Article

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Amygdalin Alleviates Airway Inflammation and Remodeling in Asthma Mice: Involvement of TGF-β1/Smads Signaling Pathway

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Background: Asthma is a common respiratory system disease characterized by airway inflammation and airway remodeling. Amygdalin, an active component of the traditional Chinese medicine Bitter Almonds, has been shown to inhibit liver fibrosis via the inactivation of the transforming growth factor-beta 1 (TGF-β1)/Smads pathway. This study aims to investigate the effects of Amygdalin on airway inflammation and remodeling in asthma, as well as its regulatory mechanisms.

Methods: An asthma mouse model was constructed using ovalbumin (OVA) induction. Mouse bronchoalveolar lavage fluid (BALF) and lung tissue were harvested for *in vivo* experiments, and airway smooth muscle cells (ASMCs) were isolated from BALB/c mice for *in vitro* experiments. The mechanism of Amygdalin and the TGF-β1/Smads signaling pathway in the mouse model was analyzed pathologically and molecularly using hematoxylin-eosin (HE) staining, Masson trichrome staining, Western blot, and enzyme-linked immunosorbent assay (ELISA).

Results: Amygdalin ameliorated the pathological abnormalities of lung tissues in the OVA-induced mouse model, reducing inflammation by downregulating OVA-specific immunoglobulin E (IgE) and inflammatory factors interleukin (IL)-4, IL-5, and IL-13 ($p < 0.001$). It also reduced lung tissue fibrosis ($p < 0.01$). Additionally, Amygdalin inhibited the levels of TGF-β1, p-Smad2, and p-Smad3 proteins ($p < 0.05$), and downregulated the fibrosis markers alpha-smooth muscle actin (α-SMA), Collagen I, and Collagen III expression in the OVA-induced asthma mouse model ($p < 0.01$).

Conclusion: Amygdalin can regulate the TGF-β1/Smads signaling pathway and alleviate airway inflammation and remodeling in an asthma model in mice.

Keywords: Amygdalin; TGF-β1/Smads signaling pathway; asthma; ovalbumin; airway inflammation; airway remodeling

Introduction

Acute asthma and exacerbations of chronic obstructive pulmonary disease (COPD) are among the most common respiratory diseases, causing significant economic burdens on global health systems [1,2]. The prevalence of COPD among those aged 30–79 years ranges from 7.6% to 10.6%, predominantly affecting low- and middle-income countries [3]. It is crucial to develop better diagnostic tools and advanced treatment options to reduce the morbidity and mortality associated with these conditions [4,5]. The formation and maintenance of airway inflammation primarily depend on the generation of inflammatory mediators and cytokines, which cause tissue damage and airway dysfunction [6,7].

Ovalbumin (OVA) is widely used for asthma modeling in BALB/c mice and has symptoms of high serum immunoglobulin E (IgE) levels, airway inflammation, airway remodeling (e.g., subepithelial and airway wall fibrosis), goblet cell hyperplasia, and airway hyperresponsiveness (AHR), which are similar to those in human asthma [8–10]. The use of the OVA allergen is extremely valuable for revealing the underlying mechanisms of the disease [11,12].

Traditional Chinese medicine (TCM) has played a significant role in our country's long history. Various Chinese herbal medicines have been proven to improve asthma symptoms [13–16]. Amygdalin, also known as vitamin B-17, is an effective component found in TCM Bitter Almonds [17]. Its molecular formula is $C_{20}H_{27}NO_{11}$, a cyano-containing glycoside compound present in the seeds of Rosaceae plants. Bitter Almonds contain the highest concentration of Amygdalin, up to 5.19% [18]. Amygdalin has a wide range of pharmacological effects, mainly used for relieving cough and reducing phlegm, immunosuppression, immune regulation, anti-inflammatory, antitumor, and anti-atherosclerosis [19].

