Blocking tumor necrosis factor-α delays progression of chronic obstructive pulmonary disease in rats through inhibiting MAPK signaling pathway and activating SOCS3/TRAF1

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Received July 28, 2020; Accepted January 25, 2021

DOI: 10.3892/etm.2021.10746

Abstract. The present study was conducted in order to study the detailed molecular mechanism of tumor necrosis factor (TNF)-α in chronic obstructive pulmonary disease (COPD). The rats were treated with cigarette smoke (CS) and lipopolysaccharide (LPS) to establish the COPD model. Next, the changes in lung injury in COPD rats with TNF-α knockdown was tested. Meanwhile, the regulation of TNF-α on MAPK pathway and its downstream molecules (SOCS3/TRAF1) was determined by western blotting. On this basis, the activation of MAPK and inhibition of SOCS3/TRAF1 was also examined. Subsequently, the lung function was tested with the plethysmograph, the cells of bronchoalveolar lavage fluid was counted and classified. Furthermore, lung tissue sections were stained with hematoxylin and eosin to verify whether the treatment of MAPK pathway and downstream molecules affected the effect of TNF-α knockdown on COPD. The present study showed that TNF-α knockdown could alleviate the decrease in the function and inflammatory injury of the lungs of rats with COPD. Western blot analysis verified that TNF-α knockdown could inhibit the activation of MAPK pathway and increase the expression of SOCS3/TRAF1. The following experimental results showed that the relief of lung injury caused by TNF-α knockdown could be deteriorated by activating MAPK pathway. It was also found that the symptom of COPD was decreased following transfection with sh-TNF-α but worsened by SOCS3/TRAF1 knockdown. Overall, TNF-α knockdown inhibited the activation of MAPK pathway and increased the expression of SOCS3/TRAF1, thus delaying the process of COPD.

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Key words: chronic obstructive pulmonary disease, tumor necrosis factor-α, MAPK, SOCS3, TRAF1

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic respiratory disease with high morbidity and mortality rates, which can be prevented but not cured (1). The main characterization of patients with COPD are emphysema and chronic bronchitis accompanied by cough, dyspnea, shortness of breath and other symptoms, and the chronic hypoxemia also occurs in moderate and severe patients (2-4). The main pathogenic factors of COPD are long-term smoking, long-term exposure to air polluted environment, and bacterial infection, among which the most important and the most wildly studied factor is cigarette smoke (5,6). Cigarette smoke exposure can induce oxidative stress (7,8). Reactive substances produced by oxidative stress can activate resident cells in the lung, such as alveolar macrophages and epithelial cells, and then the inflammatory cells (neutrophils and lymphocytes) are attracted to the lung by chemotaxis and release inflammatory mediators such as tumor necrosis factor-α (TNF-α) and various types of interleukin (1,9). The deterioration of COPD is accompanied by other physiological deterioration besides the weakness of respiratory system, which causes a heavy burden for patients (10-12). Nowadays, the treatment of COPD includes conservative treatment with slow effect and surgical treatment with high risk. Therefore, it is urgent to explore the pathogenesis of COPD and find effective and safe treatment.

It was found that there are more than 500 genes associated with COPD from several genetic studies on COPD, most of which are associated with oxidative stress and inflammatory response pathway, particularly TNF-α (13). TNF-α is a type of multi-effective inflammatory factor, which plays an important role in the physiological processes of inflammation, cytotoxicity and immune regulation (14). The study shows that the concentration of TNF-α in the lung of patients with COPD is higher compared with that of healthy people, and TNF-α is associated with the progression of COPD (15). It was also found that the overexpression of TNF-α can not only induce emphysema and pulmonary fibrosis, but also decrease the muscle quality and aggravate the physiological symptoms

of patients with COPD at the end of the period, and TNF-α inhibitors are also considered as potential therapeutic drugs for COPD (16-20). Furthermore, the TNF-α 308 gene variation is an independent factor to induce COPD, which has a higher association with Asians than other populations (13,21).

Given previous studies, it is known that TNF-α factor plays an important role in COPD. In order to study the molecular mechanism of the effect of TNF-α on COPD, a rat model of COPD was established by knocking down the gene of TNF-α, followed by a series of experiments.

Materials and methods

Animal models. Twenty-four male Wister II rats (weights, 250-290 g) were obtained from Experimental animal center of Beijing University of traditional Chinese Medicine (Beijing, China). All animal experiments were approved by the Experimental Animal Welfare Ethics Review Committee of The Affiliated Hospital of Jianghan University (grant no. AW2019091203) and adhered to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

After the rats were cultured for a week to adapt to the laboratory environment, they were fasted for 12 h and divided into four groups (six rats in each group): i) Control group, the rats were injected saline and treated with smoke-free condition; ii) COPD group, the rats were injected saline and treated with lipopolysaccharide (LPS) and cigarette smoke (CS) exposure; iii) sh-TNF-α group, these rats were given the antibody against TNF-α and were treated with LPS and CS exposure; iv) shRNA negative control (sh-NC) group, the rats were injected random antibody and were treated with LPS and CS exposure. Treatment time and method: LPS was injected into the rats' trachea on the 1st and 14th day of the experiment, and the rats were smoked with 5% CS of CHIENMEN cigarettes in a 72-l closed box from the 2nd to the 28th day of the experiment. In the sh-TNF-α group, antibodies (5 μg/g) against TNF-α-IgG (cat. no. ab6671; Abcam) were injected via caudal vein, and LPS (Sigma-Aldrich; Merck KGaA) was injected after 0.5-1 h, and the sh-NC group was injected with the same amount of anti-actin antibody (cat. no. ab11003; Abcam) and LPS with the same method. The other groups were treated with equal volume injection of saline (Procell Life Science & Technology Co., Ltd.). A series of tests were carried out on all experimental animals after four weeks of feeding.

