Original Article

Knockdown of Slfn5 alleviates lipopolysaccharide-induced pneumonia by regulating Janus kinase/signal transduction and activator of transcription pathway

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Background: In recent years, the incidence of pneumonia has been increasing, which is the main cause of death and morbidity of children and the elderly in the world. Slfn5 is implicated in multiple cancers, and Slfn5 promotes epithelial-mesenchymal transition and metastasis in lung cancer. However, the influences of Slfn5 in pneumonia have not yet been completely cleared. Herein, we aimed to explore the underlying effects and regulatory mechanisms of Slfn5 in lipopolysaccharide (LPS)-induced pneumonia in mice and A549 cells.

Methods: Mice were intratracheally administered 5 mg/kg LPS to construct pneumonia model. *In vitro*, A549 cells were treated with 10 μg/mL LPS to construct cellular pneumonia model. Slfn5 expression was detected using immunohistochemistry and western blotting. Haematoxylin and eosin staining, TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end-labelling), and western blotting were performed to assess pathological injury and inflammation. MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide], flow cytometry, and enzyme-linked immunosorbent assay analysis were performed to analyze cell viability, apoptosis, and inflammation. Gene set enrichment analysis was performed to explore the mechanism of Slfn5 in pneumonia.

Results: Slfn5 expression was upregulated in LPS-induced pneumonia in mice and A549 cells. In mice, knockdown of Slfn5 weakened LPS-induced lung injury and inflammation and decreased the expression of p-JAK2, p-JAK3, and p-STAT3. In LPS-stimulated A549 cells, downregulation of Slfn5 expression increased and Slfn5 overexpression decreased cell viability. Downregulation of Slfn5 expression decreased and Slfn5 overexpression increased cell apoptosis, inflammation and the expression of p-JAK2, p-JAK3, and p-STAT3. AG490, an inhibitor of the JAK/STAT pathway, reversed the damaging effects of Slfn5 overexpression.

Conclusions: In the LPS-induced pneumonia model, Slfn5 knockdown alleviated LPS-induced lung injury by regulating the JAK/STAT pathway.

Keywords: Pneumonia; Slfn5; JAK/STAT pathway; apoptosis; inflammatory injury

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Introduction

The incidence of pneumonia, a respiratory disease, has increased in recent years (1). Based on the investigation of disease burden, about 14% of children worldwide die of lower respiratory infections (2). Clinically, pneumonia mainly manifests as an elevated body temperature, dyspnoea, hypoxaemia, chills, chest pain, coughing, and other severe comorbidities (3-5). Various pathogens (such as bacteria, fungi, and viruses) can cause pneumonia (6,7). Currently, the primary diagnostic methods for pneumonia are through radiological technology and with multiple laboratory methods (8). The use of oxygen, assisted ventilation, antibiotics, and other supportive therapies is the primary treatment strategy for pneumonia (9,10). Owing to the complexity of the pathogenesis of pneumonia, therapeutic effects are not satisfactory. Therefore, exploring the pathogenesis and effective therapeutic targets is of paramount importance for improving the prognosis of pneumonia.

The family of schlafen (Slfn) genes is originally identified in mice, they are preferentially expressed in lymphoid tissue (11). During thymocyte maturation and T cell activation, the genes of Slfn family are differentially regulated. Moreover, in fibroblasts and thymoma cells, cell growth is inhibited by the expression of Slfn1. Recent studies have indicated that Slfn family members participate in primary cellular processes, such as proliferation, viral replication, differentiation, immune response, and growth regulation (12-14). Slfn5 is expressed in normal melanocytes, renal cells, and ovary cells (13). Abnormal Slfn5 expression affects the progression of renal cell carcinoma, gastric cancer, melanoma, and glioblastoma (13,15-17). However, the function of Slfn5 has not yet been completely understood in pneumonia.

JAK/STAT pathway is related to diverse biological processes, such as cell growth, immune response, apoptosis, and inflammatory response (18,19). Various growth factors, ligands, and cytokines can trigger the JAK pathway (20). The tyrosine of STAT can be phosphorylated by activated JAK, and the phosphorylated STAT transfers to the nucleus and binds to specific promotor, resulting in target mRNA expression (21). At present, an increasing amount of evidence has confirmed that JAK/STAT pathway is involved in lipopolysaccharide (LPS)-induced lung injury in mouse (22-24).

LPS is produced by gram-negative bacteria and is essential for inflammatory reactions related to pneumonia (25-27). TLR4 is a key receptor for LPS induction (28). The activated TLR4 can activate myeloid differentiation factor 88, extracellular signal-related kinases 1 and 2, JAK2, and interleukin-1 receptor-associated kinase 1, further promoting the nuclear translocation of nuclear factor kappa B and the phosphorylation of STAT3, consequently, upregulating the levels of inflammation-related factors, such as tumor necrosis factor (TNF) and interleukins (IL-6, IL-8, IL-1β) (29-33). Excessive accumulation of inflammatory cytokines can cause the dysfunction of immune system, thereby damage to multiple tissues, including lung tissue (34). Here, *in vivo* and *in vitro*, we first used LPS to establish a pneumonia model, and then investigated the role and underlying mechanism of Slfn5 in pneumonia. We present this article in accordance with the ARRIVE reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-889/rc).

Highlight box

Key findings

- Slfn5 expression was increased in lipopolysaccharide (LPS)-induced pneumonia model. Knockdown of Slfn5 mitigated LPS-induced mice lung injury and inflammation. Overexpression of Slfn5 enhanced apoptosis and inflammatory cytokines in LPS-treated A549 cells. Knockdown of Slfn5 weakened lung injury, apoptosis, and inflammatory response through the inhibition of JAK/STAT pathway.

What is known and what is new?

