Research article

Ubiquitin-specific protease-7 promotes expression of airway mucin MUC5AC via the NF-κB signaling pathway

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A B S T R A C T

Chronic obstructive pulmonary disease (COPD) and other respiratory diseases frequently present with airway mucus hypersecretion, which not only affects the patient's quality of life but also poses a constant threat to their life expectancy. Ubiquitin-specific protease 7 (USP7), a deubiquitinating enzyme, affects cell differentiation, tissue growth, and disease development. However, its role in airway mucus hypersecretion induced by COPD remains elusive. In this study, USP7 expression was significantly upregulated in airway epithelial samples from patients with COPD, and USP7 was also overexpressed in mouse lung and human airway epithelial cells in models of airway mucus hypersecretion. Inhibition of USP7 reduced the expression of nuclear factor kappa B (NF-κB), phosphorylated-NF-κB (p-NF-κB), and phosphonated inhibitor of nuclear factor kappa B (*p*-IκBα), and alleviated the airway mucus hypersecretion *in vivo* and *in vitro*. Further research revealed that USP7 stimulated airway mucus hypersecretion through the activation of NF-κB nuclear translocation. In addition, the expression of mucin 5AC (MUC5AC) was suppressed by the NF-κB inhibitor erdosteine. These findings suggest that USP7 stimulates the NF-κB signaling pathway, which promotes airway mucus hypersecretion. This study identifies one of the mechanisms regulating airway mucus secretion and provides a new potential target for its prevention and treatment.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by damaged airway epithelial cells, cilia inversion, glandular hyperplasia, and mucus hypersecretion, with mucus hypersecretion being the most prominent pathological feature [1,2]. In recent years, the roles of airway mucus hypersecretion in the development, progression, and prognosis of chronic airway inflammatory diseases have garnered increasing interest [3]. The prevailing view is that airway mucus hypersecretion is not only a clinical symptom but also a significant risk factor for lung dysfunction and decreased quality of life in patients with inflammatory airway diseases. Therefore, investigating the regulatory mechanism of airway mucus formation and secretion will help identify new therapeutic targets for airway mucus hypersecretion and is crucial for the development of novel agents that can effectively control mucus hypersecretion. In addition to contributing to a better understanding of these respiratory diseases, expanding fundamental research on airway mucus hypersecretion in chronic airway inflammatory diseases such as COPD can also help to identify novel therapeutic approaches. Under physiological conditions, airway mucus is an important component of the body's intrinsic immunity and plays a critical role in preventing harmful substances, such as microorganisms, from entering the respiratory system [4]. Airway mucus also remove germs and other foreign substances from the airway, thus forming a barrier for the body to defend itself against foreign damage [5]. Although the

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composition of airway mucus varies depending on the state of the body, mucin is a major component of airway mucus in various states of the body and is closely related to mucus viscosity and ciliary function. The respiratory tract is known to express 12 mucins, including MUC5AC and MUC5B. The increased expression of MUC5AC mRNA or MUC5AC protein can serve as an indicators of goblet cell hyperplasia and metaplasia, and the change in MUC5AC content can indirectly reflect the amount of mucus secretion [6].

However, in conditions such as COPD, patients' airways exhibit permanent bronchial changes, including chronic inflammation and infection, goblet cell hyperplasia and metaplasia, and excessive mucus secretion [7]. Excessive secretion of pathological mucus can inhibit ciliary oscillation of mucus, inducing airway obstruction and a further decline in lung function [8]. Moreover, inflammation of the respiratory system not only affects mucus cilia function but also reduces the secretion of alveolar surface-active substances and alters the biochemical properties of airway mucus, leading to respiratory tract infections and increased airway resistance and significantly impairing patients' lung function [9]. In the lungs of patients with COPD, accumulated mucus creates a niche environment for chronic microbial infections, with *Pseudomonas aeruginosa (P. aeruginosa)* being one of the most common pathogens causing COPD [10]. *P. aeruginosa* grows in mucus-rich airways and enhances resistance to antibiotics and removal by phagocytosis through biofilm formation. Acute exacerbation of pulmonary infections and increased excessive mucus secretion have been associated with *P. aeruginosa* infection, particularly in patients with advanced cystic fibrosis and COPD who are receiving antibiotics or require mechanical ventilation [11,12]. *P. aeruginosa* infection can promote the growth of multiple virulence factors in the mucus-rich airways, which can further lead to increased airway mucus, excessive bronchodilation, elevated hospitalization rate, and decreased quality of life [13]. As one of the most common pathogens, *P. aeruginosa* has complex virulence factors and high toxicity [14,15]. Pyocyanin (PCN), lipopolysaccharide, flagellin, alginate, and protease are the primary pathogenic factors of *P. aeruginosa* that contribute to its virulence and induce mucus hypersecretion [16,17]. Previous studies have reported that PCN can cause airway mucus hypersecretion [17–19]. Notably, PCN is essential for both acute and chronic lung infections. In addition, its detectable concentration in the diseased airways induces bronchoconstriction and reduces mucociliary transport velocity and mucociliary clearance [20]. Chronic exposure to PCN has been previously demonstrated to promote goblet cell proliferation, chemotaxis, and airway mucus hypersecretion [17,21,22].

An essential post-translational modification of proteins, ubiquitination, plays a significant role in various physiopathological processes, including airway inflammation [22,23]. In the presence of ubiquitin-activating enzyme E1, ubiquitin-binding enzyme E2, and ubiquitin ligase E3, the C-terminal amino acid of the ubiquitin molecule is linked to the lysine on the target protein via an isopeptide bond [24]. Ubiquitination and deubiquitination are dynamic equilibrium processes, and changes in related enzymes during airway inflammation or other toxic stresses can disrupt this dynamic process and affect airway mucus secretion in COPD. Ubiquitin-specific protease 7 (USP7) has recently been identified as a protein that plays a critical role in lung-related diseases, suggesting a correlation between USP7 and inflammatory pathways. However, further research is required to determine its role in *P. aeruginosa*-induced airway mucus hypersecretion. The inflammatory response can cause changes in the airway microenvironment, resulting in recurrent airway infections and even airflow limitation and airway remodeling. The nuclear transcription factor kappa B (NF-κB) family is a key regulator of the body's inflammatory and immune responses. It is also involved in regulating the expression of several immune-related genes, particularly those that encode inflammatory cytokines and chemokines, along with other genes important for the growth and development of the immune system [25,26]. When the receptor protein receives a stimulatory signal, it initially activates the inhibitor of nuclear factor-κB (IκB) kinase (IKK) and subsequently phosphorylates the serine at the IκB subunit regulatory site of the NF-κB-IκB complex, affectings the modification of IκB and decreasing NF-κB expression. Among them, the free dimer enters the nucleus and binds to target genes to initiate the transcription process. This leads to the production of numerous pro-inflammatory factors, which can amplify the inflammatory response and exacerbate lung injury [27]. In addition, E3 ubiquitin ligases and E2 ubiquitin-binding enzymes catalyze the formation of K63, K11, and linearized polyubiquitin chains. They also recruit transforming growth factor-β (TGF-β)-activated kinase 1 (TAK1) and TAK1-binding proteins (TAB), and ultimately trigger the activation of NF-κB, which is involved in the inflammatory response and immune regulation of the body [28].