In vivo gene transfection. shRNA targeting TNF-α, random gene, SOCS3 and TRAF1 (0.1 μg, 50 pmol) and 1.5 μl Entranster-R4000 were diluted to 25 μl with PBS. The sequences of shRNAs was as follows: TNF-α, 5'-GTAGCC CATGTTGTAGCAA-3'; random gene (control sequence), 5'-TTCTCCGAACGTGTCACGT-3'; SOCS3, 5'-CCAAGA ACCTGCGCATCC A-3'; and TRAF-1, 5'-GCCTTCTAC ACTGCCAAGTAT-3'. The shRNA and Entranster-R4000 were mixed fully by an oscillator to synthesize the transfection complex and were allowed to stand for 15 min. Then, shRNA-TNF-α-Entraster-R4000, shRNA-SOCS3-Entraster-R4000/shRNA-TRAF1-Entraster-R4000 and shRNA-NC-Entraster-R4000 were respectively injected into the rats through the caudal vein. In addition, the transfection efficiencies were detected by western blotting (Fig. S1). The MAPK activator (Asiatic acid; cat. no. ab143120; Abcam; 50 mg/kg) was also injected into the caudal vein of rats. Finally, the rats were executed and a series of studies were carried out after feeding for 3 days. Each treatment had three independent rats as receptor.

Measurement of respiratory function in rats. AniRes2005 lung function system (Bestlab, version 2.0, China) includes a plethysmograph chamber, a computer-controlled small animal ventilator and pressure and vacuum reservoirs, which were used to measure the parameters of rats' lung function.

Firstly, the peak expiratory flow-rate (PEF) and tidal volume of the rats was recorded by the whole-body plethysmograph when the rats were awake. Next, the rats were anesthetized by intraperitoneal injection of 1% pentobarbital sodium (50 mg/kg) and a cannula was inserted into the trachea, and then the rats were connected to a small animal ventilator. Then, adjusting the respiratory frequency of the system ventilator to match the rat's respiratory frequency, and recording the data with the computer connected to the ventilator for the next analysis. The main outcome measures were forced expiratory volume (forced expiratory volume in 1 sec, FEV1), forced vital capacity (FVC) and forced expiratory flow at 25% (FEF 25%). Respiratory function tests were repeated three times at each state to obtain an average.

Cell count and enzyme activity test of bronchoalveolar lavage fluid (BALF). The rats were killed by using cervical dislocation after intraperitoneally injecting with 150 mg/kg 1% phenobarbital sodium, and the left lung was clamped to preserve architecture for subsequent histopathological evaluation. The trachea of rats was placed with cannula, and the right lung of rats was washed with 1.0 ml PBS for 5 times. The recovered BALF was centrifuged at 4˚C at 1500 x g for 5 min. The medium RPMI-1640 (Thermo Fisher Scientific, Inc.) containing 10% FBS (Thermo Fisher Scientific, Inc.) was used to resuspend the precipitate, and the cell suspension was coated on the slide and fixed with 10% neutral formaldehyde at room temperature for 30 min. The cells were classified and counted under light microscope (magnification, x40) after staining with Wright-Giemsa (solution A for 1 min and solution B for 3 min) at room temperature. Subsequently, the level of myeloperoxidase (MPO) was detected by ELISA kit (cat. no. SEA601Ra; Cloud-Clone Corp.) according to the manufacturer's instructions. Three independent experiments were conducted in each state of rats.

Western blotting. The right upper lobe of the lungs of the rats in the experimental groups and the control groups was lysed with RIPA buffer (10 mM Tris-HCl, pH 7.2, 0.1% SDS, 1% sodium deoxycholate, 0.15 M NaCl, 1% NP-40), and the lysates were centrifuged at 1,500 x g for in 4˚C for 20 min. The concentration of total protein was determined by the BCA method. The protein concentration was measured by enzyme standard instrument at 562 nm after 30 min. Then, 10% SDS-PAGE gel electrophoresis was performed to isolate and transfer the protein to the PVDF membrane, and the membrane was sealed at room temperature for 1 h with

