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The tyrosine phosphatase, SHP-1, is involved in bronchial mucin production during oxidative stress

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ABSTRACT

Mucus hypersecretion is a clinically important manifestation of chronic inflammatory airway diseases, such as asthma and Chronic obstructive pulmonary disease (COPD). Mucin production in airway epithelia is increased under conditions of oxidative stress. Src homology 2 domain-containing protein tyrosine phosphatase (SHP)-1 suppression is related to the development of airway inflammation and increased ROS levels. In this study, we investigated the role of SHP-1 in mucin secretion triggered by oxidative stress. Human lung mucoepidermoid H292 carcinoma cells were transfected with specific siRNA to eliminate SHP-1 gene expression. Cultured cells were treated with hydrogen peroxide (H_2O_2), and Mucin 5AC(MUC5AC) gene expression and mucin production were determined. Activation of p38 mitogen activated protein kinase (MAPK) in association with MUC5AC production was evaluated. *N*-acetylcysteine (NAC) was employed to determine whether antioxidants could block MUC5AC production. To establish the precise role of p38, mucin expression was observed after pre-treatment of SHP-1-depleted H292 cells with the p38 chemical blocker. We investigated the *in vivo* effects of oxidative stress on airway mucus production in SHP-1-deficient heterozygous (mev/+) mice. *MUC5AC* expression was enhanced in SHP-1 knockdown H292 cells exposed to H_2O_2, compared to that in control cells. The ratio between phosphorylated and total p38 was significantly increased in SHP-1-deficient cells under oxidative stress. Pre-treatment with NAC suppressed both MUC5AC production and p38 activation. Blockage of p38 MAPK led to suppression of MUC5AC mRNA expression. Notably, mucin production was enhanced in the airway epithelia of mev/+ mice exposed to oxidative stress. Our results clearly indicate that SHP-1 plays an important role in airway mucin production through regulating oxidative stress.

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Introduction

Mucus production is a normal defensive mechanism that plays an important role in protecting airway epithelium from various harmful particles and microbes in contact with the respiratory tract and maintaining the normal function of epithelium [1]. However, mucus hypersecretion and airway goblet cell hyperplasia observed in chronic airway inflammatory disorders [1–3], can lead to agonizing respiratory symptoms, even life-threatening airway obstruction. Thus, appropriate control of airway mucin generation is crucial in the management of chronic inflammatory airway diseases.

While various stimuli related to airway inflammatory conditions can cause mucus hypersecretion, significant evidence shows that oxidative stress promotes mucin gene and protein expression, even in the absence of airway inflammation [1,4,5]. The respiratory tract is one of the organs most frequently exposed to oxidative stress, which may be generated either endogenously via mitochondrial respiration and activation of inflammatory cells or exogenously from air pollutants and cigarette smoking [6,7]. Oxidative stress is closely associated with the disease pathogenic mechanisms of several chronic airway inflammatory diseases [6,7]. However, its precise mechanism of action in mucus hyper-production is currently unclear.

The src homology 2-containing protein tyrosine phosphatase (SHP-1), a negative regulator in intracellular signaling, is expressed in hematopoietic, neuronal and epithelial cells [8]. SHP-1 knockout homozygous mice (mev/mev) spontaneously develop a Th2-dominant immune response and display significantly enhanced airway

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mucus secretion with no antigen challenge [9]. Moreover, increased intracellular ROS leads to inhibition of the enzymatic activities of protein tyrosine phosphatases (PTPs), such as SHP-1 [10]. Suppression of the SHP-1 function, in turn, promotes a further increase in the intracellular ROS level by eliciting amplified and prolonged activation of endogenous ROS [11].

These studies collectively suggest that SHP-1 may be involved in mucin gene expression induced under conditions of oxidative stress. Elucidation of the underlying mechanisms should thus provide novel information on the regulatory pathway of mucus hypersecretion. In the present study, we examined the role of SHP-1 in mucin hypersecretion promoted by oxidative stress using a human bronchial epithelial cell line, H292. Furthermore, the *in vivo* effect of SHP-1 on mucus hypersecretion was evaluated with SHP-1-deficient heterozygous (mev/+) mice.

Materials and methods

Cell culture and animals. The human mucoepidermoid bronchiolar carcinoma cell line, NCI-H292, was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U penicillin and 100 μg streptomycin per milliliter at 37 °C in a 5% CO_2 incubator. Experiments were performed after overnight serum starvation.

We employed 6–8 week old heterozygous female motheaten mice (mev/+) as SHP-1-deficient models and wild-type controls. All mice, purchased from the Jackson Laboratory (Bar Harbor, ME, USA), were from a C57BL/6 background. Mice were maintained under specific pathogen-free conditions. Animal experiments were approved by the institution.

