Downregulation of miR-409-3p suppresses LPS-induced inflammation in human bronchial epithelial cells through SOCS3/JAK1/STAT3 signaling: The implication for bronchopneumonia

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Abstract. In recent decades, the role of microRNAs (miRs) in the development of pneumonia has been reported by a number of researchers. The present study aimed to investigate the role of miR-409-3p in lipopolysaccharide (LPS)-induced human bronchial epithelial cells and the implication for bronchopneumonia. An *in vitro* inflammation model was established using LPS-induced BEAS-2B cells. Cell apoptosis was determined by flow cytometry. Inflammatory factors were detected by ELISA and reverse transcription-quantitative PCR. Protein levels of Janus kinase 1 (JAK1)/STAT3 and suppressor of cytokine signaling (SOCS)3 were determined by western blotting. Dual-luciferase reporter assay was performed to confirm the interaction between miR-409-3p and SOCS3. LPS treatment significantly increased miR-409-3p expression and decreased the expression levels of SOCS3 in BEAS-2B cells. Dual-luciferase reporter assay demonstrated that miR-409-3p directly targeted and negatively regulated SOCS3. Inhibition of miR-409-3p markedly decreased the levels of TNF-α, IL-6 and IL-1β, and suppressed apoptosis induced by LPS, which was reversed by SOCS3-knockdown. The inhibition of SOCS3 significantly activated JAK1/STAT3 signaling, as well as enhancing the levels of TNF-α, IL-6 and IL-1β, and promoting apoptosis, which was reversed by the JAK1 inhibitor Tofacitinib. Suppression of miR-409-3p improved LPS-induced inflammation through SOCS3 in LPS-treated BEAS-2B cells, and this may be caused by regulating JAK1/STAT3 signaling.

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Abbreviations: LPS, lipopolysaccharide; miRNA/miR, microRNA; NC, negative controls; WT, wild-type; MUT, mutant

Key words: miR-409-3p, suppressors of cytokine signaling 3, inflammation, bronchopneumonia

Introduction

Pneumonia, one of the most common pediatric respiratory diseases, is also the most prevalent cause of mortality and morbidity for children <5 years old, especially newborns (1-3). The World Health Organization reports that pneumonia accounts for a third of newborn mortality worldwide (4), and 1.1-1.4 million children succumb to pneumonia worldwide every year (5). Among the types of pneumonia, bronchopneumonia is the most common type in children and is a leading cause of child mortality, resulting in 935,000 deaths in children <5 years old in 2013 (6,7). Despite the high mortality rate of bronchopneumonia, its underlying molecular mechanisms remain to be elucidated.

In the last decade, the role of microRNAs (miRNAs/miRs) in the development of pneumonia has been reported in a number of studies (8-11). For instance, Gomez *et al* (12) detected 1,100 miRNAs in an S. pneumoniae pneumonia mouse model and identified that 31 miRNAs were significantly increased and 67 miRNAs were decreased. Additionally, miRNAs including miR-1247, miR-217 and miR-3941 have been associated with pneumonia development (13-15). A recent study reported that hsa-miR-409-3p is upregulated in whole blood of adenovirus-infected children with pneumonia (16). However, to the best of our knowledge, no study has reported the role and associated molecular mechanisms of miR-409-3p in the development of bronchopneumonia.

Suppressor of cytokine signaling (SOCS)3 is considered as an anti-inflammation factor in a number of diseases, including pneumonia (17). The inhibition of SOCS3 may facilitate the M1 macrophage polarization in childhood pneumonia (17). Additionally, the inactivation of SOCS3 is considered to be associated with the activation of NF-κB signaling, which is the key factor of inflammatory signaling (18). In asthma, SOCS3 and NF-κB expression is stimulated and associated with inflammation (19). Liu *et al* (20) identified that SOCS3 is a direct target of miR-409-3p in astrocytes and in the pathogenesis of experimental autoimmune encephalomyelitis mice. However, the association between miR-409-3p and SOCS3 in bronchopneumonia is unclear.

The present study aimed to investigate the role of miR-409-3p in lipopolysaccharide (LPS)-induced BEAS-2B

cells as an in vitro model of bronchopneumonia, in order to improve the understanding of the role of miR-409-3p in LPS-induced inflammation and to provide novel research targets for bronchopneumonia development.

Materials and methods

Cell culture and treatment. Immortalized human bronchial epithelial BEAS-2B cells were obtained from the American Type Culture Collection. The cells were cultured in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.) with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C and 5% CO_2. BEAS-2B cells were treated with 10 μM LPS (Sigma-Aldrich; Merck KGaA) for 6 h at the same condition of 37˚C and 5% CO_2 to establish the *in vitro* bronchopneumonia model (21). Untreated cells were used as controls. For inhibition of JAK/STAT3 signaling, 10 μM Tofacitinib (Sigma-Aldrich; Merck KGaA) was used to treat the BEAS-2B cells for 6 h at 37˚C and 5% CO_2 (22).