5% skimmed milk powder. Subsequently, the primary antibodies were incubated with the membrane overnight at 4˚C, and the secondary antibodies were incubated with protein for 1 h at 4˚C. Finally, the exposure and data analysis of protein bands were carried out. Primary antibodies, including anti-p-ERK (cat. no. ab79483; dilution, 1:1,000), p-p38 (cat. no. ab4822, dilution, 1:1,000), p38 (cat. no. ab31828; dilution, 1:1,000), ERK (cat. no. ab17942, dilution, 1:1,000), p-JNK (cat. no. #81E11; dilution, 1:1,000), JNK (cat. no. ab124956, dilution, 1:10,000), SOCS3 (cat. no. ab16030; concentration, 1) and TRAF1 (cat. no. ab129279; concentration, 1 μg/ml), as well as the secondary antibodies, goat anti-rabbit IgG H&L (HRP) (ab6721; dilution, 1:20,000); goat anti-mouse IgG H&L (HRP) (ab6789; dilution, 1:10,000), donkey anti-goat IgG H&L (HRP) (cat. no. ab6885, concentration, 1 μg/ml) were purchased from Abcam. Actin was used as the internal control. Three independent experiments were conducted in each state of rats.

Histopathological examination of lung. The left lung of rats was fixed with 4% paraformaldehyde at room temperature for 24 h and embedded in paraffin. The lung tissue sections (4 μm) were stained with hematoxylin (for 2-3 min) and eosin (for 1 min) at room temperature. The photos were captures at the magnifications of x100 and x400 by an optical microscope, and then, the inflammatory infiltration around alveoli or the structural damage of alveoli and airway were observed in the photos.

Statistical analysis. The data are presented as mean ± SD. The statistical significance of the differences among multiple groups of data was analyzed by one-way ANOVA followed by S multiple comparisons test in GraphPad Prism 7.0 (GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effect of TNF-α on lung function, inflammatory response and lung structure in rats with COPD. In order to test the effect of TNF-α on inflammatory response and lung structure in rats with COPD, the lung function indexes, number of inflammatory cells and structural changes in alveoli and airway were detected in rats with COPD. After the rats were treated with CS and LPS to establish COPD models, it was found that the lung function indexes of the rats treated with CS and LPS, such as FEV1, FVC, FEV1/FVC, FEF25, PEF, VE (minute ventilation at rest) and FEV0.3 were significantly lower compared with those of the normal rats (Fig. 1A-G). However, the decrease in lung function caused by LPS + CS was reversed by knocking down TNF-α (Fig. 1A-G). For inflammatory cells, the rats with COPD had a higher level of leukocyte and neutrophils, but the number of leukocytes and neutrophils were decreased after TNF-α knockdown (Fig. 1H-I). In addition, the number of alveolar macrophages, lymphocytes and monocytes of the COPD rats were decreased compared with those in normal rats, and increased following knockdown of TNF-α (Fig. 1J-L). The high level of MPO in COPD rats was decreased by TNF-α knockdown (Fig. 1M). Compared with healthy rats, COPD rats showed obvious inflammatory cell infiltration around the airway, the cystic expansion in the disordered alveolar structure increase of pulmonary bullae (Fig. 1N), indicating that the COPD models were established successfully. However, the structural and functional damage of lungs in COPD rats was decreased by the knockdown of TNF-α (Fig. 1M). All the experimental results showed that the damage of function and structure of lungs in COPD rats caused by CS + LPS could be alleviated by TNF-α knockdown.

Underlying molecular mechanism of TNF-α in COPD. TNF-α can initiate a series of molecular cascade reaction, including the activation of the MAPK pathway and some downstream signaling molecules, named as TNF signaling pathway. However, the mechanism of TNF signaling pathway in COPD is not yet well understood. To explore this issue, the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/kegg2.html) was used to identify TNF signaling pathway (Fig. S2), and the MAPK signaling pathway was chosen as well as downstream signaling molecules (TRAF1 and SOCS3) to investigate the underlying molecular mechanism. Western blotting was used to detect the changes in the expression level of TRAF1, SOCS3, as well as major proteins in the MAPK pathway in COPD rats transfected with or without TNF-α shRNA (sh-TNF-α). The results of western blotting indicated that the expression of p-ERK, p-p38 and p-JNK was increased in rats with COPD, and it was decreased after COPD model rats were transfected with sh-TNF-α (Fig. 2). However, on the contrary, the expression of SOCS3 and TRAF1 was decreased in the COPD rats and increased in TNF-α knockdown rats (Fig. 2). These results indicated that TNF-α knockdown could inhibit the activity of the MAPK pathway and upregulate the expression of SOCS3 and TRAF1.