- Abnormal Slfn5 expression affects the progression of various cancers, including lung cancer.
- Here, *in vivo* and *in vitro*, we found that knockdown of Slfn5 inhibited cell apoptosis and the production of inflammatory cytokines in LPS-induced pneumonia model. Additionally, Slfn5 had a regulatory effect on the JAK/STAT pathway.

What is the implication, and what should change now?

- Our study provides new insights for the pathogenesis of pneumonia. Slfn5 may be a promising therapeutic target for pneumonia treatment.

Methods

Bioinformatics analysis

Slfn5 expression in rat bronchoalveolar lavage fluid (BALF) was analyzed through Gene Expression Omnibus database (GSE111241). Gene set enrichment analysis (GSEA) was

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used to analyze the pathways enriched by Slfn5.

Construction of LPS-induced pneumonia mouse model

The experimental protocol of our study was performed in accordance with the Guide for the Care and Use of Laboratory Animals and was approved by the Jinan City People's Hospital (No. 2022-0621). GemPharmatech Co., Ltd (Nanjing, China) provided C57BL/6 mice (10-week-old, 25–30 g). All mice had free access to food and water under a 12-h light-dark photocycle at constant temperature and humidity. Pentobarbital sodium (50 mg/kg; Sigma, St. Louis, MO, USA) was used to anaesthetise the mice. To construct pneumonia-infected lung injury, mice (LPS group, n=10) were intratracheally administered LPS (5 mg/kg) (Sigma). An equal volume of normal saline was administered to mice in the control group (n=10). One week before the construction of the pneumonia mouse model, in the LPS + sh-NC (n=10) and LPS + sh-Slfn5 group (n=10), mice were injected with adenoviral vectors containing short hairpin RNA-negative control (sh-NC, 1×10^9 pfu/100 µL) and shRNA of Slfn5 (sh-Slfn5, 1×10^9 pfu/100 µL) via the tail vein. After injection of LPS for three days, the mice were sacrificed and then the BALF and the lung tissues were harvested for later analysis.

Immunohistochemistry

The right lung of each mouse was fixed in 10% formalin. Next, the lung tissues were embedded in paraffin. After sectioning, the lung tissues were de-paraffinized with xylene and rehydrated with ethanol (75%, 80%, 90%, 95%, and 100%). To recover the antigen, lung tissue sections were incubated with Tris-EDTA buffer. Subsequently, sections were treated with 0.3% hydrogen peroxide and blocked with 10% goat serum. The sections were incubated with Slfn5 antibody and a horseradish peroxidase (HRP) -conjugated secondary antibody. After washing with phosphate-buffered saline (PBS), the sections were stained a DAB kit (Beyotime Biotechnology, Shanghai, China) and haematoxylin solution.

Lung wet-to-dry (W/D) ratio in mice

The wet weights of the left lungs were measured. After flushing with PBS, the lungs were incubated at 65 ℃ for 4 days in an oven to obtain dried specimens, and then the dry weight was measured. Finally, the W/D ratio of the mouse lungs was calculated.

Haematoxylin and eosin (H&E) staining

After dewaxing with xylene and treatment with ethanol, the lung tissue sections were stained with haematoxylin. The sections were then treated with 5% acetic acid and eosin. Next, the sections were dehydrated in ethanol (70%, 80%, 90%, and 100%), made transparent with xylene, and sealed with neutral gum. Pathological changes in the lung tissues were observed under a light microscope (Olympus, Tokyo, Japan).

TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end-labelling) assay

After dewaxing with xylene and treatment with ethanol, sections of lung tissues were treated with 20 µg/mL proteinase K (Beyotime Biotechnology) for 20 min, and then washed with PBS. The TUNEL Apoptosis Detection Kit (Roche, Basel, Switzerland) was used to treat lung tissue sections. The sections were stained with diaminobenzidine and counterstained with haematoxylin. Finally, TUNEL-positive cells were observed under a light microscope. Brownish-yellow cells were considered apoptotic (TUNEL positive cells).

BALF collection and analysis

The mice lungs were washed three times with cooled PBS to collect the BALF. After centrifugation at 1,500 rpm for 10 min at 4 ℃, the cell pellets from BALF samples were resuspended in PBS. Total cells, neutrophils, and macrophages were counted using a haemocytometer and WrightGiemsa staining (Beyotime Biotechnology).

Cell culture and treatment

Procell Life Science & Technology Co., Ltd. (Wuhan, China) provided the A549 cell line. A549 cells were incubated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) at 37 ℃ with 5% CO_2.

To establish LPS-injured cell model, 10 µg/mL LPS was used to treat cells for 12 h. The pcDNA-Slfn5 (200 ng) and sh-Slfn5 (2 µg) and their respective negative controls [pcDNA-NC (200 ng) and sh-NC (2 µg)] were transfected into the cells using Lipofectamine 2000 (Life Technologies, Frederick, MD, USA). After transfection for 72 h, the cells in the LPS + sh-NC, LPS + sh-Slfn5, LPS + pcDNA-NC, and LPS + pcDNA-Slfn5 groups were treated with LPS.

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Cells in the LPS + pcDNA-Slfn5 + AG490 group were treated with the JAK/STAT inhibitor (AG490) and LPS.

Analysis of western blot

Total protein was extracted from the tissues and cells using radioimmunoprecipitation assay lysis buffer (Beyotime Biotechnology). Next, proteins (30 μg) were separated using 10% SDS-PAGE and transferred to PVDF (polyvinylidene difluoride) membranes (Sangon Biotech, Shanghai, China). Membranes were blocked with 5% skimmed milk for 1 h, and then membranes were probed with primary antibodies (Slfn5, TNF-α, IL-1β, IL-6, p-JAK2, JAK2, p-JAK3, JAK3, p-STAT3, and STAT3) at 4 ℃ overnight. Subsequently, the membranes were probed with secondary antibodies. Finally, an enhanced chemiluminescence kit (Beyotime Biotechnology) was used to visualise the protein bands.

