

# Psoralen inhibits the inflammatory response and mucus production in allergic rhinitis by inhibiting the activator protein 1 pathway and the downstream expression of cystatin-SN

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**Abstract.** Psoralen (PSO) exerts anti-inflammatory pharmacological effects and plays an important role in a variety of inflammatory diseases. However, the effects of PSO with allergic rhinitis (AR) are yet to be reported. In the present study, an *in vitro* AR model was generated by inducing JME/CF15 human nasal epithelial cells with IL-13, after which MTT was used to assess the cytotoxicity of PSO. The expression levels of inflammatory cytokines (granulocyte-macrophage colony-stimulating factor and Eotaxin) were determined by ELISA. Furthermore, the expression of inflammatory IL-6 and -8, as well as mucin 5AC, was assessed by reverse transcription-quantitative PCR and western blotting, and cellular reactive oxygen species were detected using a 2',7'-dichlorodihydrofluorescein diacetate fluorescent probe. Western blotting was also used to detect the expression and phosphorylation of c-Fos and c-Jun in the activator protein 1 (AP-1) pathway, as well as the expression of cystatin-SN (CST1). PSO inhibited the inflammatory response and mucus production in IL-13-induced JME/CF15 cells. Furthermore, the levels of c-Fos and c-Jun phosphorylation in the AP-1 pathway were decreased in IL-13-induced JME/CF15 cells following PSO treatment. The expression of pathway proteins was activated by the addition of PMA, an AP-1 pathway activator, which concurrently reversed the inhibitory effects of PSO on the inflammatory response and mucus formation. The addition of an AP-1 inhibitor (SP600125) further inhibited pathway activity, and IL-13-induced inflammation and mucus formation was restored. In conclusion, PSO regulates the

expression of CST1 by inhibiting the AP-1 pathway, thus suppressing the IL-13-induced inflammatory response and mucus production in nasal mucosal epithelial cells.

## Introduction

Allergic rhinitis (AR) is a common and frequently recurring disease resulting from exposure to associated allergens, which promotes allergic reactions that lead to nasal inflammation (1). Its main manifestation is the inflammatory reaction of cells and the generation of mucus (2,3). The clinical manifestations of AR are sneezing, nasal itching, a runny nose, nasal congestion and itchy eyes, which seriously affect patient quality of life (4). At present, clinical treatments for AR include histamine receptor antagonists, hormones and anticholinergic agents (5). Although the disease can be alleviated or controlled, currently available treatments are restricted by considerable side-effects, drug resistance and high recurrence rates (6). Therefore, the identification of appropriate treatments for AR has become a focus, as well as a challenge, in the otolaryngology department.

Psoralen (PSO), derived from the fruit of leguminous plants, belongs to the group of furanocoumarin compounds, and is one of the most important active components of the psoralen family (7). PSO is used in various prescriptions for tonifying the kidney and strengthening bones, and modern pharmacology has demonstrated that PSO exerts anti-inflammatory, antioxidant, antitumor and other pharmacological effects (8). PSO has been shown to reduce the expression of TGF- $\beta$ , IL-1 $\beta$  and TNF- $\alpha$  in pulmonary fibrosis models (9). Furthermore, PSO exerts a significant anti-inflammatory response, as well as protecting and activating chondrocytes to relieve osteoarthritis (10). Therefore, it was speculated that PSO may also inhibit the inflammatory response, and thus alleviate AR.

Activator protein 1 (AP-1) is an intracellular transcriptional activator composed primarily of proto-oncogene-encoded proteins Jun and Fos, which bind target DNA sequences in the form of homologous or heterodimer complexes, thus regulating the expression of target genes (11). AP-1 is a key regulator of cellular proliferation, differentiation and apoptosis (12). As AP-1 can act as a molecular switch at the transcriptional level,

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the AP-1 signal transduction pathway can be activated by changes in cellular tension, ionization effects, DNA damage, oxidative stress and UV irradiation, as well as bacterial and viral infection (13). Activated AP-1 subsequently binds to the TPA response element (TRE) to promote the expression of a variety of inflammatory factors (including IL-2, IL-8, TNF- $\alpha$ , TGF- $\beta$ 1 and IFN- $\gamma$ ), affecting physiological cell functions and in turn, influencing the occurrence of certain inflammatory diseases (14), such as inflammatory skin disease (15) and chronic obstructive pulmonary disease (16). According to the Gene Expression Omnibus (GEO) database, the expression of cystatin-SN (CST1) in individuals with AR is significantly upregulated compared with that in healthy controls (GSE19187) (17). Moreover, in patients with AR, mitogen-activated protein kinases can induce transcription factors of AP-1, thereby regulating the expression of Charcot-Leyden crystal protein or CST1 (18). However, the role of the AP-1 pathway in AR is yet to be reported. In addition, a literature review indicated that PSO regulates AP-1 pathway activation and promotes osteoclast differentiation and bone resorption (19). As IL-13 induces the release of inflammatory cytokines and excessive mucus secretion, a human nasal epithelial cell model of IL-13-induced AR has been developed and is widely used for *in vitro* research (20,21). Therefore, the aim of the present study was to investigate the role and underlying mechanisms of PSO with AR, so as to provide a theoretical basis for the treatment of AR.

