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Aquaporin 5 expression inhibited by LPS via p38/JNK signaling pathways in SPC-A1 cells

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ABSTRACT

Proper H_2O to mucin ratio of airway mucus is important for mucociliary clearance. Recent studies suggest that decreased aquaporin 5 (AQP5) is correlated with increased staining of MUC5AC in submucosal glands of COPD patients. Lipopolysaccharide (LPS) is one of the major insults in airway mucin secretion in COPD. In this study, changes in both AQP5 and MUC5AC expression levels in SPC-A1, a human airway submucosal gland cell line, were quantified after exposure of the cells to LPS. AQP5 transcription and protein expression were decreased while MUC5AC expression was increased by LPS exposure in SPC-A1 cells. Further studies revealed that AQP5 expression was down-regulated via the p38/JNK signaling pathway, while MUC5AC was up-regulated through the EGFR-p38/JNK pathway. Therefore, p38 and JNK may become promising targets to preserve AQP5 expression and prevent MUC5AC over-expression to restore proper H_2O to mucin ratio of the airway mucus, which may be beneficial to the clinical management of COPD patients.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is one of the major causes of chronic morbidity and mortality globally. It is currently the fourth leading cause of population death and is projected to rank the fifth as a worldwide burden of disease by 2020 (Rabe et al., 2007).

COPD is pathologically characterized by goblet and submucosal gland cell hyperplasia as well as mucus hypersecretion (Vestbo et al., 1996). Airway mucus is mainly composed of water and ions, within which approximately 2% is mucins (Rogers, 2003; Livraghi and Randell, 2007). Of the 20 known human MUC genes, MUC1, MUC2, MUC4, MUC5AC, MUC5B, and MUC6 are expressed in airways (Casalino-Matsuda et al., 2006). Among those mucin genes, MUC5AC and MUC5B are the major gel-forming mucin genes. Study showed MUC5AC is highly inducible while MUC5B is expressed constitutively (Rogers, 2004). In the airway, MUC5AC was thought to be produced by goblet cells rather than submucosal gland cells. However, recent studies show that MUC5AC is present in secretions from the human tracheal glandular ducts, suggesting that submucosal gland cells are also the source of MUC5AC (Roger et al., 2001; Caramori et al., 2009). In addition, it has been shown that COPD is associated with increased MUC5AC expression in submucosal glands (Ma et al., 2005; Inoue et al., 2008).

Recent studies suggested association between mucin secretion and Aquaporin 5 (AQP5) expression. The AQPs were a family of small (30 kDa monomer) integral membrane proteins that function as selective water transporters (Verkman, 2007). Thirteen related AQPs have been discovered in mammals and at least four of them were present in the lung and airway: AQP1 is expressed in microvascular endothelia, AQP3 and AQP4 are expressed in airway epithelia, and AQP5 is localized at apical membrane of type I alveolar epithelial cells, acinar epithelial cells in submucosal glands and large airway epithelia (Kreda et al., 2001; Chen et al., 2006a,b). Deletion of AQP5 resulted in more concentrated protein and mucus secretion in the upper respiratory tract of mice (Song and Verkman, 2001), possibly due to impaired fluid transport without AQP5 expression as seen in salivary gland secretion (Ma et al., 2000).

In COPD patients, decreased AQP5 expression is associated with increased staining of MUC5AC in their submucosal glands (Wang et al., 2007). Both AQP5 down-regulation and MUC5AC up-regulation could potentially result in dehydrated and viscous mucus. The mechanism for AQP5 down-regulation and MUC5AC up-regulation is not clear. AQP5 down-regulation could be an independent event or could result in MUC5AC up-regulation. Our previous study showed AQP5 down-regulation by siRNA in SPC-1 cell line was associated with MUC5AC over-expression (Chen et al., 2006b). It has been shown that bacterial endotoxins lipopolysaccharides

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(LPSs) are associated with mucin overproduction in COPD patients (Wang et al., 2009). In this study, SPC-A1, a human airway submucosal gland cell line, was used to quantify modulations of AQP5 and MUC5AC after LPS exposure, and assess possible signaling pathways leading to alterations in AQP5 and MUC5AC expression.