Transfection. For BEAS-2B cell transfection, the miR-409-3p mimics, inhibitor and the corresponding negative controls (NCs), as well as small interfering (si)RNAs against SOCS3 (si-SOCS3) and si-NC, were synthesized by Shanghai GeneChem Co., Ltd.). Scrambled sequences were used as NC. Cells were transfected with 50 nmol/l miR-409-3p mimics, miR-409-3p inhibitor or si-SOCS3 at 37˚C for 48 h using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Transfection efficiency was determined after 48 h of transfection by reverse transcription-quantitative (RT-q) PCR. The sequences were as follows: miR-409-3p mimics: 5'-GAAUGUUGCUCGGUGA ACCCCU-3'; NC inhibitor: 5'-ACTACTGAGTGACAGT AGA-3'; miR-409-3p inhibitor: 5'-GAGCUACAGUGCUUC AUCUCA-3'; inhibitor NC: 5'-UUCUCCGAACGUGUCACG UTT-3'; si-SOCS3: sense, 5'-TTCTACATGGGGGGATAG-3', antisense 5'-TGGTCCAGGAACTCCCGAAT-3'; si-NC: sense 5'-UUCUCCGAACGUGUCACGUTT-3', antisense 5'-CUU GAGGCUGUUGUCAUACTT-3'

Apoptosis. Briefly, BEAS-2B cells ($4x10^5$/well) were harvested, trypsinized and seeded into 6-well plates. The cells were stained by PI (20 μg/ml) for 20 min using an Annexin V-FITC Apoptosis Detection kit (Sigma-Aldrich; Merck KGaA) according to the manufacturer's instructions. Cell apoptosis was analyzed using a FACSort flow cytometer (BD Biosciences) with Cell Quest software 5.1 (BD Biosciences). Early and late apoptotic cells were considered for the apoptotic rate.

ELISA. Briefly, the cell suspension was centrifuged at 1,200 x g for 15 min at room temperature. The supernatants were collected and the levels of TNF-α, IL-6 and IL-1β were evaluated by ELISA using the following commercially available kits: Human TNF-α ELISA kit (cat. no. ab181421; Abcam), human IL-6 ELISA kit (cat. no. ab178013; Abcam) and Human IL-1β ELISA kit (cat. no. ab100562; Abcam).

RT-qPCR. Total RNA was extracted from cells using TRIzol® (Thermo Fisher Scientific, Inc.). The mirVana miRNA isolation kit (Ambion; Thermo Fisher Scientific, Inc.) was used for miRNA extraction according to the manufacturer's instruction. RNA was converted to cDNA using the High Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, Inc.) for mRNA and Taqman MicroRNA Reverse Transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) for miRNA according to the manufacturer's instructions. The PCR reactions were conducted in an Applied Biosystems 7500 Real Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) using SYBR-Green PCR Master Mix (Beijing Solarbio Science & Technology Co., Ltd.). Thermocycling conditions were: Initial denaturation at 94˚C for 30 sec, then 94˚C for 5 sec, 60˚C for 30 sec and 72˚C for 30 sec (40 cycles). Primer sequences were as follows: miR-409 forward (F), 5'-GAATGTTGCTCGGTGA-3' and reverse (R), 5'-GTG CAGGGTCCGAGGT-3'; SOCS3 F, 5'-CCTGCGCCTCAA GACCTTC-3' and R, 5'-GTCACTGCGCTCCAGTAGAA-3'; TNF-α F, 5'-ATGAGCACTGAAAGCATGATCCGG-3' and R, 5'-GCAATGATCCCAAAGTAGACCTGCCC-3'; IL-6 F, 5'-ATGAACTCCTTCTCCACAAGCGC-3' and R, 5'-GAAGAGCCCTCAGGCTGGACTG-3'; IL-1β F, 5'-ATGGCAGAAGTACCTGAGCTCGC-3' and R, 5'-ACA CAAATTGCATGGTGAAGTCAGTT-3'; U6 F, 5'-CTCGCT TCGGCAGCACA-3' and R 5'-AACGCTTCACGAATT TGCGT-3'; GAPDH F, 5'-CCATGGAGAAGGCTGGGG-3' and R 5'-CAAAGTTGTCATGGATGACC-3'. U6 and GAPDH were used as internal references for miRNAs and mRNAs, respectively. The relative expression level was calculated using the $2^{-\Delta\Delta Cq}$ method (23). All experiments were repeated in triplicate.

Dual-luciferase reporter assay. The binding region for SOCS3 3'-untranslated region (UTR) and miR-409-3p was predicted using TargetScan 7.2 software (http://www.targetscan.org). The wild-type (WT) or mutant (MUT) 3'-UTR of SOCS3 was sub-cloned into a pGL4.10 luciferase reporter vector (Promega Co.), followed by co-transfection with either the vectors, miR-409-3p mimics, inhibitors or the respective NCs using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.). After 48 h of transfection, luciferase assays were conducted using a Luciferase Assay System (Promega Co.) and the relative luciferase activity was calculated by normalization to *Renilla* luciferase activity.

Western blotting. Briefly, proteins were extracted using RIPA buffer (Vazyme Biotech Co., Ltd.) from BEAS-2B cells. The protein amount was determined using a Bio-Rad protein assay reagent (Bio-Rad Laboratories, Inc.). A total of 50 μg protein sample was subjected to 10% SDS-PAGE. Samples were then transferred onto PVDF membranes. After blocking with 5% non-fat milk for 1 h at room temperature, membranes were incubated at 4˚C overnight with the following primary antibodies: Anti-SOCS3 (cat. no. ab16030; 1:1,000; Abcam), anti-JAK1 (cat. no. ab133666; 1:1,000; Abcam) anti-phosphorylated (p-)JAK1 (cat. no. ab138005; 1:1,000; Abcam), anti-STAT3 (cat. no. ab119352; 1:5,000; Abcam), anti-p-STAT3 (cat. no. ab76315; 1:2,000; Abcam), anti-cleaved caspase-3 (cat. no. ab2302; 1:500; Abcam), anti-Bax (cat. no. ab32503; 1:1,000; Abcam), anti-Bcl-2 (cat. no. ab32124; 1:1,000; Abcam) and anti-GAPDH (cat. no. ab8245; 1:500; Abcam). The samples were then incubated with an HRP-conjugated goat anti-rabbit

