40 was measured using cell lysates after exposing to either hyperoxia or room air for 24-72 h. Hyperoxia increased YKL-40 mRNA expression by ~2.7, 5.2 fold at 48, 72 h respectively, compared with the time matched control (Fig. 3A).

Similarly, the result of Western blot shows increase of YKL-40 dependent on the hyperoxia exposure time (Fig. 3B).

**A**


24h
48h
72h
Room air
Hyperoxia
Propidium Iodide
Annexin V
Q1 2.13%
Q2 2.61%
Q4 89.1%
Q3 6.20%
Q1 2.58%
Q2 3.49%
Q4 88.2%
Q3 5.68%
Q1 3.08%
Q2 3.51%
Q4 84.2%
Q3 9.24%
Q1 5.20%
Q2 9.58%
Q4 80.8%
Q3 4.42%
Q1 4.51%
Q2 9.87%
Q4 75.3%
Q3 10.3%
Q1 30.7%
Q2 51.1%
Q4 15.4%
Q3 2.81%

**B**

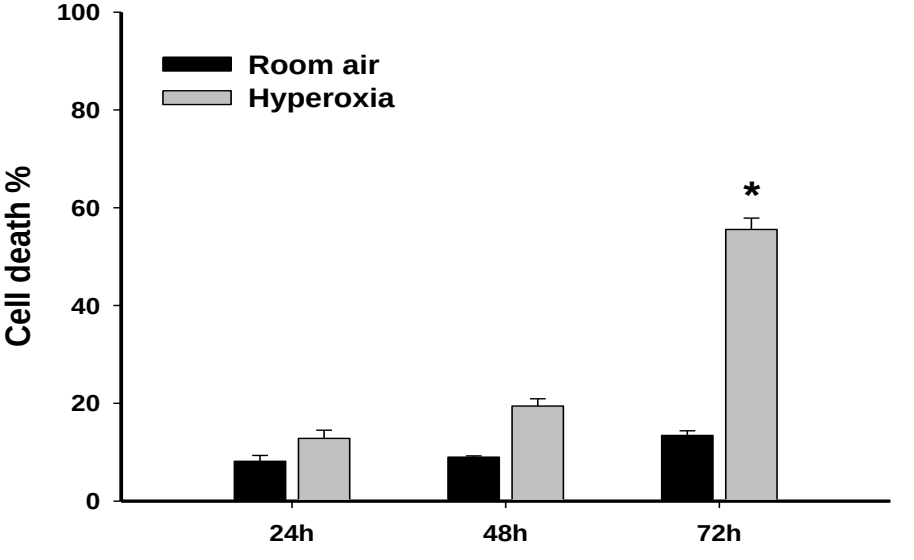
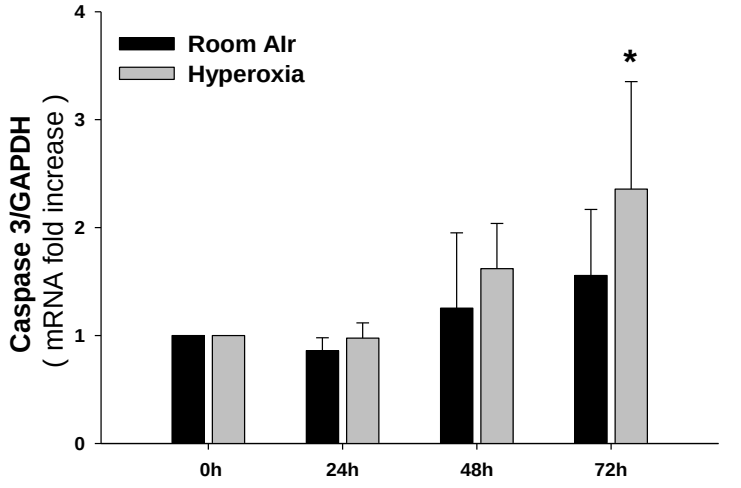
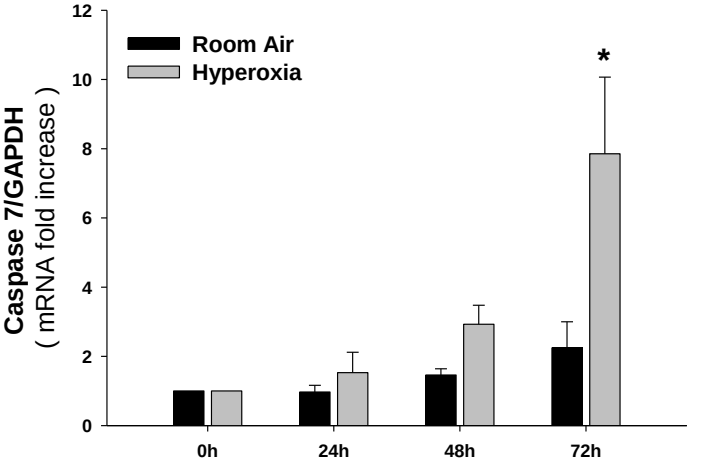