2. Methods and materials

2.1. Cell culture

SPC-A1, a human lung adenocarcinoma cell line, was provided by Shanghai Cell and Biology Institute, Chinese Academy of Science (Shanghai, China). SPC-A1 cells were cultured in RPMI 1640 medium (Gibco, USA) supplemented with 10% fetal calf serum (Gibco, USA), penicillin (100 U/ml), and streptomycin (100 U/ml) in a humidified atmosphere with 5% CO_2 at 37 °C. Before experiments, confluent SPC-A1 cells were serum-starved for 24 h to maintain low basal levels of MUC5AC and AQP5 expression.

2.2. Treatment of cells with LPS and inhibitors

After 24 h of serum starvation, SPC-A1 cells were stimulated with LPS (Sigma, USA) at various time and concentrations. To assess the role of EGFR in LPS-induced AQP5 transcription, the cells were treated with EGFR tyrosine kinase inhibitor AG1478 (Calbiochem, USA) at 2 and 10 μM. To decide how AQP5 transcription was affected by intracellular MAPK activation, the cells were treated with either ERK1/2 inhibitor PD98059 (Cell Signaling Technology, USA) at 2 and 10 μM, p38 inhibitor ML3404 (Calbiochem, USA) at 2 and 10 μM or JNK inhibitor SP600125 (Calbiochem, USA) at 2 and 10 μM. For inhibitor studies, serum-starved cells were pretreated with the inhibitors for 30 min before exposure to stimuli, the cells were then cultured for 6 h with both LPS and the inhibitors.

2.3. Quantitative real-time PCR

Total RNA was isolated from cultured cells using Trizol reagent (Invitrogen) following the manufacturer's instruction and was quantified by spectrophotometer. cDNA was reverse transcribed with MMLV first-strand synthesis kit (BBI, USA). PCR reactions were preformed in 25 μl-reaction containing 1 μl cDNA, 1 mM of each forward and reverse primer, and 0.25× SyBr green Mix. β-Actin was used as the internal control to quantitate initial cellular transcripts. Primer sequences included: β-actin—sense: 5′CCTGTACGCCAACACAGTGC3′, antisense: 5′ATACTCCTGCTTGCTGATCC3′; AQP5—sense: 5′CTGTCCATTGGCCTGTCTGTC3′, antisense: 5′GGCTCATACGTGCCTTTGATG3′; MUC5AC—sense: 5′GAGGGCAACAACGTCATCTCC 3′, antisense: 5′TCT TGGTCAGCCACCTTCACA3′. Real-time PCR conditions for amplification of target genes were as follows: pre-denaturing at 94 °C for 5 min, 40 cycles of denaturing at 94 °C for 10 s, annealing for AQP5 at 60 °C (MUC5AC at 59 °C, β-actin at 57 °C) for 15 s, extension at 72 °C for 20 s, followed by the last extension at 72 °C for 10 min. Amplification data measured by fluorescence were collected in real-time and analyzed by Rotor-Gene 6.0.14 Software.

2.4. Immunofluorescence staining

SPC-A1 cells were seeded into six-well tissue culture plates, with glass cover slips placed on the bottom. Once the cells were 70–80% confluent, the cover slips were removed. Cells were washed with PBS, fixed in 4% paraformaldehyde for 20 min, and then incubated with anti-AQP5 primary antibody (1:100 Calbiochem, USA) in a humidity chamber at 37 °C for 1 h. Cells were washed three times in PBS and incubated with goat anti-rabbit IgG antibody conjugated to FITC at room temperature for 1 h. After being washed three times in PBS, cells were visualized using a Zeiss microscope (Carl Zeiss, Germany).

2.5. Western blot analysis

Western blots were performed as described in the manufacturer's instructions (Wang et al., 2009). Briefly, western blots were performed using whole cell extracts, separated on 8–10% SDS-PAGE gels and transferred to polyvinylidine difluoride membranes (Pall Life Sciences, Pensacola, FL). The membrane was blocked with a solution of TBS containing 0.1% Tween 20 (TBS-T) and 5% nonfat milk. After three washes in TBS-T, the membrane was incubated with anti-AQP5 primary antibody (1:1000 Calbiochem, USA). After another three washes in TBS-T, the membrane was incubated with 1:2000 dilution of the corresponding secondary antibody. The membrane was reacted with chemiluminescence reagent ECL (Amersham Biosciences) to visualize the blots.

2.6. MUC5AC ELISA

After incubation of SPC-A1 cells at different conditions, levels of MUC5AC protein in cell culture supernatants and in cell lysates were measured by ELISA as previously described (Takeyama et al., 1999). The amount of MUC5AC protein in each tested portion was normalized to total protein and expressed as fold changes over control.