Effect of MAPK pathway on inflammatory response and lung structure in rats with COPD. To detect whether the inhibition of the MAPK pathway activity caused by TNF-α knockdown could affect the inflammatory response and lung structure in COPD rats, western blotting, pulmonary function test, cell count of BALF and histopathological examination of the lungs were conducted. The results of western blotting showed that the key proteins in MAPK pathway such as p-ERK, p-JNK and p-p38 were decreased by the transfection of sh-TNF-α but increased after the introduction of the activator of MAPK pathway, while ERK and p38 had no change (Fig. 3A). The results of pulmonary function test demonstrated that the increase in all pulmonary function indexes induced by TNF-α knockdown was weakened after the introduction of the activator of MAPK pathway (Fig. 3B-H). The results of cell classification and counting showed that the decrease in the number of leukocytes and neutrophils induced by sh-TNF-α transfection was reversed by adding the activator of MAPK pathway (Fig. 3I-J), and the tendency of alveolar macrophages and lymphocytes was contrary (Fig. 3K-L). And the reduction of the number of monocytes caused by TNF-α knockdown had no significant change after the introduction of the MAPK pathway activator (Fig. 3M). In addition, the decline in MPO caused by knocking down the TNF-α was reversed by activating MAPK pathway (Fig. 3N). The results of histopathology showed that the alleviation of inflammatory infiltration around alveoli or damage of alveoli and airway structures caused by sh-TNF-α transfection was deteriorated by adding the