2. Materials and methods

2.1. Data acquisition

The mRNA expression profile dataset GSE128708 was downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) and analyzed with the microarray-based platform GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array). This dataset contains 208 human airway epithelial samples (124 in the COPD group and 84 in the normal group).

2.2. Differential expression analysis of target gene

Gene expression matrix files for dataset GSE128708 were downloaded from the GEO database, and probe annotation was performed using the GPL570 microarray platform files. After normalizing the overall gene expression profile data by R (version 4.1.2) with limma package, the expression profiles of the target gene USP7 in the COPD and normal group samples were screened, followed by box plot generation for comparison.

2.3. Cell culture and treatment

NCI-H292 cells, a human pulmonary mucoepidermoid carcinoma cell line, were purchased from the American Type Culture Collection (CRL-1848; ATCC, USA). NCI-H292 cells were grown in growth medium (Roswell Park Memorial Institute 1640 medium,

HyClone; GE Healthcare, USA) supplemented with 10 % fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in 5 % CO2. After reaching 70 % confluency, the cells were preexposed to P5091/erdosteine for 6 h before PCN was added, and the cells were then incubated for an additional 24 h.

2.4. Whole-cell, cytosolic, and nuclear extract preparations

Whole-cell extracts were prepared from airway cells using the Nuclear and Cytoplasmic Protein Extraction Kit (Sangon Biotech, China). Airway cells were suspended in the extraction kit, incubated on ice, sonicated, and vortexed every 15 min for 1 h. Lysed cells were centrifuged at 14,200 rpm for 20 min, and the supernatant was collected as a whole-cell extract. The Nuclear and Cytoplasmic Extraction Kit was used to fractionate cytosolic and nuclear proteins. Briefly, airway cells were resuspended in cytoplasmic lysis buffer and incubated on ice for 10 min. The supernatant was collected as cytoplasmic fraction following centrifugation at 14,200 rpm for 10 min. The resulting pellets were resuspended in the nuclear lysis buffer, incubated on ice for 1 h, and sonicated every 15 min. The lysates were centrifuged at 14,200 rpm for 20 min and the supernatant was collected as the nuclear fraction. Protein concentration was determined by the Bicinchoninic acid Protein Assay Kit (Beyotime, China).

2.5. Western blotting analysis

Western blotting analysis was performed using two types of gels, agarose-acrylamide hybrid gel to resolve large molecular weight MUC5AC mucins; and regular 10 % sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel for the other proteins. Briefly, agarose-acrylamide hybrid gels were assembled as follows: 1 % agarose in Tris-borate-EDTA buffer was mixed with 3–6 % acrylamide, 150 mM Tris-HCl (pH 8.8), and 10 % glycerol. The gel mixture was dissolved by heating it in a microwave. Prior to solidifying the gel, SDS, ammonium persulfate, and tetramethylethylenediamine were added to the mixture. The separated protein was then transferred onto a polyvinylidene fluoride membrane (Thermo Fisher Scientific, USA). Other proteins separated on SDS-PAGE gels were transferred onto a nitrocellulose membrane (Thermo Fisher Scientific, USA). Transferred membranes were blocked with 5 % skimmed milk dissolved in Tris-buffered saline with Tween-20 and incubated overnight at 4 °C with primary antibodies against MUC5AC (#sc-2170, Santa, USA), USP7 (#sc-377,147, Santa, USA), NF-κB (#AF-1234, Beyotime, China), phosphorylated-NF-κB (p-NF-κB, #310013, ZEN BIO, China), IκBα (#AF1282, Beyotime, China), and *p*-IκBα (#AF5851, Beyotime, China). Immune complexes were visualized by the enhanced chemiluminescence Western blotting detection system (Thermo Fisher Scientific, USA). All primary antibodies were diluted to 1:1000 for Western blotting analyses.

2.6. Immunofluorescent staining

For immunofluorescent staining, NCI-H292 cells were cultured on coverslips and fixed with 3.7 % paraformaldehyde (10 min, 25–28 °C) in phosphate-buffered saline (PBS) (pH 7.4). After washing thrice with PBS, the cells were permeabilized with 0.1 % Triton-X100 (Sigma-Aldrich) for 10 min at room temperature. After washing thrice with PBS for 5 min, the cells were incubated with a blocking solution (3 % bovine serum albumin in PBS) for 30 min. A rabbit anti-NF-κB p65 antibody was utilized as the primary antibody, which was utilized to incubate the cells overnight at 4 °C. The following day, the cells were washed five times with PBS for 5 min each and then incubated with the secondary antibody for 1 h. These cells were then washed with PBS thrice for 5 min each. The cell nuclei were then stained with 10 μg/mL 4′,6-diamidino-2-phenylindole for 5 min at room temperature. Following three washes with PBS, the coverslips were mounted on slides using Fluoro-GEL (Electron Microscopy Sciences, USA). Confocal imaging was performed sequentially for different fluorophore channels to obtain a series of axial images. Image contrast and brightness were adjusted using the Zen software (Carl Zeiss).