2.7. Statistical analysis

Data were expressed as means ± SEM. Statistical differences among multiple groups were calculated by using one-way analysis of variance (ANOVA). If a certain ANOVA was statistically significant, Student–Newman–Keuls test was used in multiple comparisons. Statistically significant differences were accepted at $p < 0.05$.

3. Results

3.1. LPS exposure progressively reduced AQP5 mRNA and protein expression in SPC-A1 cells

The indirect immunofluorescence staining showed that AQP5 was located in the membrane of SPC-A1 cells (Fig. 1A). The cells were incubated in media supplemented with 0, 5, 10, 20, and 40 μg/ml LPS for 6 h in real-time PCR or for 24 h in western blotting. AQP5 mRNA and protein levels decreased significantly with 10, 20 or 40 μg/ml LPS treatment in a dose-dependent manner (Fig. 1B). AQP5 mRNA and protein decreased maximally with 20 μg/ml LPS, thus 20 μg/ml LPS was used for subsequent experiments. We have routinely checked cell viability by MTT and found no evidence that the dose of LPS used in this study was harmful to the cultures (data not shown here).

To analyze the time course of LPS-mediated inhibition on AQP5 expression, SPC-A1 cells were treated with LPS for various periods of time before total RNA and protein isolation. AQP5 mRNA decreased significantly to about 20% of the control level after 6 h LPS treatment. Similarly, AQP5 protein decreased dramatically to about 20% of the control level after 12, 24 or 48 h of LPS treatment (Fig. 1C). AQP5 protein did not return to baseline level after 48 h.

3.2. LPS exposure progressively increased MUC5AC mRNA and protein expression in SPC-A1 cells

To determine changes of MUC5AC with different LPS doses, the cells were treated with different concentrations of LPS, i.e.