Detection of cell viability

Cell viability was detected using a MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-h-tetrazolium bromide] assay. After indicated treatment, A549 cells in each well were treated with 20 μL MTT solution (Sigma) for 3 h. Next, cells were treated with 150 μL dimethyl sulfoxide (DMSO) for 15 min. Finally, the optical density was measured at 490 nm using a microplate reader (Bio-Rad, Hercules, CA, USA) and then cell viability was calculated.

Evaluation of cell apoptosis

Apoptosis was evaluated using the Annexin V-FITC Apoptosis Kit (Beyotime Biotechnology). Briefly, after the indicated treatments, A549 cells were resuspended in 1 × binding buffer. Subsequently, the cells were incubated with Annexin V-FITC and PI. Apoptotic cells were analyzed using flow cytometry (BD Biosciences, San Jose, CA, USA).

Enzyme-linked immunosorbent assay (ELISA)

After the indicated treatment, the levels of IL-6, IL-1β, and TNF-α were measured in A549 cell culture supernatant using ELISA kits (Beyotime Biotechnology) following the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader.

Statistical analysis

Data from three independent experiments were presented as the mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism 7 and SPSS software. Student's *t*-test and one-way analysis of variance (ANOVA) with Tukey's test were used to compare significance. P value <0.05 indicated statistical significance.

Results

Slfn5 was increased in LPS-induced pneumonia in mice

GSE111241 database analyzes the abnormal expression genes in BALF of control (rats with normoxia) and LPS group (rats with LPS treatment with normoxia). An increase in Slfn5 expression was observed in LPS-treated rats (*Figure 1A*, P=0.0043). Next, we established a mouse model of pneumonia by treating with LPS. As shown in *Figure 1B,1C*, Slfn5 expression in the lung tissues was enhanced compared to that in the control group.

LPS-induced mouse lung injury and inflammation were mitigated by knockdown of Slfn5

To further explore the regulatory role of Slfn5 in pneumonia, sh-Slfn5 and sh-NC were used to treat the mouse pneumonia model. As shown in *Figure 2A*, sh-Slfn5 decreased Slfn5 expression in the mouse lung tissues compared to that in the LPS + sh-NC group. Compared to the control group, LPS stimulation increased the lung W/D ratio, whereas knockdown of Slfn5 eliminated this increase (*Figure 2B*). H&E staining showed that LPS led to inflammatory cell infiltration, alveolar haemorrhage, and structural damage to the lung tissue, but knockdown of Slfn5 alleviated these LPS-induced changes (*Figure 2C*). TUNEL staining revealed a higher apoptosis ratio in LPS-treated mouse lung tissues; however, the apoptosis ratio was reduced by Slfn5 knockdown (*Figure 2D*). Next, we collected BALF and analyzed the number of total cells, macrophages, and neutrophils. LPS increased the number of total cells, neutrophils, and macrophages, whereas Slfn5 knockdown inhibited aggregation (*Figure 2E-2G*). Moreover, in LPS-treated lung tissues, knockdown of Slfn5 reduced the production of TNF-α, IL-1β, and IL-6 (*Figure 2H*).

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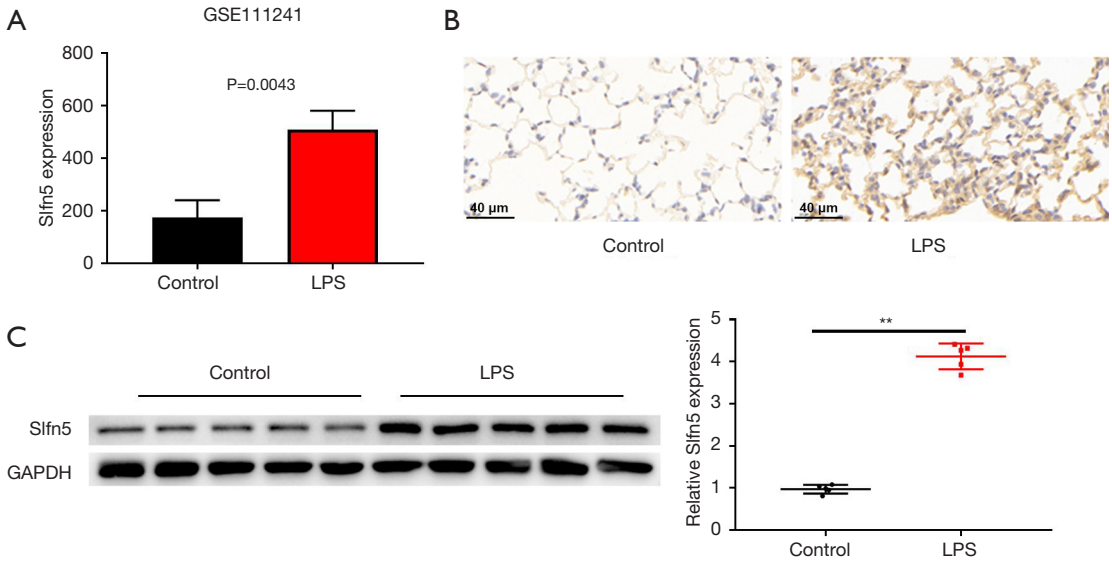

Figure 1 Slfn5 was increased in LPS-induced pneumonia in mice. (A) GSE111241 database showed an increase of Slfn5 in BALF of LPS-treated rats. Mice were intratracheally administered 5 mg/kg LPS to construct pneumonia model. Immunohistochemistry (B) and western blot (C) were used to assess Slfn5 expression in mice lung tissues (n=5). The data was from three independent experiments. Compared to control group, **, P<0.01. LPS, lipopolysaccharide; GSE, gene expression omnibus series; BALF, bronchoalveolar lavage fluid.