SHP-1 knockdown in H292 cells with specific siRNA. SHP-1 siRNA was purchased from Santa Cruz Biotechnology Inc. (CA, USA sc-29478). The sequences were as follows: CUGGUGGAGCAUUUCAAG ATT (Duplex 1 Sense Strand), CGCAGUACAAG UUCAUCUATT (Duplex 2 Sense Strand), CAACCCUUCUCCUCUUGUATT (Duplex 3 Sense Strand), mRNA Accession Number NM_002831). H292 cells were seeded in a 6-well plate (5–7 × 10^5 cells/well) 1 day before transfection. At 70% confluence, the medium was altered to serum/antibiotic-free medium. Cells were maintained in this medium for 24 h prior to transfection experiments. SHP-1 siRNA (0.5, 1, 1.5 μg) and scrambled siRNA as a control was gently mixed with 250 μL of Opti-MEM-reduced serum medium. Lipofectamine 2000 (5 μL, Invitrogen Inc., Carlsbad, CA, USA) was diluted with Opti-MEM (250 μL) for 5 min at room temperature. Each siRNA/Lipofectamine 2000-diluted solution was mixed for 30 min at room temperature. The mixture was added to cells, and the medium was changed to antibiotic-free growth medium.

At first, we determined whether the SHP-1 siRNA was effective in suppression of SHP-1 expression in H292 cells by assessing both SHP-1 mRNA and protein levels 24 and 48 h after transfection. SHP-1 mRNA expression was determined using reverse transcription-polymerase chain reaction (RT-PCR), and adjusted according to the GAPDH mRNA level. The primer sequences for SHP-1 mRNA were as follows: downstream primer, 5′-TGGCGTGGCAGGAGAACAG-3′ (forward) and upstream primer, 5′-GCAGTTGGTCACAGAGTAGGGC-3′ (reverse). The primer sequences for GAPDH were 5′-ACCACAGTCCATGCCATCAD-3′ (downstream) and 5′-TCCACCACCCTGTTGCTGTA-3′ (upstream). PCR products were resolved on 2% agarose gels, and visualized with ethidium bromide under a transilluminator. SHP-1 protein level was assessed by using Western blot with SH-PTP antibody (1:1000, Santa Cruz Biotechnology Inc.).

SHP-1 expression was significantly suppressed upon treatment with specific siRNA. Efficiency of suppression was effectively maintained till the 48 h after treatment (Fig. 1A). Accordingly, subsequent experiments were performed at 24 h after transfection with 1 μg siRNA.

Treatment of H292 cells with hydrogen peroxide, antioxidant and p38 inhibitor. SHP-1 siRNA-transfected and scrambled RNA infected control cells were treated with hydrogen peroxide (H_2O_2, Sigma, St. Louis, MO, USA) to induce oxidative stress. Following preliminary experiments to determine the optimal concentration, 1 mM H_2O_2 was used for subsequent experiments. Cells were treated with various concentrations (1, 5, and 10 mM) of the antioxidant, *N*-acetylcysteine (NAC, Sigma), 1 h before H_2O_2 stimulation. Pre-treatment with the p38 inhibitor, SB203580 (20 μM; Calbiochem, San Diego, CA, USA), was performed 30 min prior to the application of H_2O_2.

Determination of MUC5AC expression. In the present study, MUC5AC mRNA and mucin protein levels were measured at 4 and 12 h after stimulation with H_2O_2, respectively. RNA was extracted from H292 cells treated with H_2O_2, NAC and a p38 inhibitor using TRI reagent. Extracted RNA was quantified as 200–400 ng/μL on average on the ND spectrophotometer (NanoDrop Technologies, LLC, Wilmington, Delaware, USA). Following RNA extraction, 1–20 μg of RNA was used for cDNA synthesis with the RT kit (iNtRON Biotechnology Inc., Seoul, Korea). MUC5AC mRNA expression was determined using PCR, and adjusted according to the GAPDH mRNA level. The primer sequences for MUC5AC were as follows: downstream primer, 5′-TGATCATCCAGCAGCAGGGCT-3′ (forward) and upstream primer, 5′-CCGAGCTCAGAGGACATATGG-3′ (reverse).

Mucin protein production in H292 cells treated with various compounds was assessed by measuring MUC5AC protein levels in the supernatant using ELISA, as described previously [12]. Briefly, 50 μL of supernatant was incubated with bicarbonate–carbonate buffer (50 μL) at 40 °C in a 96-well plate until dry. Plates were washed three times with PBS, and blocked with 2% BSA, fraction V (Sigma), for 1 h at room temperature. Next, plates were rewashed three times with PBS, and incubated with 50 μL of monoclonal MUC5AC antibody (1:100, Abcam Ltd., Cambridge, UK, ab24070) diluted in PBS-T (PBS–0.05% Tween 20) containing 1% BSA. After 1 h, wells were washed three times with PBS-T, and 100 μL of horseradish peroxidase-conjugated anti-mouse IgG (diluted 1:2500 with PBS-T containing 1% BSA) was dispensed into each well. After 1 h, plates were washed six times with PBS-T. The color reaction was developed with substrate reagent (3,3′,5,5′-tetramethylbenzidine peroxidase solution, R&D systems, Minneapolis, MN, USA, DY999), and terminated with 2N H_2SO_4. Optical density was measured at 450 nM using a microplate reader.