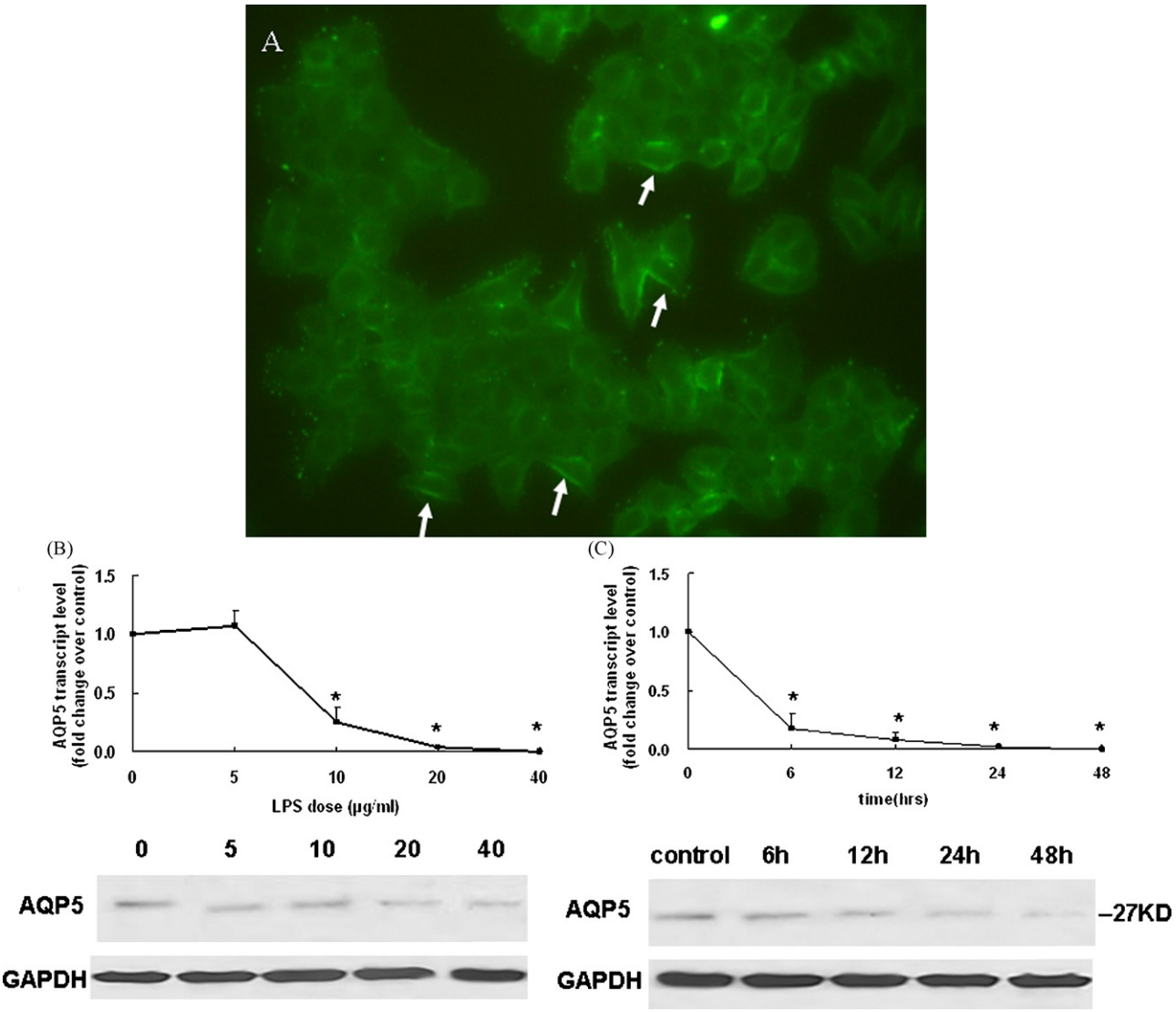

Fig. 1. (A) AQP5 in the membrane of SPC-A1 cells (200×) (indirect immunofluorescence staining). (B) Dose-dependent decrease in AQP5 mRNA and protein along with LPS treatment. Cells were incubated in media supplemented with LPS for 6 h (real-time PCR) or 24 h (western blot). (C) Time-dependent decrease in AQP5 mRNA and protein expression along with LPS treatment. Representative blots from three independent experiments. Results (mean ± SEM, $n = 5$ for each group) were expressed as fold changes over the control. *Significant difference from the control ($p < 0.05$).

0, 5, 10, 20 and 40 μg/ml, for 6 h. MUC5AC mRNA and protein increased proportionally in a dose-dependent manner and both peaked at 40 μg/ml LPS (Fig. 2A). To determine changes of MUC5AC at different time points, the cells were exposed to LPS (20 μg/ml) for 6, 12, 24 or 48 h. Increase of MUC5AC gene and protein level initiated after 6 h and peaked after 24 h (Fig. 2B).

3.3. LPS-induced down-regulation of AQP5 transcript levels was mediated through p38/JNK signaling pathway

To find out which signaling pathway was involved in AQP5 down-regulation with LPS exposure, inhibitors of different kinases were applied to the cells with or without LPS. Decrease of AQP5 transcript was significantly inhibited by both p38 inhibitor (ML3404) and JNK inhibitor (SP600125) pretreatment in a dose-dependent manner. At the concentration of 2 μM, either ML3404 or SP600125 significantly inhibited ($p < 0.05$) the down-regulation of AQP5 mRNA induced by LPS exposure. The inhibition was almost completely reversed at the concentration of 10 μM ($p < 0.05$). Pretreatment of the cells with AG1478 and ERK1/2 inhibitors (PD98059) did not result in an inhibition to the down-regulation of AQP5 transcript levels (Fig. 3) even when the maximum dose recommended by the manufacturer was used. The concentration ranges for each of these agents have been demonstrated to be non-cytotoxic in cell culture proved by using MTT assay (data not shown here).

3.4. LPS-induced up-regulation of MUC5AC transcript levels was mediated through EGFR-p38/JNK signaling pathway

SPC-A1 cells at confluence were pretreated with 10 μM AG1478, 10 μM PD98059, 5.0 μM ML3404, or 10 μM SP600125 for half an hour before being incubated with 20 μg/ml LPS or PBS for 6 h. By using real-time PCR for quantification, LPS-induced up-regulation of MUC5AC transcript was found to be significantly inhibited by EGFR inhibitor AG1478, p38 inhibitor (ML3404) or JNK inhibitor (SP600125) pretreatment (Fig. 4A) while ERK1/2 inhibitor (PD98059) pretreatment did not inhibit MUC5AC transcript increase.