## Materials and methods

**Cell culture.** The JME/CF15 nasal epithelial cell line was obtained from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences, and cultured at 37°C (5% CO<sub>2</sub>) in DMEM supplemented with 10% FBS (both Gibco; Thermo Fisher Scientific, Inc.) and 1% (v/v) penicillin/streptomycin (Sigma-Aldrich; Merck KGaA) at a density of 5x10<sup>6</sup> cells per well.

**Reagents.** PSO (batch no. 110739-201115; National Institutes for Food and Drug Control) was dissolved in DMSO (Sigma-Aldrich; Merck KGaA) to prepare a psoralen-conditioned medium stock solution. Then, 100, 20, 10 and 1  $\mu$ M working stocks of PSO were prepared with low-glucose DMEM containing 10% FBS. After 2 h of PSO pretreatment, JME/CF15 cells were stimulated with 10 ng/ml IL-13 for 24 h at 37°C to generate a cell-based AR model. In this paper, cells were pretreated with 10 nM PMA for 24 h and 15  $\mu$ l SP600125 for 24 h at 37°C, as outlined in previous studies (22,23). After giving different concentrations (1, 10 and 20  $\mu$ M) of PSO, the cells were divided into the control, IL-13, 1  $\mu$ M PSO + IL-13, 10  $\mu$ M PSO + IL-13 and 20  $\mu$ M PSO + IL-13 groups. The control group was given the same dose of DMEM. In another set of experiments, a concentration of 20  $\mu$ M PSO was selected, and the cells were divided into the control, IL-13, PSO + IL-13, PMA + PSO + IL-13 and SP600125 + PMA + PSO + IL-13 groups.

**Database.** According to the GEO (<https://www.ncbi.nlm.nih.gov/geo/>) database, the expression level of CST1 in individuals with AR was detected (GSE19187).

**Cell Counting Kit-8 (CCK-8) assay.** The CCK-8 system (Dojindo Molecular Technologies, Inc.) was used to assess cell viability. Cells were seeded into 96-well plates at a density of 5x10<sup>3</sup> cells per well. After the relevant treatment, 10  $\mu$ l CCK-8 solution was added to each well and incubated for 2 h, after which cell viability was assessed using a Benchmark microplate spectrometer (Bio-Rad Laboratories, Inc.).

**ELISA.** Quantification of granulocyte-macrophage colony-stimulating factor (GM-CSF; cat. no. SGM00; R&D Systems, Inc.) and Eotaxin (cat. no. MME00; R&D Systems, Inc.) in cell supernatants (300 x g; 4°C; 10 min) was performed using an ELISA kit according to the manufacturer's instructions (24).

**Reverse transcription-quantitative (RT-q)PCR.** Cells (1x10<sup>3</sup> cells/well) were cultured in 6-well plates and total RNA was extracted using TRIzol<sup>®</sup> reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA (1  $\mu$ g) was reverse-transcribed into first-strand complementary DNA using the SuperScript<sup>™</sup> III Reverse Transcriptase kit (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol, and then amplified in triplicate by qPCR (cobas Z 480 system; Roche Diagnostics) using SYBR Green (final reaction volume, 20  $\mu$ l; Takara Biotechnology Co., Ltd.), according to the manufacturer's protocol. The following thermocycling conditions were used for qPCR: Initial denaturation at 95°C for 10 min; followed by 40 cycles of 95°C for 10 sec and 60°C for 60 sec. The following primers (GenScript) were used for qPCR: GM-CSF forward, 5'-CAGCCACTACAAGCAGCACT-3' and reverse, 5'-GGG GATGACAAGCAGAAAGT-3'; Eotaxin forward, 5'-TGT CTCGTTCTCCCTCTGCT-3' and reverse, 5'-CTCCGCTCA CAGTCATTTCC-3'; IL-6 forward, 5'-GGCCCTTGCTTT CTCTTCG-3' and reverse, 5'-ATAATAAAGTTTTGATTA TGT-3'; IL-8 forward, 5'-ATGGCTGCTGAACCAGTAGA-3' and reverse, 5'-CTAGTCTTCGTTTTGAACAG-3'; mucin 5AC (MUC5AC) forward, 5'-ATCACCGAAGGCTGCTTC TGTC-3' and reverse, 5'-GTTGATGCTGCACACTGTCCA A-3'; and GAPDH forward, 5'-AGCCACATCGCTCAGACA C-3' and reverse, 5'-GCCCAATACGACCAATCC-3'. When evaluating the effects of treatment, the expression level of each control was assigned an arbitrary value of 1, and those of the treated cells were evaluated as a fold-change above the control, and calculated using the 2<sup>- $\Delta\Delta$ C<sub>q</sub></sup> method (25). GAPDH was used as the internal control gene.