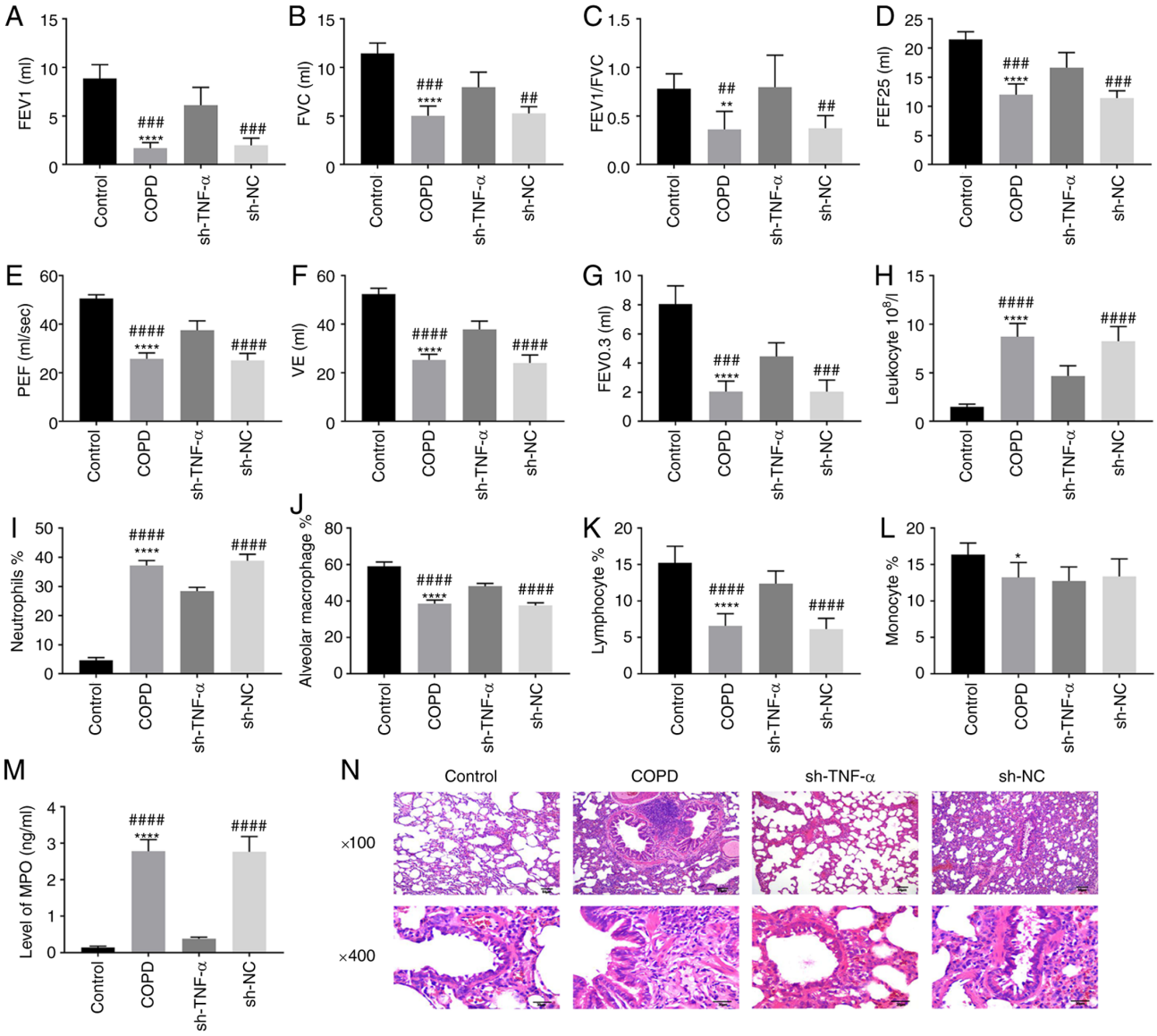

Figure 1. Effect of TNF-α on inflammatory response and lung structure in rats with COPD. The rats were treated with LPS + CS and transfected with sh-TNF-α. (A-G) The pulmonary functions of rats, including (A) FEV1, (B) FVC, (C) FEV1/FVC, (D) FEF 25, (E) PEF, (F) VE and (G) FEV0.3, were measured by the plethysmograph. (H-L) BALF was obtained by fiberoptic bronchoscopy and normal saline, and the cells and enzyme in BALF such as (H) leukocyte, (I) neutrophils, (J) alveolar macrophage, (K) lymphocyte and (L) monocyte were detected. (M) The level of MPO in BALF was detected by enzyme-linked immunosorbent assay. (N) The lung tissues of rats were sectioned and stained with hematoxylin and eosin to observe the characteristics of alveoli and airway. *P<0.05; **P<0.01; ****P<0.0001, vs. control group. $^{\#\#}$P<0.01; $^{\#\#\#}$P<0.001; $^{\#\#\#\#}$P<0.0001, vs. sh-TNF-α group/sh-RNA group. TNF, tumor necrosis factor; LPS, lipopolysaccharide; COPD, chronic obstructive pulmonary disease; CS, cigarette smoke; FEV, forced expiratory volume; FVC, forced vital capacity; FEF, forced expiratory flow; PEF, peak expiratory flow; VE, minute ventilation at rest; BALF, bronchoalveolar lavage fluid; MPO, myeloperoxidase; shRNA, short interfering RNA; NC, negative control.

activator of MAPK pathway (Fig. 3O). Therefore, the disease progression of COPD could be accelerated by activating the MAPK pathway, and TNF-α could affect the inflammatory response and lung structure in COPD through regulating MAPK pathway.

Effect of SOCS3 and TRAF1 on inflammatory response and lung structure in rats with COPD. To detect whether the increased expression of SOCS3 and TRAF1 induced by TNF-α knockdown could affect the inflammatory response and lung structure in COPD rats, these two molecules were knocked down and the pulmonary function, the number of inflammatory cells and the pathological changes in the lung were detected. Western blotting showed that the expression of SOCS3 and TRAF1 decreased after the rats were treated with LPS + CS, but this trend was reversed after TNF-α was knocked down, and their expression was weakened after the rats co-transfected with sh-TNF-α and sh-SOCS3 or sh-TRAF1 (Fig. 4A). The increased values of pulmonary function of COPD model rats caused by sh-TNF-α was weakened after the introduction of sh-SOCS3 or sh-TRAF1 (Fig. 4B-H). The cell classification and counts of BALF from COPD rats indicated that the decrease in the number of leukocytes and neutrophils by sh-TNF-α was reversed by sh-SOCS3 and sh-TRAF1 (Fig. 4I-J). However, the increase in alveolar macrophages and lymphocytes induced by sh-TNF-α was

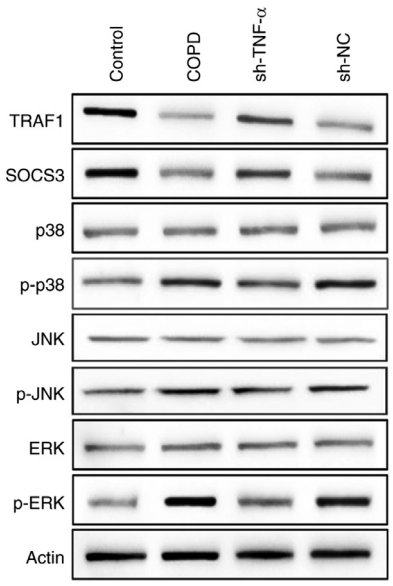

Figure 2. Underlying molecular mechanism of TNF-α in COPD. The expression of proteins and downstream signaling molecules associated with MAPK pathway in rats of each group were detected by western blotting. TNF, tumor necrosis factor; COPD, chronic obstructive pulmonary disease; sh-, short interfering RNA; NC, negative control.

decreased by sh-SOCS3 and sh-TRAF1 (Fig. 4K-L), and the number of monocytes had no significant change (Fig. 4M). In addition, the decline of MPO in COPD rats caused by knocking down TNF-α was reversed by transfecting with sh-SOCS3/TRAF (Fig. 4N). It was observed through the histopathology that knockdown of SOCS3 and TRAF1 could aggravate the damage of alveoli and airway structure and the inflammatory infiltration around alveoli (Fig. 4O). All results showed that the condition of COPD would be aggravated by knocking down SOCS3 and TRAF1, and TNF-α knockdown could delay the progression of COPD through up-regulating the expression of SOCS3 and TRAF1.

Discussion

The present study found that CS + LPS exposure caused COPD in rats, and the symptoms include reduced lung function, increased inflammatory cells and the structure of alveoli and airway was disordered; but these symptoms could be relieved by TNF-α knockdown. Furthermore, TNF-α knockdown could delay the progression of COPD through regulating the activity of the MAPK pathway and the expression of SOCS3 and TRAF1.

COPD is an incurable but preventable lung disease characterized by persistent respiratory symptoms and airflow restriction caused by bronchitis, emphysema and apoptosis of alveolar epithelial cells (22-24). The diagnosis of COPD requires vital capacity measurement (FEV1/FVC), which is at risk of over-diagnosis in older people or under-diagnosis in younger people (25). The treatment of COPD can be divided into conservative treatment and surgical treatment: The conservative treatment mainly depends on bronchodilator, anti-inflammatory agents and long-term oxygen therapy with a long cycle and slow effect; the surgical treatment includes lung volume reduction and lung transplant with high risk and high cost (25). Therefore, we urgently need a new diagnosis and treatment methods of COPD.