2.7. Mouse lung exposure

Animal experiments were performed in strict accordance with the protocol approved by the Institutional Animal Care and Ethics Committee of Southwest Medical University. Sixty 6-week-old male C57BL/6 mice were housed in positively ventilated microisolator cages with automatic recirculating water, located in a room with laminar, high-efficiency particle accumulation-filtered air. The animals received autoclaved food, water, and bedding. The mucus hypersecretion models were constructed using information from previous studies [17,29]. The mice were randomly divided into six groups (n = 10 per group): Con group, PCN group, PB group, PF group, PCN + PB group and PCN + PF group. After mice were anesthetized with isoflurane, mice in the PCN group received PA (25 μL) nasal drops, whereas those in the Con group received PBS (25 μL) nasal drops at the same time. Mice in the PB group received P5091 (2 mg/kg) nasal drip, whereas those in the PF group received P5091 (10 mg/kg) intraperitoneal injection. Mice in the PCN + PB/PF group were initially pretreated with P5091 and then PA intervention after 3 h. For PA infection, mice were intranasally-infected with 1×10^6 CFU of wild-type PA strain PAO1 bacteria on Days 1, 3, 5, and 7 as previously described [17,30]. Mouse lungs were collected on Day 8.

2.8. Immunohistochemical (IHC) analysis of mouse lung tissues

Following euthanasia, lung tissues were collected for IHC analyses. The method of lung tissue sampling was implemented according to the methods described in previous literature [17,30]. For IHC analysis, paraffin-embedded lung sections (5 mm thick) were stained using the primary antibodies against MUC5AC and NF-κB.

2.9. Statistical analysis

Quantitative data were expressed as mean ± standard error of the mean. Data were analyzed for statistical significance using the one-way analysis of variance, followed by Tukey's post-hoc test. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. USP7 was significantly upregulated in human airway epithelial samples of COPD

In June 2019, GEO provided the clinical and gene expression data. The data included 208 human airway epithelial samples (124 patients with COPD and 84 normal controls). According to Fig. 1, USP7 expression significantly correlated with COPD. Compared with normal controls, USP7 expression increased significantly in patients with COPD.

3.2. USP7 was associated with airway mucus hypersecretion

To investigate the time- and dose-dependent effects of PCN on MUC5AC and USP7 expression, NCI-H292 cells were exposed to different concentrations of PCN for 24 or 48 h, and the findings are presented in Fig. 2A–D. Western blotting analysis results revealed that PCN significantly increased MUC5AC and USP7 protein expression (Fig. 2A, B, and D), and their expression peaked following exposure to 10 μg/mL of PCN. Furthermore, the results revealed that although the maximum intervention time of PCN was 48 h, the expression of MUC5AC and USP7 proteins was still considerable at 24 h of intervention. This was probably due to PCN significantly inhibiting the growth and activity of the cells. Subsequently, IHC was performed to further validate the findings to determine the extent of this alteration in MUC5AC and USP7 proteins. As presented in Fig. 2C, mice in the control group had fewer areas of airway epithelium and lumen that stained positive for MUC5AC and USP7, and the staining was light brown in intensity. The MUC5AC and USP7 positive staining areas on the airway epithelium surface of mice in the PCN group increased significantly, and the staining degree was deep, presenting dark yellow and brownish yellow. Therefore, we hypothesized that USP7 may be involved in the regulation of airway mucus hypersecretion.