SHP-1 expression and MAPK activity. Mitogen activated protein kinase (MAPK) expression was examined 15 min after H_2O_2 treatment. H292 cells treated with various compounds were disrupted in cell lysis buffer containing protease and phosphatase inhibitors. Cell lysates were centrifuged at 4 °C, 27,000*g* for 30 min, and the concentration of the proteins in the supernatant was determined using the Bradford method. Each protein sample was separated by 10% SDS–PAGE, and transferred to PVDF membrane in Tris-buffered saline (TBS) for 2 h. Blots were incubated with phospho-p44/42 (ERK) MAPK antibody (1:1000, Cell Signaling Technology, Beverly, MA, USA) and p44/42 MAPK antibody (1:1000, Cell signaling Technology) diluted in 2.5% skimmed milk and phospho-p38 MAPK antibody (1:1500, Cell signaling Technology) or p38 MAPK antibody (1:1500, Santa Cruz Biotechnology Inc.) diluted in 5% BSA for 3 h. After washing with TBS-T (0.5% Tween 20 in TBS), blots were incubated with anti-mouse IgG or anti-rabbit IgG secondary antibody conjugated with horseradish peroxidase (HRP) for 1 h. Following a wash with TBS-T, blots were visualized using chemiluminescent substrate.

Induction of oxidative stress in lungs of heterozygous mev/+ mice. Paraquat dichloride (Riedel de Haen, Seelze, Germany), which is a well known chemical agent inducing oxidative stress *in vivo,* was