**Western blotting.** Total cellular protein was extracted using RIPA buffer, and quantified using a BCA assay (both Beyotime Institute of Biotechnology). Cell lysates containing 50-100  $\mu$ g protein were resolved by electrophoresis on 10% SDS-PAGE gels (Beyotime Institute of Biotechnology). The separated proteins were subsequently transferred to PVDF membranes (Thermo Fisher Scientific, Inc.), which were then blocked in 5% non-fat milk for 1 h at room temperature, and subsequently incubated overnight at 4°C in 0.25% non-fat milk containing the appropriate primary antibodies. Primary antibodies against the following targets were used in the present study: IL-6 (1:1,000; cat. no. ab6672), IL-8 (1:1,000; cat. no. ab18672), MUC5AC (1:1,000; cat. no. ab3649), phosphorylated (p)-c-Fos (1:1,000;

cat. no. ab27793), c-Fos (1:1,000; cat. no. ab222699), p-c-Jun (1:1,000; cat. no. ab30620), c-Jun (1:1,000; cat. no. ab32137), CST1 (1:1,000; cat. no. ab124281) and GAPDH (1:1,000; cat. no. ab8245). All primary antibodies were purchased from Abcam. On the second day, the membranes were incubated with the secondary antibody (1:5,000; cat. no. ab150077; Abcam) for 2 h at room temperature. The protein bands were visualized using enhanced chemiluminescence reagents (Thermo Fisher Scientific, Inc.). Protein band intensity was determined using ImageJ software (version 146; National Institutes of Health).

**Quantification of reactive oxygen species (ROS).** Cellular ROS levels were detected using a fluorescent probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA; Sigma-Aldrich; Merck KGaA), which is rapidly oxidized into the highly fluorescent 2',7'-dichlorofluorescein (DCF) in the presence of intracellular ROS. Fluorescence was monitored with a laser scanning confocal microscope (magnification, x200; Leica Microsystems GmbH) at 488 nm. ROS levels were quantified as the relative fluorescence intensity of DCF per cell in the scanned area.

**Statistical analysis.** All experiments were repeated three times. The data were analyzed using SPSS version 19.0 (IBM Corp) and GraphPad 6.0 (GraphPad Software, Inc.), and are presented as the mean  $\pm$  SD. 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

**PSO inhibits inflammation in IL-13-induced JME/CF15 cells.** JME/CF15 cells were induced using different concentrations of PSO (0, 1, 10, 20 and 100  $\mu$ M), and cell viability was assessed using an MTT assay. As shown in Fig. 1A, normal cells exhibited marked damage following treatment with 100  $\mu$ M PSO; therefore, a concentration gradient of 1, 10, and 20  $\mu$ M PSO was used for subsequent experimentation. The cells were divided into the control, IL-13, 1  $\mu$ M PSO + IL-13, 10  $\mu$ M PSO + IL-13 and 20  $\mu$ M PSO + IL-13 groups, and inflammatory responses were detected by ELISA. Compared with the control group, the expression levels of GM-CSF (Fig. 1B and C) and Eotaxin (Fig. 1D and E) were significantly increased in the IL-13 group, indicating that an inflammatory response had occurred. Compared with the IL-13 group, GM-CSF and Eotaxin expression in the 1  $\mu$ M PSO + IL-13, 10  $\mu$ M PSO + IL-13 and 20  $\mu$ M PSO + IL-13 groups were downregulated in a dose-dependent manner. RT-qPCR (Fig. 1F) and western blotting (Fig. 1G) were used to detect the mRNA and protein expression of IL-6 and -8, and the expression trends were the same as those observed for GM-CSF and Eotaxin. Cellular ROS expression was detected using a DCFH-DA probe, and was found to be increased in the IL-13 group compared with the control group. Compared with the IL-13 group, ROS expression in the PSO-treated groups was decreased in a dose-dependent manner (Fig. 1H and I). These results indicated that PSO exerted dose-dependent inhibition of the IL-13-induced inflammatory response in JME/CF15 cells.

**PSO inhibits mucus production in IL-13-induced JME/CF15 cells.** Cellular expression of MUC5AC, a representative mucus-producing protein, was also detected to determine whether PSO was able to influence IL-13-induced mucus production. Compared with the control group, MUC5AC expression was significantly increased in the IL-13 group, and decreased in a dose-dependent manner following treatment with different concentrations of PSO (Fig. 2A and B). The results confirmed that IL-13 induced mucus production in JME/CF15 cells, which was subsequently inhibited by PSO.

**PSO inhibits inflammation and mucus production in IL-13-induced JME/CF15 cells by suppressing the AP-1 signaling pathway.** In the present study, phosphorylation levels of the AP-1 pathway-associated proteins c-Fos and c-Jun were found to be abnormally altered. Compared with the control group, the levels of p-c-Fos and p-c-Jun were significantly increased in the IL-13 group, indicating that the AP-1 pathway had been activated. After the addition of PSO, the levels of p-c-FOS and p-c-Jun were decreased in a dose-dependent manner, compared with those in the IL-13 group (Fig. 3A). Subsequently, the AP-1 pathway activator PMA and the pathway inhibitor SP600125 were used to further assess whether the regulatory effects of PSO on AR were achieved through the AP-1 pathway. A concentration of 20  $\mu$ M PSO was selected, and the cells were divided into the control, IL-13, PSO + IL-13, PMA + PSO + IL-13 and SP600125 + PMA + PSO + IL-13 groups. Western blot analysis was used to detect the expression of AP-1 pathway proteins. Compared with the PSO + IL-13 group, the levels of p-c-Fos and p-c-Jun were significantly increased in the PMA + PSO + IL-13 group (Fig. 3B), and the expression of GM-CSF and Eotaxin were also increased (Fig. 4A-D). Furthermore, the expression levels of IL-6 and -8 (Fig. 4E and F), ROS (Fig. 4G and H) and MUC5AC (Fig. 5A and B) were all increased. These results indicated that PMA reversed the inhibitory effects of PSO on p-c-FOS, p-c-Jun, IL-13-induced inflammation and mucus production in JME/CF15 cells. Compared with the PMA + PSO + IL-13 group, the levels of c-Fos and c-Jun phosphorylation in the SP600125 + PMA + PSO + IL-13 group were reduced (Fig. 3B), and the expression of GM-CSF, Eotaxin (Fig. 4A-D), inflammatory cytokines IL-6 and IL-8 (Fig. 4E and F), ROS (Fig. 4G and H) and MUC5AC (Fig. 5A and B) were all decreased. These results suggested that PSO suppressed inflammation and mucus production in IL-13-induced JME/CF15 cells by inhibiting the AP-1 signaling pathway.

