Salvianolic acid B inhibits myofibroblast differentiation and extracellular matrix accumulation in nasal polyp fibroblasts via the TGF-β1 signaling pathway

ZHENFENG SUN, DAN LUO, SANG WANG, RUOFEI HUANG and PIN DONG

Department of Otolaryngology, Head and Neck Surgery, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 201620, P.R. China

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Abstract. In the process of nasal tissue remodeling, nasal fibroblasts serve an important role via myofibroblast differentiation and the production of extracellular matrix (ECM). Nasal fibroblast abnormalities can lead to conditions such as chronic rhinosinusitis. Salvianolic acid B (Sal B), a water-soluble active pharmaceutical compound extract from the root of the traditional Chinese medicine *Salvia miltiorrhiza*, displays antioxidative, antiproliferative and antifibrosis properties. The present study aimed to investigate the mechanism underlying the effects of Sal B on nasal polyp fibroblast (NPF) myofibroblast differentiation and ECM accumulation. Primary NPFs were obtained from nasal polyps of patients with chronic sinusitis. The proliferative and cytotoxic effects of Sal B on NPFs were evaluated by performing the Cell Counting Kit-8 assay. The Transwell assay was conducted to assess cell migration. α-smooth muscle actin (α-SMA), TGF-β1 receptor (TβR)-I, TβR-II, Smad2/3 mRNA and protein expression levels and (p)-Smad2/3 phosphorylation levels were measured via reverse transcription-quantitative PCR and western blotting, respectively. Type III collagen and fibronectin levels were analyzed by ELISA. The results indicated that Sal B significantly downregulated TGF-β1-induced α-SMA, fibronectin and collagen III expression levels in NPFs. Similarly, Sal B significantly decreased TGF-β1-induced TβR-I, TβR-II, p-Smad2/3, MMP-2 and MMP-9 mRNA and protein expression levels in NPFs. Furthermore, Sal B significantly decreased TGF-β1-induced NPF migration. Therefore, the present study indicated that Sal B inhibited myofibroblast differentiation and ECM accumulation in nasal fibroblasts, suggesting that Sal B may inhibit nasal polyp formation via certain mechanisms.

Correspondence to: Dr Pin Dong, Department of Otolaryngology, Head and Neck Surgery, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, 100 Haining Road, Shanghai 201620, P.R. China
E-mail: dongpin64@126.com

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Introduction

Chronic rhinosinusitis (CRS) is a chronic inflammatory condition that affects the mucosa of the nasal and paranasal sinuses for >12 weeks (1). CRS is a highly prevalent chronic disease with a heavy socioeconomic burden, characterized by ≥2 of the following symptoms: Blockage or discharge affecting the mucosa of the nasal and/or paranasal sinuses, facial pain or pressure, and problems with smell (1). Clinically, endoscopy or CT scans are typically used as diagnostic tools and according to the phenotypical differentiation of the disease, CRS is divided into two types: i) CRS with nasal polyps (CRSwNP); and ii) CRS without nasal polyps (2).

CRSwNP is the most common type of CRS, but the mechanism underlying nasal polyp development is not completely understood (3). However, the differentiation of fibroblasts into myofibroblasts, which results in an accumulation of extracellular matrix (ECM) proteins and fibrosis, leads to nasal tissue remodeling, which has been attributed to polyp development (4-6). Myofibroblasts expressing α-smooth muscle actin (α-SMA) can promote ECM protein secretion and collagen deposition, and α-SMA is a key indicator of myofibroblast differentiation (7). TGF-β1 increases fibroblast collagen secretion and promotes fibroblast differentiation into myofibroblasts, leading to ECM accumulation and a significant increase in nasal polyps (7). The key components of TGF-β1 signaling include TGF-β1 receptor (TβR)-I, TβR-II and the transcription factor Smad2/3 (8). As an important transcription factor in TGF-β1 signaling, Smad2/3 serves an important role in TGF-β1-induced myofibroblast differentiation and phosphorylated (p)-Smad2/3 expression in nasal polyp fibroblasts (NPFs) (8). Matrix metallopeptidases (MMPs) are key proteins involved in ECM remodeling that belong to the family of zinc and calcium-dependent endopeptidases (9). MMP expression levels are increased in chronic inflammatory diseases, including CRS (10). Several reports have demonstrated the expression levels of MMP-2 and MMP-9 in CRSwNP are increased compared with healthy individuals (11-13). Moreover, MMP-2 and MMP-9 are involved in the pathophysiology of CRSwNP via degrading the ECM and promoting inflammatory cell migration to the ECM (8,14).

Salvia miltiorrhiza (Danshen), a traditional Chinese medicine, has been widely used in the clinical setting due to its extensive reported therapeutic effects (15). Salvianolic acid B (Sal B; Fig. 1A) is the major water-soluble extract of *Salvia miltiorrhiza* Bge. The key structural constituents of Sal B are the trimolecular-3, 4-dihydroxybenyl lactic acids and a molecule of caffeic acid (15,16). Previous studies have demonstrated that Sal B displays a wide range of pharmacological effects, including antioxidation, antiatherosclerosis, antitumor, antifibrosis and protective effects in the liver and heart (17). Sal B has also been reported to be effective in attenuating cardiac, hepatic, renal and lung fibrosis, and functions by degrading the ECM and preventing myofibroblast differentiation (18-21). To the best of our knowledge, whether Sal B alters tissue remodeling in CRSwNP has not been previously reported. Therefore, the present study assessed whether Sal B influenced NPF myofibroblast differentiation and ECM production, and investigated the mechanisms underlying the therapeutic effects of Sal B.