It has been reported that Amygdalin can protect against epithelial-mesenchymal transition in mice with experimental COPD disease [20] and reduce D-galactosamine- and lipopolysaccharide D-galactosamine-triggered acute liver injury [21]. Additionally, berberine at-

tenuates cigarette smoke extract-induced airway inflammation in mice by regulating the transforming growth factor-beta 1 (TGF-β1)/Smads pathway [22]. Amygdalin can inhibit liver fibrosis via TGF-β1/Smads pathway inactivation [23], but its mechanism of action on airway inflammation and remodeling in asthma is still unclear.

The induction and activation of TGF-β family members (TGF-β1, -β2, and -β3) have been observed in various diseases. TGF-β regulates a range of biological processes, which are essential for lung organogenesis and homeostasis, as well as for epithelial-interstitial interactions during lung branching morphogenesis and alveolation [24]. TGF-β also plays a multifunctional role in T cell differentiation and homeostasis, thereby influencing the immune system in the airway of asthma [25–27]. Lung fibrosis, commonly seen in the pathogenesis of severe and chronic asthma, is considered an irreversible consequence of asthma-induced airway inflammation and remodeling [28]. Additionally, fibrosis results from chronic inflammation and is characterized by excessive extracellular matrix (ECM) deposition, primarily type I collagen [29]. Disruption of the TGF-β1/Smad pathway is a contributing factor to tissue fibrosis [30,31]. TGF-β1 activates the phosphorylation of Smad2 and Smad3 (downstream mediators), leading to the overexpression of pro-fibrotic genes such as alpha-smooth muscle actin (α-SMA), Collagen I, and Fibronectin [32–34]. Current research highlights the potential of targeting the TGF-β1/Smad signaling pathway to prevent and treat tissue fibrosis [31].

In this study, OVA-induced asthma mouse models and airway smooth muscle cells (ASMCs) were utilized to observe pathological phenotypes and inflammation in mice. Additionally, protein levels related to fibrosis and the TGF-β1/Smad signaling pathway were assessed. Our focus was on investigating the effect of Amygdalin on airway inflammation and remodeling in asthma.

Materials and Methods

Experimental Animals

Forty female BALB/c mice (6–7 weeks old, 20 $\pm$ 3 g) free of specific pathogens were purchased from Hangzhou Medical College (Hangzhou, China) and were acclimated for 2 weeks under specified pathogen-free (SPF) conditions (23 $\pm$ 2 °C, 40–70% humidity, and a controlled light/dark cycle).

Groups

The mice were randomly divided into three groups, with 10 mice in each group. An allergic asthma model was induced using OVA (No. P0010, Baomanbio, Shanghai, China) as follows [35]: Mice were sensitized by intraperitoneal injection of 0.2 mL of sensitization solution on days 0, 7, and 14. On the 7th day after the final sensitization, the mice were subjected to daily nebulization with a 1% OVA solution for 30 minutes over a period of 7 days.

For the Amygdalin administration group (SA8250, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China): The procedure was similar to that of the OVA asthma model, except that mice were additionally treated with an intraperitoneal injection of 20 mg/kg/day Amygdalin 30 min before each challenge for 7 consecutive days.

In the control group: OVA was replaced with an equal volume of saline (S6546-1L, Merck KGaA, Darmstadt, Germany). Mice in the control group exhibited normal mental function and a good appetite. In contrast, mice in the OVA and Amygdalin groups displayed various symptoms such as wheezing, increased breathing rate, sneezing, reduced activity, and mental dysfunction, similar to those of patients with acute asthma [35].

The success rate of the asthmatic mouse model was 90%. Mice were euthanized within 24 hours after modeling using anesthesia (Pentobarbital solution, P-010-1ML, 150 mg/kg, Merck KGaA, Germany). Following euthanasia, bronchoalveolar lavage fluid (BALF) and lung tissues were collected.