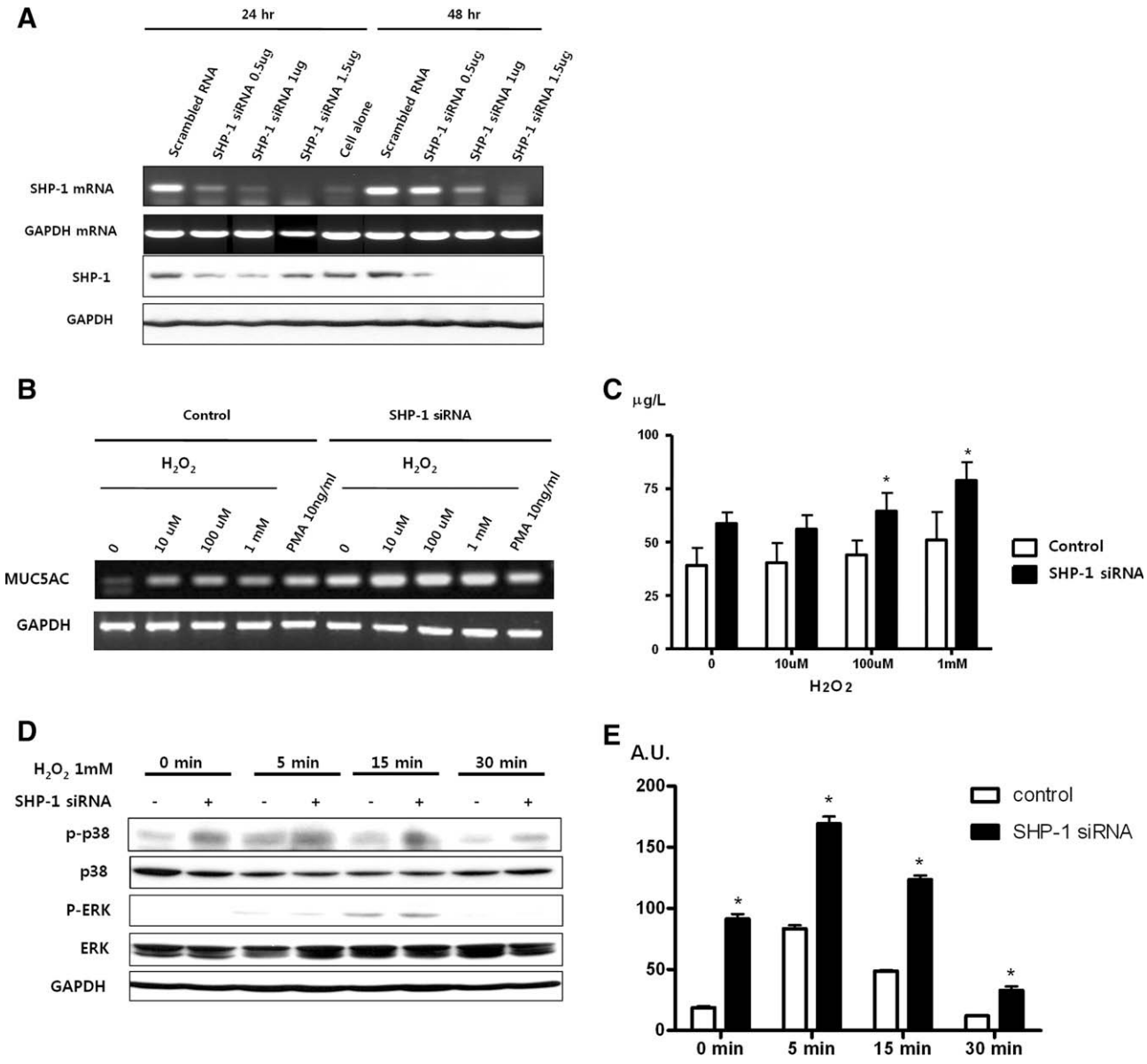

Fig. 1. MUC5AC expression and p38 MAPK activation are enhanced in SHP-1-deficient H292 cells exposed to H_2O_2. The inhibitory effect of siRNA on SHP-1 expression in H292 cells was determined by RT-PCR and Western blot analysis at 24 and 48 h after transfection of H292 cells with scrambled siRNA and SHP-1 siRNA (0.5, 1, 1.5 μg) (A). MUC5AC mRNA and protein levels were determined using RT-PCR (B) and ELISA (C), after H_2O_2 treatment of SHP-1-suppressed and control H292 cells. Phosphorylated p38/p38 and phosphorylated ERK/ERK expression ratio were determined by Western blot analysis after stimulation of SHP-1-depleted and control H292 cells with 1 mM H_2O_2 (D). Phosphorylated p38/p38 MAPK ratio was semi-quantitatively estimated by using densitometry analysis (E) (*$p < 0.05$, compared with the control group). Data are presented as means ± SEM of three separate experiments. Results were adjusted according to GAPDH expression. PMA (*para*-methoxyamphetamine). A.U. means arbitrary unit.

administered intraperitoneally in mice at 2.5 μg/g body weight twice a week for 2 weeks in mev/+ and wild-type mice. Mice were anesthetized and sacrificed by cardiac puncture 24 h after the final administration of paraquate. Lung tissue was obtained for histologic assessment and investigation of mucus production, fixed in Streck solution (Streck Laboratory, La Vista, NE, USA), embedded in paraffin, sliced, and stained with periodic acid schiff (PAS). Histology and mucus production were evaluated under an optical microscope (Olympus, Japan). The quantity of mucin production in the airway was assessed by measuring the mucus secreting goblet cells stained with PAS and the percentage of PAS-positive cells was determined in the largest visible airway. We categorized each animal according to a semilogarithmic scale as grade 0 (<1% positive cells), grade 1 (1–3% positive cells), grade 2 (4–10% positive cells), grade 3 (11–30% positive cells), and grade 4 (>31% positive cells).

Statistical analysis. For comparison between groups, Student *t*-test or ANOVA with Bonferroni post hoc analysis was performed using SPSS (Version 13.0 software). Data were expressed as means ± SEM, and statistical significance defined as $p < 0.05$.

Results

MUC5AC expression is enhanced in SHP-1-deficient H292 cells exposed to H_2O_2

First, MUC5AC mRNA expression was examined in control cells and in SHP-1-suppressed H292 cells after treatment with H_2O_2 in order to evaluate the role of SHP-1 in mucus gene expression during oxidative stress. SHP-1-suppressed H292 cells had increased amount of MUC5AC mRNA compared to control cells. Following induction of oxidative stress with 10, 100, and

1000 μM H_2O_2, MUC5AC mRNA expression was markedly increased in SHP-1-suppressed cells, compared with that in control cells, which also responded to H_2O_2 (Fig. 1B). Moreover, data from ELISA experiments disclosed that MUC5AC secretion in the culture supernatant was significantly enhanced in SHP-1 deficient cells exposed to H_2O_2, compared to control cells (Fig. 1C).

p38 MAPK is further activated in SHP-1 deficient H292 cells treated with H_2O_2

To establish the intracellular mechanisms of mucus production in SHP-1-suppressed H292 cells, we determined whether p38 MAPK and ERK activities were associated with mucin generation. Phosphorylated p38/p38 and phosphorylated ERK/ERK expression ratios following H_2O_2 stimulation were analyzed by Western blot analysis. Exposure to oxidative stress led to a significant increase in p38 phosphorylation in SHP-1-suppressed cells, compared with that in control cells. In contrast, the phosphorylated ERK/ERK ratios were not significantly different between the two groups (Fig. 1D and E).