Room air
Hyperoxia
Cell death %
100
80
60
40
20
0
*
24h
48h
72h

**Figure 1. Hyperoxia induced apoptosis in BEAS-2B cells.** BEAS-2B cells were exposed to hyperoxia (95% $O_2$ , 5% $CO_2$ ) or room air (95% air and 5% $CO_2$ ) conditions for 24-72 h. Cells were harvested at the indicated time, stained with Annexin V-FITC and analyzed by flow cytometry. (A) Dot plots of flow cytometry analysis. Annexin V-FITC vs propidium idodide plots show the populations corresponding to viable and non-apoptotic (Annexin $V^-PI^-$), early (Annexin $V^+PI^-$), and late (Annexin $V^+PI^+$) apoptotic cells. (B) Quantification graph of apoptotic cells. Data represent the mean ± SEM of the three independent experiments. *$p<0.05$ determined by control versus hyperoxia.

A


Room AIr
Hyperoxia
Caspase 3/GAPDH
( mRNA fold increase )
*
0
1
2
3
4
0h
24h
48h
72h


Room Air
Hyperoxia
Caspase 7/GAPDH
( mRNA fold increase )
*
0
2
4
6
8
10
12
0h
24h
48h
72h

B

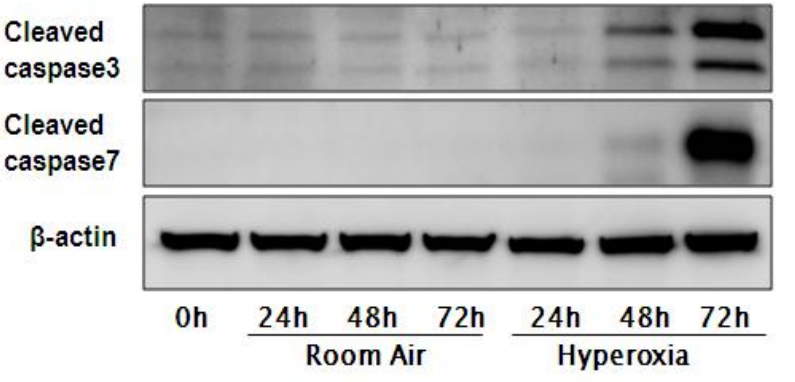

Cleaved caspase3
Cleaved caspase7
β-actin
0h
24h
48h
72h
24h
48h
72h
Room Air
Hyperoxia

**Figure 2. Hyperoxia increased the expression level of caspase 3 and 7 .** Expression level of caspase 3 and 7 was measured in cell lysates after exposing to hyperoxia or room air for 24-72 h. (A) mRNA transcription was detected by real-time PCR. The data represents the mean ± SEM of the four independent experiments. *$p<0.05$ vs room air. (B) Cell lysates were prepared and subjected to Western blot analysis using antibodies against cleaved caspase 3, cleaved caspase 7 and β-actin. The results shown are representative of three separate expreiments.