Cells Isolation and Grouping

Airway smooth muscle cells (ASMCs) were isolated from 10 BALB/c mice. The airway tissue was digested for 30 min with trypsin (25300054, Thermo Fisher Scientific, Waltham, MA, USA), then neutralized with Dulbecco's Modified Eagle's Medium (DMEM) (A4192101, Thermo Fisher Scientific, USA) and centrifuged at 1000 $\times$g to remove the supernatant. The remaining pellet was further digested for 15 min, followed by digestion termination and centrifugation, as the previously step. The separated ASMCs were incubated in DMEM containing 10% fetal bovine serum (16140063, Thermo Fisher Scientific, USA) and 1% penicillin/streptomycin (10378016, Thermo Fisher Scientific, USA) at 37 °C in a 5% CO_2 incubator (Steri-Cycle i250, Thermo Fisher Scientific, USA) [36]. The ASMCs tested negative for mycoplasma.

Experimental groups were as follows: Control group: Cells cultured normally for 48 hours; TGF-β1 group: Cells treated with TGF-β1 (10 ng/mL, 14834262, Thermo Fisher Scientific, USA) for 48 hours; TGF-β1+Amygdalin group: Cells treated with 10 ng/mL TGF-β1 and 400 µg/mL Amygdalin for 48 hours.

Immunofluorescence Assay

ASMCs (1×10^6) were fixed with 4% paraformaldehyde (441244, Sigma-Aldrich, St. Louis, MO, USA) for 15 min and permeabilized with 0.1% Triton X-100 (93443, Sigma-Aldrich, USA) for 10 min at room temperature. Following phosphate-buffered saline (PBS) washing, the cells were incubated with 5% bovine serum albumin (V900933, Sigma-Aldrich, USA) at 37 °C for 30 minutes. The ASMCs were then incubated overnight at 4 °C with an α-SMA primary antibody (14-9760-95, Thermo Fisher Scientific, USA), followed by a 30-min incuba-

tion at 4 °C with a fluorescence-labeled secondary antibody (A-10667, Thermo Fisher Scientific, USA). Cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, D9542, Sigma-Aldrich, USA) for 10 minutes in the dark. Observations were made using a confocal microscope (FV3000, Olympus, Tokyo, Japan) at 200× magnification.

Cell Viability Assay

Amygdalin, upon entering the body, decomposes into hydrocyanic acid, which is toxic. Excessive intake can inhibit cell function and potentially lead to cell death [37]. To evaluate cell toxicity, we designed 7 concentration groups and used a Cell Counting Kit-8 (CCK-8) kit (ab228554, Abcam, Cambridge, UK). ASMCs were suspended and cell numbers were counted. Cells (1.6×10^4) were plated in each well of a 96-well plate (CLS3879-50EA, Merck KGaA, Germany) with 100 µL of culture medium. A total of 6 parallel experiments were conducted for each concentration group. Amygdalin was applied at concentrations of 0, 50, 100, 200, 400, 600, and 800 µg/mL, with cells incubated for 48 hours at 37 °C in a 5% CO_2 incubator (Steri-Cycle i250, Thermo Fisher Scientific, USA). CCK-8 solution was then added to detect cytotoxicity, and the cells were incubated for an additional 2 h (37 °C, 5% CO_2). The optical density (OD) value was measured at 460 nm (A51119600DPC, Thermo Fisher Scientific, USA).

Histological Section Observation

Lung tissues were fixed for 72 h with 4% paraformaldehyde (441244, Sigma-Aldrich, USA), followed by dehydration and clearing before embedding and sectioning. Histological sections were then stained with hematoxylin and eosin (hematoxylin for 4 min and eosin for 35 s; 60524ES60, Yeasen, Shanghai, China) to observe pathological changes under a microscope (200× and 400× magnification, DM3000 & DM3000 LED Microscope, Leica, Wetzlar, Germany).

Masson trichrome staining kit (G1340, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) was performed to evaluate collagen fibrils in lung tissues. Sections were developed using Weigert's iron hematoxylin solution for 7 min, Masson's blue solution for 4 min, ponceau magenta solution for 6 min, and aniline blue solution for 100 s.