MUC5AC expression and p38 activation in SHP-1 suppressed cells are oxidant-dependent

As shown in Fig. 2, phosphorylation of p38 induced in SHP-1-suppressed H292 cells under conditions of oxidative stress was reduced upon NAC pre-treatment in a dose-dependent manner. Moreover, MUC5AC mRNA expression stimulated by H_2O_2 in SHP-1-suppressed H292 cells was dose-dependently inhibited by NAC (Fig. 2C). These results indicate that these responses are caused by oxidants.

MUC5AC expression induced by oxidative stress is suppressed by a p38 inhibitor in SHP-1-depleted cells

We additionally examined the effects of a p38 inhibitor on p38 activation and MUC5AC expression by pre-treatment of SHP-1-suppressed and control cells with SB203580, prior to H_2O_2 exposure. The p38 inhibitor blocked p38 phosphorylation and MUC5AC mRNA expression induced by H_2O_2 in SHP-1-suppressed cells in a dose-dependent manner (Fig. 3), indicating that p38 signaling is critical in MUC5AC expression.

Mucus production in mev/+ mouse airways exposed to oxidative stress

Finally, we examined whether SHP-1 participates in mucus gene expression induced by oxidative stress *in vivo*, using SHP-1 deficient heterozygous mutant (mev/+) mice. In resting state, mev/+ mice showed no phenotypical abnormalities. Notably, a significant increase in mucus expression was observed in the airway epithelia of mev/+ mice, compared with wild-type mice treated with the oxidant inducer paraquat for 2 weeks (Fig. 4).

Discussion

In the present study, we demonstrate that bronchial mucin production is closely associated with SHP-1 expression during oxidative stress via p38 MAPK activation. It is speculated that enhanced oxidative stress is a critical initiator of mucus production, even in the absence of specific airway inflammatory conditions, supporting the possible clinical importance of antioxidants as effective medication to control mucus secretion. Our data indicate that SHP-1 participates in the regulation of mucin gene expression induced by oxidative stress, and may thus be utilized as a novel treatment target to control mucus hypersecretion.

Mucus hypersecretion in the airway is currently of significant concern, as it is an important pathological change occurring in chronic airway inflammatory diseases, including asthma, COPD, and cystic fibrosis [3,13]. Postmortem studies and biopsies reveal that mucus plugging is a major cause of airway obstruction and impairment of gas exchange that may lead to cell death [14]. Other reports show that mucus secretion is predictive of mortality, particularly in patients with lowered baseline lung functions [15]. Despite the clinical importance of mucus hypersecretion, which induces airflow limitation, symptom aggravation, and mortality of asthma and COPD, this feature is relatively undervalued, compared with airway inflammation and bronchial hyperresponsiveness. Notably, anti-inflammatory agents may be utilized as a pivotal therapeutic strategy for the management of mucus hypersecretion in chronic inflammatory airway diseases, since various