Knockdown of Slfn5 inhibited the JAK/STAT pathway in LPS-induced pneumonia in mice

To further explore the mechanism of Slfn5 in pneumonia, we performed GSEA and found that Slfn5 activated the JAK/STAT pathway (*Figure 3A*). We then assessed the changes in JAK/STAT pathway-related proteins. As shown in *Figure 3B*, LPS increased p-JAK2, p-JAK3, and p-STAT3 expression compared with the control group (P<0.01), whereas knockdown of Slfn5 decreased p-JAK2, p-JAK3, and p-STAT3 expression compared with the LPS + sh-NC group (P<0.01).

Overexpression of Slfn5 enhanced apoptosis and inflammatory factors in LPS-treated A549 cells

A549 cell is a human alveolar epithelial type II cell line. For various studies associated with respiratory diseases including pneumonia, A549 cells treated with LPS have been used as a cellular model (35-37). To investigate the role of Slfn5 in LPS-induced injury, sh-Slfn5 was transfected into A549 cells to construct low-expressed cells (*Figure 4A*). The MTT assay results showed that LPS reduced cell viability, whereas downregulation of Slfn5 expression increased cell viability in LPS-treated A549 cells (*Figure 4B*). In addition, the results of flow cytometry and ELISA showed an increased cell apoptosis and the levels of TNF-α, IL-1β, and IL-6 in LPS-treated cells, whereas downregulation of Slfn5 expression decreased cell apoptosis and the levels of TNF-α, IL-1β, and IL-6 compared to those in the LPS + sh-NC group (*Figure 4C-4F*). What's more, pcDNA-Slfn5 was transfected into A549 cells to construct over-expressed cells (*Figure 4G*). As shown in *Figure 4H-4L*, compared to the LPS + pcDNA-NC group, overexpression of Slfn5 decreased cell viability and increased cell apoptosis and the levels of TNF-α, IL-1β, and IL-6.

Knockdown of Slfn5 inhibited the JAK/STAT pathway in LPS-treated A549 cells

Changes in JAK/STAT pathway-related proteins were examined in LPS-treated A549 cells. The expression levels of p-JAK2, p-JAK3, and p-STAT3 were enhanced in LPS-stimulated cells (*Figure 5A,5B*). Compared with the LPS group, Slfn5 downregulation reduced p-JAK2, p-JAK3, and p-STAT3 expression (*Figure 5A*), whereas Slfn5 overexpression further enhanced p-JAK2, p-JAK3, and

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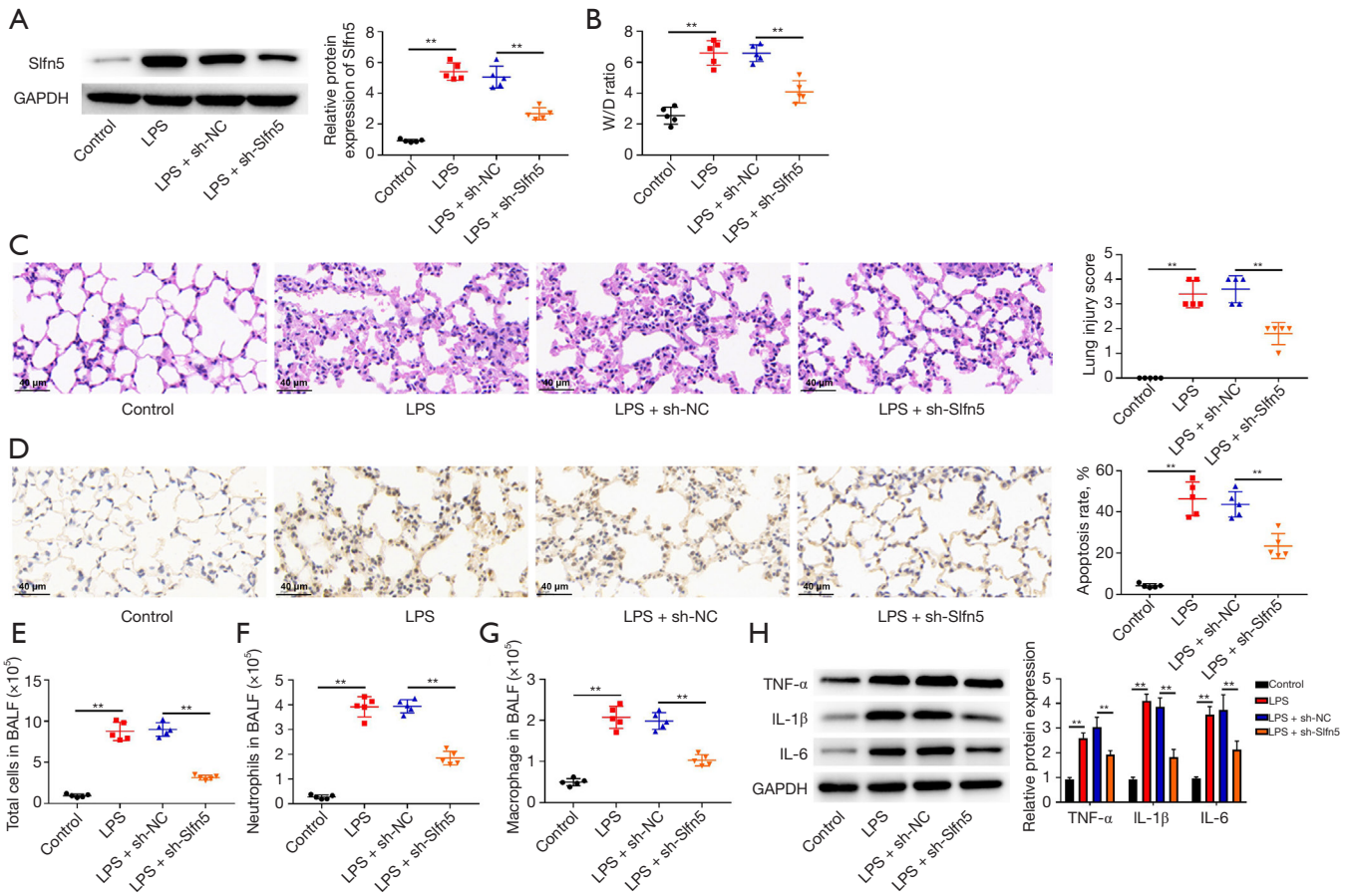