**PSO downregulates CST1 expression by inhibiting the AP-1 signaling pathway.** CST1 is a targeted regulator of the AP-1 pathway (6). In the present study, the expression of CST1 in IL-13-induced cells was significantly increased compared with that in the control group. Compared with the IL-13 group, the expression of CST1 in the PSO + IL-13 group was decreased, indicating that PSO inhibited the expression of CST1. Furthermore, compared with the PSO + IL-13 group, CST1 expression was increased in the PMA + PSO + IL-13 group, and compared with the PMA + PSO + IL-13 group, CST1 expression was decreased in the SP600125 + PMA + PSO + IL-13 group (Fig. 6). Collectively, these findings indicated that PSO

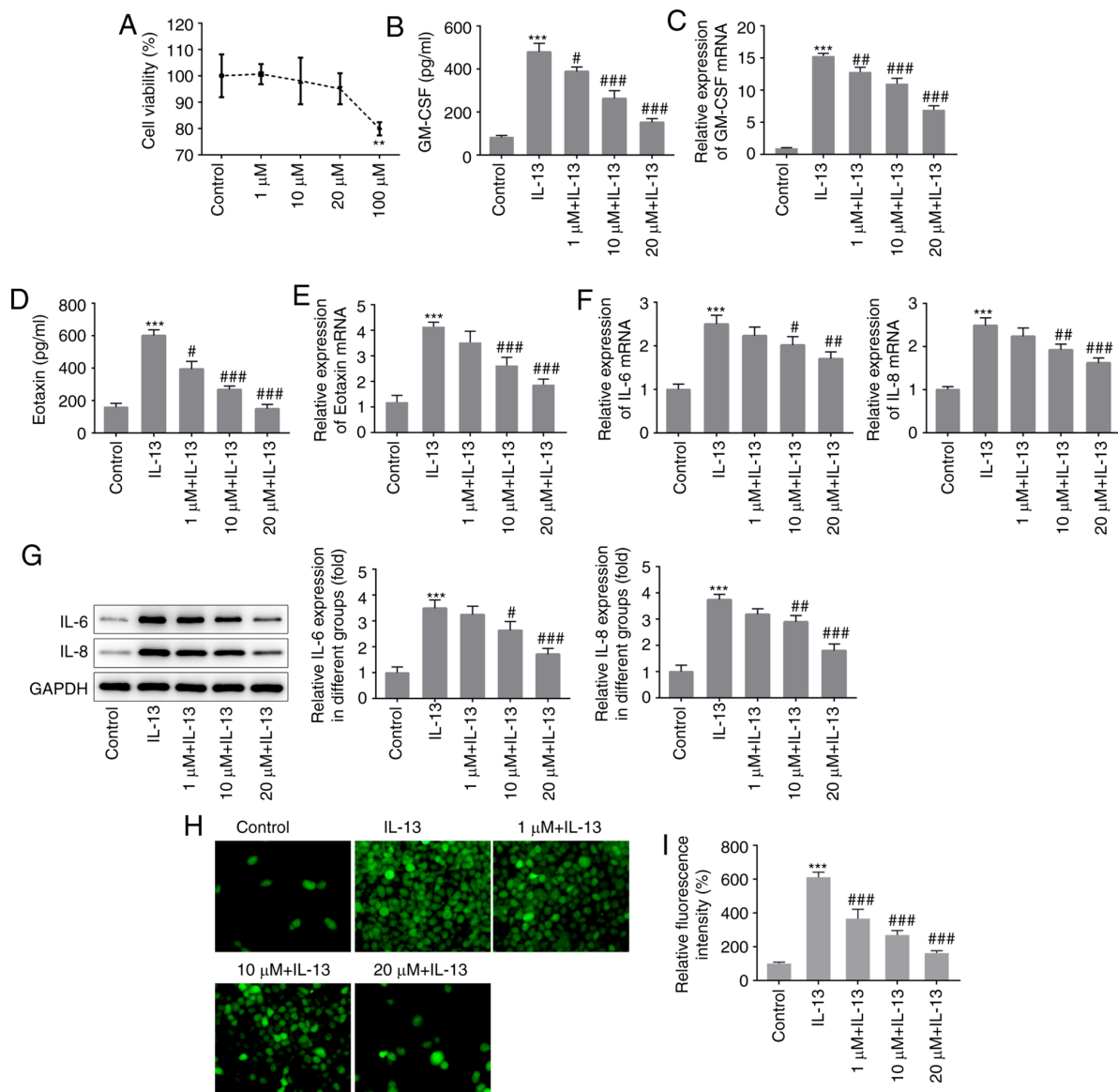


Figure 1. PSO inhibits inflammation in IL-13-induced JME/CF15 cells. (A) A Cell Counting Kit-8 assay was performed to detect cell viability (n=5). The expression of GM-CSF was detected via (B) ELISA and (C) RT-qPCR (n=5). The expression of Eotaxin was detected via (D) ELISA and (E) RT-qPCR. (F) RT-qPCR was performed to determine the expression of IL-6 and IL-8 (n=5). (G) Western blotting was conducted to detect the expression of IL-6 and IL-8 (n=5). (H) The expression of reactive oxygen species was detected using a 2',7'-dichlorodihydrofluorescein diacetate fluorescent prob (n=3). (I) Statistical analysis of fluorescence intensity. n=3. \*\*P<0.01, \*\*\*P<0.001 vs. control group; #P<0.05, ##P<0.01, ###P<0.001 vs. IL-13 group. PSO, psoralen; GM-CSF, granulocyte-macrophage colony-stimulating factor; RT-qPCR, reverse transcription-quantitative PCR.

downregulated the expression of CST1 by inhibiting the AP-1 signaling pathway, thus suppressing the IL-13-induced inflammatory response and mucus production in nasal mucosal epithelial cells.