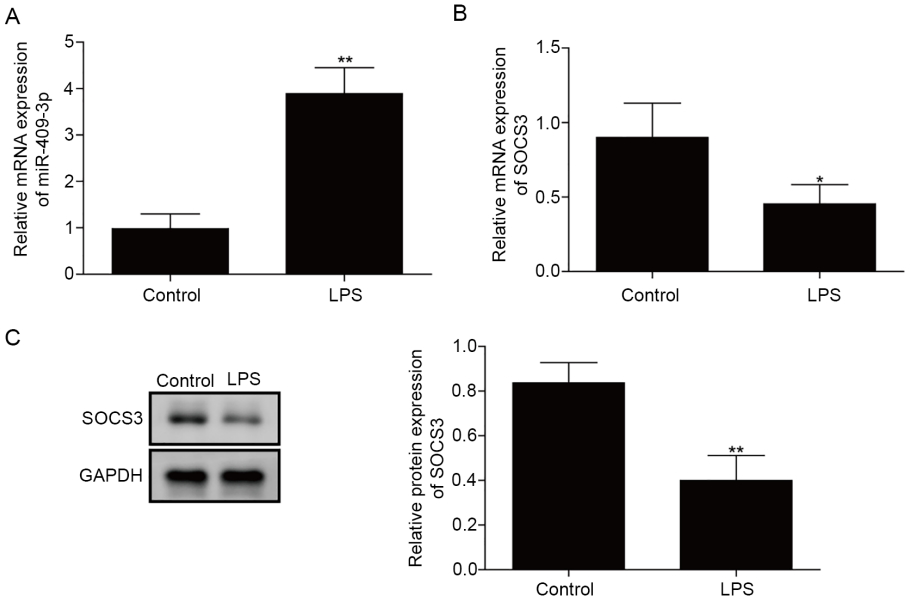

Figure 1. miR-409-3p expression is increased and SOCS3 expression is downregulated in LPS-induced BEAS-2B cells. BEAS-2B cells were treated with LPS (10 μM for 6 h). (A) miR-409-3p and (B) SOCS3 expression was determined by reverse transcription-quantitative PCR. (C) The protein levels of SOCS3 were measured by western blotting. *P<0.05, **P<0.01, vs. control. miR, microRNA; SOCS, suppressor of cytokine signaling; LPS, lipopolysaccharide.

IgG secondary antibody (cat. no. ab205718; 1:1,000; Abcam) or an HRP-conjugated goat anti-mouse IgG H&L secondary antibody (cat. no. ab205719; 1:1,000; Abcam) at 37˚C for 45 min. The blots were scanned and images were captured using the Super Signal West Pico Chemiluminescent Substrate kit (Pierce; Thermo Fisher Scientific, Inc.). Image-Pro Plus software 6.0 (Media Cybernetics, Inc.) was used to calculate the relative protein expression.

Statistical analysis. At least three independent experiments were performed for all procedures. All statistical analyses were performed using SPSS v22.0 (IBM Corp.). Comparisons among ≥3 groups were performed using one-way ANOVA followed by Tukey's post-hoc test. Comparison between two groups was made by t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

miR-409-3p expression is increased and SOCS3 expression is downregulated in LPS-induced BEAS-2B cells. First, the expression levels of miR-409-3p and SOCS3 in the *in vitro* bronchopneumonia model were measured. As demonstrated in Fig. 1A, miR-409-3p expression was significantly upregulated in LPS-treated BEAS-2B cells compared with in control cells (P<0.01). By contrast, mRNA and protein levels of SOCS3 were significantly downregulated in LPS-induced cells compared with in control cells (P<0.05; Fig. 1B and C). These results indicated that miR-409-3p and SOCS3 were abnormally expressed in LPS-induced BEAS-2B cells.

Inhibition of miR-409-3p suppresses the LPS-induced inflammatory response in BEAS-2B cells. To further investigate the role of miR-409-3p in LPS-induced inflammation, miR-409-3p inhibitor was used to knockdown miR-409-3p expression, and the results demonstrated that miR-409-3p expression was successfully suppressed in BEAS-2B cells using the inhibitor (P<0.05; Fig. 2A). Additionally, the transfection of miR-409-3p inhibitor resulted in inhibition of LPS-stimulated miR-409-3p expression in BEAS-2B cells (P<0.01; Fig. 2B). mRNA and protein expression levels of TNF-α, IL-6 and IL-1β were significantly upregulated in LPS-induced BEAS-2B cells, and inhibiting miR-409-3p significantly decreased these effects (P<0.05; Fig. 2C and D). Apoptosis analysis identified that LPS treatment significantly enhanced the apoptosis rate; however, miR-409-3p inhibition was able to rescue the LPS-induced apoptosis (P<0.05; Fig. 2E). Similar results were identified for apoptosis-related proteins. The protein levels of cleaved caspase-3 and Bax were markedly enhanced, while Bcl-2 expression was significantly decreased in LPS-induced BEAS-2B cells, and these effects were significantly reversed by inhibition of miR-409-3p (P<0.05; Fig. 2F). The present results suggested that knockdown of miR-409-3p inhibited LPS-induced inflammation in BEAS-2B cells.