Materials and methods

Reagents. Sal B (98.0% purity; Sigma-Aldrich; Merck KGaA) and human recombinant TGF-β1 (PeproTech, Inc.) were freshly prepared in DMSO and diluted into working concentrations using DMEM (Thermo Fisher Scientific, Inc.).

Cell culture. Primary nasal fibroblasts were isolated from nasal polyps of eight patients with nasal polyposis (4 female patients and 4 male patients; mean age, 40.2±3.4 years) from January 2019 to January 2020 at the Department of Otorhinolaryngology, Shanghai General Hospital of Shanghai Jiao Tong University (Shanghai, China). The present study was approved by the ethics committee of Shanghai First People's Hospital (approval no. 2018KY008). All patients provided written informed consent. The following inclusion criteria were used: i) No prior nasal surgery; ii) no active inflammation, allergies or aspirin hypersensitivity; iii) no oral or topical antibiotics, antihistamines, steroids or other medications for at least 4 weeks prior to surgery; and iv) no oral antiallergy medications for ≥2 weeks prior to surgery.

Polyp tissues were mechanically milled and enzymatically digested in DMEM containing 500 U/ml collagenase, 30 U/ml hyaluronidase, 10 U/ml DNAse, 500 U/ml penicillin and 500 μg/ml streptomycin. At 80-90% confluence, the culture medium was replaced to remove non-adherent cells. Subsequently, 0.05% EDTA (Sigma Aldrich; Merck KGaA) was used to separate the cells from the plate. Cells were subcultured in DMEM containing 10% FBS (Thermo Fisher Scientific, Inc.), 500 U/ml penicillin and 500 μg/ml streptomycin at 37˚C with 5% CO_2 with humidity. The purity of NPFs was assessed by observing the characteristic spindle-shaped cell morphology using an optical microscope and by performing immunocytochemistry using vimentin as a positive marker and pan-cytokeratin as a negative marker (Fig. 1B). Cells from passage 4-7 were used for subsequent experiments.

Cells were divided into the following five groups: i) Sal B group, cells cultured in DMEM containing 10 ng/ml Sal B; ii) TGF-β1 group, cells cultured in DMEM containing 5 ng/ml TGF-β1; iii) TGF-β1 + Sal B group, cells cultured in DMEM containing 5 ng/ml TGF-β1 and 10 ng/ml Sal B; iv) control group, cells cultured in DMEM; and v) DMSO group, cells were cultured in DMEM containing DMSO.

Cell proliferation and cytotoxicity. The CCK-8 (Beyotime Institute of Biotechnology) assay was performed to assess the effects of Sal B on NPF proliferation. NPFs were seeded ($5x10^3$ cells/well) into 96-well plates. Cells were incubated with Sal B (0-40 ng/ml) or DMSO (an equal volume to that used for 40 ng/ml Sal B) for 24, 48 or 72 h at 37˚C. Subsequently, CCK-8 solution was added to each well for 2 h. The optical density was measured at a wavelength of 450 nm using iMark™ Microplate Absorbance Reader (Bio-Rad Laboratories, Inc.).

Cell migration assay. Cell migration was assessed by performing Transwell migration assays. NPFs were seeded ($1x10^5$ cells/ml) into the upper chamber with DMEM containing TGF-β1 (5 ng/ml), Sal B (10 ng/ml) or DMSO (an equal volume to that used for 10 ng/ml Sal B). Subsequently, 600 μl DMEM containing 20% FBS was added to the lower chamber. After incubation for 48 h at 37˚C, migratory cells were fixed with methanol at room temperature for 30 min, followed by crystal violet staining for 10 min at room temperature. Stained cells were observed using an inverted light microscope (magnification, x100).

Cell cycle analysis via flow cytometry. Cells were seeded into 6-well culture plates and incubated at 37˚C with 5% CO_2. The cell culture medium was replaced with DMEM containing Sal B (0-40 ng/ml) or DMSO (an equal volume to that used for 40 ng/ml Sal B). Following incubation for 48 h at 37˚C, cells were collected, washed twice with PBS, resuspended in 250 μl PBS and vortexed. Subsequently, cells were added to 750 μl pre-cooled absolute ethanol for fixation at 4˚C overnight. Cells were centrifuged twice at 1800 x g for 5 min at room temperature, the supernatant was discarded and the pellet was resuspended in 500 μl PBS. The cells were then incubated with 20 μg/ml RNase and 50 μg/ml PI at room temperature for 30 min in the dark with gentle agitation. Cells were analyzed via flow cytometry (Beckman Coulter FC500 analyzer; Beckman Coulter, Inc.).