Figure 2 LPS-induced mouse lung injury and inflammation were mitigated by knockdown of Slfn5. To construct pneumonia-infected lung injury, mice were intratracheally administered 5 mg/kg LPS. One week before the construction of the pneumonia mouse model, in the LPS + sh-NC and LPS + sh-Slfn5 group, mice were injected with adenoviral vectors containing sh-NC and sh-Slfn5 via the tail vein. (A) In mice lung tissues, Slfn5 expression was analyzed using western blot. (B) Lung W/D ratio of mice was calculated. (C) Histopathological changes of mice lung tissues were analyzed by H&E staining. (D) In mice lung tissues, TUNEL staining was taken to evaluate cell apoptosis. In BALF of mice, the number of total cells (E), neutrophils (F), and macrophages (G) was calculated. (H) In mice lung tissues, TNF-α, IL-1β, and IL-6 expression was assessed by western blot (n=5). The data was from three independent experiments. Compared to control or LPS + sh-NC groups, **, P<0.01. LPS, lipopolysaccharide; sh, short hairpin; NC, negative control; W/D, wet-to-dry; H&E, haematoxylin and eosin; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end-labelling; BALF, bronchoalveolar lavage fluid.

p-STAT3 expression (*Figure 5B*).

Slfn5 regulated apoptosis and inflammatory damage through the JAK/STAT pathway in LPS-treated A549 cells

To further verify whether Slfn5 regulates apoptosis and inflammatory damage through the JAK/STAT pathway, AG490 (an inhibitor of the JAK/STAT pathway) was used to treat LPS-stimulated A549 cells. As expected, compared to the LPS + pcDNA-Slfn5 group, AG490 inhibited p-JAK2, p-JAK3, and p-STAT3 expression in LPS-stimulated A549 cells (*Figure 6A*). Moreover, in LPS-stimulated A549 cells, AG490 mitigated the influences of Slfn5 overexpression on cell viability, apoptosis, and the production of TNF-α, IL-1β, and IL-6 (*Figure 6B-6F*).

Discussion

As one of the lower respiratory tract illnesses, pneumonia is

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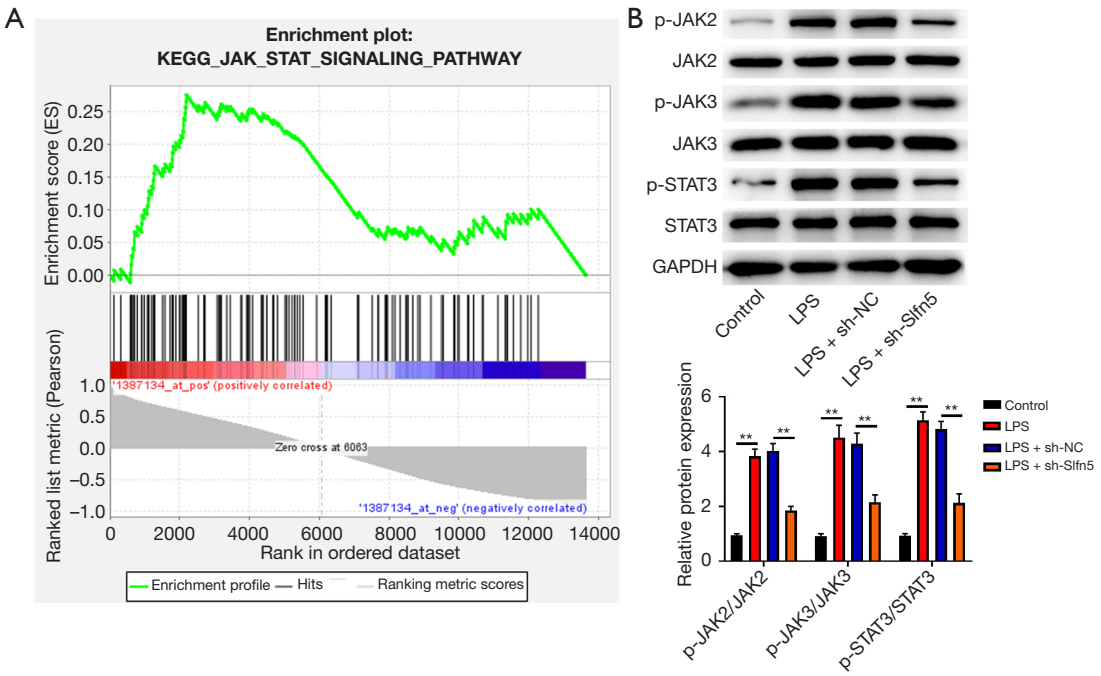

Figure 3 Knockdown of Slfn5 inhibited the JAK/STAT pathway in LPS-induced pneumonia in mice. (A) GSEA analysis (permutation = geneset, metric = Diff_of_classes, metric = weighted, #permutation = 2500) showed that Slfn5 activated JAK/STAT signaling pathway. (B) In mice lung tissues, the change of JAK/STAT pathway-related proteins was determined using western blot (n=5). The data was from three independent experiments. Compared to control or LPS + sh-NC groups, **, P<0.01. LPS, lipopolysaccharide; GSEA, gene set enrichment analysis; NC, negative control.