miR-409-3p directly targets and negatively regulates SOCS3. The interaction between miR-409-3p and SOCS3 was further explored. First, the binding between miR-409-3p and SOCS3 was predicted using bioinformatics (Fig. 3A). As demonstrated in Fig. 3B, miR-409-3p overexpression significantly inhibited the relative luciferase activity, while knockdown of miR-409-3p markedly enhanced the relative luciferase activity in SOCS3-WT (P<0.01). However, no significant difference was identified in SOCS3-MUT (Fig. 3B). The expression of miR-409-3p was markedly downregulated by transfection of miR-409-3p inhibitor and significantly upregulated following transfection with miR-409-3p mimics (Fig. 3C). Meanwhile, overexpressing miR-409-3p significantly inhibited mRNA and protein levels of SOCS3 in BEAS-2B cells, while miR-409-3p inhibition led to the opposite results (Fig. 3D and E). The present results indicated that miR-409-3p directly targeted SOCS3 and negatively regulated its expression.

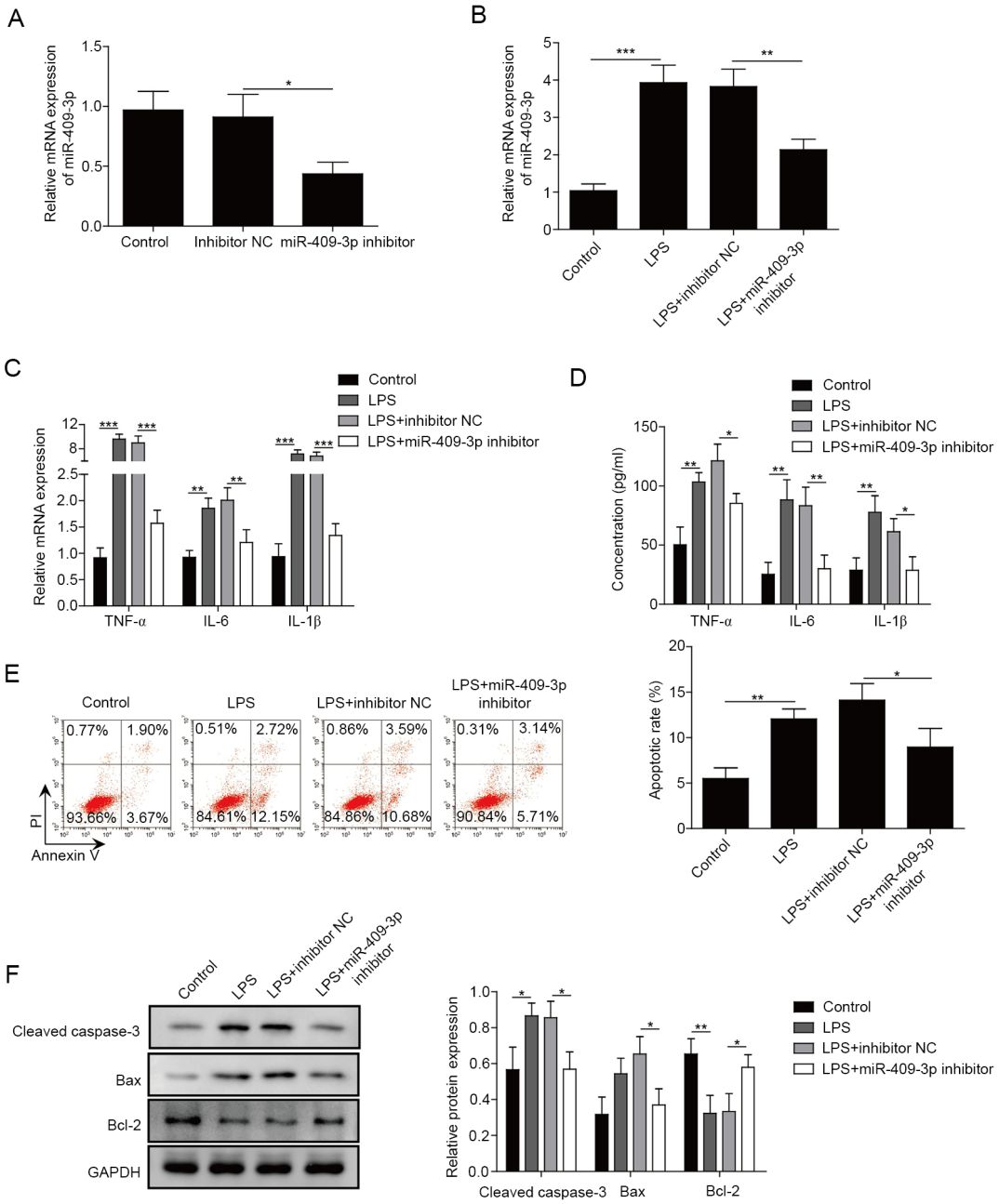

Figure 2. Inhibition of miR-409-3p suppresses LPS-induced inflammatory response in BEAS-2B cells. (A) Transfection efficiency of miR-409-3p inhibitor was confirmed by RT-qPCR. (B) miR-409-3p expression was detected by RT-qPCR in LPS-treated BEAS-2B cells transfected with inhibitor NC and miR-409-3p inhibitor. (C) The expression levels of inflammatory factors TNF-α, IL-6 and IL-1β were determined by RT-qPCR in LPS-treated BEAS-2B cells transfected with inhibitor NC and miR-409-3p inhibitor. (D) Protein expression levels of TNF-α, IL-6 and IL-1β were determined by ELISA. (E) Apoptosis was measured by flow cytometry. (F) Apoptosis-related proteins Bax, cleaved caspase-3 and Bcl-2 were determined by western blotting. *P<0.05, **P<0.01, ***P<0.001. miR, microRNA; LPS, lipopolysaccharide; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control.