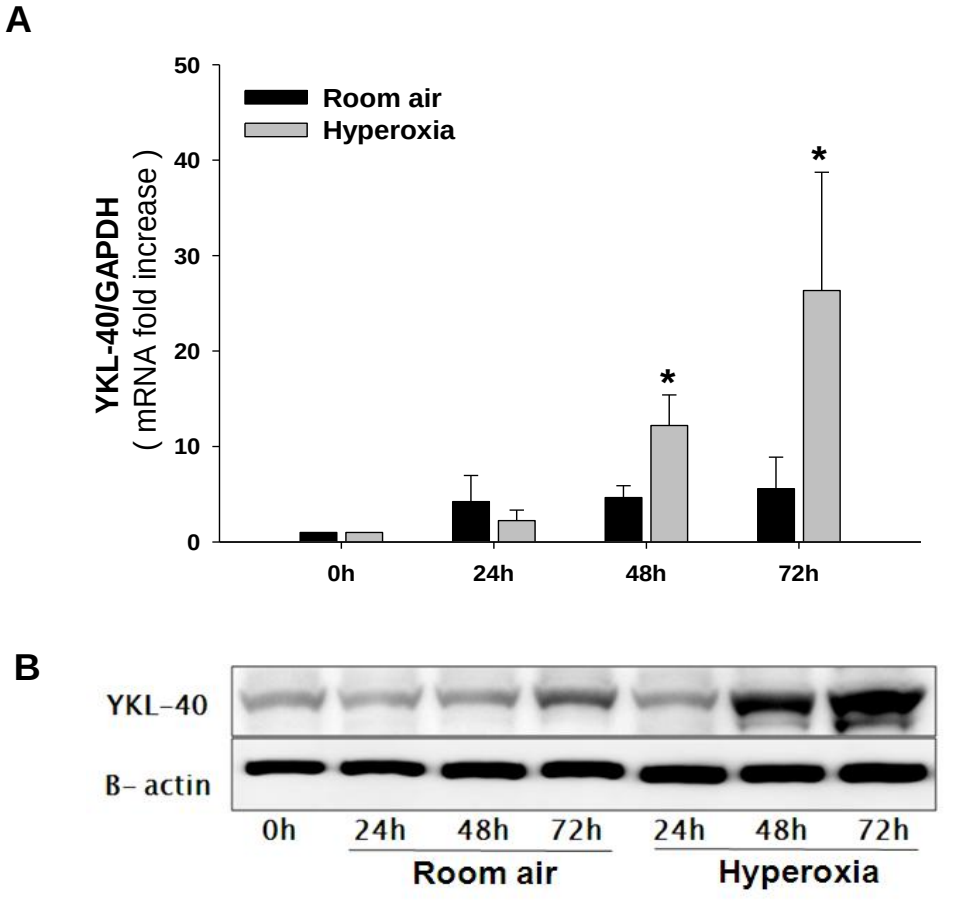


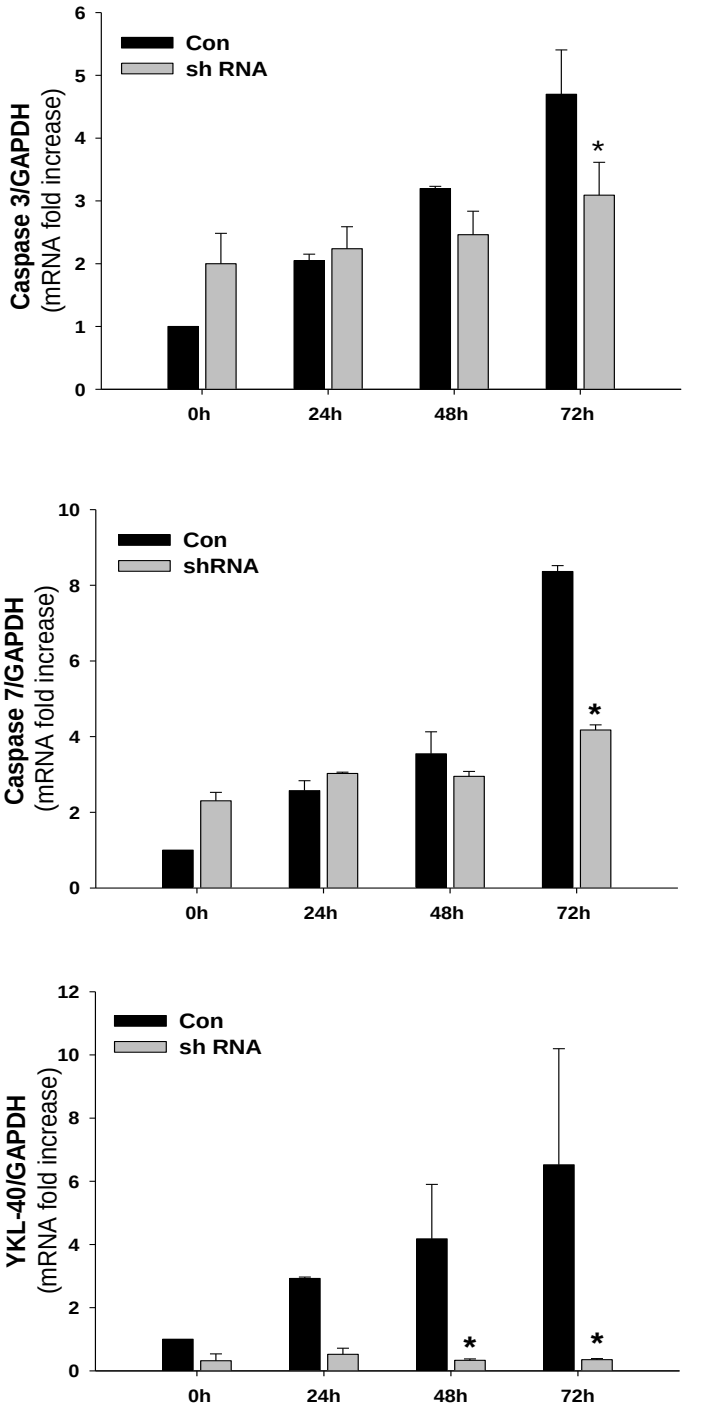

**Fig. 3. Hyperoxia multiplied YKL-40 expression.** BEAS-2B cells were exposed to hyperoxia or room air for 24-72h. After exposure, cell lysates were obtained and the level of YKL-40 was measured. (A) YKL-40 mRNA expression was analyzed by Real-time PCR. The data represents the mean ± SEM of the four independent experiments. *$p<0.05$ vs room air. (B) YKL-40 protein expression was detected by Western blot. The results shown are representative of three separate experiments.

**3. Silencing of YKL-40 reduces caspase 3 and 7 expression in hyperoxia exposed BEAS-2B cells**

Apoptosis and YKL-40 expression were induced and increased by hyperoxia in airway epithelial cells. To determine whether the apoptosis induced by hyperoxia is affected by presence of YKL-40, lentiviral short hairpin RNA (shRNA) transduction was conducted, as described in *Materials and Methods.* After transfection of shRNA that contains either anti-sense sequence of YKL-40 or non-targeting sequnce, cells were exposed to hyperoxia for 24, 48 and 72 h, then YKL-40 expression was measured to confirm the