Cytoprotective Effect of Kaempferol on Paraquat-Exposed BEAS-2B Cells via Modulating Expression of MUC5AC

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Received March 17, 2014; accepted June 19, 2014

Mucins are highly glycosylated secretory proteins produced by most epithelial cells. Hypersecretion of mucins is one of the prominent symptoms of several airway diseases, including asthma, cystic fibrosis, nasal allergy, rhinitis, and sinusitis. Paraquat (PQ), a common herbicide, has been associated with pulmonary damage and is a potent reactive oxygen species (ROS) producer. However, until now the role of PQ on mucin overproduction has not been studied. The aim of this study is to explore how kaempferol (KM), a widely used dietary flavonoid, affects the protection of human PQ-exposed bronchial epithelium BEAS-2B cells by suppressing Mucin gene expression via nuclear factor-kappa B (NF-κB). We observed that PQ generates intracellular ROS, and also induces lipid peroxidation in BEAS-2B cells. Additionally, we found that PQ effectively induces the expression of the MUC5AC gene; however, co-treatment of PQ with KM drastically reduces its expression. Furthermore, we observed that PQ activates NF-κB, while co-treatment with KM occludes its nuclear translocation, and additionally KM repressed the PQ phosphorylation of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) in BEAS-2B cells. Based on our data, we believe that KM can suppress the over-expression of the MUC5AC gene. This would contribute to the protection of PQ cytotoxicity to exposed BEAS-2B cells, and allow further study toward a better understanding of ROS-associated diseases.

Key words MUC5AC; kaempferol; reactive oxygen species; human bronchial epithelial BEAS-2B cell; nuclear factor-kappa B

Several studies have been carried out demonstrating hypersecretion of mucous from respiratory epithelial cells a major pathological event in chronic airway diseases such as chronic obstructive pulmonary diseases or asthma. 1,2 These pathological manifestations are governed by the interaction between underlying genetic factors and xenobiotics, which together are responsible for intercellular reactive oxygen species (ROS) production. 3 Findings have shown that ROS such as: hydrogen peroxide, superoxide anion, hydroxyl ion are responsible for the initiation and progression of chronic airway diseases via over-expression of Mucin genes. 4–6 ROS is a major etiological factor in the pathogenesis of chronic inflammatory airway diseases as it triggers mucin secretion in epithelia. 7

Twenty different MUC genes have been characterized in human airway epithelia, these are then subdivided into membrane-bound and secreted forms. 8–11 MUC5AC is a hallmark gene, and considered the most phenotypical gene in respiratory diseases; it accumulates in respiratory tract can lead to symptoms such as chronic cough and excessive sputum production. Previous studies showed that paraquat (PQ) is a potent ROS inducer in different epithelial cell lines 12–14 and is associated with cellular cytotoxicity. However, there has been no study into whether PQ can induce MUC gene expression or not, so in this study we investigated the effects of PQ on Bronchial epithelial BEAS-2B cells.

Kaempferol (KM) is a natural flavonoid that exists in broccoli, chives, kale, propolis and various other vegetable sources. 15,16 We chose KM due to its beneficial effects such as an antioxidant, anti-inflammatory, anti-hypertensive, and is an anti-carcinogenic agent. 17–20 It has been shown that KM can provide maximum attenuation of neuronal diseases progression in ROS-mediated cellular toxicity in both in vitro and in vivo. 17,21

Nuclear factor-kappa B (NF-κB) is a redox-sensitive transcription factor that conduct different physiological event involving inflammatory process, cellular development, and apoptosis. 22 It is known that several xenobiotics can activate NF-κB and others can restrain NF-κB signaling on inflammation and carcinogenesis. 23,24 A previous study demonstrated that MUC5AC is activated through NF-κB stimulation involving mitogen-activated protein (MAP) kinase inhibition. 25,26 Song et al. has been reported that ROS are accountable for activating MUC5AC gene expression by stimulating the MAP kinase pathway. 27 A detailed study on the mechanisms of MUC5AC induction in normal human PQ-exposed bronchial epithelial cells had not been undertaken. Ample evidence supports the hypothesis that MUC5AC induced by PQ may mediate by the MAP kinase-NF-κB signaling pathway. We also hypothesized that antioxidant compound KM could abate the overexpression of the MUC5AC gene.