RNA isolation and reverse transcription-quantitative PCR (qPCR). Following treatment for 24 h at 37˚C, total RNA was isolated from fibroblasts using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

Total RNA was reverse transcribed into cDNA using the ReverTra Ace qPCR RT kit (Takara Bio, Inc.) according to the manufacturer's protocol. Subsequently, qPCR was performed using a MiniOpticon Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The following primers were used for qPCR: GAPDH forward, 5'-GATGC CCCCATGTTCGTCAT-3' and reverse, 5'-TCTTCTGGGTGG CAGTGATG-3'; α-SMA forward, 5'-TAGCACCCAGCACCAT GAAG-3' and reverse, 5'-TCTTCTGGGTGGCAGTGATG-3'; TβR-I forward, 5'-GTGACAGATGGGCTCTGCTT-3' and reverse, 5'-GCAATGGTCCTGATTGCAGC-3'; Smad2 forward, 5'-GAACTTCCGCCTCTGGATGA-3' and reverse, 5'-CTGGAGAGCCTGTGTCCATAC-3'; Smad3 forward, 5'-CCATCTCCTACTACGAGCTGAA-3' and reverse, 5'-CAC TGCTGCATTCCTGTTGAC-3'; MMP-2 forward, 5'-GGTTC ATTTGGCGGACTGTG-3' and reverse, 5'-CACAGCCTTCT

CCTCCTGTG-3'; and MMP-9 forward, 5'-CCTGGGCAGATT CCAAACCT-3 and reverse, 5'-GTACACGCGAGTGAAGG TGA-3'; TβR-II forward, 5'-CGTGTGGAGGAAGAACG ACA-3' and reverse, 5'-CGTGGGAGAAGTGGCATCTT-3'.

The following thermocycling conditions were used for qPCR: Initial denaturation at 95˚C for 10 min; followed by 40 cycles of denaturation for 15 sec at 95˚C and annealing for 30 sec at 58˚C. mRNA expression levels were normalized to the internal reference gene GAPDH. Analysis of relative gene expression data was carried out using the $2^{-\Delta\Delta Ct}$ method (22).

Western blotting. Following culture in DMEM supplemented with 10% FBS for 24 h at 37˚C, NPFs were treated with Sal B (10 ng/ml) and/or TGF-β1 (5 ng/ml) for 48 h at 37˚C. Cells were collected and washed twice with PBS. Total protein was isolated from cells using RIPA lysis buffer (Thermo Fisher Scientific, Inc.) containing 1% PMSF and 1% protease inhibitor. Proteins (30 μg) were quantitatively analyzed by using BCA method and separated via SDS-PAGE on 10% gels and transferred onto PVDF membranes. After blocking with 5% skimmed milk for 1 h at room temperature, the membranes were incubated at 4˚C overnight with primary antibodies targeted against: MMP-2 (cat. no. 40994S; Cell Signaling Technology, Inc.; 1:1,000), MMP-9 (cat. no. 13667S; Cell Signaling Technology, Inc.; 1:1,000), p-Smad2 (cat. no. 3104s; Cell Signaling Technology, Inc.; 1:5,00), p-Smad3 (cat. no. 9520S; Cell Signaling Technology, Inc.; 1:5,00), α-SMA (cat. no. sc-53142; Santa Cruz Biotechnology, Inc.; 1:1,000), TβR-I (cat. no. sc-518018; Santa Cruz Biotechnology, Inc.; 1:1,000), TβR-II (cat. no. sc-17792; Santa Cruz Biotechnology, Inc.; 1:1,000), Smad2 (cat. no. sc-101153; Santa Cruz Biotechnology, Inc.; 1:1,000) and Smad3 (cat. no. sc-101154; Santa Cruz Biotechnology, Inc.;1:500). Following primary antibody incubation, the membranes were incubated with HRP-linked anti-rabbit IgG (cat. no. 7074P2; Cell Signaling Technology, Inc.; 1:3,000) or mouse IgGκ light chain binding-protein (cat. no. sc-516102; Santa Cruz Biotechnology, Inc.; 1:2,000) secondary antibodies for 1 h at room temperature. Protein bands were visualized using an ECL system (Thermo Fisher Scientific, Inc.). GAPDH (cat. no. 2118S; Cell Signaling Technology, Inc.; 1:1,000) was used as the loading control.

Immunofluorescence staining. NPFs were fixed with 4% paraformaldehyde for 30 min at room temperature and soaked in 1% BSA (cat. no. A1933-25g; Sigma-Aldrich; Merck KGaA) containing 0.2% Triton X-100 for 10 min. After blocking with 3% BSA for 1 h at room temperature, NPFs were incubated with an anti-α-SMA primary antibody (cat. no. sc-53142; Santa Cruz Biotechnology, Inc.; 1:1,000) overnight at 4˚C. Subsequently, NPFs were incubated with a mouse IgGκ light chain binding-protein secondary antibody (cat. no. sc-516102; Santa Cruz Biotechnology, Inc.; 1:2,000) for 1 h at room temperature. Stained NPFs were observed using a confocal laser scanning microscope.

Immunocytochemistry. The slides were placed in an oven for 30 min for dewaxing, then hydrated with gradient alcohol. A total of 1 ml 30% H_2O_2 and 9 ml methanol were used to remove endogenous catalase. Subsequently, 5% BSA blocking solution (cat. no. A1933-25g; Sigma-Aldrich; Merck KGaA) was added to block non-specific binding in a 37˚C incubator for 30 min. The slides were incubated with primary antibody against vimentin (cat. no. 5741; Cell Signaling Technology, Inc.; 1:200) or CK (cat. no. 13063; Cell Signaling Technology, Inc.; 1:200) in 1% BSA overnight at 4˚C. Subsequently, NPFs were incubated with a HRP-conjugated rabbit secondary antibody (SignalStain® Boost IHC Detection Reagent; cat. no. 8114; Cell Signaling Technology, Inc.) for 1 h at room temperature, then wash three times in PBS for 2 min each. DAB substrate was used to detect HRP activity. The results were observed using an inverted microscope (Nikon Corporation; magnification, x400).