silencing effectiveness of shRNA. The expression level of YKL-40 in control cells was significantly increased dependent on hyperoxia exposure time, whereas considerably small amount of YKL-40 expression was detected in shRNA transfected cells (FIg. 4). To ascertain the impact of YKL-40 on hyperoxia induced apoptosis in airway epithelial cells, caspase 3 and 7, which represent the common executor of apoptosis, expression was measured by Western blot and real-time PCR (Fig. 4). Silencing of YKL-40 protected the airway epithelial cells against hyperoxia-induced caspase 3 and 7 activation. YKL-40 silenced cells expressed significantly lower level of caspase 3 and 7 after exposure to hyperoxia for 72 h. These results confers proapoptotic function of YKL-40 after hyperoxia exposure in airway epithelial cells.

**4. Over-expression of YKL-40 augmented the expression of caspase 3 & 7 in hyperoxia exposed BEAS-2B cells**

**A**


Caspase 3/GAPDH
(mRNA fold increase)
Con
sh RNA
0
1
2
3
4
5
6
*
0h
24h
48h
72h
Caspase 7/GAPDH
(mRNA fold increase)
Con
shRNA
0
2
4
6
8
10
*
0h
24h
48h
72h
YKL-40/GAPDH
(mRNA fold increase)
Con
sh RNA
0
2
4
6
8
10
12
*
*
0h
24h
48h
72h