In order to test this hypothesis, we examined the cytoprotection mechanisms by which KM contributed to cell survivability in the human BEAS-2B bronchial epithelial cells treated with PQ. Here we show that two different MAP kinases (c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK)) and NF-κB signaling cascade are essential for PQ-induced MUC5AC gene expression in BEAS-2B cells. We also show that KM significantly reduces the gene expression level of MUC5AC where cells were co-treated with PQ. Moreover, KM dramatically revives cell injury when BEAS-2B cells were co-treated with PQ. Collectively, the goal of this study is to contemplate the effect of PQ on pathological event and likewise protection effect of KM by suppressing MUC5AC expression.
MATERIALS AND METHODS

Chemicals, Inhibitors, and Antibodies  Kaempferol, silymarin, alpha lipoic acid, paraquat, 3-(4,5-dimethythiazol-2-yl)-2,5-diphenylicetrazolium bromide (MTT), trypan blue stain solution, and Triton-X100 were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Chemical inhibitors (SP600125, PD98059) were bought from LC Laboratories (Woburn, MA, U.S.A.). BAY11-7082 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Bronchial epithelial cell basal medium (BEBM) was obtained from Lonza (Walkersville, MD, U.S.A.). Fetal bovine serum was purchased from HyClone (Logan, UT, U.S.A.) and antibiotic/antimycotic (100 U/mL penicillin, 100 µg/mL streptomycin and 0.25 µg/mL amphotericin B) were obtained from Gibco (Grand Island, NY, U.S.A.). Lactate dehydrogenase (LDH) was purchased from Roche (Pleasanton, CA, U.S.A.) and all polymerase chain reaction (PCR) primers were purchased from Bioneer (Daejeon, Korea). All primary antibodies except for β-actin were purchased from Cell Signaling Technology (Danvers, MA, U.S.A.), and the β-actin specific antibody was obtained from Abcam (Cambridge, MA, U.S.A.). The anti-rabbit immunoglobulin G (IgG)-horseradish peroxidase (HRP) and anti-mouse IgG-HRP were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.).

BEAS-2B Cell Culture and Measurement of Cellular Toxicity  The human bronchial epithelial BEAS-2B cells were purchased from American Type Culture Collection (Manassas, VA, U.S.A.). BEAS-2B cells were cultured in BEBM and maintained for 10 to 15 passages in a 37°C humidified incubator containing 5% CO₂. The PQ and KM were dissolved water and ethanol, respectively. The PQ and KM were applied to the culture plates at different concentrations for various times as indicated. The cytotoxicity was determined using MTT assay. The level of LDH releases in the culture media was quantified using a colorimetric test based on NADPH. The measurement technique was followed exactly as per the supplier’s protocol. The absorbance was read using a Victor X3 multilabel reader (PerkinElmer, Inc.). The measurement was followed exactly as per the supplier’s protocol. The cytotoxicity was determined using MTT assay. The level of LDH releases in the culture media was quantified using a colorimetric test based on NADPH. The measurement technique was followed exactly as per the supplier’s protocol.

Measurement of Intracellular ROS and Lipid Peroxidation Level  An ROS detection kit (Enzo Life Sciences, Farmingdale, NY, U.S.A.) was used to measure the total ROS generated by PQ as, previously described in our previous study. The BEAS-2B cells were seeded in 12-well tissue culture plates at a density of 1×10⁵ cells/mL and grown to 50–70% confluency. Then the cells were co-treated with KM and PQ. After stimulation, the medium was replaced with a ROS wash buffer containing a ROS detection solution. The fluorescence intensity was measured using an Axiovert 25 Microscope (Carl Zeiss, Jena, Germany). The measurement technique was followed exactly as per the supplier’s protocol. The absorbance was read using a Victor X3 multilabel reader (PerkinElmer, Inc.). The measurement was followed exactly as per the supplier’s protocol.

A lipid peroxidation assay was performed as described earlier. The cells were co-treated with PQ and KM for 24 h then cells were lysed with malondialdehyde (MDA) lysis buffer. A 200 µL supernatant was collected by centrifugation to quantifying the amount of MDA. After that, the supernatant was mixed with a 600 µL thiobarbituric acid (TBA) solution and then the absorbance was taken at 530 nm using a VictorTM X3 multilabel reader (PerkinElmer, Inc.). The peroxidation was measured from a standard curve and expressed as nmol/mg protein.