## Discussion

Since allergic diseases are usually caused by a variety of inflammatory mediators, the pathological mechanisms underlying AR are complex. However, IL-13 is a human lymphoid factor that regulates inflammatory and immune responses, and a study has reported that IL-4 and -13 produced by T-helper 2 cells are the primary instigators of AR (26). In addition, IL-13 promotes mucus secretion and eosinophil production in patients with asthma (27). Therefore, an IL-13-induced model has been widely used to conduct basic

AR-associated research. The human JME/CF15 epithelial cell line induced by IL-13 has been used in previous studies to construct an AR model (28,29). Therefore, in the current study, IL-13 was used to induce inflammation and mucus production in human nasal epithelial cells. In addition, AR mainly refers to the non-infectious inflammatory disease of nasal mucosa caused by individual exposure to atopic allergens (1). The primary manifestation of AR is the inflammatory reaction of cells and the generation of mucus (2,3). Therefore, the present study focused on the detection of cellular inflammatory response and mucus production indicators, in order to determine the severity of AR.

PSO is widely used in a variety of prescriptions for tonifying the kidney and strengthening the bones. As such, modern pharmacological studies have demonstrated the anti-inflammatory, antibacterial and antioxidant



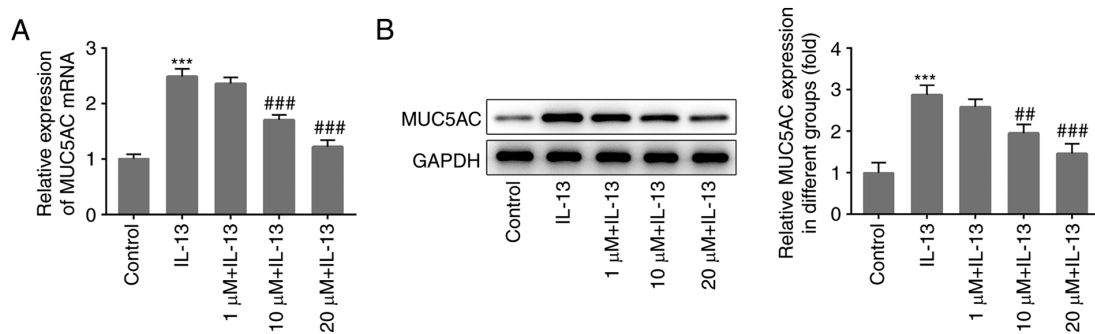


Figure 2. PSO inhibits mucus production in IL-13-induced JME/CF15 cells. (A) Reverse transcription-quantitative PCR was performed to detect the mRNA expression of MUC5AC (n=5). (B) Western blotting was performed to determine the protein expression of MUC5AC (n=3). \*\*\*P<0.001 vs. control group; ##P<0.01, ###P<0.001 vs. IL-13 group. PSO, psoralen; MUC5AC, mucin 5AC.