3.3. USP7 activated NF-KB signaling pathway in the airway mucus hypersecretion model

To investigate the possible mechanism of PCN -induced MUC5AC protein expression, changes in protein expression of inflammation, apoptosis, and autophagy-related pathways were examined after PCN and P5091 intervention. The results showed that the expression of NF-κB pathway-related proteins and ERK proteins changed significantly (Fig. 3A–K). However, changes in ERK protein expression did not yield significant outcomes. Several studies have demonstrated that USP7 can stabilize the expression of NF-κB and play a pro-inflammatory role [29,31]. Western blotting analysis revealed that NF-κB/p-NF-κB and p-IκBα increased following treatment with PCN (Fig. 3A–F, I, and K), indicating that PCN stimulation activates the NF-κB pathway. Additionally, si-USP7 treatment significantly inhibited PCN -induced overexpression of NF-κB/p-NF-κB and p-IκBα at the protein level (Fig. 4A–E).
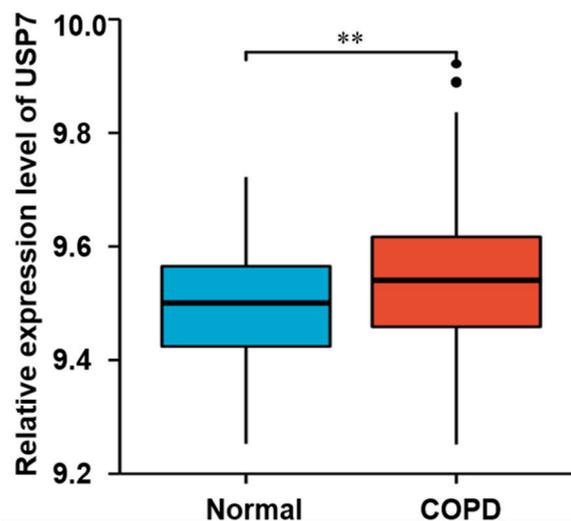
Fig. 1. USP7 was significantly upregulated in human airway epithelial samples of the COPD. (Based on the information of 124 COPD patients and 84 normal controls in the GEO database). (**$P < 0.01$).
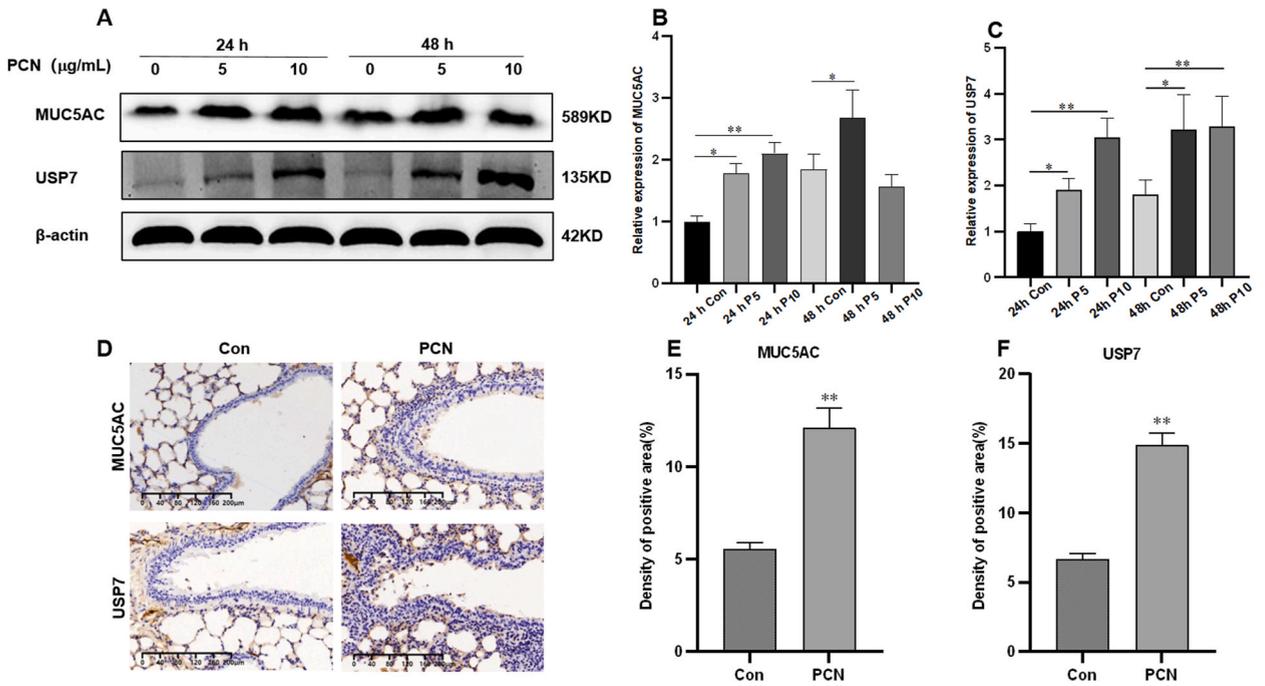
Fig. 2. USP7 was associated with airway mucus hypersecretion. (A) Activation the expressions of MUC5AC and USP7 by PCN. Proteins of NCI-H292 cells previously exposed to PCN for 24 h or 48 h were probed with anti-MUC5AC and USP7 antibody, n = 3. (B and C) The expression levels of MUC5AC and USP7 were quantified using the ImageJ program. (D) Immunohistochemistry analysis of MUC5AC and USP7 expression in lung tissue (n = 3). (E) Analysis of MUC5AC positive area of the immunohistochemistry results. (F) Analysis of USP7 positive area of the immunohistochemistry results. (*$P < 0.05$, **$P < 0.01$).
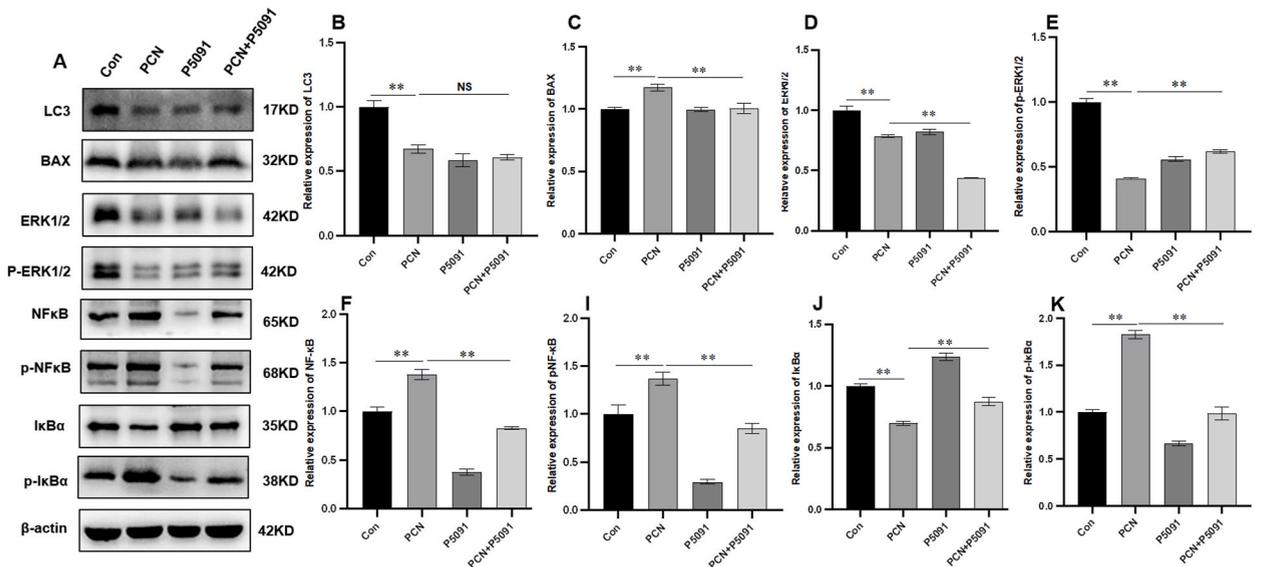
Fig. 3. USP7 activated NF-κB signaling pathway in airway mucus hypersecretion model. (A) NCI-H292 cells from different groups were pretreated with P5091 and then exposed to PCN, the expressions of LC3, BAX, ERK1/2, *p*-ERK1/2, NF-κB, p-NF-κB, IκBα and p-IκBα by Western blot (n = 3). (B-K) The expression levels of LC3, BAX, ERK1/2, *p*-ERK1/2, NF-κB, p-NF-κB, IκBα and *p*-IκBα were quantified using the ImageJ program (*$P < 0.05$, **$P < 0.01$).

3.4. PCN affects the protein stability of NF-κB

To investigate the mechanism of PCN-induced upregulation of NF-κB protein levels, airway epithelial cells were treated with the protein biosynthesis inhibitor cycloheximide (CHX), and the effect of PCN on NF-κB protein stability was analyzed (Fig. 5A and B). The results of Western blotting analysis revealed that PCN enhanced the protein stability of NF-κB and inhibited its degradation. Because proteasome-dependent degradation is the predominant mode of intracellular protein degradation, the involvement of the proteasome in NF-κB degradation was investigated. MG132, a proteasome-specific inhibitor, was utilized to inhibit the proteolytic activity of the proteasome in airway epithelial cells (Fig. 5C and D). Our findings indicated that PCN increased the expression level of NF-κB protein and significantly attenuated the decrease of NF-κB protein expression level. To investigate the effect of PCN on ubiquitination, the Western blotting analysis was performed, revealing that PCN significantly increased ubiquitin protein expression (Fig. 5E), which peaked following exposure to 10 µg/mL of PCN.