Overexpression of TNF-α in the lung can cause oxidative stress and neutrophil inflammatory infiltration (4). Yao *et al* (26) indicated that the expression of TNF-α is closely associated with the occurrence and development of COPD, suggesting that TNF-α may be a potential biomarker of lung function and inflammation in patients with COPD. TNF-α-238 G/A is associated with airway remodeling, and its combination with TGF-β1 can aggravate the severity of airflow limitation in patients with COPD (27). However, the study on the molecular mechanism of TNF-α in COPD was is insufficient, and so the present study focused on the molecular mechanism. A COPD model was successfully constructed, and found that TNF-α knockdown could improve the decrease in lung function and inflammatory damage of lung structure.

To explore the specific molecular mechanism of TNF-α in COPD, the KEGG database was used to identify TNF signaling pathway, and the MAPK signaling pathway, as well as downstream signaling molecules (TRAF1 and SOCS3), were chosen to investigate the underlying molecular mechanism. MAPKs pathways include p38-MAPK, ERK1/2, JNK1/2, and MAPKs are the most common inflammatory signaling pathway of TNF-α to regulate diseases (28). For example, Chen *et al* (29) found that the inhibitor of p38-MAPK can significantly alleviate the apoptosis induced by TNF-α. Studies also demonstrate that MAPK inhibitors have great potential in the treatment of chronic inflammatory diseases (30,31). For example, inhibition of p38-MAPK can decrease the expression level of cytokines in the lung and blood cells of COPD, suggesting that MAPK inhibitors play a role in local and systemic inflammation (32). In agreement with previous reports, the present study also found that the activation of MAPK pathway could make the pathological manifestations of COPD more serious, whilst TNF-α knockdown retarded the process of COPD.

The existing literature shows that the expression of SOCS3 is positively correlated with TNF-α, and that SOCS3 promotes apoptosis (33,34). However, other studies have found that SOCS3 were transiently induced by TNF-α but inhibited over time, therefore, it is speculated that the activation of TNF pathway is inhibited by the combination of SOCS3 and TRAF1, thus reverse inhibiting the expression of SOCS3 (35,36). Furthermore, Mori *et al* (37) found that TNF-α can activate the IL-6/STAS3 signaling pathway, and Huang *et al* (38) observed that the IL-6/STAT3 pathway can negatively regulate the expression of SOCS3. In the present study, it was found that the expression of SOCS3 was increased after TNF-α knockdown. And Springer *et al* (39) also found that SOCS3 has a low expression in COPD. Combined with previous research literature, it was speculated that IL-6/STAT3 pathway was inhibited by TNF-α knockdown and lost the inhibition for SOCS3, thus increasing the expression of COCS3. Another possibility is that SOCS3 reversely inhibits its own expression by combining with TRAF1 to inhibit the activation of MAPK. These hypotheses need to be confirmed with experiments, and thus are the future research directions. Additionally, the present study also indicated that the expression of TRAF1 was increased after TNF-α knockdown. Furthermore, the literature shows that TRAF1 is a negative regulator of TNF signaling pathway (40). A study found that TRAF1 can inhibit NF-κB and JNK 1/2 pathway, and the apoptotic induced by TNF-α can be enhanced