Enzyme-Linked Immunosorbent Assay (ELISA)

Bronchoalveolar lavage fluids (BALFs) were centrifuged at 1500 ×g to obtain the supernatant, which was then analyzed for inflammatory factors using commercially available ELISA kits (interleukin (IL)-4, ab100710; IL-5, ab204523; and IL-13, ab219634, Abcam, UK). Total IgE and OVA-specific IgE levels were measured using an IgE ELISA kit (ab157718, Abcam, UK). All reagents were equilibrated to room temperature (RT). Samples were added to each well of a mouse-specific 96-well ELISA microplate and incubated at RT. Following incubation, the wells were aspirated and washed four times with washing solution. Then, 100 µL of diluted enzyme-antibody conjugate was added and incubated in the dark for 30 min, followed by 100 µL of Chromogen Substrate Solution for 10 min. The reaction was stopped using the stop solution, and absorbance was read at 450 nm. The average background value was subtracted, duplicate readings for each standard were averaged, and a standard curve was constructed using ReaderFit v2.0 (MiraiBio Group, Hitachi Solutions America, Ltd., Irvine, CA, USA).

Western Blot

In Vivo Sample Processing

Milled mouse lung tissue was homogenized at RT for 6 min in 600 µL of Immunol precipitation (IP) lysis solution (87788, Pierce, Thermo Fisher Scientific, USA) with added protease inhibitor (M5293, AbMole, Houston, TX, USA). The samples were then sonicated (FB705, Thermo Fisher Scientific, USA) and centrifuged at 12,000 ×g. Protein samples were mixed with 5× protein loading buffer (C508320-0001, Sangon Biotech, Shanghai, China), and separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, P0012A, Beyotime, Shanghai, China), and transferred to nitrocellulose membranes (FFN53, Beyotime, Shanghai, China).

In Vitro Sample Processing

Five million lysed (5×10^6) cells were resuspended in 200 µL of IP lysis buffer with protease inhibitor and incubated for 4 min at RT. The lysate was then sonicated, centrifuged at 12,000 ×g, and mixed with 5× protein loading buffer. The samples were subsequently separated by SDS-PAGE and transferred to nitrocellulose membranes.

Antibodies

The membranes were blocked with blocking solution (P0023B-100ml, Beyotime, Shanghai, China) and gently shaken (SHKE416HP, Thermo Fisher Scientific, USA) at RT for 2 h. They were then incubated overnight at 4 °C with primary antibodies, followed by washing with 1× Tris-Buffered Saline with Tween 20 (TBST, ab64204, Abcam, Cambridge, UK) and incubation with secondary antibodies. All antibodies were from Abcam (Cambridge, UK). The primary antibodies used were: α-SMA (ab5694, 1:10,000, 42 kDa); Collagen I (ab6308, 1:1000, 130 kDa); Collagen III (ab7778, 1:1000, 138 kDa); TGF-β1 (ab92486, 1:500, 44 kDa); Smad2 (ab33875, 1:1000, 58 kDa); Smad3 (ab40854, 1:1000, 55 kDa); p-Smad2 (ab53100, 1:500, 58 kDa); p-Smad3 (ab63403, 1:500, 48 kDa); β-actin (ab8226, 1:1000, 42 kDa).

The secondary antibodies used were goat anti-rabbit immunoglobulin G (IgG)-horseradish peroxidase (HRP) (ab205718, 1:50,000) and goat anti-mouse IgG-HRP (ab205719, 1:5000). After incubation with secondary anti-