3.5. USP7 attenuates MUC5AC via the suppression of NF-κB nuclear translocation in NCI-H292 cells

As a nuclear transcription factor, NF-κB typically exerts its pro-inflammatory effect via nuclear translocation. Hence, the levels of NF-κB in the cytoplasmic and nuclear extracts of NCI-H292 cells were assessed by Western blotting analysis to further investigate whether USP7 promoted hypersecretion of airway mucus by inducing NF-κB nuclear translocation. The expression levels of NF-κB in the nucleus were elevated while those in the cytoplasm decreased following treatment with PCN (Fig. 6A–C), which is consistent with our immunofluorescence findings (Fig. 6D). NF-κB nuclear translocation was significantly reduced after pretreating cells with si-USP7 and further treating them with PCN (Fig. 6D). When cells were pretreated with erdosteine and then treated with PCN, the expression of MUC5AC was significantly reduced compared with that in the PCN group (Fig. 6E and F). These findings confirmed that si-USP7 attenuated the hypersecretion of airway mucus by suppressing NF-κB nuclear translocation.

3.6. Inhibiting USP7 alleviated airway mucin MUC5AC

The animal experimental flow diagram is illustrated in Fig. 7A. IHC was performed to detect the expression level of MUC5AC protein on the airway surface of mice in each group. In comparison to mice in the PCN group, which had significantly higher levels of MUC5AC expression than those in the P5091 intervention group, mice in the Con, PB, and PF groups expressed lower levels of MUC5AC on the surface of their airways, as depicted in Fig. 7B. The expression of NF-κB was lowest in the Con, PB, and PF groups, and increased significantly following PCN treatment. However, NF-κB expression decreased following P5091 intervention, with the P5091 nasal drip group demonstrating the most significant changes (Fig. 7C).
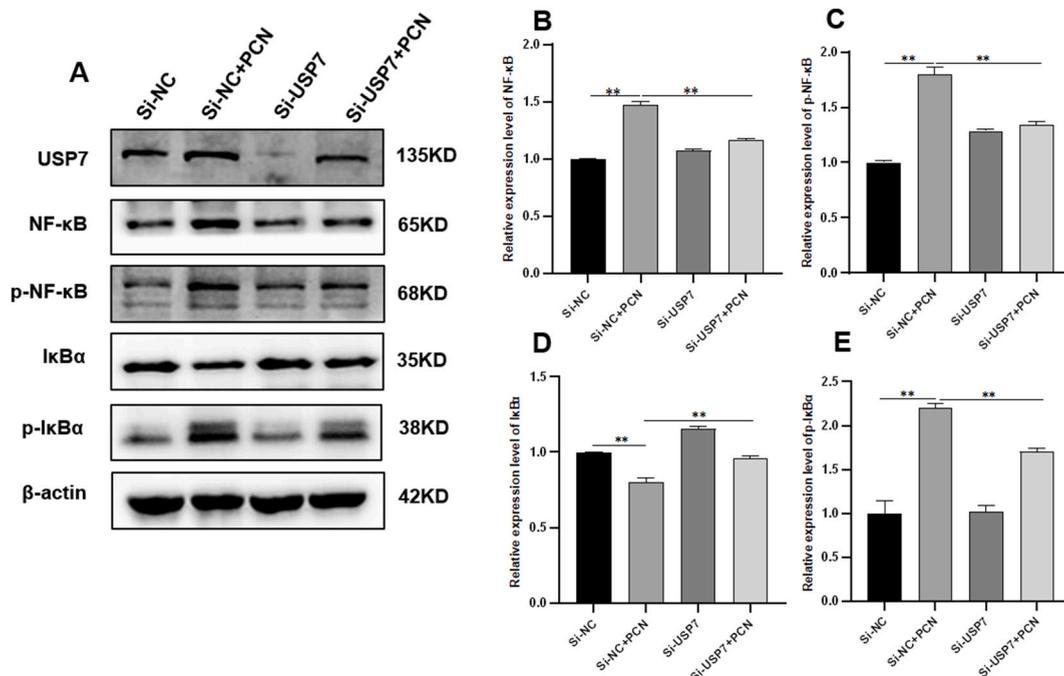
Fig. 4. USP7 activated NF-κB signaling pathway in airway mucus hypersecretion model. (A) NCI-H292 cells from different groups were pretreated with SiRNA and then exposed to PCN, the expressions of NF-κB, p-NF-κB, IκBα and *p*-IκBα by Western blot (n = 3). (B–E) The expression levels of NF-κB, p-NF-κB, IκBα and *p*-IκBα were quantified using the ImageJ program (*$P < 0.05$, **$P < 0.01$).
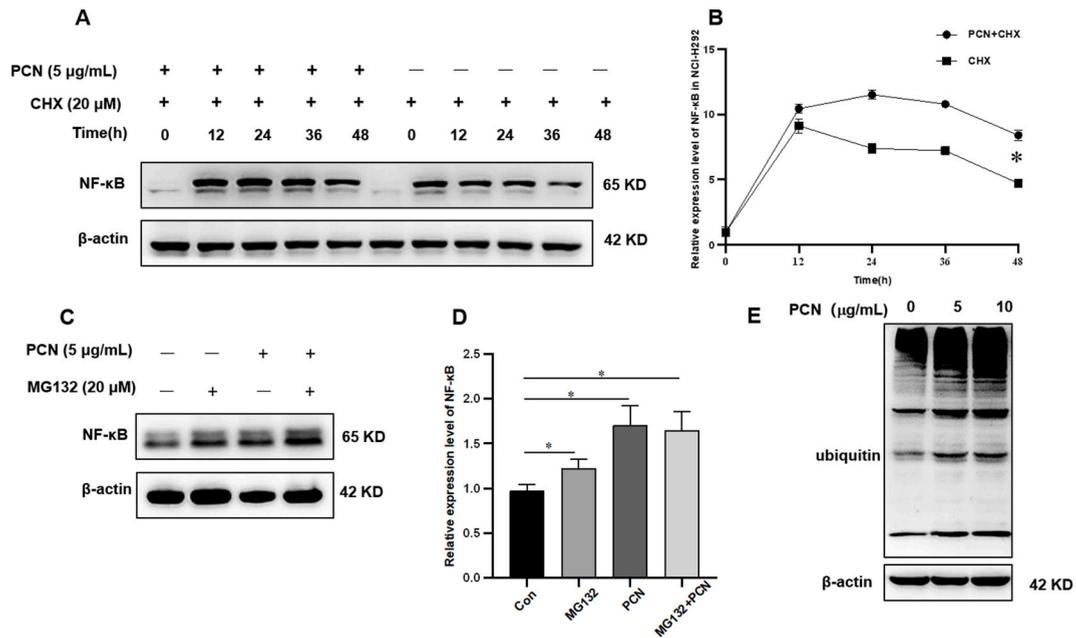
Fig. 5. PCN affects the protein stability of NF-κB. (A) The protein expression of NF-κB treated with PCN and CHX was measured by Western blot (n = 3). (B) The expression level of NF-κB was quantified using Line chart. (C) The protein expression of NF-κB treated with PCN and MG132 was measured by western blotting (n = 3). (D) The expression level of NF-κB was quantified using the ImageJ program. (E) The protein expression of ubiquitin treated with PCN was measured by western blotting (n = 3). (*$P < 0.05$, **$P < 0.01$).