a common fatal infection in hospital (7,38). The pathogenesis of pneumonia is complex, and it is often accompanied by an immune response and inflammation, thus seriously damaging the function of the lung tissues (39). Severe pneumonia weakens the immune system, eventually leading to damage to the respiratory system, multiple organ failure, and even shock (40). Slfn5 was reported to be upregulated in non/low-invasive cancer cell lines (MCF7, HCT116, and A549 cells) compared with highly invasive cancer cell lines (HT1080 cells and 786-0 cells), and knockdown of Slfn5 increased the migration and invasion of MCF7, HCT116, and A549 cells (41). A previous study reported that Slfn5 expression was lower in lung adenocarcinoma (LUAC) tissues, and that overexpression of Slfn5 inhibited LUAC cell proliferation and promoted LUAC cell apoptosis (42). Here, in a LPS-induced pneumonia model, we found that Slfn5 expression was upregulated. Thus, we speculate that Slfn5 is involved in the development of pneumonia.

The most direct consequence of pneumonia is lung injury, characterized by the production of inflammatory cytokines and inflammatory damage to the alveolar capillary membrane (43,44). Animal experiments have shown that acute inflammatory reactions can be stimulated by LPS, with early histopathological changes observed in the lung tissues (45,46). Here, we injected LPS into mice to induce pulmonary inflammation and found that Slfn5 knockdown eliminated the influence of LPS on the lung W/D ratio, histopathological changes, apoptosis, and the number of total cells, macrophages, and neutrophils. Cytokine accumulation affects pneumonia progression. The release of TNF-α was reported to promote cell apoptosis and tissue necrosis (47). As a pro-inflammatory cytokine, IL-1β increases the permeability of pulmonary epithelium and neutrophils accumulation, causing lung oedema (48). In the plasma of patients with pneumonia, IL-6 levels are increased; high IL-6 levels in circulating leukocytes increase incidence of secondary infection in patients with pneumonia (49). In our study, in LPS-treated lung tissues, knockdown of Slfn5 reduced TNF-α, IL-1β, and IL-6 levels. Moreover, *in vitro*, downregulation of Slfn5 increased cell viability and decreased cell apoptosis and TNF-α, IL-1β, and IL-6 levels, whereas overexpression of Slfn5 led

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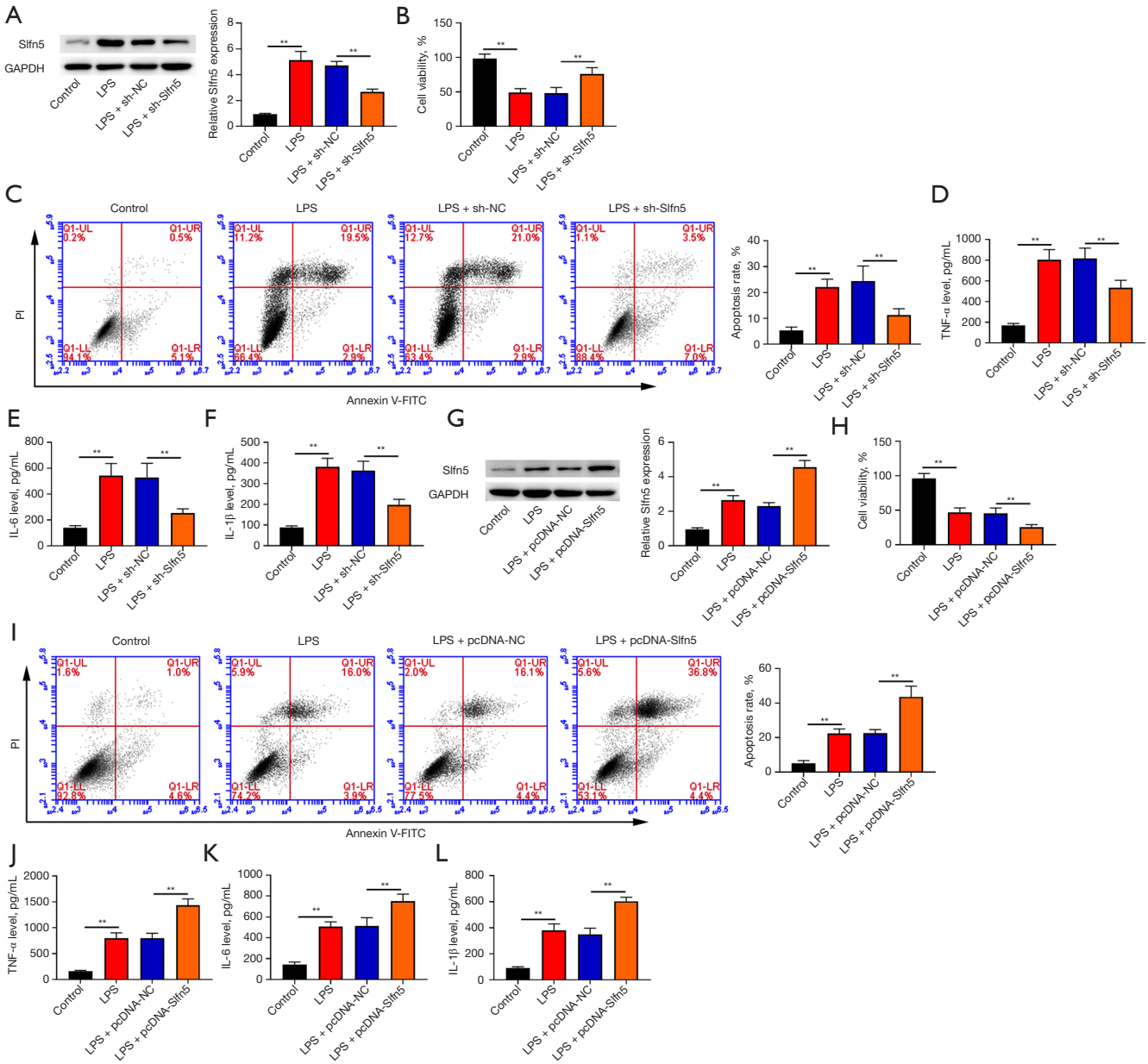