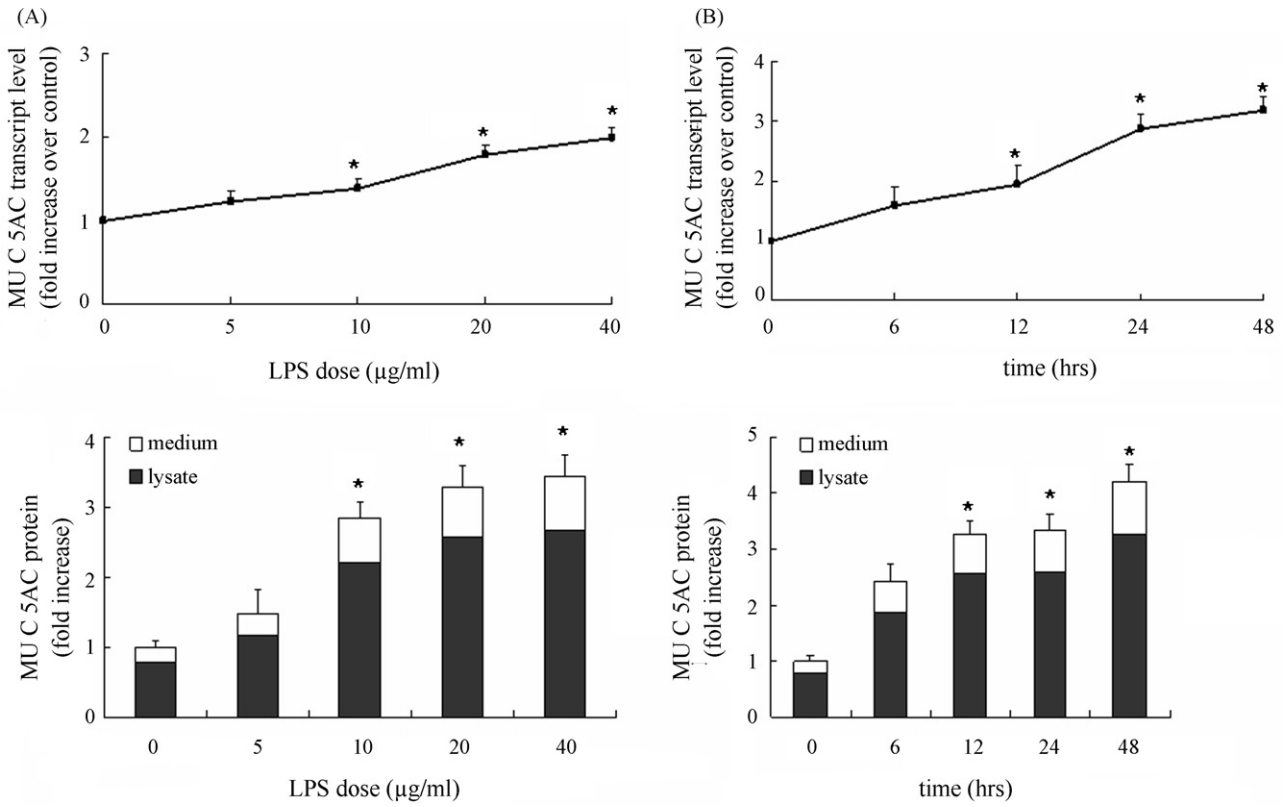

Fig. 2. Concentration- and time-course study of LPS-induced MUC5AC production. (A) MUC5AC gene expression and protein upon LPS treatment, measured by real-time PCR and ELISA. (B) Upon exposure to LPS (20 μg/ml), increase of MUC5AC gene and protein expression. Results (mean ± SEM, $n = 5$ for each group) were expressed as fold changes over the control. *Significant difference from the control ($p < 0.05$).

4. Discussion

Acting as a viscoelastic gel that spreads over the airway luminal surface to form mucociliary clearance system to protect against infectious and exogenous agents, mucus plays an important role in airway defense. However, excessive airway mucus production, termed mucus hypersecretion (Rogers, 2003), may significantly impair mucociliary clearance and facilitate bacteria colonization (Mall, 2008).

The fluid component of gland secretion is driven by active transepithelial ion secretion. Cl^- is actively transported from gland acina cells to apical lumen, then Na^+ comes out for neutral electricity through paracellular pathway and water molecule comes out following ion gradient through apical membrane water channel AQP5 and paracellular pathway, as seen in upper airway submucosal glands and salivary glands (Ma et al., 2000; Song and Verkman, 2001). Deletion of AQP5 significantly reduces glands fluid secretion. As a result, secreted fluid becomes more viscous and dehydrated. Airway clearance, which is critical in COPD patients, may be impaired by viscous and dehydrated glands fluid (Randell et al., 2006; Mail et al., 2008). Attenuated AQP5 staining has been shown to be present in COPD airways (Wang et al., 2007), suggest-