miR-409-3p regulates LPS-induced inflammation through SOCS3 in BEAS-2B cells. To further clarify the mechanisms for miR-409-3p in LPS-induced BEAS-2B cells, cells were transfected with miR-409-3p inhibitor, si-SOCS3 or miR-409-3p inhibitor and si-SOCS3. The transfection efficiency of si-SOCS3 was confirmed by both RT-qPCR and western blotting (Fig. 4A and B). The results demonstrated that miR-409-3p expression was significantly decreased by miR-409-3p inhibitor (P<0.05), but was not affected by inhibition of SOCS3 using si-SOCS3 (Fig. 4C). Co-transfection of miR-409-3p inhibitor and si-SOCS3 showed similar effects to mono-transfection of miR-409-3p inhibitor. By contrast, inhibition of miR-409-3p significantly enhanced SOCS3 expression (P<0.01; Fig. 4D). SOCS3 expression was significantly decreased by transfection with si-SOCS3 and was then reversed by miR-409-3p inhibitor (P<0.01; Fig. 4D). For TNF-α, IL-6 and IL-1β expression, the LPS-induced upregulation of the inflammatory factors was significantly decreased by suppression of miR-409-3p compared with inhibitor NC group and was significantly increased by si-SOCS3 compared with si-NC group. SOCS3 inhibition by co-transfection of si-SOCS3 reversed the effects

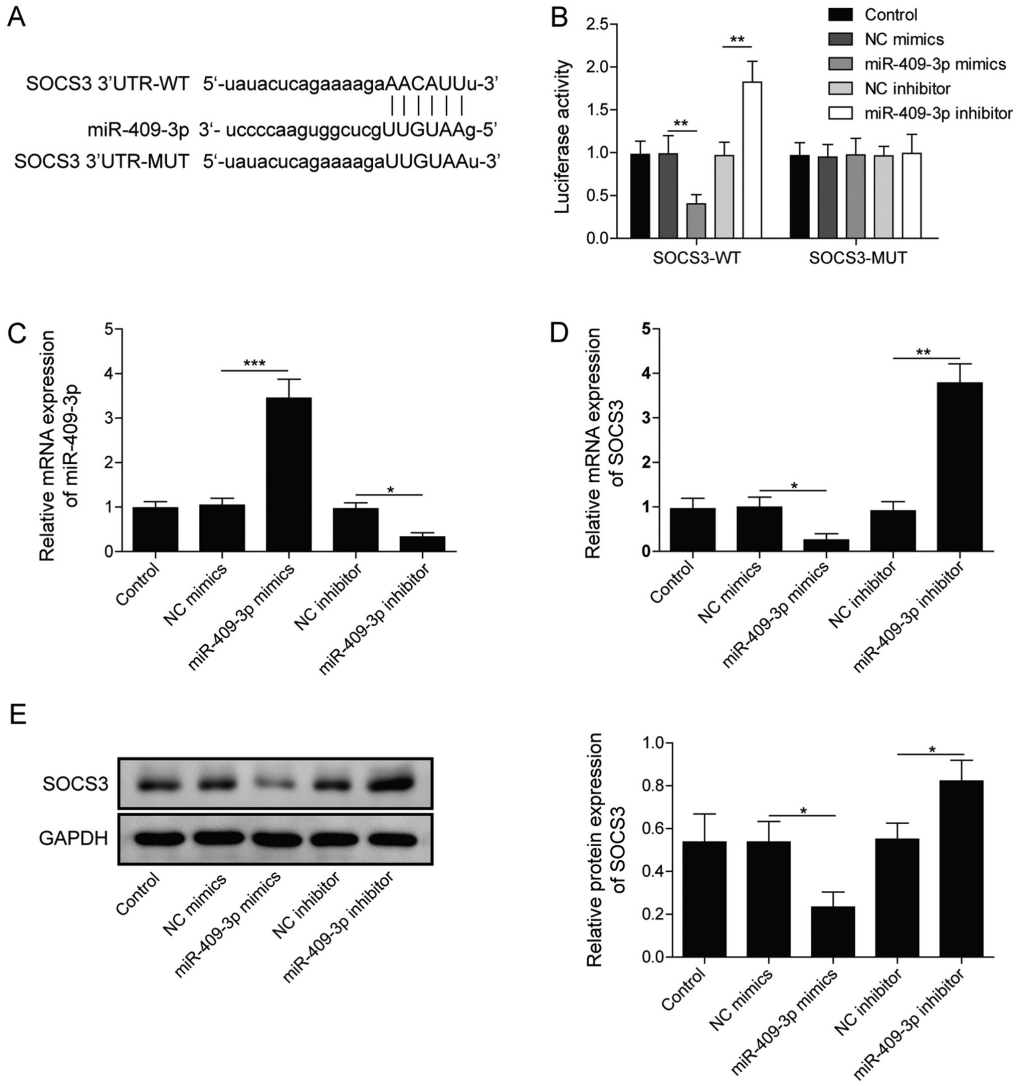

Figure 3. miR-409-3p directly targets and negatively regulates SOCS3. (A) The predicted binding region for SOCS3 3'-UTR and miR-409-3p was obtained from TargetScan 7.2. (B) Relative luciferase activity was determined by dual-luciferase reporter assay. (C) Expression of miRNA-409-3p in cells transfected with miR-409-3p inhibitor or mimics was determined using RT-qPCR. (D) mRNA and (E) protein expression levels of SOCS3 in cells transfected with miR-409-3p inhibitor or mimics were determined using reverse transcription-quantitative PCR and western blotting, respectively. *P<0.05, **P<0.01 and ***P<0.001. miR, microRNA; SOCS, suppressor of cytokine signaling; UTR, untranslated region; WT, wild-type; MUT, mutant; NC, negative control; RT-qPCR, reverse transcription-quantitative PCR.