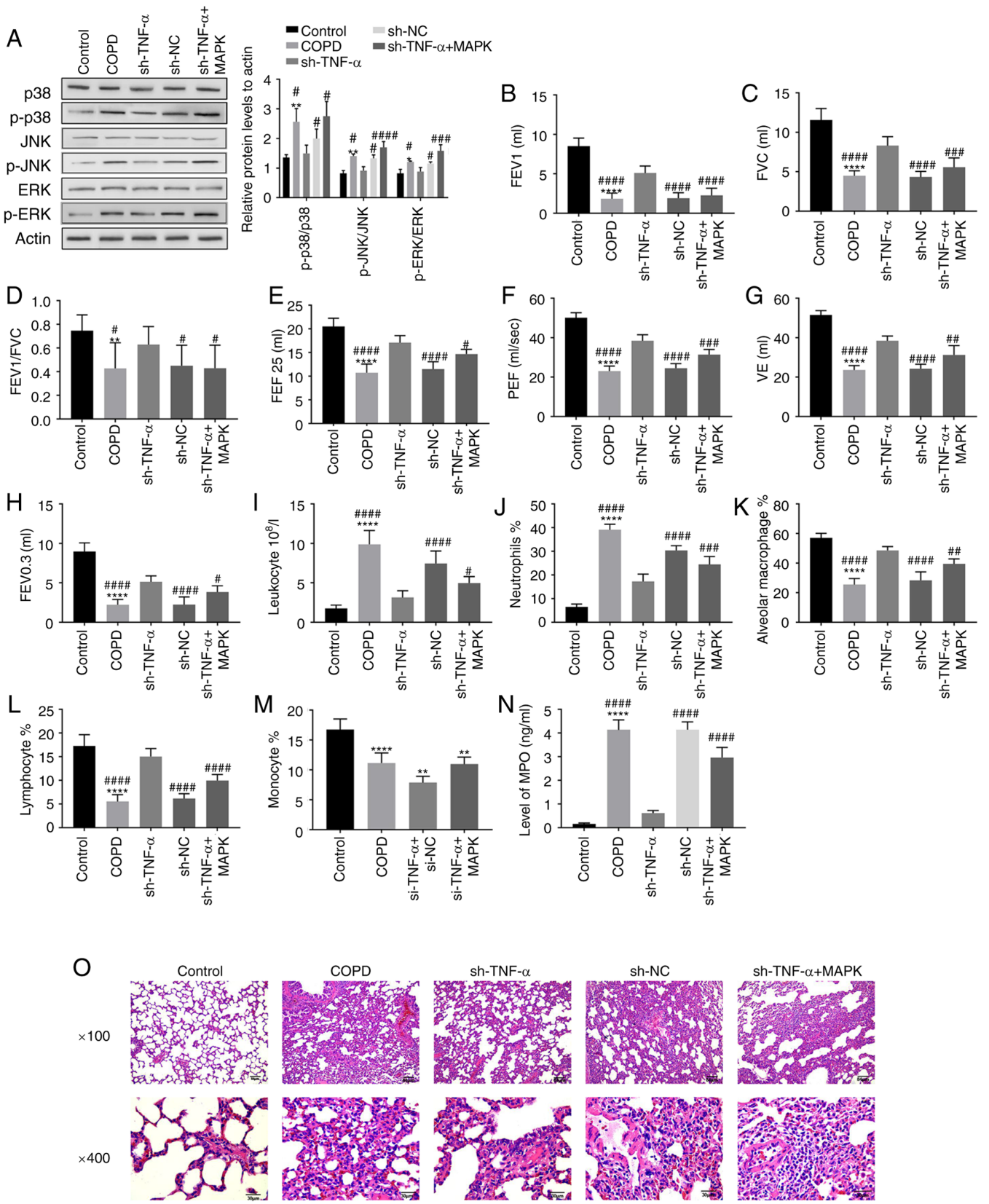

Figure 3. Effect of MAPK pathway on inflammatory response and lung structure in rats with COPD. The rats were treated with the activator of MAPK pathway. (A) The expression of proteins associated with the MAPK pathway in rats with various treatments was detected by western blotting. (B-H) The changes in pulmonary function such as (B) FEV1, (C) FVC, (D) FEV1/FVC, (E) FEF 25, (F) PEF, (G) VE and (H) FEV 0.3 of rats were detected by using a plethysmograph after adding the activator of MAPK pathway. (I-M) The number of pulmonary inflammatory cells, such as (I) leukocyte, (J) neutrophils, (K) alveolar macrophage, (L) lymphocyte and (M) monocyte in the lungs of rats was measured by classifying and counting cells of BALF. (N) In addition, the level of MPO in BALF was detected by enzyme-linked immunosorbent assay. (O) The lung tissue of rats was sectioned and stained with hematoxylin and eosin to observe the changes in alveoli and airway of rats after the activator of MAPK pathway was added. *P<0.05, **P<0.01, ****P<0.0001, vs. Control group; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, $^{\#\#\#\#}$P<0.0001, vs. sh-TNF-α group/sh-RNA group. COPD, chronic obstructive pulmonary disease; FEV, forced expiratory volume; FVC, forced vital capacity; FEF, forced expiratory flow; PEF, peak expiratory flow; VE, minute ventilation at rest; BALF, bronchoalveolar lavage fluid; p-phosphorylated; sh-, short interfering RNA; NC, negative control.