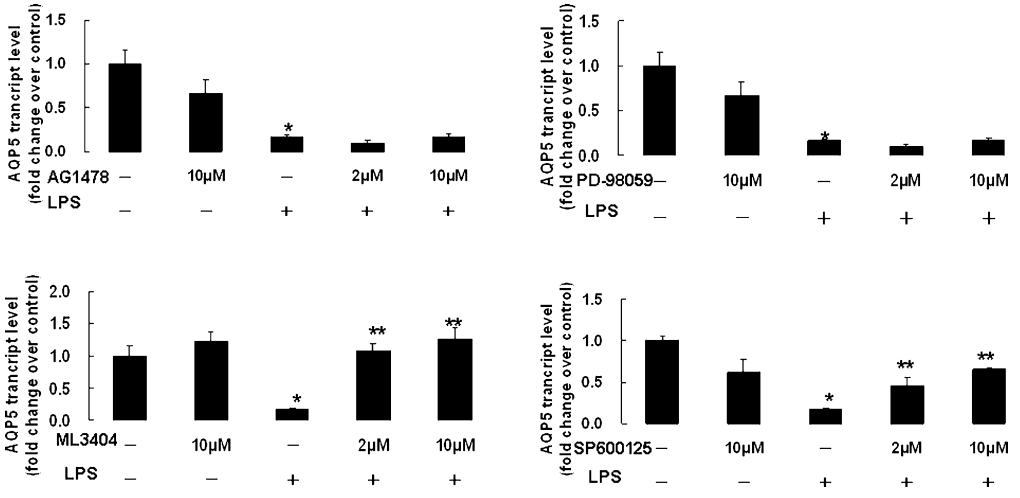

Fig. 3. Effect of AG1478, PD-98059, SP600125 and ML3404 on LPS-induced decrease in AQP5 transcript level. *Significant difference when compared with the control ($p < 0.05$). **Significant difference when compared with the LPS-treated control group ($p < 0.05$).

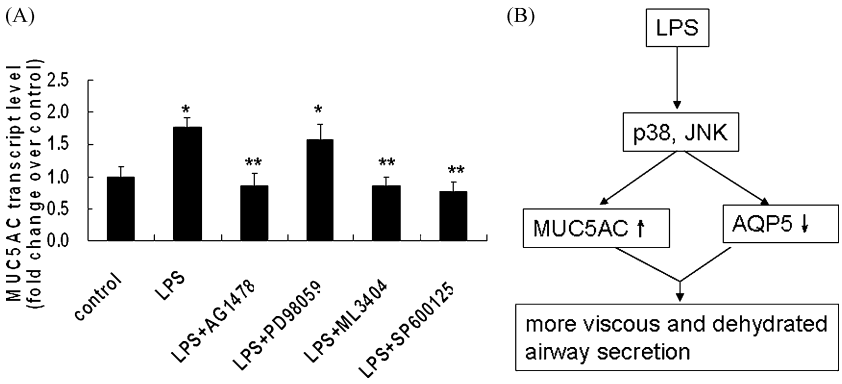

Fig. 4. (A) Effect of various inhibitors on LPS-induced MUC5AC mRNA increase. (B) Signaling pathways mediating MUC5AC and AQP5 expression. *Significant difference when compared with the untreated control ($p < 0.05$). **Significant difference when compared with the LPS-treated control group ($p < 0.05$).

ing that viscous mucus may be related to AQP5 down-regulation. In this study, LPS exposure was found to be associated with decreased AQP5 expression at both mRNA and protein level in dose- and time-dependent manners, and AQP5 was down-regulated via p38/JNK pathway.

Not only AQP5, but also MUC5AC expression may be affected by LPS. As shown in this study, MUC5AC expression increased significantly with LPS exposure in both time- and dose-dependent manners, and MUC5AC expression was up-regulated via EGFR-p38/JNK pathways. P38/JNK seemed to be the common signaling pathways of AQP5 down-regulation and MUC5AC up-regulation by LPS. Combination of AQP5 down-regulation and MUC5AC up-regulation was speculated to be involved in induction of excessive viscous mucus production in COPD patients.

Our previous studies suggested that there may be a direct interaction between AQP5 and MUC5AC expression (Chen et al., 2006a,b). It has been showed that siRNA-induced AQP5 down-regulation was associated with MUC5AC over-expression in SPC-1 cell line. However, the mechanism underlying this association has not been fully understood yet.