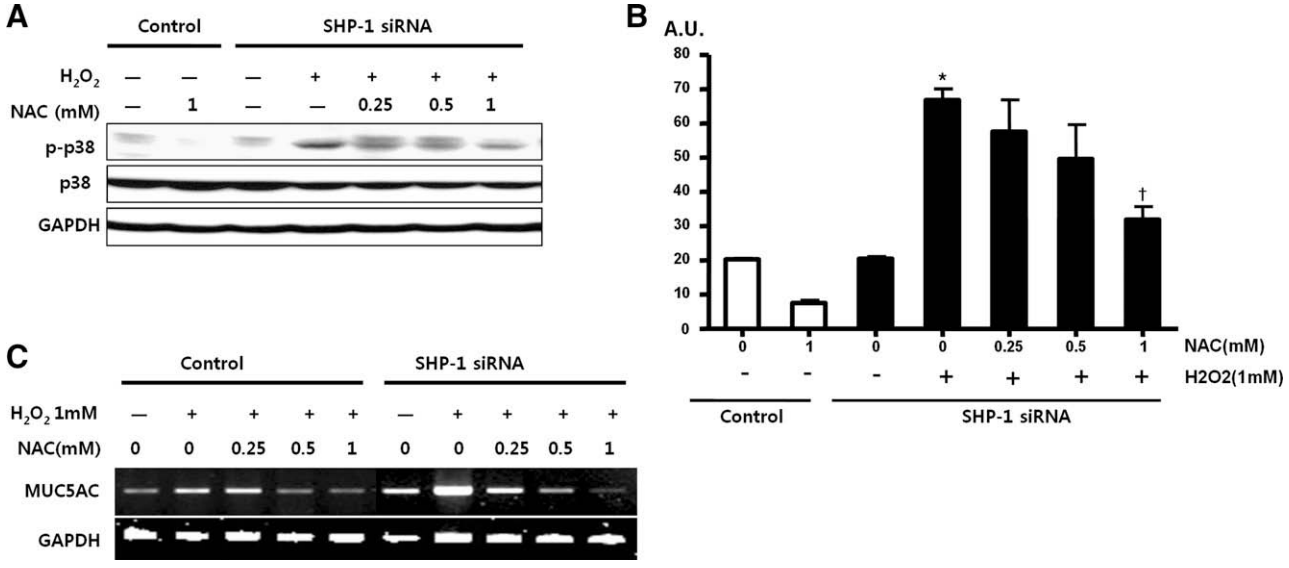

Fig. 2. MUC5AC expression and p38 activation induced by H_2O_2 after pre-treatment with NAC. The effects of pre-treatment with NAC on the phosphorylated p38/p38 expression ratio were evaluated by Western blotting after stimulation of SHP-1-suppressed H292 cells with 1 mM H_2O_2 (A,B). Results were adjusted according to GAPDH. Effects of NAC pre-treatment on MAC5AC mRNA expression were determined by RT-PCR (C) (*$p < 0.01$, compared with control cells not exposed to H_2O_2, †$p < 0.01$, compared with SHP-1 knockdown cells not pre-treated with NAC). Data are presented as means ± SEM of three separate experiments. A.U. means arbitrary unit.