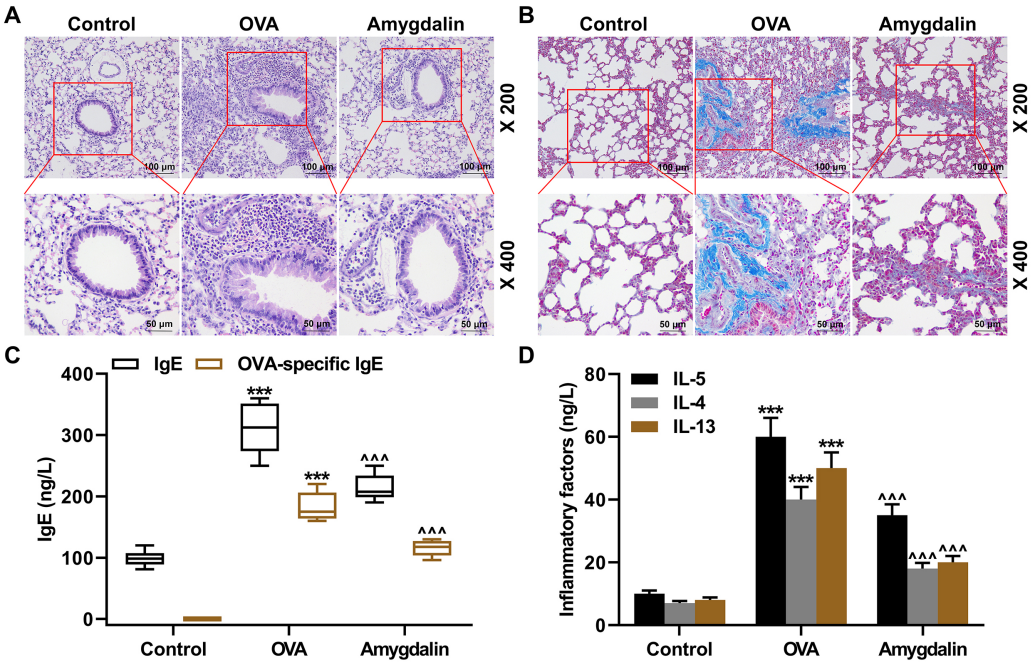

Fig. 1. The influences of Amygdalin on ovalbumin (OVA)-induced asthma in mice. (A) Infiltration of inflammatory cells (hematoxylin-eosin (HE) staining). Scale: 100 µm, 50 µm. (B) Distribution of collagen fibers in alveolar tissue (Masson trichrome staining). Scale: 100 µm, 50 µm. (C) Quantification of immunoglobulin E (IgE) and OVA-specific IgE levels (ng/L) in bronchoalveolar lavage fluid (BALF) (enzyme-linked immunosorbent assay (ELISA)). (D) The effect of Amygdalin on inflammatory factors interleukin (IL)-4, IL-5 and IL-13 in lung tissue. *** $p < 0.001$ vs Control; ^^^ $p < 0.001$ vs OVA. n = 3.

bodies, the membranes were gently shaken (2 h, RT), rinsed with TBST, and developed using Chromogenic Liquid (34577, Thermo Fisher Scientific, USA) in the dark. Detection was performed using a Bio-Rad ChemiDoc Touch (Bio-Rad, USA).

Statistical Analysis

Data from experiments repeated three times were described by mean ± standard deviation (SD) and dissected by SPSS 21.0 software (SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) was exploited for data comparison. $p < 0.05$ was deemed of statistical significance.

Results

Amygdalin Ameliorated the Pathological Phenotype and Inflammation of Mouse Models in OVA Group

The schedule for the establishment of the asthma model and the administration of Amygdalin is shown in **Supplementary Fig. 1**. Mouse lung tissues were collected for HE and Masson trichrome staining. In the control group, normal lung tissue structure was observed, with no significant inflammatory cell infiltration or morphological changes (Fig. 1A,B). In contrast, the pathological section of the OVA group exhibited a significantly thickened airway wall and airway smooth muscle, along with substantial inflammatory cell infiltration around the bronchus (Fig. 1A). Additionally, the bronchial and alveolar structures were distorted, the lumen was stenosed, and the airway was stained blue, indicating a significant increase in collagen fibers (Fig. 1B). Following Amygdalin treatment, improvements were noted in lung structure. Pathological changes such as inflammatory cell infiltration were alleviated, and the deposition of collagen around the airway and thickening of smooth muscle were reduced (Fig. 1A,B).