TLRs are well known cell surface receptors recognizing invading microbes and initiating cellular signaling pathways against bacterial components (Hajjar et al., 2002; Erridge et al., 2004). Studies showed that LPS lead to transcription and translation of a variety of proinflammatory cytokines such as TNF-α through the TLR2/TLR4 (Gon et al., 2004; Raoust et al., 2009). Proinflammatory cytokine TNF-α has been shown not only to induce MUC5AC expression (Song et al., 2003) but also to inhibit AQP5 expression in vitro (Towne et al., 2001). Therefore we could not exclude the possibility that LPS may decrease AQP5 production and increase MUC5AC through TLR2/TLR4 signaling pathway.

To summarize, LPS exposure led to decreased AQP5 transcription and protein expression as well as increased MUC5AC expression in SPC-A1 cells. AQP5 expression was down-regulated by LPS exposure via p38/JNK signaling pathways, while MUC5AC was up-regulated through EGFR-P38/JNK pathways. Therefore, p38/JNK, their common signaling pathways, may become a promising target to preserve AQP5 expression and prevent MUC5AC over-expression to restore proper H_2O to mucin ratio of airway mucus and may be beneficial to the clinical management of COPD patients.

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References

Caramori, G., Casolari, P., Di Gregorio, C., Saetta, M., Baraldo, S., Boschetto, P., Ito, K., Fabbri, L.M., Barnes, P.J., Adcock, I.M., Cavallesco, G., Chung, K.F., Papi, A., 2009. MUC5AC expression is increased in bronchial submucosal glands of stable COPD patients. Histopathology 55, 321–331.

Casalino-Matsuda, S.M., Monzon, M.E., Forteza, R.M., 2006. Epidermal growth factor receptor activation by epidermal growth factor mediates oxidant-induced goblet cell metaplasia in human airway epithelium. American Journal of Respiratory Cell and Molecular Biology 34, 581–591.

Chen, Z.H., Wang, X.D., Gao, L., Bai, L., Zhu, R., Bai, C.X., 2006a. Regulation of MUC5AC mucin secretion by depletion of AQP5 in SPC-A1 cells. Biochemical and Biophysical Research Communications 342, 775–781.

Chen, Z.H., Zhu, R., Bai, L., Bai, C.X., 2006b. Downregulation of aquaporin 5 induced by vector-based short hairpin RNA and its effect on MUC5AC gene expression in human airway submucosal gland cells. Respiratory Physiology & Neurobiology 152, 197–203.

Erridge, C., Pridmore, A., Eley, A., Stewart, J., Poxton, I.R., 2004. Lipopolysaccharides of Bacteroides fragilis, Chlamydia trachomatis and Pseudomonas aeruginosa signal via Toll-like receptor 2. Journal of Medical Microbiology 53, 735–740.

Gon, Y., Asai, Y., Hashimoto, S., Mizumura, K., Jibiki, I., Machino, T., Ra, C., Horie, T., 2004. A20 inhibits toll-like receptor 2-and 4-mediated interleukin-8 synthesis in airway epithelial cells. American Journal of Respiratory Cell and Molecular Biology 31, 330–336.

Hajjar, A.M., Ernst, R.K., Tsai, J.H., Wilson, C.B., Miller, S.I., 2002. Human Toll-like receptor 4 recognizes host-specific LPS modifications. Nature Immunology 3, 354–359.

Inoue, D., Kubo, H., Watanabe, M., Sasaki, T., Yasuda, H., Numasaki, M., Sasaki, H., Yamaya, M., 2008. Submucosal gland cells in human lower airways produce MUC5AC protein. Respirology 13, 285–287.

Kreda, S.M., Gynn, M.C., Fenstermacher, D.A., Boucher, R.C., Gabriel, S.E., 2001. Expression and localization of epithelial aquaporins in the adult human lung. American Journal of Respiratory Cell and Molecular Biology 24, 224–234.

Livraghi, A., Randell, S.H., 2007. Cystic fibrosis and other respiratory diseases of impaired mucus clearance. Toxicologic Pathology 35, 116–129.

Ma, R., Wang, Y., Cheng, G., Zhang, H.-z., Wan, H.-y., Huang, S.-g., 2005. MUC5AC expression up-regulation goblet cell hyperplasia in the airway of patients with chronic obstructive pulmonary disease. Chinese Medical Sciences Journal 20, 181–184.

Ma, T.H., Fukuda, N., Song, Y.L., Matthay, M.A., Verkman, A.S., 2000. Lung fluid transport in aquaporin-5 knockout mice. Journal of Clinical Investigation 105, 93–100.