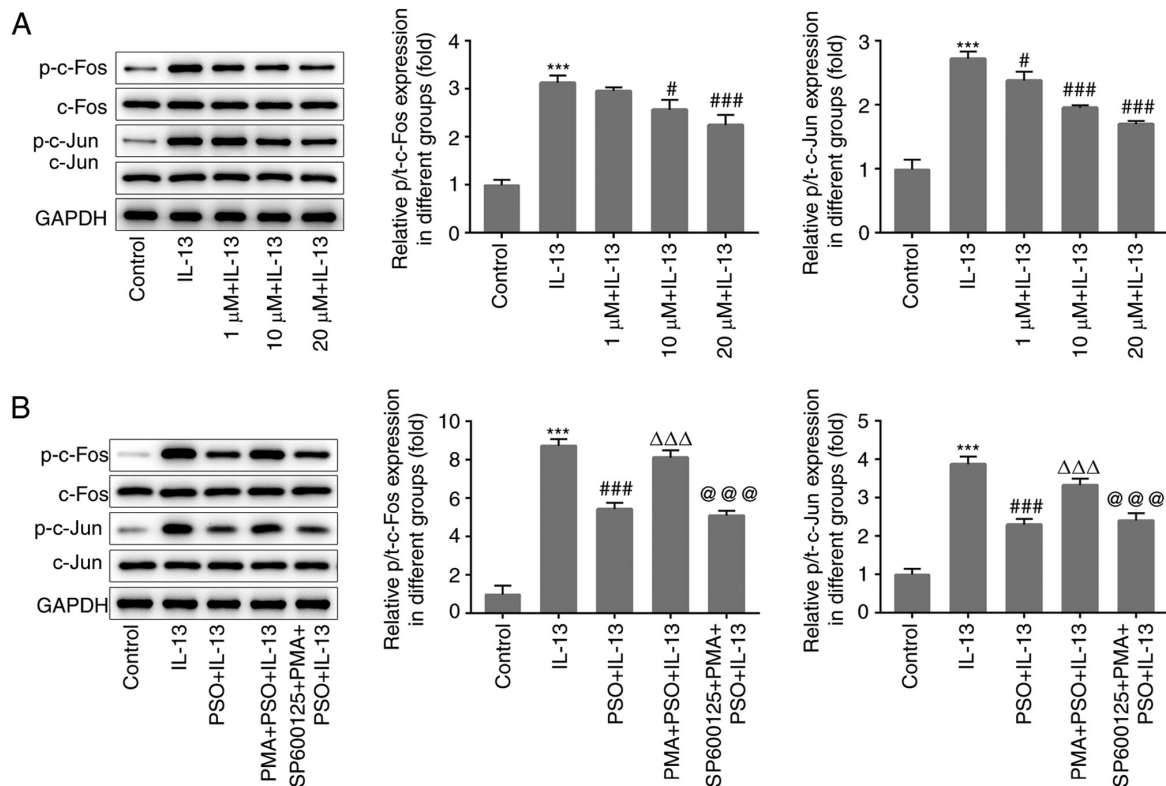


Figure 3. PSO inhibits inflammation and mucus production in IL-13-induced JME/CF15 cells by inhibiting the activator protein 1 signaling pathway. (A) Western blotting was performed to detect the protein expression of AP-1 signaling pathway after the addition of PSO (n=3). (B) Western blotting was conducted to determine the protein expression of AP-1 signaling pathway after the addition of PMA and SP600125 (n=3). \*\*\*P<0.001 vs. control group; #P<0.05, ###P<0.001 vs. IL-13 group; ΔΔΔP<0.001 vs. PSO + IL-13 group; @@@P<0.001 vs. PMA + PSO + IL-13 group. PSO, psoralen; p-, phosphorylated; t-, total.

pharmacological effects of PSO, which exerts therapeutic effects in AR (9,30). In allergic asthma, PSO can significantly inhibit inflammatory infiltration and mucus secretion in the lung tissue, and inhibit cellular IL-13 expression (31). In mice with periodontitis, PSO dose-dependently reduced mRNA expression in THP-1 cells, as well as the expression of inflammatory factors such as IL-8 (7). In the current study, PSO was found to inhibit the IL-13-induced inflammatory response, oxidative stress and mucus production. These findings suggested that PSO may have a significant therapeutic effect on AR.

In the present study, the expression levels of IL-6 and IL-8 were detected in the cells to explore the effect of PSO on the

inflammatory response in IL-13-induced JME/CF15 cells. The IL-6 and IL-8 proteins in the cell supernatant were very small and difficult to collect, so the expression of IL-6 and IL-8 in the cells was measured (32). The expression levels of IL-6 and IL-8 in cells have been detected in numerous previous studies, in which the expression of IL-6 and IL-8 in the cell supernatant was not detected (32-34).

In addition, it has been reported that IL-13 can induce the production of ROS in human bronchial epithelial cell line 16 (35). In the PM2.5-induced human nasal mucosa epithelial cell model, ROS production was also significantly increased (36). Therefore, in the present study, the expression of ROS in JME7CF15 cells induced by IL-13 was detected, and