4. Discussion

It has been reported that increased expression of MUC5AC mRNA or protein can be used as an indicator of goblet cell proliferation and chemotaxis, and changes in MUC5AC content can indirectly reflect mucus secretion [6]. Therefore, MUC5AC was selected as the main observational index of airway mucus hypersecretion in the present study. Airway mucus hypersecretion is associated with various factors including bacteria and their products, air pollutants, cytokines, inflammatory mediators, and reactive oxygen species [32]. *P. aeruginosa* is considered to be the most important pathogenic agent of COPD, with various virulence factors. These factors can induce airway inflammation, which can impair lung function and even cause lung tissue destruction [33]. A previous study has reported that among several virulence factors, PCN induces the most significant effect on airway goblet cell proliferation, chemotaxis, and mucus hypersecretion [17]. Therefore, the use of PCN to construct an airway mucus hypersecretion model is consistent with the pathophysiological condition. In the present study, the method described by Woosuk et al. was utilized to successfully construct a cellular and animal model of airway mucus hypersecretion in COPD [17,29]. To investigate the effect of the difference in the mode of administration on the therapeutic effect, we set up a transnasal administration group and an intraperitoneal injection group in the animal experimental part. The results proved that both modes of administration had a significant effect on relieving airway mucus hypersecretion.

Based on data analysis, which demonstrated that USP7 expression was significantly upregulated in the lung tissue of patients with COPD, we hypothesized that USP7 may be a novel target for airway mucus hypersecretion. Following PCN treatment, we discovered that USP7 expression was increased in mice and NCI-H292 cells. *In vitro* and *in vivo* experiments showed that inhibiting USP7 expression significantly suppressed NF-κB and MUC5AC expression. Furthermore, USP7 can regulate the NF-κB pathway and stimulate MUC5AC expression in PCN-treated NCI-H292 cells. NOD-like receptor protein 3 (NLRP3) is a key regulator of the host innate immune response. A previous study has demonstrated that USP7 is upregulated in hypertrophic myocardial tissue, and by inhibiting the NF-κB/ NLRP3 signaling pathway, its inhibitor P22077 attenuates angiotensin II-induced myocardial hypertrophy, fibrosis, inflammation, and oxidative enzyme stress [30]. However, another study reported that USP7 promotes chondrocyte proliferation and inhibits apoptosis and tumor necrosis factor-α-induced inflammatory response by inhibiting the BiP-eIF2α-ATF4-CHOP signaling pathway and NF-κB/p65 signaling pathway of endoplasmic reticulum stress [34]. These differences in the outcomes may be attributed to differences in the tissues. Interestingly, in the present study, USP7 activated the NF-κB signaling pathway. P5091 and erdosteine are specific inhibitors of USP7 and NF-κB, respectively. In the present study, P5091 treatment dramatically decreased the induction of NF-κB protein expression by PCN, providing additional evidence that USP7 controls airway mucus secretion via NF-κB. Notably, deubiquitination was not used in this study when attempting to regulate NF-κB via USP7. However, whether USP7 contributes to COPD inflammation and mucus hypersecretion through other signaling pathways requires further study. For example, pyroptosis plays a role in the development of several lung inflammatory diseases, such as asthma, COPD, acute lung injury, silicosis, pulmonary hypertension, and cancer [35–37]. In addition, previous studies have indicated that USP7 can upregulate pyroptosis through several pathways, such
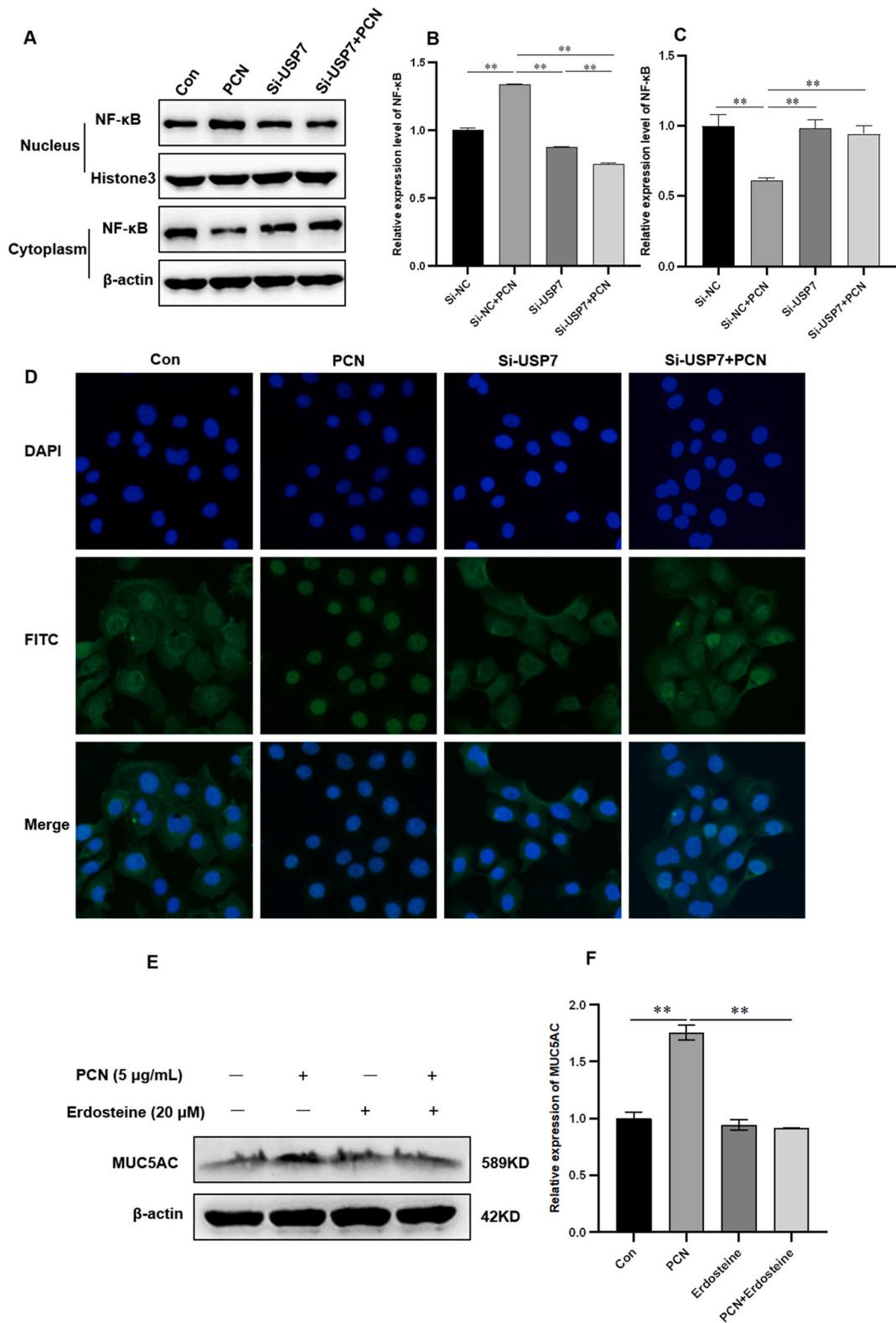
(caption on next page)