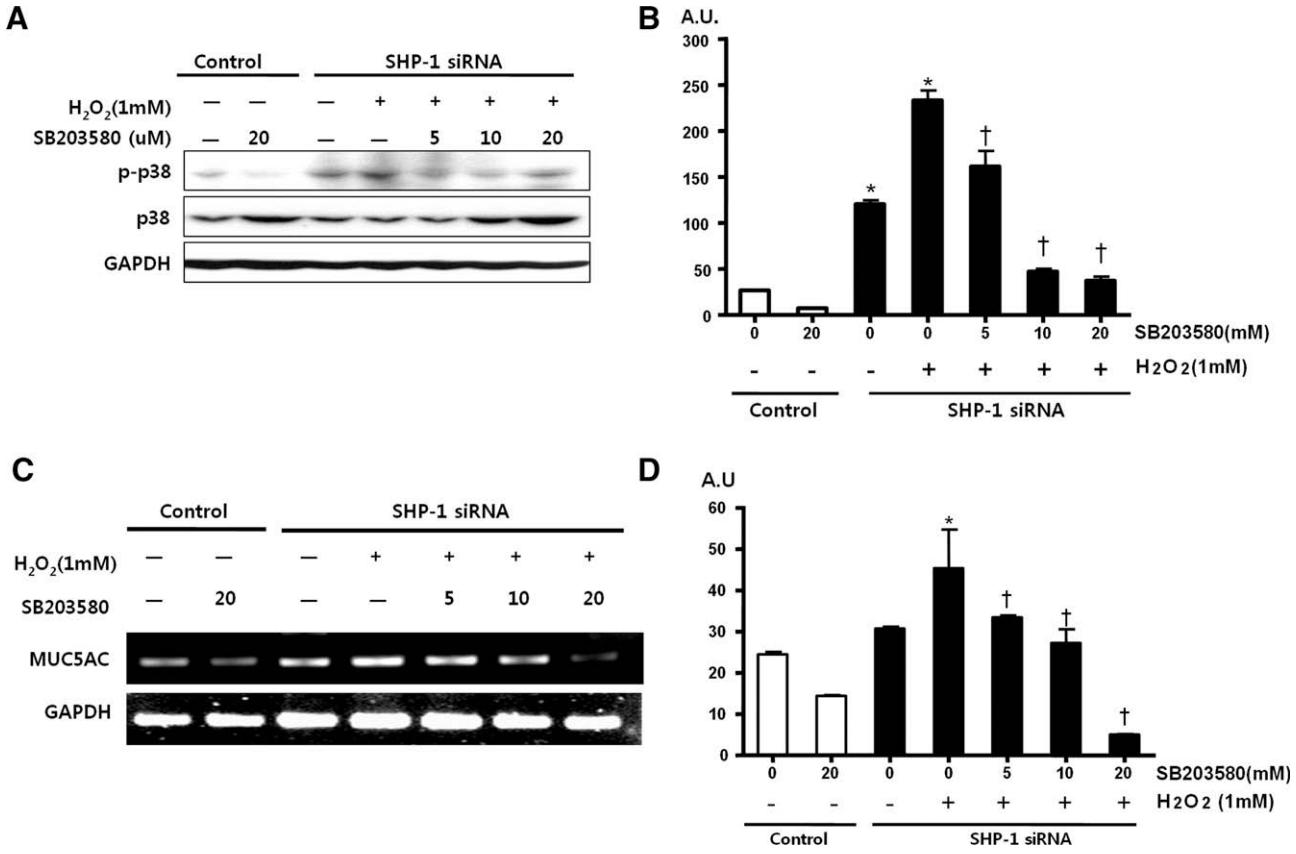

Fig. 3. Effects of the p38 inhibitor, SB203580, on MUC5AC expression and p38 activation induced by H_2O_2. SHP-1-suppressed H292 cells were pre-treated with p38 inhibitor, followed by exposure to 1 mM H_2O_2, and the phosphorylated p38/p38 expression ratio determined by Western blot analysis (A,B) (*$p < 0.01$, compared with control cells not exposed to H_2O_2, †$p < 0.01$, compared with SHP-1 knockdown cells not pre-treated with SB203580). The effects of SB203580 pre-treatment on *MAC5AC* mRNA expression were confirmed by RT-PCR (C,D). The expression of *MAC5AC* mRNA was adjusted according to GAPDH expression and semi-quantitatively estimated using densitometry (*$p < 0.01$, compared with control cells not exposed to H_2O_2, †$p < 0.01$, compared with H_2O_2 exposed SHP-1 knockdown cells not pre-treated with SB203580). Data are presented as means ± SEM of three separate experiments. A.U. means arbitrary unit.

inflammatory mediators are important causative factors. However, several anti-inflammatory drugs, including inhaled corticosteroids, a principal first-line therapy for asthma, are largely ineffective in controlling mucus production [1,3]. Thus, it appears that mucus hypersecretion is caused, not simply by airway inflammation, but a combination of different mechanisms.

Gene expression of the large, heavily glycosylated protein, mucin, a major component of mucus, is upregulated by inflammatory cytokines, bacterial products, and environmental chemicals, causing oxidative stress [1,13,16]. The organs of all living creatures that use oxygen are inevitably exposed to various levels of oxidative stress, particularly the lung. Recent studies demonstrate that oxidative injuries induced by either endogenously generated ROS or exogenously inhaled oxidants play an important role in mucin gene expression, as well as airway inflammation in human epithelia [4,5,17]. In fact, the recent experimental finding that mice depleted of Nrf2, a transcription factor regulating several antioxidant genes, display amplified asthmatic allergic inflammation, including mucus cell hyperplasia, confirm the important function of oxidative stress in mucus production by the asthmatic airway [18].

SHP-1 is involved in the regulation of cellular development, survival, and activation via several signaling pathways, including MAPK, Janus tyrosine kinase2 (Jak2) and signal transducer and activator of transcription (STAT), phosphatidylinositol 3-kinase (PI3-K), and NF-κB [8]. SHP-1 additionally inhibits the intrinsic generation of oxidative stress by counteracting NADH oxidase and iNOS activities [11,19]. In addition, SHP-1 is inactivated by ROS through transient reversible oxidation of its active site containing cysteine residues [20]. Our previous study shows that SHP-1 deficient heterozygous mutant mice (mev/+) susceptible to oxidative stress display allergic airway inflammation induced by low-dose antigen without adjuvant, which does not generally stimulate inflammation [21]. The results collectively indicate that SHP-1 plays an important role in the regulation of intracellular oxidative stress and development of airway pathology. However, whether SHP-1 is directly involved in mucin expression during oxidative stress and the precise underlying mechanism remains to be established.

To our knowledge, this is the first study to report a role of SHP-1 in the pathogenesis of mucin hyper-production. In our experiments, MUC5AC expression was markedly enhanced in the SHP-1 suppressed H292 cell line after exposure to H_2O_2, compared to control cells. This finding can be explained by the theory that SHP-1 deficient cells, which are more sensitive to oxidative stress, permit the amplification of endogenous ROS production. In turn, mucin gene expression is increased through ROS-induced intracellular signaling. Accordingly, we propose that SHP-1 functions in the regulation of mucin gene expression in the context of enhanced oxidative stress.

The cellular mechanism of mucin gene expression during oxidative stress is associated with numerous signaling pathways, including MAPK (extracellular signal-related kinase (ERK) or p38 MAPK) and receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR) [22]. In our study, p38 MAPK activity was associated with mucus hypersecretion in SHP-1 suppressed H292 cells