ELISA. After culturing in DMEM containing 10% FBS for 24 h, NPFs were treated with Sal B (10 ng/ml) and/or TGF-β1 (5 ng/ml) for 24 h at 37˚C. Subsequently, the cells were collected following centrifugation at 1,800 x g for 5 min at room temperature, and phase-contrast microscopy and a hemocytometer were used to count cells.

ELISA kits were used to measure the concentrations of secreted type-III collagen (cat. no. CSI 007-01-02; Thermo Fisher Scientific, Inc.) and fibronectin (cat. no. BMS2028TEN; Thermo Fisher Scientific, Inc.) concentrations in the supernatants were measured by performing ELISA, according to the manufacturer's protocols.

Statistical analysis. Statistical analyses were performed using SPSS software (version 20.0; IBM Corp.). Data are presented as the mean ± SD of three independent experimental repeats. Comparisons among multiple groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Sal B inhibits cell viability and TGF-β1-induced cell migration in NPFs. The CCK-8 assay was performed to examine the cytotoxicity of Sal B in NPFs. Compared with in the control group, fibroblast viability was significantly suppressed by treatment with 10 ng/ml Sal B for 72 h (Fig. 1C). In addition, the cell cycle analysis results indicated that Sal B induced cell cycle arrest in G1 phase in a concentration-dependent manner, indicating that Sal B mediated cell viability by altering cell proliferation (Fig. 1D). Therefore, 10 ng/ml Sal B was identified as the optimum concentration for subsequent experiments.

Myofibroblasts serve an important role in wound tissue repair by altering cell migration (8). Therefore, a Transwell assay was performed to assess the effect of Sal B on myofibroblast migration. Sal B significantly decreased TGF-β1-induced NPF migration (Fig. 1E). These results indicated that Sal B inhibited myofibroblast biological activity by regulating cell viability and migration.

Sal B suppresses NPF myofibroblast differentiation. To investigate whether Sal B inhibited myofibroblast differentiation, the present study assessed α-SMA expression in NPFs treated with Sal B and/or TGF-β1. The results demonstrated that compared with in the control group, TGF-β1 treatment significantly increased α-SMA mRNA and protein expression levels, which were significantly downregulated by Sal B (Fig. 2A and B). In addition, the immunofluorescence staining results indicated that α-SMA was abundantly expressed in