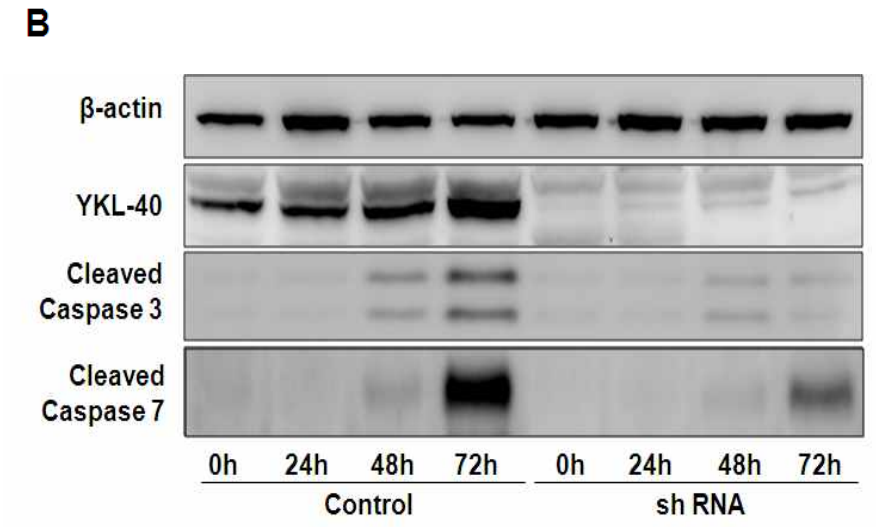



**Figure 4. YKL-40 short hairpin RNA(shRNA) depleted the expression of capase 3 and 7.** YKL-40 lentiviral shRNA transduction was done as described in *materials and methods*. shRNA transfected cells and control cells were exposed to hyperoxia for 24-72 h. After exposure, cell lysates were obtained at the indicated time and the level of YKL-40, caspase 3 and 7 was measured. (A) mRNA expression was analyzed by real-time PCR. The data represents the mean ± SEM of four independent experiments. *$p<0.05$ vs control (B) Western blot analysis of cell lysates probed with YKL-40, cleaved capase 3 and cleaved caspase 7. The results shown are representative of three separate experiments.

YKL-40 over-expression was used to further validate of the proapoptotic function of YKL-40. As shown in Fig. 5, YKL-40 over-expression resulted in a distinct increase of cleaved caspase 3 and 7 proteins compared with untransfected cells and control vector transfected cells after hyperoxia exposure. This results corroborate the proapoptotic function of YKL-40 in airway epithelial cells after hyperoxia exposure.

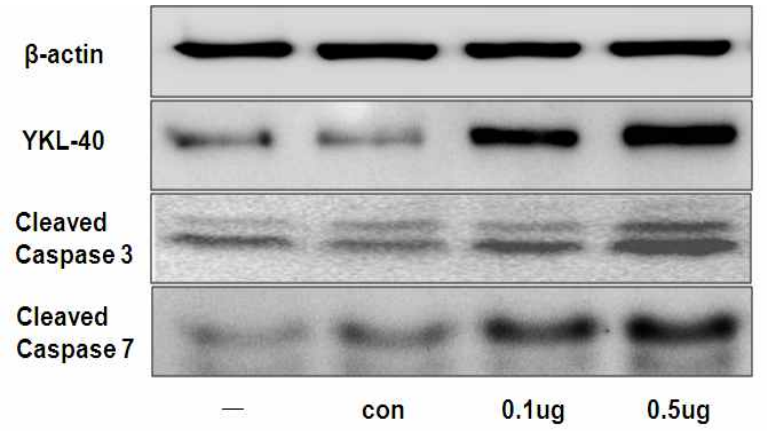



**Figure 5. YKL-40 over-expression augmented caspase 3 and 7 expression.** YKL-40 over-expression vector was transfected to BEAS-2B cells, and the cells were exposed to hyperoxia for 48h. - : untrasfected cells, con: control DNA transfected cells, 0.1 μg, 0.5 μg: YKL-40 over-expression vector transfected cells. After exposure, cell lysates were obtained and the level of YKL-40, cleaved caspase 3 and 7 was analysed by Western blot. The results shown are representative of three separate expreiments.

# Ⅳ. DISCUSSION

YKL-40 is chitinase like protein which is involved in evolutionarily conserved 18 glycosyl hydrolase family. Elevated concentrations of YKL-40 were demonstrated in a variety of diseases that are pathologically characterized by tissue inflammation and remodeling. Because of its strong association with disease, YKL-40 is regarded as a useful prognostic or diagnostic marker and potential therapeutic target[36,37]. However, the molecular processes governing the induction of YKL-40 and the roles of YKL-40 remain poorly understood.

Hyperoxia-induced lung epithelial cell apoptosis is a distinguishing characteristic of hyperoxia induced acute lung injury[38-41]. In vitro studies have demonstrated that oxygen-exposed cells present the oxidation of DNA, lipids and protein, and growth inhibition followed by cell death after prolonged exposure[42-45]. Biochemical and morphological features of apoptosis have been detected in hyperoxia-induced murine macrophages, fibroblastic cell lines, primary lung fibroblasts, and endothelial cells[46-48]. In contrast, hyperoxia induced cell death in the mouse lung epithelial cells and in cultured human A549 cells has been reported to show morphorlogical feature of necrosis and involve a cellular death signaling pathway of both apoptosis and necrosis[45,49]. In the present study, we demonstrated that YKL-40 regulates hyperoxia induced apoptosis in human airway epithelial cells.