Nuclear and Cytosolic Protein Preparation  Cytosolic and nuclear protein fractions were analyzed as per manufacturer’s protocol (Biovision, Mountain View, CA, U.S.A.). The detailed procedure is explained in our previous study. Briefly, the cells were exposed with PQ and KM in 6 well-plates. After treatment, the cells were washed twice with cold 1× phosphate buffered saline (PBS) and lysed with a cytosol extraction buffer. The supernatant was collected by centrifugation at maximum speed for 5 min. The nuclear fraction was separated using a nuclear extraction buffer.

Western Blot Analysis  Cells were washed twice with cold PBS buffer and lysed with RIPA lysis buffer (Santa Cruz Biotechnology, CA, U.S.A.) containing protease inhibitors including dithiothreitol (DTT). Lysates were separated by centrifugation at 1400×g for 15 min at 4°C. The protein samples were separated by 10% sodium dodecyl sulfate (SDS) polyacrylamide gels and transferred to a polyvinylidene difluoride (PVDF) membrane. The membranes were blocked in 5% skimmed milk for 1 h at room temperature. Then, the membranes were incubated with the indicated primary antibodies, and then incubated with appropriate HRP-conjugated secondary antibody for 90 min. Immunoreactive proteins were detected using an enhanced chemiluminescence reagent, and images were captured using a ChemiDoc XRS+ imaging system with Image Lab™ software (Bio-Rad, Hercules, CA, U.S.A.).

Quantitative Real-Time Reverse Transcription (RT)-PCR (qRT-PCR) Assay  Cells were co-treated with indicated doses of silymarin (Sily), alpha lipoic acid (LA), and KM for 24 h. The total RNA was collected from the BEAS-2B cells using a mini RNA isolation kit (Qiagen, Valencia, CA, U.S.A.) according to the manufacturer’s instructions, and 1 μg RNA was converted into cDNA using the Maxime RT PreMix kit (Intron Biotechnology, Seoul, Korea). The detailed primer information is listed in Table 1. MUC5AC, DUOX1, and NOX4 (AccuTarget™ qRT-PCR Primer set) primer sets were purchased from Bioneer (Daejeon, Korea). Quantitative RT-PCR was performed using a CFX96™ Real-Time PCR Detection System with iQTM SYBR Green Supermix (Bio-Rad). The ΔΔCt values of the genes were represented as relative fold induction and were normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an endogenous control.

Small Interfering RNA (siRNA) Assay  siRNA (sc-29380, Santa Cruz Biotechnology, CA, U.S.A.) against JNK Kinase was used to occlude JNK specific expressions. The cells were seeded in an antibiotic-free normal growth medium and incubated at 37°C in CO₂ until the cells got to around 50% confluent.

Table 1. Primer Sets Used in the Study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences</th>
<th>Amplicon sizes</th>
</tr>
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<tr>
<td>MUC5AC (F)</td>
<td>5'-CGACCTGTGTCTGATCCAT-3'</td>
<td>140bp</td>
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<tr>
<td>MUC5AC (R)</td>
<td>5'-CCACCTCGGTAGCTGAA-3'</td>
<td>79bp</td>
</tr>
<tr>
<td>DUOX1 (F)</td>
<td>5'-CCCTGGCTTACGATGACAC-3'</td>
<td>140bp</td>
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<td>DUOX1 (R)</td>
<td>5'-CTGCACTTCCCACGAATG-3'</td>
<td>380bp</td>
</tr>
<tr>
<td>GAPDH (F)</td>
<td>5'-TCCATACATTCTTCACA-3'</td>
<td>140bp</td>
</tr>
<tr>
<td>GAPDH (R)</td>
<td>5'-CATCACGCCAGTTCCT-3'</td>
<td>140bp</td>
</tr>
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</table>
ency. For each transfection, 6 µL siRNA duplex and siRNA transfection reagent (sc-29528) were diluted in 100 µL siRNA transfection medium (sc-36868). The siRNA duplex and siRNA transfection reagent were mixed and incubated for 30 min at room temperature. The mixture of siRNA duplex and siRNA reagent was then added to the cells with 800 µL of transfection reagent.
tion medium, and then overlaid onto the cells. The cells were incubated at 37°C in CO₂ for 6h. The transfection reagent did not interfere with the viability of the cells. After incubation, the transfection medium was aspirated, and replaced by normal growth medium for additional 24h incubation under normal cell culture conditions.