Quantitative analysis of total OVA-specific IgE and inflammatory factors in BALF using ELISA revealed an upregulation of OVA-specific IgE, IL-4, IL-5, and IL-13 in the OVA group (Fig. 1C,D, $p < 0.001$). Amygdalin treatment significantly reduced OVA-specific IgE (Fig. 1C, $p < 0.001$) and levels of inflammatory factors (Fig. 1D, $p < 0.001$), suggesting that Amygdalin alleviates lung structural changes, reduces cell inflammation, and mitigates fibrosis in the OVA group mouse model.

The Influence of Amygdalin on TGF-β1/Smads Signaling Pathway in Vivo

α-SMA, Collagen I, and Collagen III are markers of fibrosis [38]. Western blot analysis revealed that the levels of α-SMA, Collagen I, and Collagen III proteins were upregulated in the lung tissue of the OVA group. However, the Amygdalin treatment resulted in a downregulation of these proteins (Fig. 2A,B, $p < 0.01$).

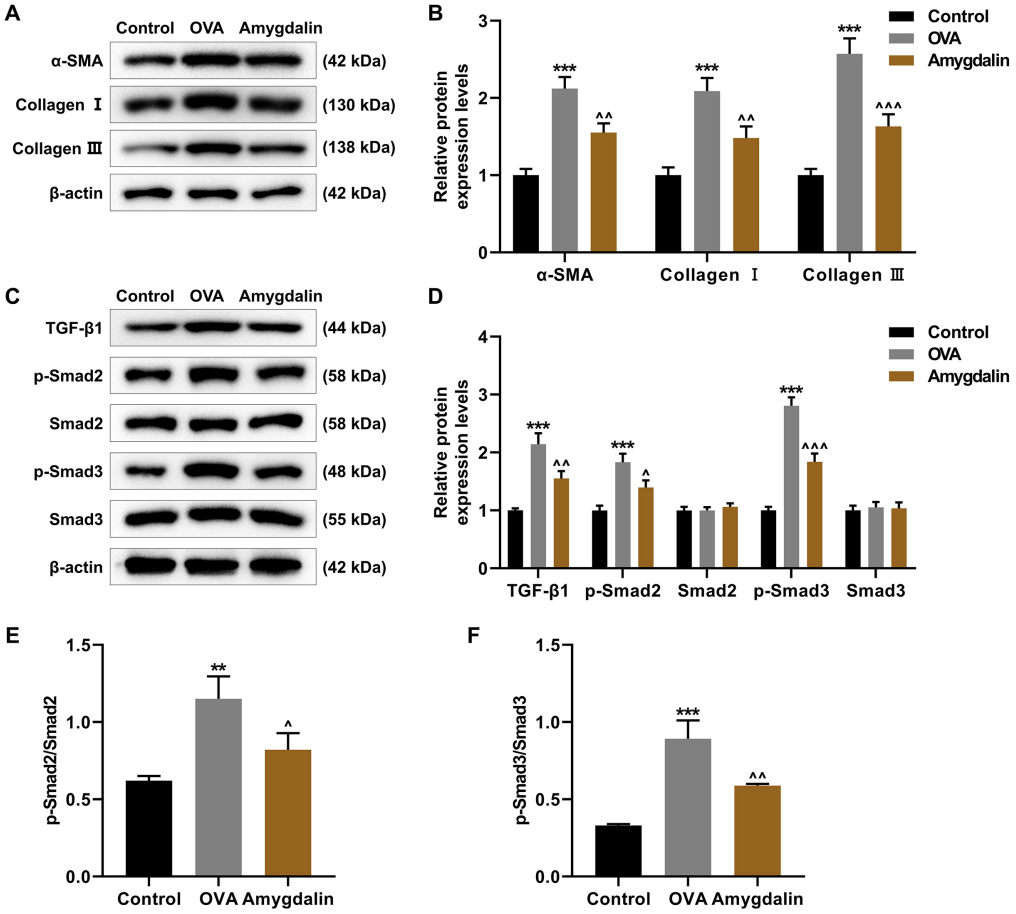

Fig. 2. The impact of Amygdalin upon the fibrosis markers and transforming growth factor-beta 1 (TGF-β1)/Smads signaling pathway in lung tissue. (A,B) Alpha-smooth muscle actin (α-SMA), Collagen I, and Collagen III protein expressions in lung tissue (Western blot). (C,D) Levels of TGF-β1/Smads signaling pathway-related proteins in lung tissue (Western Blot). (E,F) Influences of Amygdalin on p-Smad2/Smad2 and p-Smad3/Smad3. ** $p < 0.01$, *** $p < 0.001$ vs Control; ^ $p < 0.05$, ^^ $p < 0.01$, ^^^ $p < 0.001$ vs OVA. n = 3.