Figure 4 Overexpression of Slfn5 enhanced apoptosis and inflammatory factor in LPS-treated A549 cells. A549 cells were transfected with sh-NC, sh-Slfn5, pcDNA-NC, or pcDNA-Slfn5 followed by LPS stimulation. (A,G) In A549 cells, western blot was performed to measure Slfn5 expression. (B,H) A549 cell viability was assessed by MTT assay. (C,I) A549 cell apoptosis was assessed by flow cytometry. (D-F,J-L) In A549 cells, the levels of TNF-α, IL-1β, and IL-6 were assessed using ELISA assay. The data was from three independent experiments. Compared to control, LPS + sh-NC, or LPS + pcDNA-NC groups, **, P<0.01. LPS, lipopolysaccharide; NC, negative control; ELISA, enzyme-linked immunosorbent assay.

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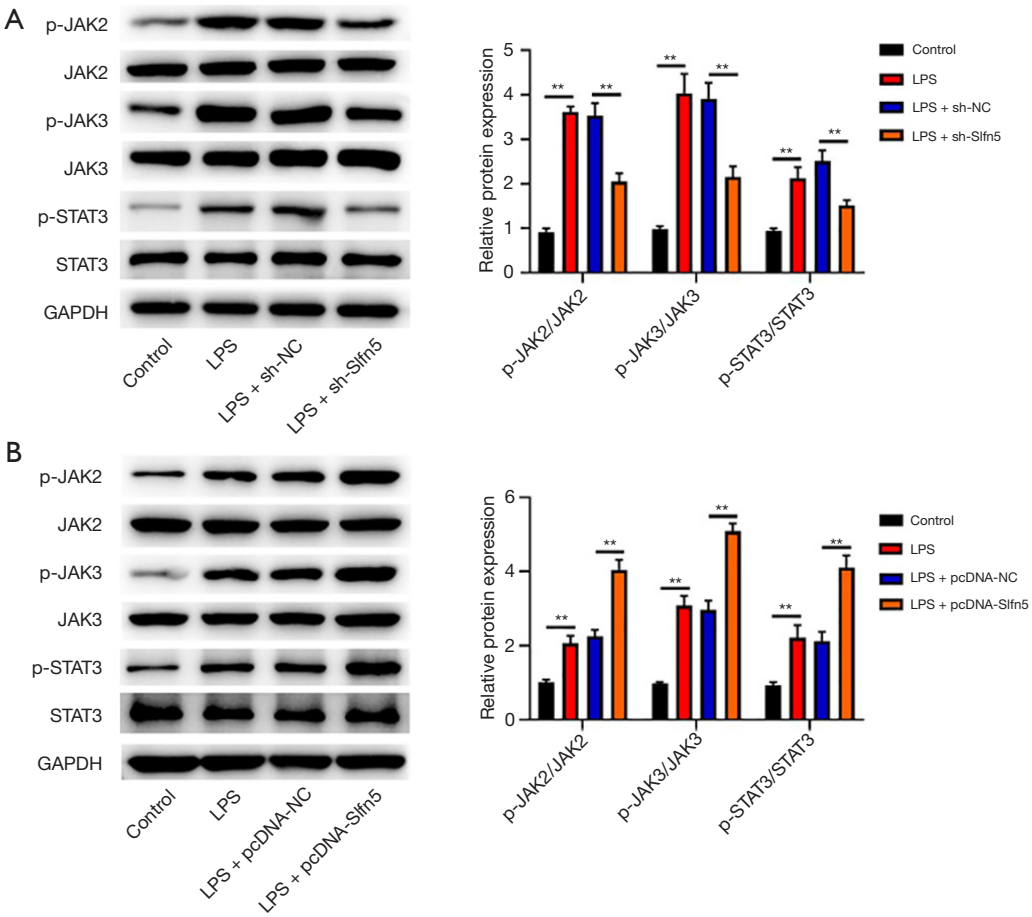

Figure 5 Knockdown of Slfn5 inhibited the JAK/STAT pathway in LPS-treated A549 cells. A549 cells were transfected with sh-NC, sh-Slfn5, pcDNA-NC, or pcDNA-Slfn5 followed by LPS stimulation. (A,B) In A549 cells, the change of JAK/STAT pathway-related proteins was analyzed using western blot. The data was from three independent experiments. Compared to control, LPS + sh-NC, or LPS + pcDNA-NC groups, **, P<0.01. LPS, lipopolysaccharide; NC, negative control.

to the opposite results. Taken together, Slfn5 knockdown mediated the improvement of LPS-induced pneumonia.

One of the classic inflammation-related pathways, the JAK/STAT pathway participates in cytokine-mediated biological response (50). A previous study has reported that JAKs affects cytokine receptors and are involved in intracellular responses (51). STAT3 activation plays an important role in many oncogenic signaling pathways and promotes inflammatory cytokine production (52). Zhu-Ge *et al.* found that LPS exposure activated the JAK/STAT pathway, and knockdown of CRNDE alleviated inflammatory injury and reduced the activity of the JAK/STAT pathway (53). Moreover, knockdown of lncRNA SNHG16 attenuated LPS-induced WI-38 cell injury, which was related to the inhibition of the JNK pathways (54). In the present study, *in vivo* and *in vitro*, we observed that LPS increased p-JAK2, p-JAK3, and p-STAT3 expression, whereas Slfn5 knockdown decreased p-JAK2, p-JAK3, and p-STAT3 expression. Moreover, in LPS-stimulated A549 cells, AG490, an inhibitor of the JAK/STAT pathway, mitigated the influence of Slfn5 overexpression on cell viability, apoptosis, and the production of TNF-α, IL-1β, and IL-6. These results illustrate that Slfn5 regulates apoptosis and inflammatory damage via the JAK/STAT pathway in the LPS-induced pneumonia model. Although A549 cells are widely used for *in vitro* pneumonia research, a human normal non-cancerous lung cell line should be used to validate the results of this study.