of miR-409-3p inhibitor compared with cells only transfected with miR-409-3p inhibitor (all P<0.05; Fig. 4E and F). Inhibition of miR-409-3p suppressed the LPS-induced cell apoptosis rate, which was increased by SOCS3-knockdown, and this effect was reversed by inhibition of both miR-409-3p and SOCS3 in LPS-induced BEAS-2B cells (P<0.05; Fig. 4G). Similarly, the LPS-induced upregulation of cleaved caspase-3 and Bax was markedly decreased by miR-409-3p inhibitor and the downregulation of Bcl-2 was notably increased by miR-409-3p inhibitor, while si-SOCS3 transfection resulted in the opposite effects (P<0.05; Fig. 4H). Co-transfection of si-SOCS3 markedly reversed the effects of transfection of miR-409-3p inhibitor on apoptosis-related proteins. Additionally, protein levels of p-JAK1 and p-STAT3 were significantly upregulated by LPS treatment, which were then downregulated by miR-409-3p-knockdown and significantly augmented by SOCS3-knockdown (P<0.05; Fig. 4I). Notably, the effects of miR-409-3p inhibition on p-JAK1 and p-STAT3 levels were significantly reversed by si-SOCS3 (P<0.05; Fig. 4I). The aforementioned results suggested that miR-409-3p regulated LPS-induced inflammation by regulating SOCS3 and that JAK1/STAT3 signaling may be involved.

miR-409-3p regulates inflammation through SOCS3/JAK1/STAT3 in LPS-induced BEAS-2B cells. Finally, it was attempted to confirm the effect of JAK1/STAT3 signaling on miR-409-3p/SOCS3 axis-regulated LPS-induced inflammation. Inhibition of SOCS3 significantly increased p-JAK1 and p-STAT31 protein levels, which were then significantly suppressed by treatment with Tofacitinib, a JAK/STAT3 inhibitor (P<0.05; Fig. 5A). Protein and mRNA levels of inflammatory factors TNF-α, IL-6 and IL-1β were significantly enhanced by transfection with si-SOCS3; however, treatment with Tofacitinib significantly decreased these effects (P<0.01; Fig. 5B and C). Additionally, the apoptosis rate was significantly increased by si-SOCS3 in LPS-induced BEAS-2B