Subsequently, we examined the levels of TGF-β1, p-Smad2, and p-Smad3 in the TGF-β1/Smads signaling pathway. The levels of TGF-β1, p-Smad2, p-Smad3, p-Smad2/Smad2 and p-Smad3/Smad3 were elevated in the model mice but were significantly reduced following Amygdalin treatment (Fig. 2C–F, $p < 0.05$). These findings indicate that Amygdalin can suppress TGF-β1 and the phosphorylation of its downstream mediators, Smad2 and Smad3, thereby inhibiting pulmonary fibrosis.

The Effect of Amygdalin on TGF-β1/Smads Signaling Pathway in Vitro

To further verify the results, we isolated ASMCs from BALB/c mice and cultured them for *in vitro* experiments, the morphological image of ASMCs was shown in **Supplementary Fig. 2**. The isolated cells exhibited prominent α-SMA staining (Fig. 3A), confirming their identity as ASMCs. We then treated the ASMCs with Amygdalin at concentrations of 0, 50, 100, 200, 400, 600, and 800 µg/mL for 48 h, and assessed cell viability using the CCK-8 assay. Cell viability remained relatively stable at Amygdalin concentrations from 0 to 400 µg/mL. However, at concentrations above 600 µg/mL, cell viability began to decline (Fig. 3B, $p < 0.05$). Based on these results, we selected 400 µg/mL of Amygdalin for subsequent experiments.

We then treated ASMCs with TGF-β1 alone or with TGF-β1 combined with Amygdalin for 48 h. Western blot analysis was performed to detect intracellular fibrosis markers and TGF-β1/Smads signaling pathway-related gene levels. In cells treated with TGF-β1, the protein expressions of α-SMA, Collagen I, Collagen III, TGF-β1, p-Smad2, p-Smad3, p-Smad2/Smad2 and p-Smad3/Smad3 were significantly increased (Fig. 4A–F, $p < 0.001$). These expressions were significantly reduced following Amygdalin treatment (Fig. 4A–F, $p < 0.01$).

Discussion

Asthma is an allergic disease characterized by the involvement of multiple cells and inflammatory factors, and