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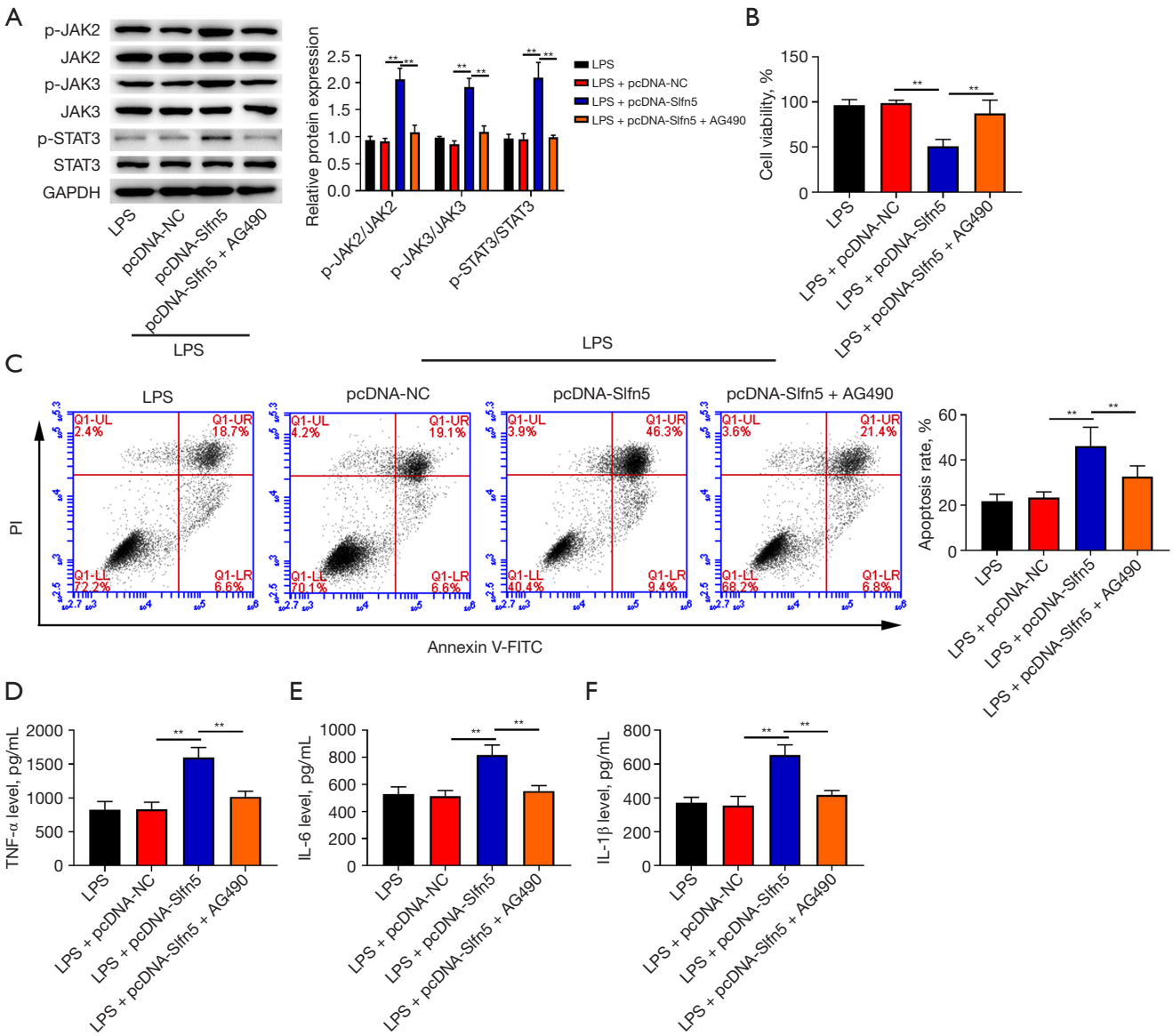

Figure 6 Slfn5 regulated apoptosis and inflammatory damage through the JAK/STAT pathway in LPS-treated A549 cells. A549 cells were transfected with pcDNA-NC or pcDNA-Slfn5 followed by AG490 and LPS stimulation. (A) In A549 cells, the change of JAK/STAT pathway-related proteins was assessed via western blot. (B) A549 cell viability was assessed by MTT assay. (C) Flow cytometry was utilized to assess A549 cell apoptosis. (D-F) In A549 cells, ELISA assay was utilized to assess TNF-α, IL-1β, and IL-6 levels. The data was from three independent experiments. Compared to LPS + pcDNA-Slfn5, LPS + pcDNA-NC groups, **, P<0.01. LPS, lipopolysaccharide; NC, negative control; ELISA, enzyme-linked immunosorbent assay.

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Conclusions

Collectively, our results indicated that Slfn5 expression was upregulated in the LPS-induced pneumonia model. Knockdown of Slfn5 reduced lung injury, apoptosis, and inflammatory response, which might be related to the inhibition of the JAK/STAT pathway. Our study identified a promising therapeutic target for the treatment of pneumonia.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-889/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-889/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-889/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The experimental protocol of our study was performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Jinan City People's Hospital (2022-0621).

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