Mail, M.A., Hlarkema, J.R., Trojanek, J.B., Treis, D., Livraghi, A., Schubert, S., Zhou, Z., Kreda, S.M., Tilley, S.L., Hudson, E.J., O'Neal, W.K., Boucher, R.C., 2008. Development of chronic bronchitis and emphysema in beta-epithelial Na+ channel-overexpressing mice. American Journal of Respiratory and Critical Care Medicine 177, 730–742.

Mall, M.A., 2008. Role of cilia, mucus, and airway surface liquid in mucociliary dysfunction: lessons from mouse models. Journal of Aerosol Medicine and Pulmonary Drug Delivery 21, 13–24.

Rabe, K.F., Hurd, S., Anzueto, A., Barnes, P.J., Buist, S.A., Calverley, P., Fukuchi, Y., Jenkins, C., Rodriguez-Roisin, R., van Weel, C., Zielinski, J., 2007. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease—GOLD executive summary. American Journal of Respiratory and Critical Care Medicine 176, 532–555.

Randell, S.H., Boucher, R.C., Univ, N.C.V.L.G., 2006. Effective mucus clearance is essential for respiratory health. American Journal of Respiratory Cell and Molecular Biology 35, 20–28.

Raoust, E., Balloy, V., Garcia-Verdugo, I., Touqui, L., Ramphal, R., Chignard, M., 2009. Pseudomonas aeruginosa LPS or flagellin are sufficient to activate TLR-dependent signaling in murine alveolar macrophages and airway epithelial cells. Plos One, 4.

Roger, P., Gascard, J.P., Bara, J., Dulmet, E., Brink, C., 2001. EGTA treatment of human airways in vitro unmasks M1/MUC5AC mucin in submucosal glands. European Respiratory Journal 18, 176–183.

Rogers, D.F., 2003. The airway goblet cell. International Journal of Biochemistry & Cell Biology 35, 1–6.

Rogers, D.F., 2004. Airway mucus hypersecretion in asthma: an undervalued pathology? Current Opinion in Pharmacology 4, 241–250.

Song, K.S., Lee, W.J., Chung, K.C., Koo, J.S., Yang, E.J., Choi, J.Y., Yoon, J.H., 2003. Interleukin-1 beta and tumor necrosis factor-alpha induce MUC5AC overexpression through a mechanism involving ERK/p38 mitogen-activated protein kinases-MSK1-CREB activation in human airway epithelial cells. Journal of Biological Chemistry 278, 23243–23250.

Song, Y.L., Verkman, A.S., 2001. Aquaporin-5 dependent fluid secretion in airway submucosal glands. Journal of Biological Chemistry 276, 41288–41292.

Takeyama, K., Dabbagh, K., Lee, H.M., Grattan, K., Ueki, I., Lausier, J., Nadel, J.A., 1999. Mucin MUC5AC gene and protein expression is up-regulated by the epidermal growth factor system. American Journal of Respiratory and Critical Care Medicine 159, A35–A135.

Towne, J.E., Krane, C.M., Bachurski, C.J., Menon, A.G., 2001. Tumor necrosis factor-alpha inhibits aquaporin 5 expression in mouse lung epithelial cells. Journal of Biological Chemistry 276, 18657–18664.

Verkman, A.S., 2007. Role of aquaporins in lung liquid physiology. Respiratory Physiology & Neurobiology 159, 324–330.

Vestbo, J., Prescott, E., Lange, P., Jensen, G., Schnohr, P., Appleyard, M., Nyboe, J., Gronbaek, M., 1996. Association of chronic mucus hypersecretion with FEV(1) decline and chronic obstructive pulmonary disease morbidity. American Journal of Respiratory and Critical Care Medicine 153, 1530–1535.

Wang, K., Feng, Y.L., Wen, F.Q., Chen, X.R., Ou, X.M., Xu, D., Yang, J., Deng, Z.P., 2007. Decreased expression of human aquaporin-5 correlated with mucus overproduction in airways of chronic obstructive pulmonary disease. Acta Pharmacologica Sinica 28, 1166–1174.

Wang, Y.H., Yao, S., Ka, L., Zhang, P.H., Wang, G.F., Lei, G., Bai, C.X., 2009. Role of matrix metalloproteinase-9 in lipopolysaccharide-induced mucin production in human airway epithelial cells. Archives of Biochemistry and Biophysics 486, 111–118.