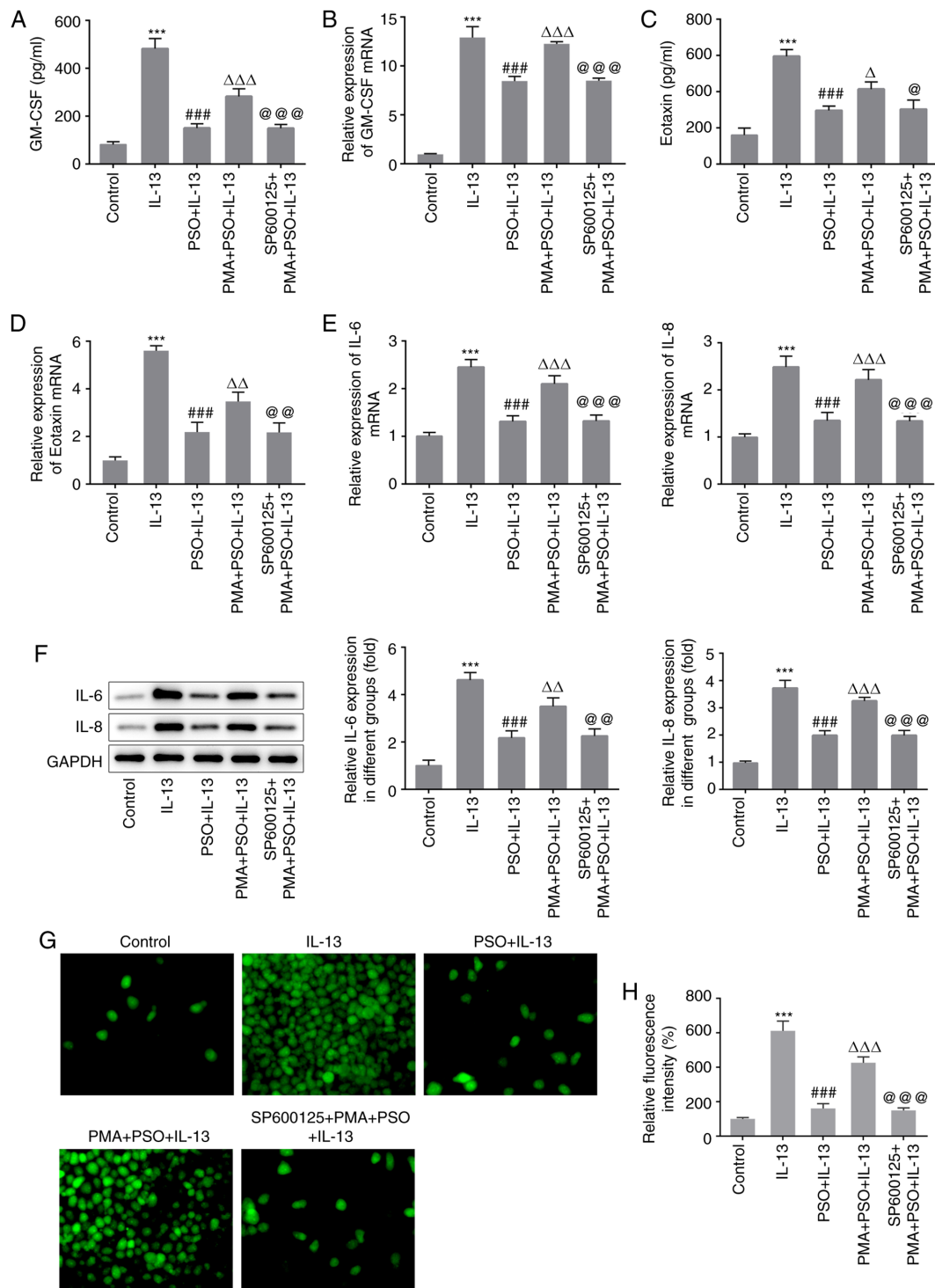


Figure 4. PSO inhibits inflammation in IL-13-induced JME/CF15 cells by inhibiting the activator protein 1 signaling pathway. The expression of GM-CSF was detected via (A) ELISA and (B) RT-qPCR (n=5). The expression of Eotaxin was detected via (C) ELISA and (D) RT-qPCR (n=5). (E) RT-qPCR was performed to determine the expression of IL-6 and IL-8 (n=5). (F) Western blotting was performed to determine the expression of IL-6 and IL-8 (n=3). (G and H) The expression of reactive oxygen species was detected using a 2,7'-dichlorodihydrofluorescein diacetate probe (n=3). \*\*\*P<0.001 vs. control group; ###P<0.001 vs. IL-13 group;  $\Delta$ P<0.05,  $\Delta\Delta$ P<0.01,  $\Delta\Delta\Delta$ P<0.001 vs. PSO + IL-13 group; @P<0.05, @@P<0.01, @@@P<0.001 vs. PMA + PSO + IL-13 group. PSO, psoralen; GM-CSF, granulocyte-macrophage colony-stimulating factor; RT-qPCR, reverse transcription-quantitative PCR.

ROS expression was found to be significantly increased after IL-13 induction, whereas ROS decreased in a dose-dependent manner after PSO administration.