**Statistical Analysis** All experiments were performed in triplicate. The results were expressed as means±S.D. and were analyzed by Student’s t-test and assuming equal variance. A significant value was defined as \( p < 0.05 \).

**RESULTS**

**PQ Induces MUC5AC mRNA Expression through MAP Kinase-NF-κB Signaling Pathway** To investigate whether PQ can actuate the secretary MUC gene expression in human epithelium BEAS-2B cells, we assessed quantitative real-time RT-PCR using MUC5AC specific primers (Table 1). As shown in Fig. 1, PQ can effectively induce the expression of MUC5AC mRNA. The induction of MUC5AC mRNA was detected with time and dose-dependent manner of PQ treatment (Figs. 1A, B). In order to determine which MAP kinase signal pathway responded in the BEAS-2B cells stimulated by PQ, we performed immunoblot analysis using MAP kinase antibodies. JNK and ERK MAP kinases were maximally induced at 15 min, and this effect declined after 30min (Figs. 2A, D). Nevertheless, no change was observed in the activation of p38 kinase (data not shown). In order to test JNK and ERK MAP
kinases signaling in PQ-induced MUC5AC gene expression, we applied to 20µM of PD98059 and SP600125 inhibitors, prior to treatment with PQ. The Western blot analysis showed that SP600125 inhibitor significantly impedes JNK but not by PD98059 (Fig. 2B). To confirm the significance of JNK inhibition upon the expression level of MUC5AC, we checked the MUC5AC gene expression by JNK siRNA (Fig. 2C). Interestingly, JNK siRNA specifically reduced MUC5AC gene expression compared with transfection using control siRNA (Fig. 2C). This data suggests that phosphorylation of JNK kinase is involved in PQ-induced MUC5AC expression. It has been known that NF-κB signal pathway is directly associated with MUC5AC expression. In order to assess NF-κB signaling on PQ condition, BEAS-2B cells were treated with 0.2mM of PQ (Fig. 2E). The phosphorylation of NF-κB was immensely induced by treatment of PQ, and the phosphorylation of NF-κB was peaked at 30min after PQ exposure and decreased afterward (Fig. 2E). To further confirm that NF-κB signaling pathway is involved closely in PQ condition, BEAS-2B cells were exposed with NF-κB inhibitor BAY 11–7082 for 1h prior to PQ exposure, the effect of NF-κB inhibitor on MUC5AC gene expression was observed by qRT-PCR (Fig. 2F). The gene expression level of MUC5AC was significantly reduced by BAY 11–7082 treatment. These results suggest that PQ-induced MUC5AC activation is depended on NF-κB signaling.

**KM Attenuates PQ-Induced Cellular Injury** In order to search for a functional ingredient for treatment of PQ induced injury, we screened several candidate molecules including phytochemicals. KM is known as a potent antioxidant, and used various diseases including cancer. In Fig. 3A, KM has no cytotoxicity itself up to 5µM concentration. However, interestingly, PQ with 5µM of KM can significantly attenuate PQ-induced cell death by 5.20%, 33.85%, 19.76%, and 11.10% at 24, 48, 72, and 96h, respectively (Fig. 3B). Morphological studies showed that KM has a protective effect on PQ-treated BEAS-2B cells (Fig. 3C). We observed that BEAS-2B cells exhibited a round and shrunken shape in PQ-treated condition, while KM co-treatment can maintain cell shape as in the control cells (Fig. 3C). We further determined membrane integrity; LDH releasing was measured from PQ treatment alone and co-treatment of PQ and KM in BEAS-2B cells. As shown in Figs. 3D and E, cells were co-treated with 5µM KM and PQ, the PQ-induced LDH releases significantly abated by: 18.91%, 18.91%, and 19.89% at 6, 12,
and 24 h, respectively. KM can reduce LDH release in a dose dependent manner, and 5 µM of KM reduced PQ-induced LDH release level by 35.91% (Figs. 3D, E).

To examine whether KM regulates ROS-related gene expression in BEAS-2B cells, we evaluated the expression of DUOXs and NOXs genes by qRT-PCR (Figs. 3F, G). PQ treatment increases DUOX1 and NOX4 with 3.5 fold and 2.5 fold, respectively. However, the expression significantly diminished on KM/PQ co-treated cells both at 15 min and 30 min. The data suggests that KM suppresses ROS-related gene expression in PQ-exposed cells.