Fig. 6. USP7 attenuates the hypersecretion of airway mucus via the suppression of the nuclear translocation of NF-κB in NCI-H292 cells. (A) The levels of NF-κB protein in the nucleus and cytoplasm were measured using Western blot and normalized to histone3 (n = 3). (B) And β-actin (C), respectively (*P < 0.05, **P < 0.01). (D) The expression of NF-κB protein in different cell groups was detected by cellular immunofluorescence staining. Scale bar: 50 μm. (E) The protein expression of MUC5AC treated with PCN and Erdosteine was measured by western blotting (n = 3). (F) The expression level of MUC5AC was quantified using the ImageJ program. (**P < 0.01).
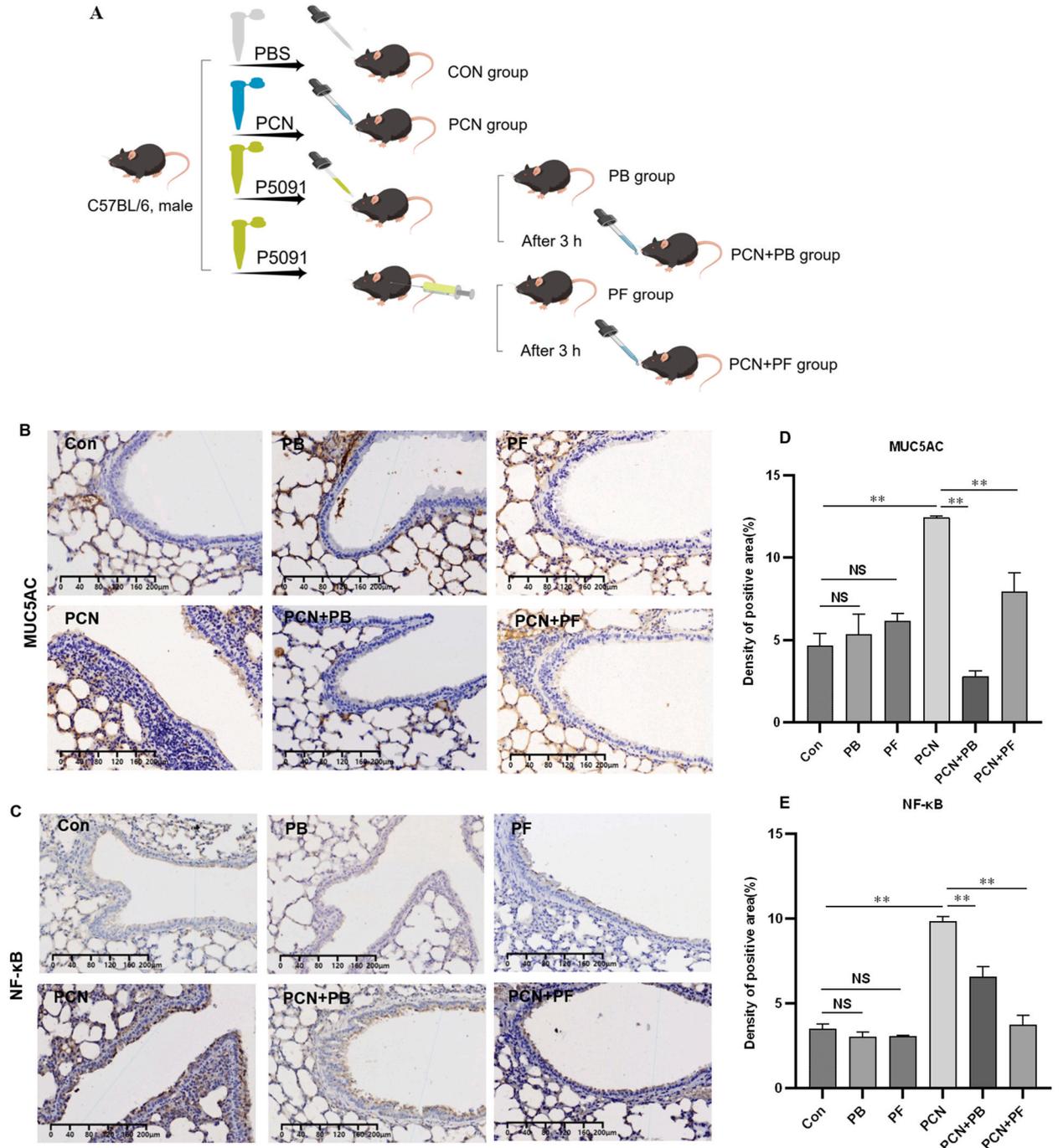
Fig. 7. Inhibiting USP7 alleviated the airway mucus hypersecretion of COPD. (A) Experimental flow diagram and animal groups (By figdraw). (B) Immunohistochemistry analysis of MUC5AC expression in lung tissue (n = 3). (C) Immunohistochemistry analysis of NF-κB expression in lung tissue (n = 3). (D) Analysis of MUC5AC-positive area in the immunohistochemistry results. (E) Analysis of NF-κB positive area in the immunohisto-chemistry results. (*P < 0.05, **P < 0.01).