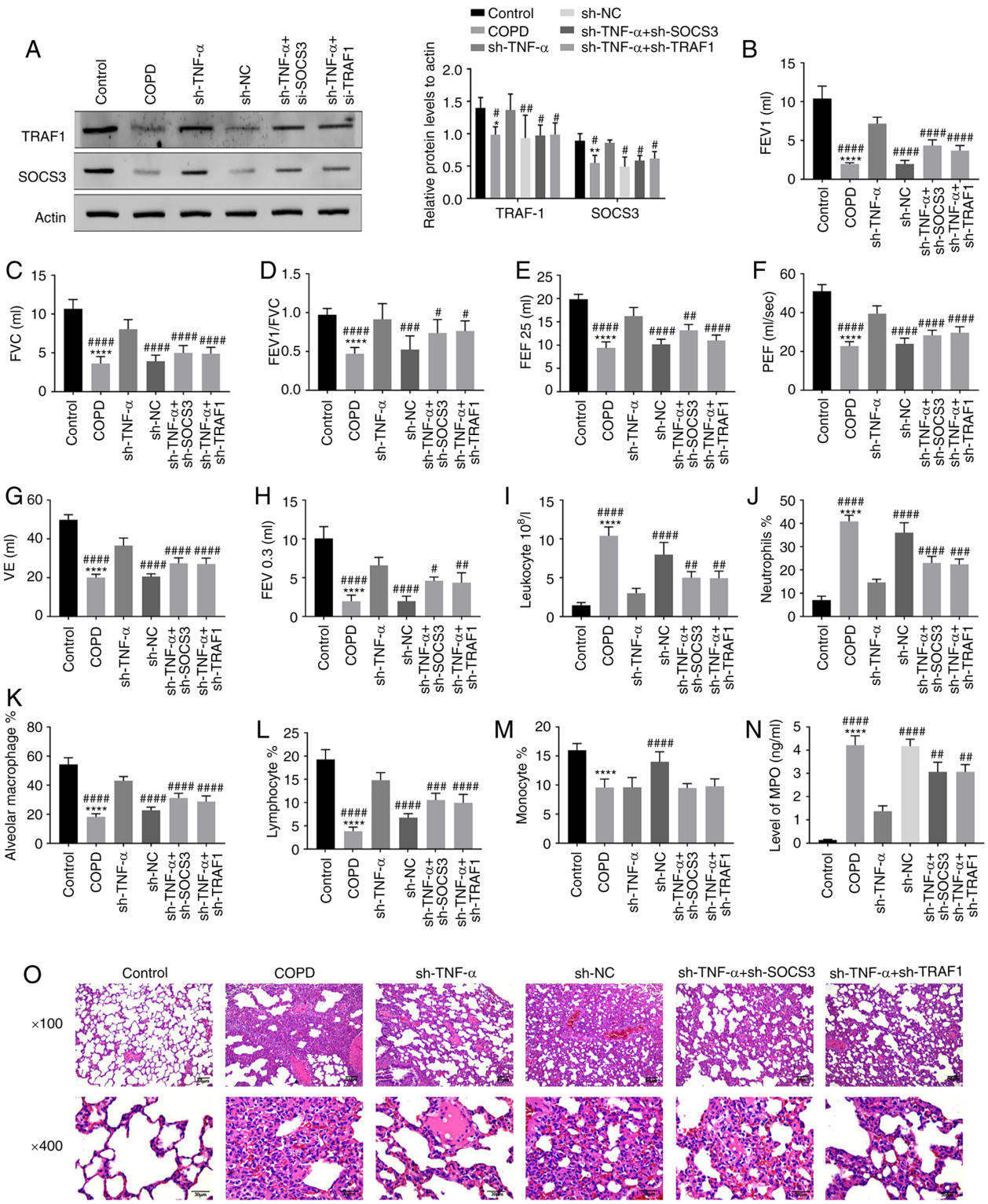

Figure 4. Effect of SOCS3 and TRAF1 on inflammatory response and lung structure in rats with COPD. The rats were transfected with sh-SOCS3 and sh-TRAF1. (A) The transfection efficiency of shRNA and the expression of SOCS3/TRAF1 in rats were detected by western blotting. (B-G) The pulmonary function of the rats, containing (B) FEV1, (C) FVC, (D) FEV1/FVC, (E) FEF 25%, (F) PEF, (G) VE and (H) FEV0.3, were detected with a plethysmograph. (I-M) The number of inflammatory cells such as (I) leukocyte, (J) neutrophils, (K) alveolar macrophage, (L) lymphocyte and (M) monocyte in the lung of rats was measured by collecting the BALF and counting the cells. (N) The level of MPO in BALF was detected by enzyme-linked immunosorbent assay. (O) The lung tissue of rats was sectioned and stained with hematoxylin and eosin to observe for changes in alveoli and airway after SOCS3 and TRAF1 were knocked down. *P<0.05, **P<0.01, ****P<0.0001, vs. Control group; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, $^{\#\#\#\#}$P<0.0001, vs. sh-TNF-α group/sh-RNA group. COPD, chronic obstructive pulmonary disease; FEV, forced expiratory volume; FVC, forced vital capacity; FEF, forced expiratory flow; PEF, peak expiratory flow; VE, minute ventilation at rest; BALF, bronchoalveolar lavage fluid; sh-, short interfering RNA; NC, negative control.

by knocking down TRAF1 (41). Therefore, it was postulated that TRAF1, as a reverse regulator of TNF signaling pathway, can reverse regulate its own expression by inhibiting the activation of MAPK pathway; this will be explored in a follow-up study.

In conclusion, the present results indicate that TNF-α knockdown could delay the procession of COPD. The molecular mechanism was that TNF-α knockdown inhibited the activating of MAPK pathway to increase the expression of downstream molecules SOCS3/TRAF1, thus alleviating the inflammation and structural damage of alveoli and airways. Furthermore, degenerative feedback regulation mechanism of SOCS3 and TRAF1 in TNF signaling pathway of COPD is the next research topic.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

QF, YZY and QHM designed the experiments; YZY and QHM carried out experiments; QF and QHM analyzed experimental results. QF and YZY wrote the manuscript; QHM approved the manuscript. All authors read and approved the final manuscript and confirmed the authenticity of all the raw data.

Ethics approval and consent to participate

All animal experiments were approved by the Experimental Animal Welfare Ethics Review Committee of the Affiliated Hospital of Jianghan University (grant no. AW2019091203) and adhered to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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