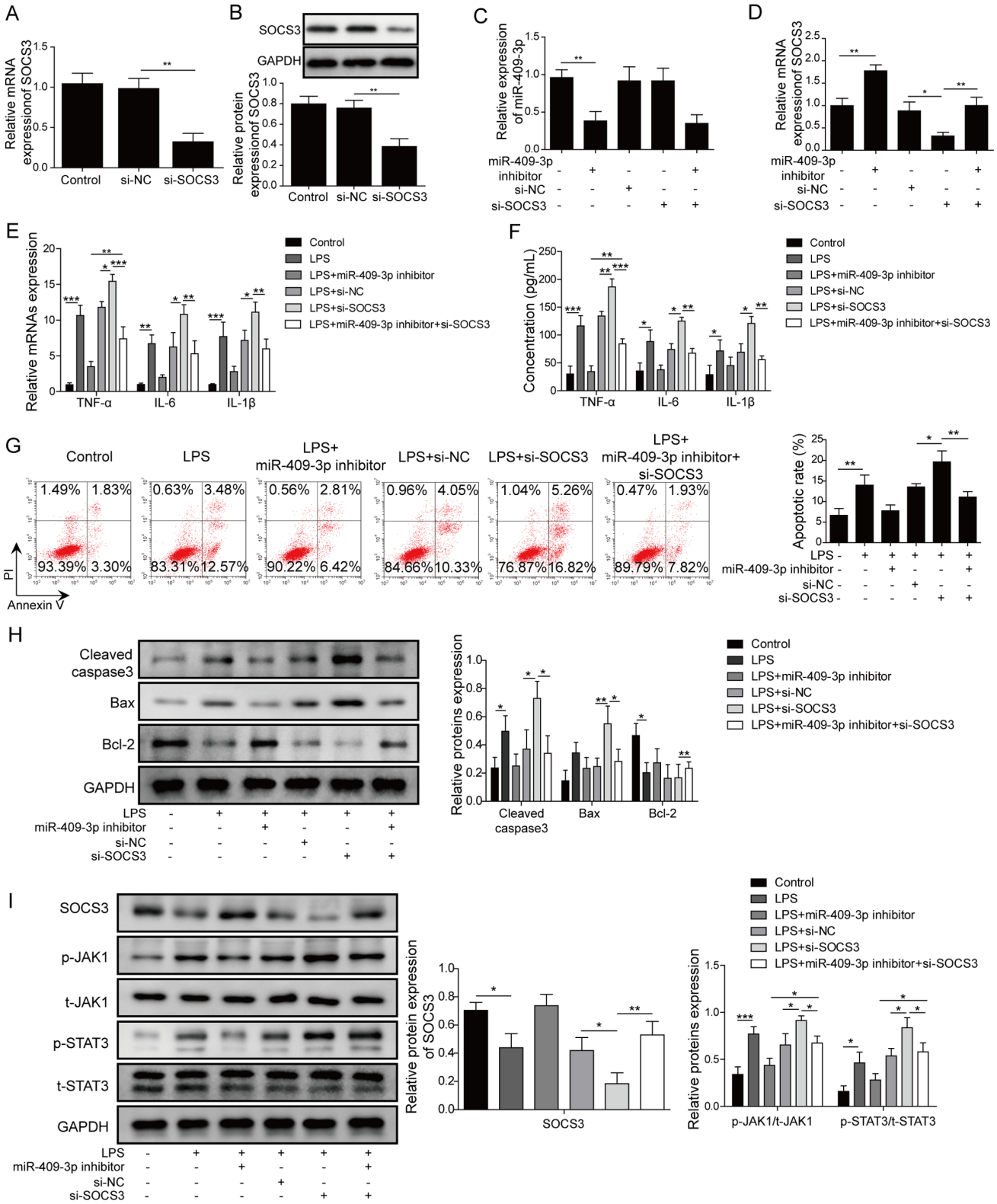

Figure 4. miR-409-3p regulates LPS-induced inflammation through SOCS3 in BEAS-2B cells. (A) mRNA and (B) protein expression levels of SOCS3 in cells transfected with si-SOCS3, si-NC, or control cells. (C) miR-409-3p and (D) SOCS3 expression in different groups of LPS-treated BEAS-2B cells was determined by RT-qPCR. (E) mRNA expression levels of inflammatory factors TNF-α, IL-6 and IL-1β were measured by RT-qPCR. (F) Protein levels of TNF-α, IL-6 and IL-1β were determined using ELISA. (G) Apoptosis was measured by flow cytometer analysis. (H) Apoptosis-related proteins Bax, cleaved caspase-3 and Bcl-2 were determined using western blotting. (I) Protein levels of SOCS3 and JAK1/STAT3 signaling-related proteins in different groups of cells were determined by western blotting. *P<0.05, **P<0.01, ***P<0.001. miR, microRNA; LPS, lipopolysaccharide; SOCS, suppressor of cytokine signaling; RT-qPCR, reverse transcription-quantitative PCR; p-, phosphorylated; t-, total; si, small interfering; NC, negative control.

cells (P<0.05), while Tofacitinib decreased the apoptosis rates (P<0.01; Fig. 5D). The protein expression levels of Bax and cleaved caspase-3 were significantly elevated by silencing of SOCS3, while Bcl-2 expression was significantly decreased,