**KM Inhibits PQ-Induced Intracellular ROS Generation, and LPO Level** To evaluate the role of KM in PQ-induced ROS generation, we also measured intracellular ROS generation. In this experiments we used NAC is a negative control and pyocyanin (PC) as a positive control for ROS inducer. As shown in Figs. 4A and B, we observed that PQ has induced intracellular ROS (labeled with PC and PQ in Fig. 4A), while KM itself did not induce ROS generation. However, PQ/KM-treated and NAC-treated cells have significantly reduced the total ROS level (Figs. 4A, B). This data suggests that KM can successfully suppress intracellular PQ-exposed intracellular ROS generation.

To test the hypothesis that KM treatment contributes to decreasing the level of lipid peroxidation in PQ exposed condition, the lipid peroxidation assay was performed (Fig. 4C). KM (5 µM) co-treatment in PQ-treated cells can notably suppress total MDA level while KM itself did not interfere.
KM Involves in PQ-Induced Phosphorylation of JNK Kinase and NF-κB Signaling In order to investigate the role of KM on molecular signaling pathway in MUC5AC regulation, we further scrutinized the effect of KM on PQ-exposed MAPK/NF-κB signaling pathway. PQ treatment can effectively induce phosphorylation of JNK and ERK kinases while 5 μM KM itself has induction of JNK kinase but not ERK kinase (Fig. 5A). Interestingly, the phosphorylation of both ERK and JNK kinases were significantly suppressed by co-treatment of 5 μM KM with PQ (Fig. 5A). These data suggests that KM can effectively block this phosphorylation of MAP kinases. Next we further investigate the effect of KM on PQ-induced NF-κB signaling (Fig. 5B). PQ treatment led to NF-κB nuclear translocation but not by KM. This nuclear translocation of NF-κB was diminished or blocked by KM cotreatment (Fig. 5B). We also observed that the phosphorylation of IκBα manifested when cells were exposed to only PQ for 30 min in cytosol protein (Fig. 5B). Data suggested that KM interfered IκB/NF-κB dissociation and NF-κB nuclear translocation. Then we further confirmed the interfering role of KM in JNK/NF-κB signaling using various inhibitors (Fig. 5C). PQ treatment induced phosphorylation of NF-κB beside activation of NF-κB was abolished by treatment of NF-κB selective inhibitor BAY11-7082. However, in PQ with either JNK or ERK kinase inhibitors, phospho-NF-κB was dramatically reduced (Fig. 5C).

We then examined the potential effect of KM on PQ-induced MUC5AC expression (Fig. 5D). Interestingly, the expression of MUC5AC in the co-treatment of KM (5 μM) with PQ (0.2 mM) in BEAS-2B cells was significantly reduced (Fig. 5D) while KM itself did not alter MUC5AC expression. In order to confirm that an antioxidant can repress the expression of MUC5AC, the cells were co-treated with 10 μM N-acetyl-L-cysteine (NAC) in a PQ treated condition. It has shown that NAC markedly suppressed MUC5AC gene expression in presence of PQ (Fig. 5E). However, other potent antioxidants, Silymarin and alpha-lipoic acid have not repressed MUC5AC gene expression which was induced PQ-treatment (Supplemental Fig. 1). This data suggests that KM has a potent abating effect on PQ-mediated MUC5AC gene induction.

The collective data suggests that KM suppressed MUC5AC expression via interfering in MAP kinase-NF-κB signaling pathway on PQ-exposed BEAS-2B cells.

DISCUSSION

Hypersecretion of mucin is the pathophysiologial consequences of various pulmonary diseases including lung cancers and is induced in response to a variety of stimuli viruses, bacteria and other noxious chemicals. One of the mucins, secreted from MUC5AC gene, is known as a major MUC gene.29 and its protein content and transcription levels in airway epithelial cells represent the magnitude of mucous secretion. ROS is known to be one of the major stimuli for over production of mucus in lung epithelial cells.29 It was shown that oxidative stress is known as an inflammatory stimulant to induce MUC5AC gene expression in upper respiratory tract.7 MUC5AC gene was up-regulated in H2O2-exposed cell lines including NHNE and NCI-H292 cells.6,7 However, there is not much known about the molecular mechanism of MUC5AC gene regulation by PQ-exposed ROS in human bronchial epithelial cells. This idea allowed us to speculate that PQ effectively expresses the MUC5AC gene. In this study, we investigated whether PQ can effectively do that and any possible signaling pathway might involve in MUC5AC regulation. In addition, we also tried to find out any functional chemicals may use for treat of MUC5AC regulation.