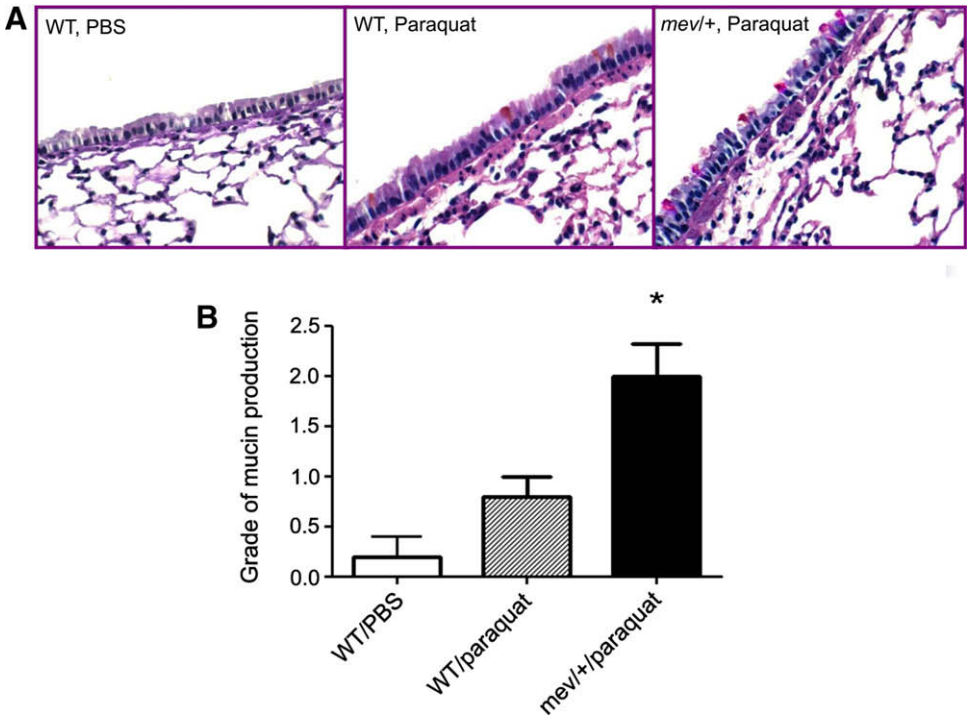

Fig. 4. Mucus production determined by PAS staining in the mev/+ mouse airway after exposure to oxidative stress. Lung histology of normal control mice, wild-type mice, and mev/+ mice after paraquat challenge for 2 weeks (PAS staining 100×) (A). The pictures are representatives of each study group in which 3–5 mice were used for the experiment. Mucus production was quantification by grading. Bars represent means ± SEM (B) (*$p < 0.05$, compared with the control and wild-type mice with paraquat treatment).

exposed to oxidative stress. These results indicate that activation of p38 MAPK is an important signaling pathway in mucin production associated with the decreased function of SHP-1. However, previous studies showed that H_2O_2 induced mucus hypersecretion has been associated with ERK1 and two signaling rather than p38 MAPK in H292 cells [5]. At this moment, it is not clear whether this is the consequence of differences in experimental conditions or the specific feature of SHP-1 inhibition which could lead to the phosphorylation of a variety of intracellular targets. Exact role of ERK activation in this process remains to be established.

Tyrosine kinase activation via EGFR is known to be a key mechanism of mucin gene expression induced by ROS [4,22]. Both ROS induced by dualoxidase-1 and proteases, such as human neutrophil elastase and matrix metalloproteinase, activate MUC5AC gene expression through the protease intracellular signaling cascade involving EGFR transactivation [17]. In the present study, the relationship between SHP-1 and EGRF signaling was not evaluated. The mechanism of EGFR action in SHP-1-related mucin gene regulation requires further evaluation.

Heterozygous mev/+ mice, in which the expression level of SHP-1 has been known to be less than half of that in wild-type control mice, were phenotypically and immunologically normal in resting state. Interestingly, SHP-1 deficient (mev/+) mice exposed to oxidative stress in the absence of factors causing immune responses, such as antigens, exhibited markedly increased mucus secretion. These results indicate that oxidative stress triggers mucus secretion independently of airway inflammation caused by immune response, and SHP-1 is critically involved in this process. Thus, SHP-1 may be a potential therapeutic target in the control of mucus hyper-production in certain airway disorders.

Finally, pre-treatment with the antioxidant, NAC, effectively suppressed MUC5AC gene expression stimulated by ROS in a dose-dependent manner through the p38 MAPK pathway. Regulation of excessive mucus secretion is clinically important in severely ill patients with chronic inflammatory airway diseases. However, currently available anti-inflammatory medications, mucolytics, and expectorants are limited in terms of effective control of mucus hypersecretion. Based on the study results, we suggest that antioxidant treatment to control oxidative stress is an effective strategy in managing mucus hypersecretion in specific clinical situations.

In conclusion, SHP-1 in the bronchial epithelium plays an important role in airway mucin production via regulating oxidative stress. Thus, antioxidants and agents modulating SHP-1 may be effectively employed as novel therapeutic strategies to regulate airway mucus hypersecretion.

Disclosure statement

All authors declare that we have no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations that could inappropriately influence the manuscript entitled.

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