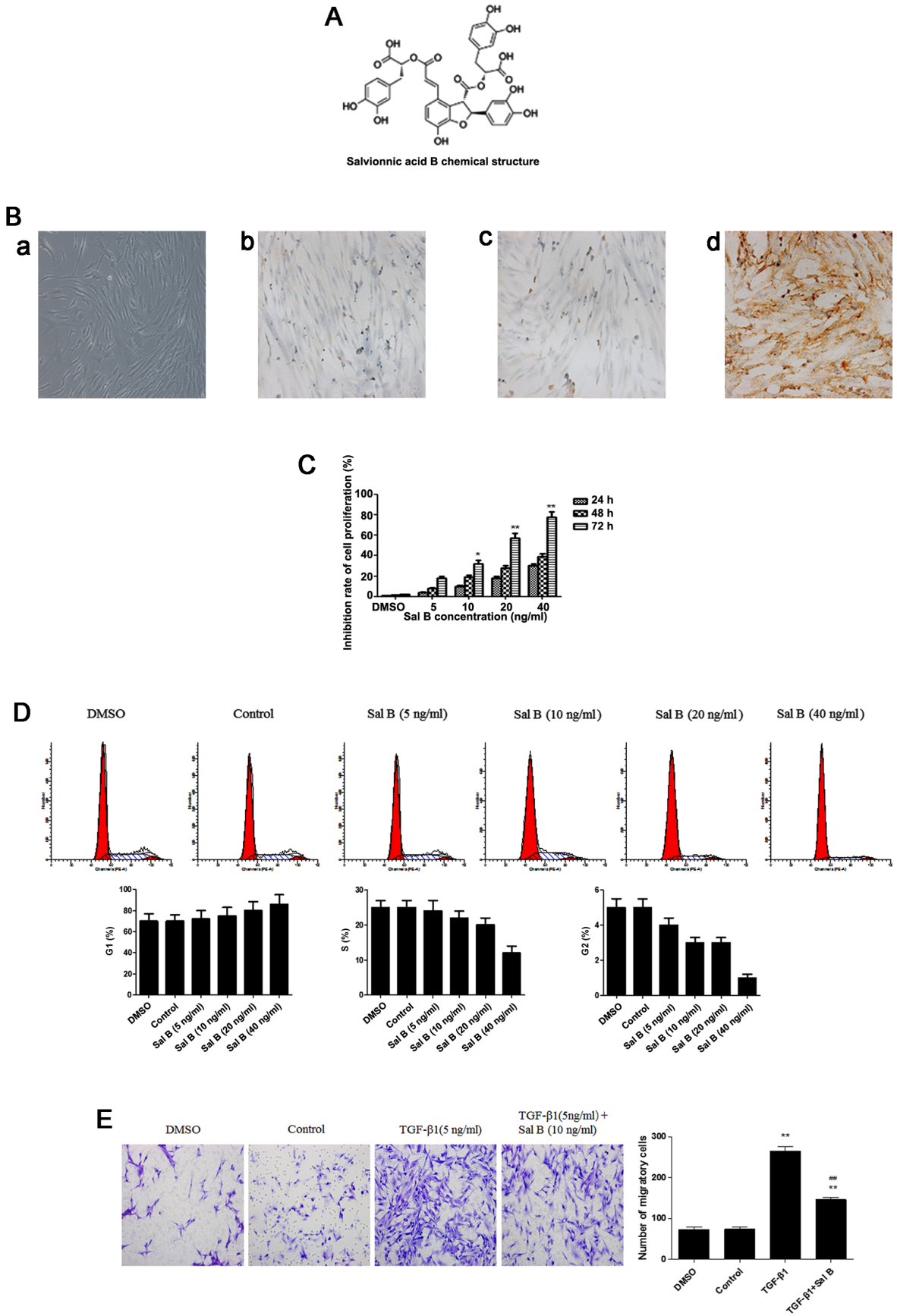

Figure 1. Sal B inhibits cell viability and TGF-β1-induced cell migration in NPFs. (A) Chemical structure of Sal B. (B-a) NPF morphology observed using an optical microscope. Immunohistochemical staining was performed to identify NPFs using (B-b) PBS (control), (B-c) pan-cytokeratin as a negative marker and (B-d) vimentin as a positive marker. Magnification, x400. (C) NPF viability following treatment with Sal B for 24, 48 or 72 h. NPFs were treated with 10 ng/ml Sal B and/or 5 ng/ml TGF-β1 for 48 h. (D) Cell cycle distribution was detected by performing flow cytometry. (E) Cell migration was assessed by performing Transwell migration assays. Magnification, x100. Data are presented as the mean ± SD of three independent experiments. *P<0.05 and **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. TGF-β1. Sal B, salvianolic acid B; NPF, nasal polyp fibroblast.

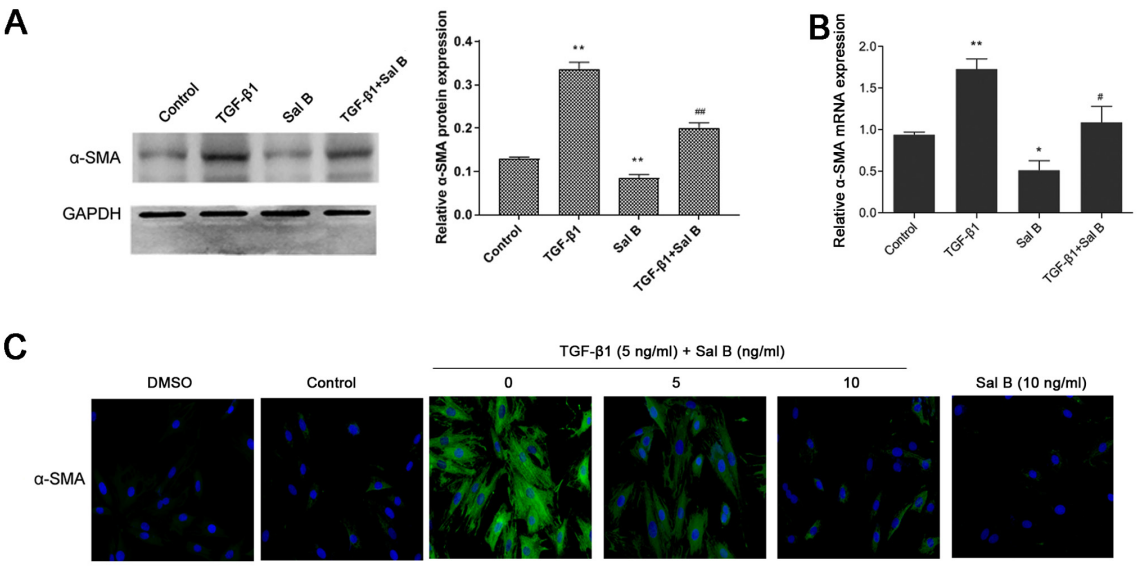

Figure 2. Effect of Sal B on NPF myofibroblast differentiation. NPFs were treated with 10 ng/ml Sal B and/or 5 ng/ml TGF-β1. (A) α-SMA protein expression levels were measured via western blotting. (B) α-SMA mRNA expression levels were measured via reverse transcription-quantitative PCR. (C) Representative immunocytochemistry images for α-SMA staining (green) with nuclear DAPI staining (blue). Magnification, x100. Data are presented as the mean ± SD of three independent experiments. *P<0.05 and **P<0.01 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. TGF-β1. Sal B, salvianolic acid B; NPF, nasal polyp fibroblasts; α-SMA, α-smooth muscle actin; Ctr, control.