First, our results showed the induction of apoptosis after exposing to hyperoxia in human airway epithelial cells. Apoptotic cell death was observed by Annexin-V FITC staining in hyperoxia exposed cells.

Caspases, a family of cysteine protease, are the central regulators of apoptosis. Exposure to hyperoxia up-regulated the expression of caspase 3 and 7 mRNAs, and the production of activated caspase 3 and 7 proteins in a time dependant manner.

Second, we demonstrated the significant increase of YKL-40 mRNA transcription and YKL-40 protein production in human airway epithelial cells depending on the exposure time to hyperoxia.

To further investigation of the association between YKL-40 and apoptosis, we examined whether absent of YKL-40 affects apoptosis induced by hyperoxia. Our results showed that silencing of YKL-40 diminished hyperoxia induced apoptosis in human airway epithelial cells manifested by considerably decreased level of caspase 3 and 7 mRNA expression and protein production. YKL-40 over-expression, on the other hand, augmented the level of caspase 3 and 7 depending on concetration after exposure to hyperoxia. These results indicate that YKL-40 performs as one of apoptosis regulators in hyperoxia exposed human airway epithelial cells.

Recent studies demonstrated that BRP-39 and YKL-40 regulate inflammatory cell apoptosis/cell death through inhibition of Fas expression and by inducing the phosphorylation of protein kinase B (PKB)/Akt in Th2 inflammation response[50]. In virto assays with connective tissue cells demonstrated the ability of YKL-40 to activate the mitogen-activated protein (MAP) kinnase and PKB/Akt pathways[51].

In summary, our finding demonstrated the proapoptotic role of YKL-40 in hyperoxia exposed human airway epithelial cells. The results have

shown that YKL-40 accomplishes its proapoptotic effect at least in part by effect on expression of caspase 3 and 7. Further studies are in progress to investigate the signaling pathway by which YKL-40 performs proapoptotic role in hyperoxia exposed human airway epithelial cells.

## Ⅴ. CONCLUSION

We concluded that hyperoxia induced apoptosis associates with YKL-40 expression and that YKL-40 could be considered as one of proapoptotic regulators in oxidative stressed human airway epithelial cells.

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**ABSTRACT (IN KOREAN)**

# 고농도 산소에 의한 인체 기도상피세포 손상에서 YKL-40 의 apoptosis 조절

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YKL-40는 enzyme activity를 가지지 않는 chitinase로 선천면역반응에서 apoptosis 를 조절하며 중요한 역할을 하는 것으로 알려져 있다. 산소는 인간의 생존에 필수적인 요소이나, 생리학적 필요 농도 이상의 지속적인 산소 노출은 폐를 포함한 많은 장기에 조직 손상을 일으키며, 이러한 손상은 상피세포의 손상과 사멸로 특징지어진다.

본 연구에서는 인간의 기도 상피세포에서 YKL-40가 고농도 산소에 의한 상피세포 손상 및 apoptosis를 조절한다는 가정 하에 기도 상피세포를 고농도 산소에 24-72시간 동안 노출한 후 annexin V-PI staining, real-time PCR, Western blot, short hairpin RNA (shRNA), over-expression 등의 방법을 통해 YKL-40와 apoptosis 조절 인자들의 발현 양상을 비교하고, YKL-40의 역할을 규명해 보고자 하였다.

BEAS-2B 세포에 고농도 산소를 처리하여 주었을 때 apoptosis의 발생이 유도 되며 산소 처리 시간에 따라 apoptosis가 증가함을 FITC-

Annecxin V에 양성을 나타내는 세포의 증가와 reai-time PCR, Western blot을 통한 capase 3와 7의 발현양 증가로 확인 할 수 있었으며, 고농도 산소를 처리해 주었을 때 시간에 따라 YKL-40의 발현양도 증가하는 것을 확인 할 수 있었다. 또한 YKL-40 shRNA와 over-expression vector를 각각 transfection 한 후 세포를 고농도 산소에 노출 시켰을 때, YKL-40 shRNA에 의해 caspase 3와 7의 발현량이 감소하고, 반대로 over-expression 에 의해서는 증가하는 것을 확인 할 수 있었다.

결론적으로 YKL-40가 고농도 산소에 의해 인체기도 상피에서 유발된 apoptosis에서 proapoptotic 조절 인자 중의 하나로 작용 하는 것으로 사료 된다.

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핵심 되는 말 : 고농도 산소, YKL-40, Chitinase-3 like 1, Apoptosis, 호흡기 상피세포, BEAS-2B