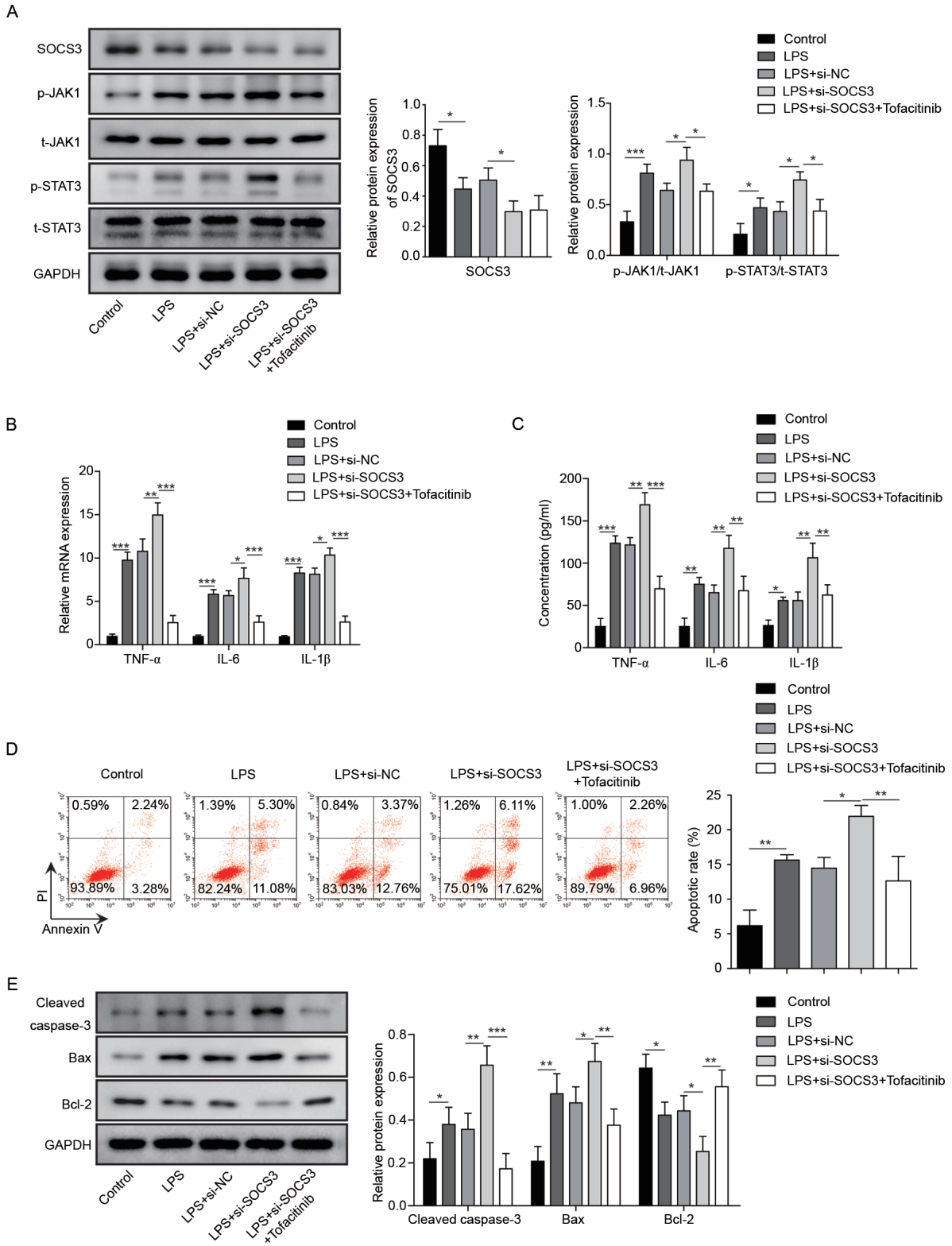

Figure 5. miR-409-3p regulates inflammation through SOCS3/JAK1/STAT3 in LPS-induced BEAS-2B cells. (A) Protein levels of SOCS3 and JAK1/STAT3 signaling-related proteins in different groups of LPS-treated BEAS-2B cells were determined by western blotting. (B) mRNA expression levels of inflammatory factors TNF-α, IL-6 and IL-1β were measured by RT-qPCR. (C) Protein levels of TNF-α, IL-6 and IL-1β were determined using ELISA. (D) Apoptosis was measured by flow cytometer analysis. (E) Apoptosis-related proteins Bax, cleaved caspase-3 and Bcl-2 were determined using western blotting. *P<0.05, **P<0.01, ***P<0.001. miR, microRNA; SOCS, suppressor of cytokine signaling; LPS, lipopolysaccharide; p-, phosphorylated; t-, total; si, small interfering; NC, negative control.

and these effects were reversed by Tofacitinib (P<0.01; Fig. 5E). These results indicated that miR-409-3p regulated LPS-induced inflammation through SOCS3/JAK1/STAT3 signaling in BEAS-2B cells.

Discussion

Despite previous studies on bronchopneumonia for both diagnosis and treatment (6,7), the molecular mechanisms for

bronchopneumonia remain to be elucidated. In recent years, the role of miRNAs in the development of inflammatory responses, as wellas pneumonia, has been noted in several studies (8-12). However, to the best of our knowledge, no studies have reported the role of miR-409-3p in bronchopneumonia. The present study identified that in LPS-induced inflammation in BEAS-2B cells, miR-409-3p targeted and suppressed SOCS3 expression and further influenced the inflammatory response by regulating JAK1/STAT3 signaling.

It has been reported that miR-409-3p serves important roles in a number of diseases, such as glioblastoma and osteosarcoma (24,25). In addition, miR-409-3p in inflammation-related diseases, as well as in pneumonia, has been identified in several studies. For example, Dai *et al* (26) noted that miR-409 expression is upregulated in idiopathic thrombocytopenic purpura. Additionally, miR-409 expression is upregulated in tissue plasminogen activator-treated K562 cells and is considered as a biomarker for megakaryocytopoiesis, which serves a crucial role in inflammatory activation (27). Similarly, the present study demonstrated that miR-409-3p expression was elevated in LPS-induced BEAS-2B cells. Functionally, it was observed that miR-409-3p inhibition improved LPS-induced inflammation and stimulated apoptosis, suggesting that miR-409-3p served anti-inflammatory functions in LPS-induced BEAS-2B cells.

SOCS3 is considered an anti-inflammation factor. In the present study, the data demonstrated that SOCS3 expression was downregulated in LPS-induced BEAS-2B cells and inhibition of SOCS3 promoted LPS-induced inflammation. Other studies are consistent with these findings. For instance, Kim *et al* (28) demonstrated that the activation of SOCS3 leads to inactivation of the IL-6 signaling pathway. Dai *et al* (29) demonstrated that Kallikrein-binding protein suppressed LPS-induced inflammation through upregulation of SOCS3. Mechanically, the present study confirmed that SOCS3 was a direct target of miR-409-3p, which exerted its function of anti-inflammation through inhibition of SOCS3.

A number of studies have demonstrated that the activation of JAK1/STAT3 signaling is a key process in inflammatory response. Shien *et al* (30) showed that the activation of the proinflammatory cytokine pathway leads to activation of JAK1/STAT3. Aloin can inhibit LPS-induced inflammation by suppressing JAK1/STAT1/3 activation (31). The association between SOCS3 and JAK1/STAT3 signaling has also been reported. Andoh *et al* (32) identified that JAK1/STAT3/SOCS3 signaling is activated in inflammatory bowel disease. Additionally, JAK/STAT/SOCS3 signaling is activated in colon and rectal cancer (33). Similarly, the present study verified that JAK1/STAT3 signaling was activated in LPS-induced inflammation and that SOCS3 could regulate the inflammatory response by regulating JAK1/STAT3 signaling in LPS-induced BEAS-2B cells.

In conclusion, the present study used an in vitro model to investigate the role of miR-409-3p in LPS-induced BEAS-2B cells and revealed that inhibition of miR-409-3p improved LPS-induced inflammation through SOCS3/JAK1/STAT3 signaling. The present study may provide insight into the molecular mechanisms for the role of miR-409-3p in LPS-induced inflammation, as well as in the development of bronchopneumonia.

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Availability of data and materials

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

Authors' contributions

LH conceived the study and methodology, performed bioinformatic analysis and wrote the original draft of the paper. LT collected, analyzed and interpreted the data, supervised the study, reviewed the manuscript, and was involved in drafting the manuscript and revising it critically for important intellectual content. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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