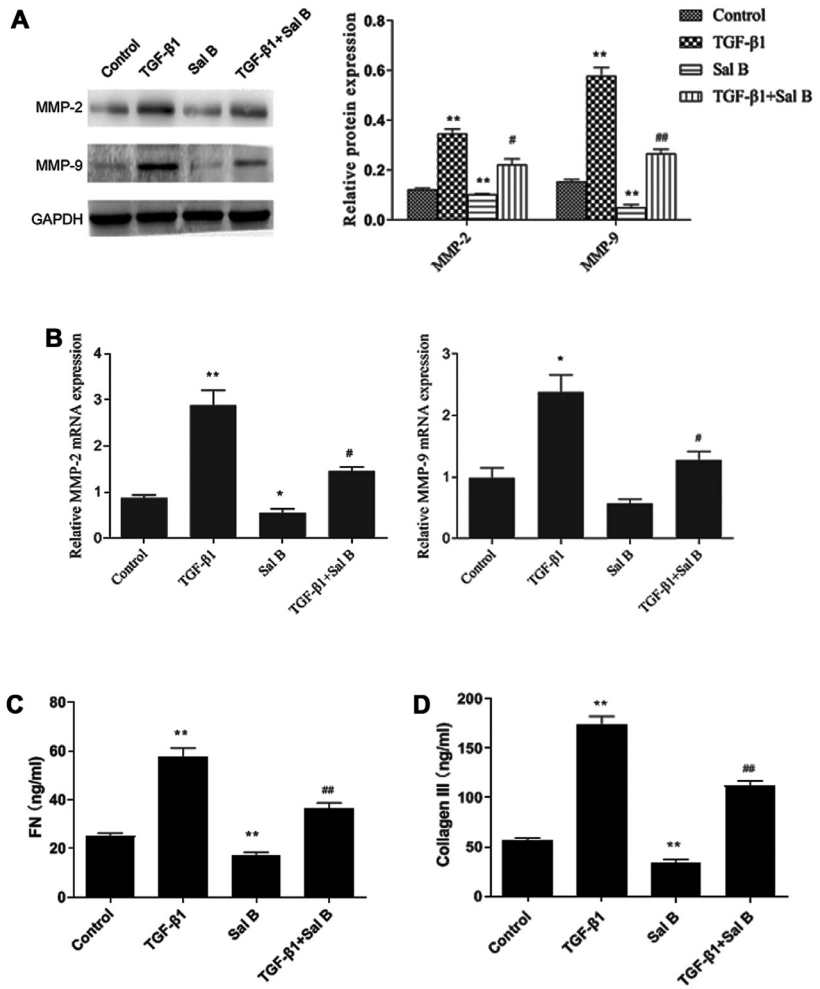

Figure 3. Sal B blocks TGF-β1-induced extracellular matrix and collagen production in NPFs. NPFs were treated with 10 ng/ml Sal B and/or 5 ng/ml TGF-β1. (A) MMP-2 and MMP-9 protein expression levels were measured via western blotting. (B) MMP-2 and MMP-9 mRNA expression levels were measured via reverse transcription-quantitative PCR. (C) FN and (D) collagen III secretion was quantified by ELISA. Data are presented as the mean ± SD of three independent experiments. *P<0.05 and **P<0.01 vs control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. TGF-β1. Sal B, salvianolic acid B; NPF, nasal polyp fibroblast; MMP, matrix metallopeptidase; Ctr, control; FB, fibronectin.