as the USP7-SOX9-miR-96-5p-NLRP3 network in myocardial injury and cardiomyocyte pyroptosis [38], and the NOX4/NLRP3 pathway in H_2O_2-induced injury of chondrocytes [39]. It is rational to hypothesize that USP7 may contribute to pyroptosis-induced COPD inflammation and mucus hypersecretion. Pyroptosis is an important research direction and may be a potential target for the clinical treatment of lung inflammatory diseases and mucus hypersecretion induced by USP7.

Numerous small-molecule inhibitors of USP7 exist, such as P5091, HBX19818, GNE-6640, and XL188. Furthermore, some natural substances, such as spongiacidin C, xestoquinone, and Sulawesin A, have significant antagonistic properties against USP7. In conjunction with this study, these drugs may be used via various modes of administration (nebulization and intravenous administration) to alleviate airway mucus hypersecretion caused by COPD or other chronic inflammatory conditions and to reduce airway mucus secretion in clinically intubated patients, especially those who are critically ill requiring long-term mechanical ventilation. However, clinically relevant trials of these drugs are currently sparse, and more research is required to demonstrate their clinical significance.

In summary, our research revealed that USP7 expression was significantly elevated in the lung tissue of patients with COPD. Moreover, the elevation of USP7 and NF-κB was significantly correlated with the severity of airway mucus hypersecretion. USP7 inhibition downregulated NF-κB expression and thus alleviated airway mucus hypersecretion. These findings suggest that USP7 plays a critical role in the development of airway mucus hypersecretion disease by activating the NF-κB signaling pathway (Fig. 8). This study identifies one of the mechanisms regulating airway mucus secretion and provides a novel potential target for preventing and treating airway mucus hypersecretion.

Ethics statement

This study was reviewed and approved by the Institutional Animal Care and Ethics Committee of Southwest Medical University (2020261).

Funding

Data availability

The dataset supporting the conclusions of this article is included within this article and is available from the corresponding author
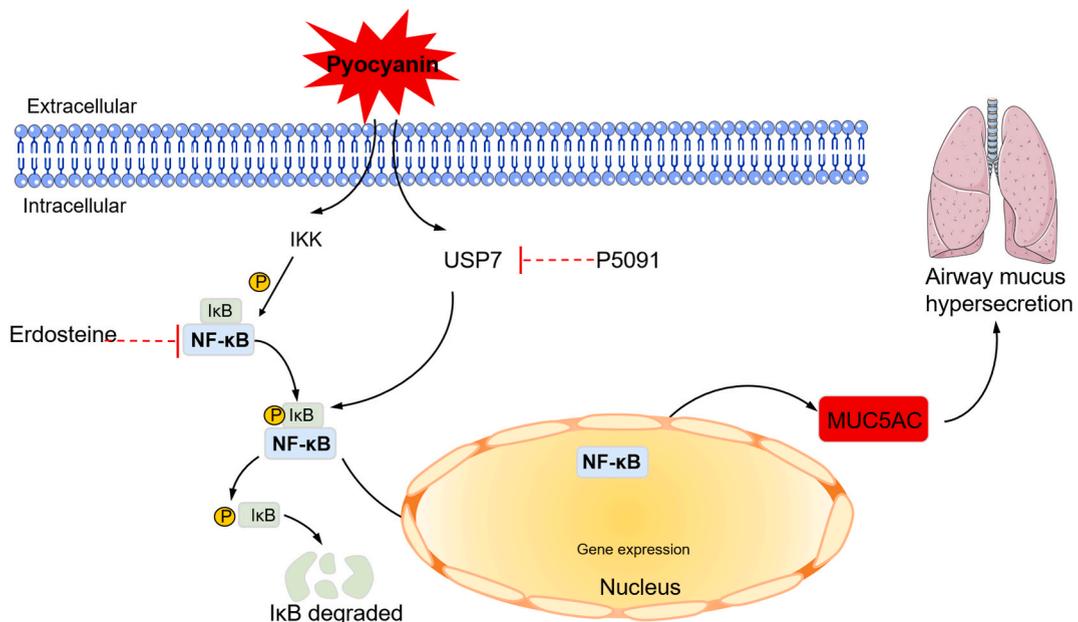
Fig. 8. USP7 promotes airway mucus hypersecretion through NF-κB Signaling Pathway. USP7 expression is elevated by PCN insult in NCI-H292 cells and mice airway epithelial cells, lead to NF-κB activation and entry the nucleus, thus aggravate the NF-κB-based inflammatory activities, promote the expression of MUC5AC.

upon request.

CRediT authorship contribution statement

Yi-Jing He: Writing – original draft, Investigation, Conceptualization. **Yi-Rong Chen:** Writing – original draft, Investigation, Conceptualization. **Jia-Rui Song:** Formal analysis. **Jin-Xiu Jiang:** Investigation. **Ting-Ting Liu:** Formal analysis. **Jia-Yao Li:** Formal analysis. **Liu Li:** Writing – review & editing. **Jing Jia:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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