Initially, the abnormal expression of AP-1 signaling pathway-related proteins was identified. Furthermore, following IL-13 induction, AP-1 pathway proteins p-c-Jun and p-c-Fos

were abnormally activated in JME/CF15 cells. A previous literature review revealed that the combination of activated AP-1 and TREs promoted the expression of a variety of inflammatory factors, thus affecting the physiological functions of cells and influencing the occurrence of certain diseases (11). In addition, AP-1 regulated the expression of IL-4, -5 and -13 by central

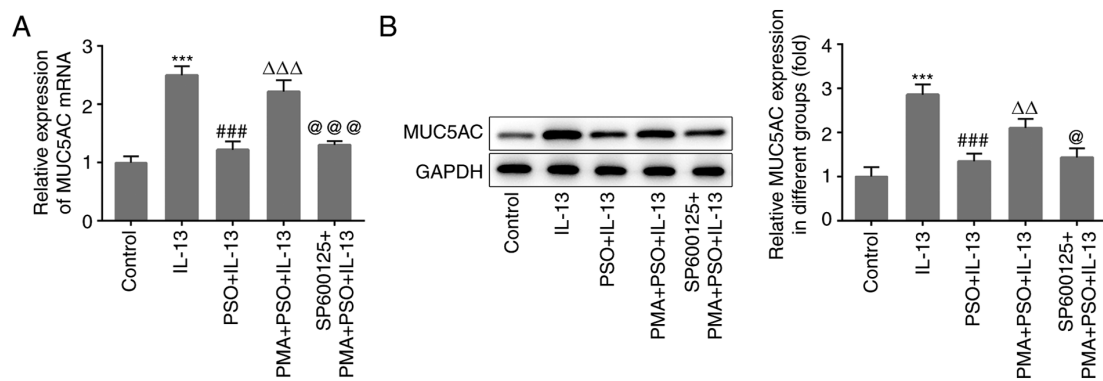


Figure 5. PSO inhibits mucus production in IL-13-induced JME/CF15 cells by inhibiting the activator protein 1 signaling pathway. (A) Reverse transcription-quantitative PCR was performed to detect the mRNA expression of MUC5AC (n=5). (B) Western blotting was performed to detect the protein expression of MUC5AC (n=3). \*\*\*P<0.001 vs. control group; ###P<0.001 vs. IL-13 group; ΔΔP<0.01, ΔΔΔP<0.001 vs. PSO + IL-13 group; @P<0.05, @@@P<0.001 vs. PMA + PSO + IL-13 group. PSO, psoralen; MUC5AC, mucin 5AC.

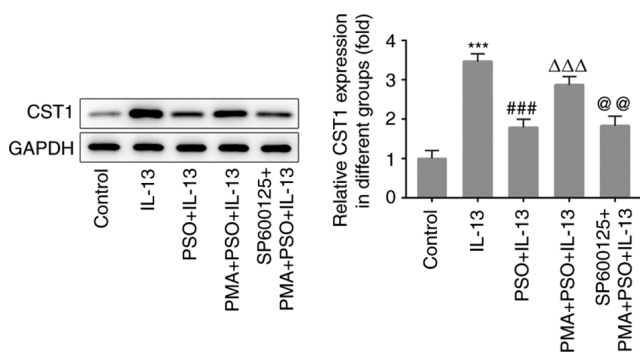


Figure 6. PSO downregulates the expression of CST1 by inhibiting the activator protein 1 signaling pathway. Western blotting was performed to detect the expression of CST1 (n=5). \*\*\*P<0.001 vs. Control group; ###P<0.001 vs. IL-13 group; ΔΔΔP<0.001 vs. PSO + IL-13 group; @@P<0.01 vs. PMA + PSO + IL-13 group. PSO, psoralen; CST1, cystatin-SN.

effector cells of airway inflammation in asthma (37). The results of the present study indicated that the AP-1 signaling pathway was activated in AR. PSO has been shown to promote osteoclast differentiation and bone resorption in osteoporosis by regulating the AP-1 signaling pathway (13). In the current study, the levels of c-Jun and c-Fos phosphorylation were dose-dependently decreased following PSO administration. The mechanism by which PSO regulates AR was further investigated using the AP-1 agonist PMA and the inhibitor SP600125. The addition of PMA was found to reverse the inhibitory effects of PSO on IL-13-induced inflammation and mucus production, while the further addition of SP600125 inhibited these processes in JME/CF15 cells. A possible explanation for this finding is that PMA and SP600125 exert opposing effects on the AP-1 pathway (thus inhibiting the effects of each other), while PSO inhibits AP-1 pathway activation, and thus suppresses IL-13-induced cellular inflammation and mucus production.

In addition, the aberrant expression of CST1 protein was also detected in the current study. Using the GEO database, CST1 protein expression was revealed to be significantly upregulated with AR (data not shown), which was also verified by *in vitro* experimentation. A study previous study indicated that the AP-1 signaling pathway regulates CST1 expression in patients with AR (12). In the present study, PSO was found

to inhibit the expression of CST1, while PMA reversed the inhibitory effects of PSO on CST1. This indicated that PSO downregulates the expression of CST1 by inhibiting AP-1 signaling, thus regulating AR.

The present study only investigated the effect of PSO on IL-13-induced nasal epithelial inflammation at the juvenile cellular level, the specific effect of PSO on AR at the animal level was not studied. This is a major limitation of this study. Our laboratory will further study the specific effect of PSO on AR *in vivo* in future experiments.

In conclusion, PSO was found to inhibit the inflammatory response and mucus production in AR by inhibiting the AP-1 pathway and the downstream expression of CST1.

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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

YX and WG wrote the manuscript and analyzed the data. ZJ and YZ carried out the experiments, supervised the present study, searched the literature and revised the manuscript. All authors read and approved the final manuscript. YX and WG confirm the authenticity of all the raw data.

## 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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