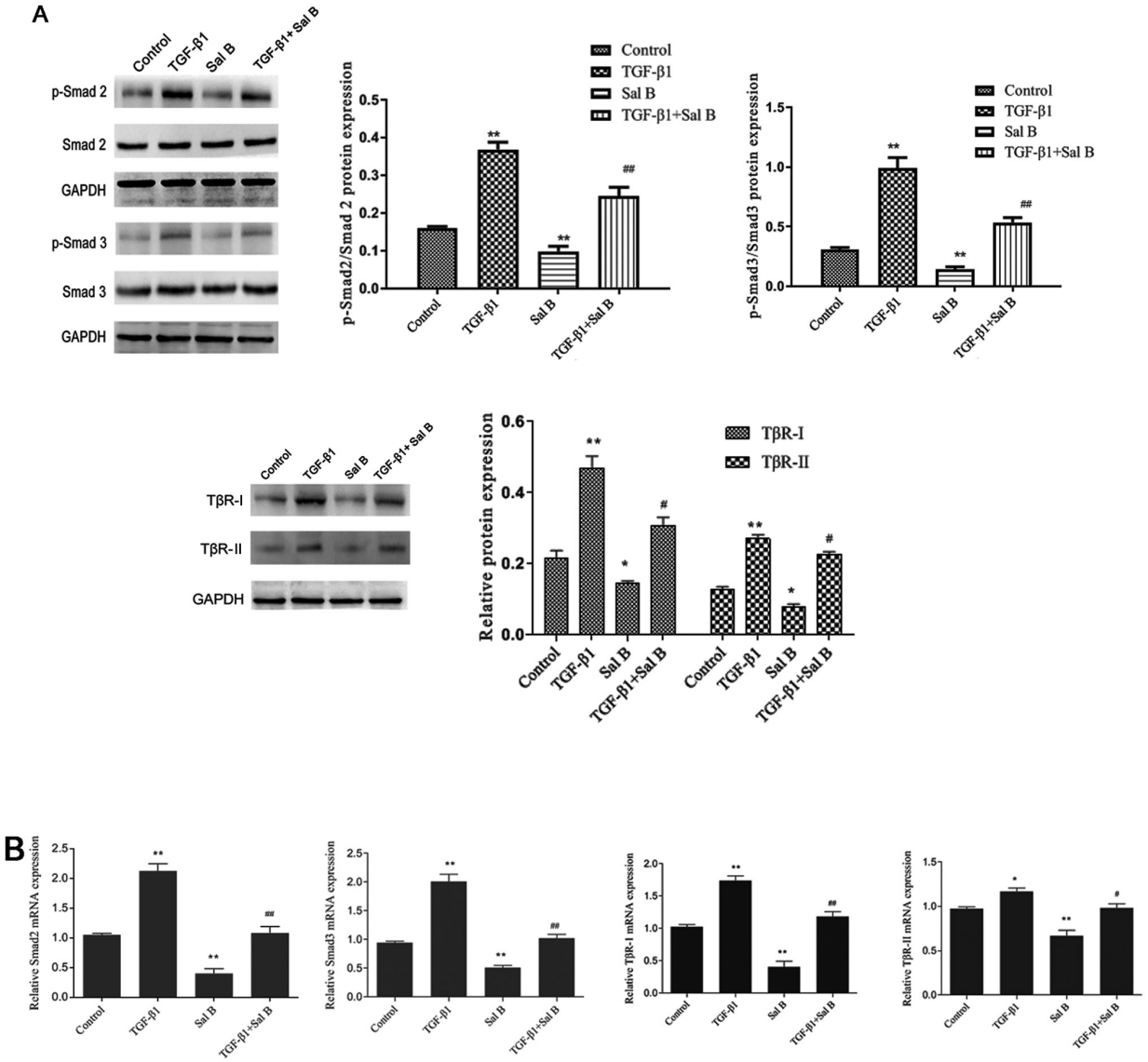

Figure 4. Sal B inhibits NPF myofibroblast differentiation via the TGF-β1 signaling pathway. NPFs were treated with 10 ng/ml Sal B and/or 5 ng/ml TGF-β1. (A) TβR-I, TβR-II, p-Smad2/Smad2 and p-Smad3/Smad3 protein expression levels were measured via western blotting. (B) Smad2, Smad3, TβR-I and TβR-II mRNA expression levels were measured via reverse transcription-quantitative PCR. Data are presented as the mean ± SD of three independent experiments. *P<0.05 and **P<0.01 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. TGF-β1. Sal B, salvianolic acid B; NPF, nasal polyp fibroblast; TβR, TGF-β1 receptor; p, phosphorylated; Ctr, control.

the cytoplasm of TGF-β1-stimulated NPFs, whereas Sal B markedly inhibited α-SMA expression in TGF-β1-stimulated NPFs (Fig. 2C). These results suggested that Sal B inhibited TGF-β1-induced NPF myofibroblast differentiation.

Sal B blocks TGF-β1-induced ECM and collagen production in NPFs. The present study investigated whether Sal B blocked ECM production in NPFs. TGF-β1 treatment significantly increased MMP-2 and MMP-9 expression levels compared with in the control group. Sal B treatment significantly decreased MMP-2 and MMP-9 mRNA and protein expression levels in TGF-β1-treated NPFs, with a larger inhibitory effect detected on MMP-9 expression compared with MMP-2 expression (Fig. 3A and B). In addition, the ELISA results demonstrated that compared with in the control group, collagen III and fibronectin levels were significantly increased by TGF-β1, and Sal B significantly inhibited TGF-β1-induced effects (Fig. 3C and D). These results suggested that Sal B inhibited TGF-β1-induced ECM accumulation and collagen production in NPFs.

Sal B inhibits NPF myofibroblast differentiation via the TGF-β1 signaling pathway. Smad2/3 is one of the primary transcription factors in the TGF-β1 signaling pathway (8). To determine the mechanism underlying the inhibitory effects of Sal B on myofibroblast differentiation and ECM accumulation, the present study measured TβR-I, TβR-II and Smad2/3 expression levels (Fig. 4). After stimulation with TGF-β1, p-Smad2/3 protein expression levels were significantly increased in NPFs compared with in control cells. Moreover, Sal B significantly decreased p-Smad2/3 protein levels in NPFs treated with or without TGF-β1. The western blotting results indicated that compared with the control group, total Smad2/3 expression levels were not notably altered by TGF-β1 or Sal B. Compared