We found that PQ can induce of MUC5AC expression in BEAS-2B cells. Furthermore, for effective induction of MUC5AC, JNK kinase and NF-κB signaling pathway are involved in PQ exposed condition in human tracheal epithelial cells. It is known that various transcriptional factors such as NF-κB, AP-1, and SP1 are responsible for regulation of MUC5AC gene expression in different airway epithelial cells in response to stimuli.25,26,30 In particular NF-κB sites on MUC promoter site perform a crucial role in regulation of MUC expression in human bronchial epithelial cells.31 In this study, PQ can induce phosphorylation of NF-κB and its nuclear translocation along with MUC5AC gene expression. The induction of MUC5AC expression was totally removed by NF-κB inhibitor (BAY11-7082). This data suggests that MUC5AC induction by PQ is the activation of NF-κB signaling.

Lora and colleagues report have shown that MUC5AC is up-regulated by NF-κB activation.32 Thus, taken together, it is conceivable that the intracellular signaling coordination by NF-κB activation may be essential for PQ-induced MUC5AC gene expression. Nie et al., demonstrated that MAP kinases signaling pathway is directly implicated in regulation of MUC expression.25 We observed that PQ can induce phosphorylation of JNK and ERK kinases but not p38 kinase. The event...
was further evaluated by specific inhibition of JNK expression by an in vitro siRNA JNK kinase. Although MUC5AC activation by MAP Kinase has been established by previous studies in other cells lines, degradation of A549 cells. Therefore, we suggest that the regulation of MUC5AC has distinct pathway depending on the type of stresses and cell types. The defined molecular mechanism needs to be investigated in-depth.

KM has been highlighted over the past years due to its multifunctional efficacies in various diseases such as Parkinson’s disease and cancer. It has been deemed as a drug for the management of inflammatory processes of airway diseases due to its potent anti-inflammatory and antioxidant characteristics. In addition, other studies have also been demonstrated that KM can block not only lipopolysaccharide-induced NF-κB signaling but also suppress stress-induced MUC expression. However, the beneficial effects of KM on PQ-mediated ROS stress in BEAS-2B cells have not been well elucidated yet.

In this study, we found that KM has a great cytoprotective effect on PQ-treated BEAS-2B cells via scavenging of ROS and reducing LDH activity and lipid peroxidation. We suggest that KM can interfere with polar head groups of phospholipids at the lipid–water interface of the membrane and membrane integrity against PQ-induced ROS stress. It has been suggested that DUOX and NOX families are critical roles in regulation of intracellular ROS generation in cells. The expression of NOX4 and DUOX2 can be induced by treatment various reagents including H2O2, and subsequently generate intracellular ROS and induction of MUC5AC in human bronchial epithelial cells. In this study, we observed that the expression of NOX4 and DUOX1 was induced by PQ treatment in BEAS-2B cells. Interestingly, we also found that co-treatment with KM in condition of PQ can eliminate the induction of NOX4 and DUOX1 mRNA expression. Researchers have demonstrated that the expression and regulation of DUOX2 and NOX4 are important factors to regulate ROS generation and MUC5AC expression in airway cells.

Interestingly, other well-known phytochemicals, such as Silymarin and alpha-lipoic acid could not effectively repress the expression of MUC5AC on PQ-treated BEAS2B cells. Data suggested that the effect of KM on MUC5AC induction is a distinct. Therefore, we propose that KM may be a good target molecule for ROS-induced airway diseases.

In summary, we provide evidence for the first time that PQ can induce MUC5AC gene expression via JNK/ NF-κB signaling. In addition, KM can protect PQ-induced cellular toxicity and regulate MUC5AC mRNA expression in human bronchial epithelial cells. Although further studies on the signaling pathways are required for understanding of inhibitory effect of KM on PQ-activated MUC secretion in airway diseases, collective data suggested that KM may be a good candidate molecule in ROS-related and MUC5AC expression-related airway diseases.

Acknowledgments This research was partly supported by the National Research Foundation of Korea (NRF-2012R1A1A2040626 for Y.-S. Kim) and by Soonchunhyang University Research Grant.

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