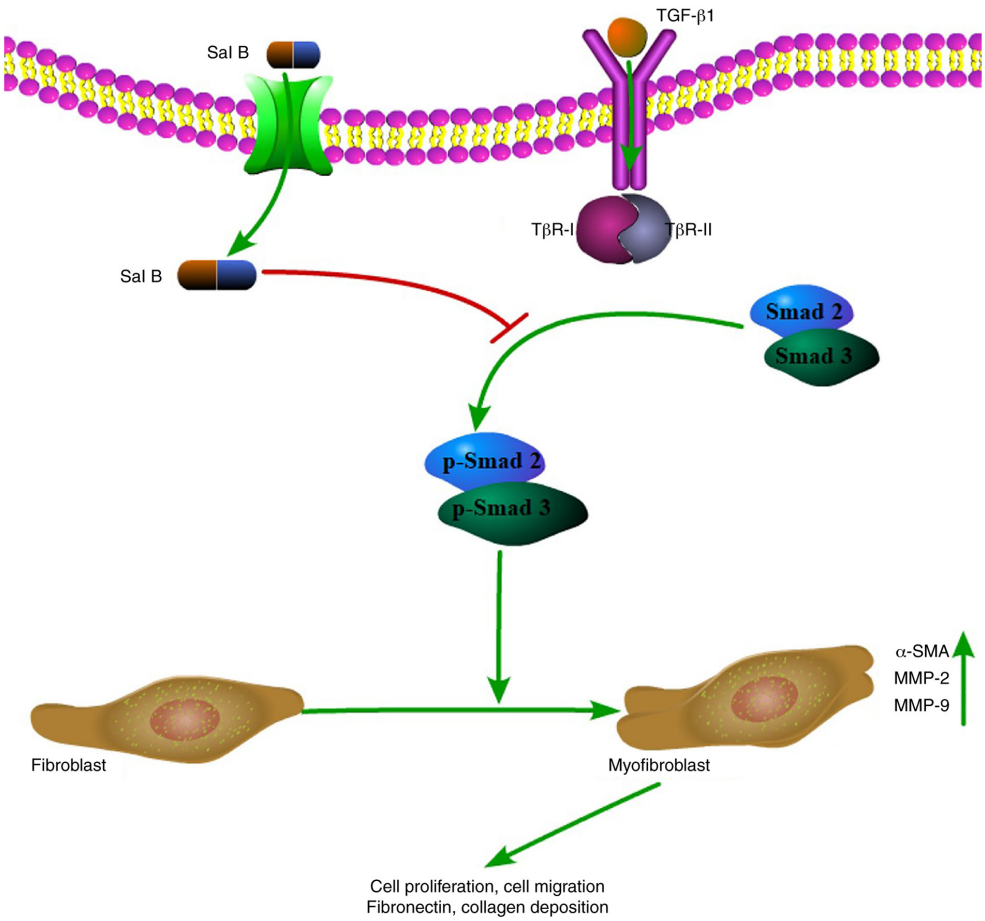

Figure 5. Sal B serves an anti-fibrotic role in nasal polyp fibroblasts via the TGF-β1 signaling pathway. Sal B, salvianolic acid B; TβR, TGF-β1 receptor; p, phosphorylated; MMP, matrix metallopeptidase; α-SMA, α-smooth muscle actin.

with in the control group, TGF-β1 significantly increased TβR-I and TβR-II expression levels, increasing TβR-I expression to a higher level compared with TβR-II. Sal B significantly decreased TβR-I and TβR-II expression levels in NPFs treated with or without TGF-β1. These results suggested that Sal B inhibited myofibroblast differentiation and ECM accumulation via the TGF-β1 signaling pathway.

Discussion

CRSwNP develops due to abnormal growth of the mucous membranes of the nasal cavity or paranasal sinuses (23), with the majority of research indicating that airway remodeling and complex inflammatory reactions are involved in the underlying mechanisms (24-27). The current treatment for CRSwNP comprises short-term oral steroids and long-term topical steroids (1). Other forms of anti-inflammatory and antiproliferative agents, including traditional Chinese extracts, have been studied as they display low toxicity and minimal side effects (28). It was hypothesized that the antifibrotic effect of Sal B occurred via inhibition of the TGF-β1 signaling pathway (17,19,20).

To the best of our knowledge, the present study reported the role and potential molecular mechanisms underlying Sal B in blocking airway remodeling by inhibiting NPF myofibroblast differentiation and ECM production for the first time. A previous studies have demonstrated that TGF-β1 may be involved in the production of α-SMA protein and the accumulation of ECM, and could be considered an inducer of myofibroblast differentiation (29). Therefore, blocking TGF-β1 activity could inhibit ECM deposition and regulate the fibrotic process of nasal polyp formation (29). The present study demonstrated that Sal B suppressed TGF-β1-induced α-SMA expression levels, and fibronectin and collagen III levels. Furthermore, Sal B significantly downregulated TGF-β1-induced TβR-I, TβR-II and p-Smad2/3 expression levels. Collectively, the results indicated that Sal B regulated the TGF-β1 signaling pathway, which may serve as the mechanism underlying the antifibrotic actions of Sal B (Fig. 5).

MMPs are key players in the process of airway remodeling by restructuring basement membranes and regulating extracellular components (30). Several studies have suggested that when patients with CRSwNP are infected with *Staphylococcus aureus*, MMP secretion increases and nasal polyp formation may also be affected (6,30,31). Thus, the present study demonstrated that Sal B decreased TGF-β1-induced MMP-2 and MMP-9 mRNA and protein expression levels in NPFs, which was consistent with the results of previous studies (6,32,33). Collectively, the results of the present study suggested that the anti-inflammatory effect of Sal B might be associated with NPF myofibroblast differentiation and ECM production.

In summary, the present study indicated that Sal B mediated effects via multiple molecular mechanisms to inhibit the development of nasal polyposis due to its anti-inflammatory activity. Therefore, Sal B may serve as a novel therapeutic target for nasal polyposis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZS contributed to study design and wrote the manuscript. DL, SW and RH performed the data analysis. PD participated in drafting the work and data analysis and revised it critically for important intellectual content. All authors read and approved the final manuscript. ZS, DL, SW, RH and PD confirm the authenticity of the data.

Ethics approval and consent to participate

The present study was approved by the ethics committee of Shanghai First People's Hospital (approval no. 2018KY008). All patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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