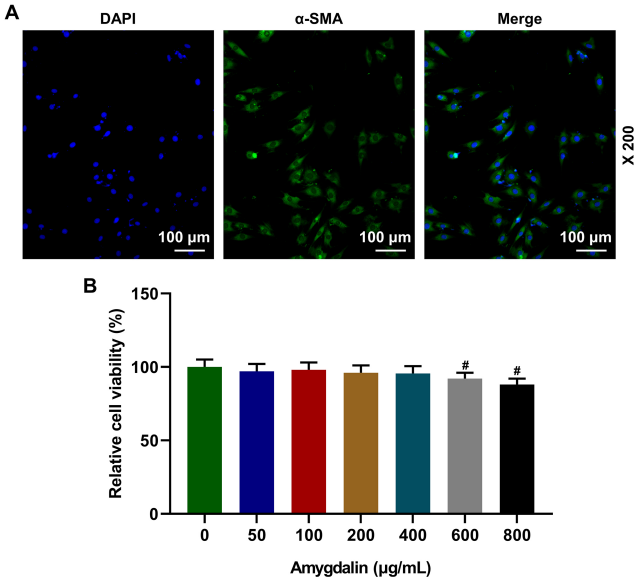

Fig. 3. Cell identification and the influence of Amygdalin on the viability of airway smooth muscle cells (ASMCs). (A) An immunofluorescence assay was performed to identify ASMCs. (B) Cytotoxicity test. $^{\#}$ $p < 0.05$ vs 0 μg/mL. n = 3. DAPI, 4′,6-diamidino-2-phenylindole.

its long-term recurrence can damage airway wall cells, leading to structural and functional changes in the airway and irreversible airflow restriction and affecting approximately 358 million people worldwide [39]. Amygdalin, known for its medicinal value, exhibits various biological activities, particularly anti-inflammatory and anti-fibrotic effects [20,21,40]. Although progress has been made in understanding its role in liver-related diseases, this study is the first to investigate the potential mechanisms of Amygdalin in asthma.

Airway inflammation is a primary cause of asthma, marked by leukocytosis, eosinophil infiltration, and excessive mucus production [41]. Our experiments revealed significant inflammatory cell infiltration, increased collagen fibers, and thickened airway walls and smooth muscle in the OVA-induced asthma mouse model. These findings are consistent with the typical characteristics of asthma.

Various cytokines play critical roles in the pathogenesis of asthma. IL-4 promotes IgE synthesis and the release of various inflammatory mediators, while IL-5 enhances allergic reactions and contributes to inflammation in asthma. IL-13, a key Th2 cytokine, activates collagen type I alpha-2 chain (*COL1A2*) mRNA expression and collagen deposition [42]. Elevated levels of immunoglobulin IgE, a powerful indicator of allergic diseases, can exacerbate asthma symptoms. In our study, levels of inflammatory factors (IL-4, IL-5, IL-13) and OVA-specific IgE in the BALF of model mice were significantly increased. Amygdalin treatment led to improved lung structure and reduced levels of these inflammatory markers in BALF, indicating that Amygdalin can alleviate lung inflammation and fibrosis in OVA-induced asthma models. These results suggest that Amygdalin holds potential as an anti-inflammatory drug for asthma treatment.

Airway remodeling, a consequence of chronic injury and repeated repair, involves pathological changes such as epithelial injury, subepithelial fibrosis, mucous gland hyperplasia, and an increase in smooth muscle soft tissue [43]. These changes result in excessive airway narrowing. Airway remodeling can be driven by airway inflammation or occur independently [44]. TGF-β1 plays a critical role in airway remodeling in asthma [45], through its interactions with the Smad protein family. The TGF-β1/Smads and Nuclear factor kappa B (NF-κB) signaling pathways can mediate fibrosis and inflammation [46].

Smad3, a downstream regulator of TGF-β signal transduction, is particularly important in mediating fibrotic diseases. In *in vivo* and *in vitro* experiments, Amygdalin was found to inhibit the expression of α-SMA, Collagen I and Collagen III, as well as the activation of Smad2 and Smad3 in the TGF-β1/Smads signaling pathway. These data indicate Amygdalin mitigated OVA-induced airway inflammation and airway remodeling likely by inhibiting Smad3 phosphorylation. Thus, Amygdalin appears to reduce airway inflammation and remodeling in model mice by targeting the TGF-β1/Smads signaling pathway.

Additionally, Wang *et al.* [36] have identified that a weak inducer of tumor necrosis factor-like apoptosis can work with TGF-β1/Smads signaling pathway to reduce airway remodeling. Beyond the Smad3 pathway, TGF-β1 can also induce myofibroblast formation through epithelial-mesenchymal transition (EMT) and promote fibrogenesis by inhibiting matrix metalloproteinases (MMPs) while promoting the tissue inhibitor of TIMP to inhibit ECM degra-

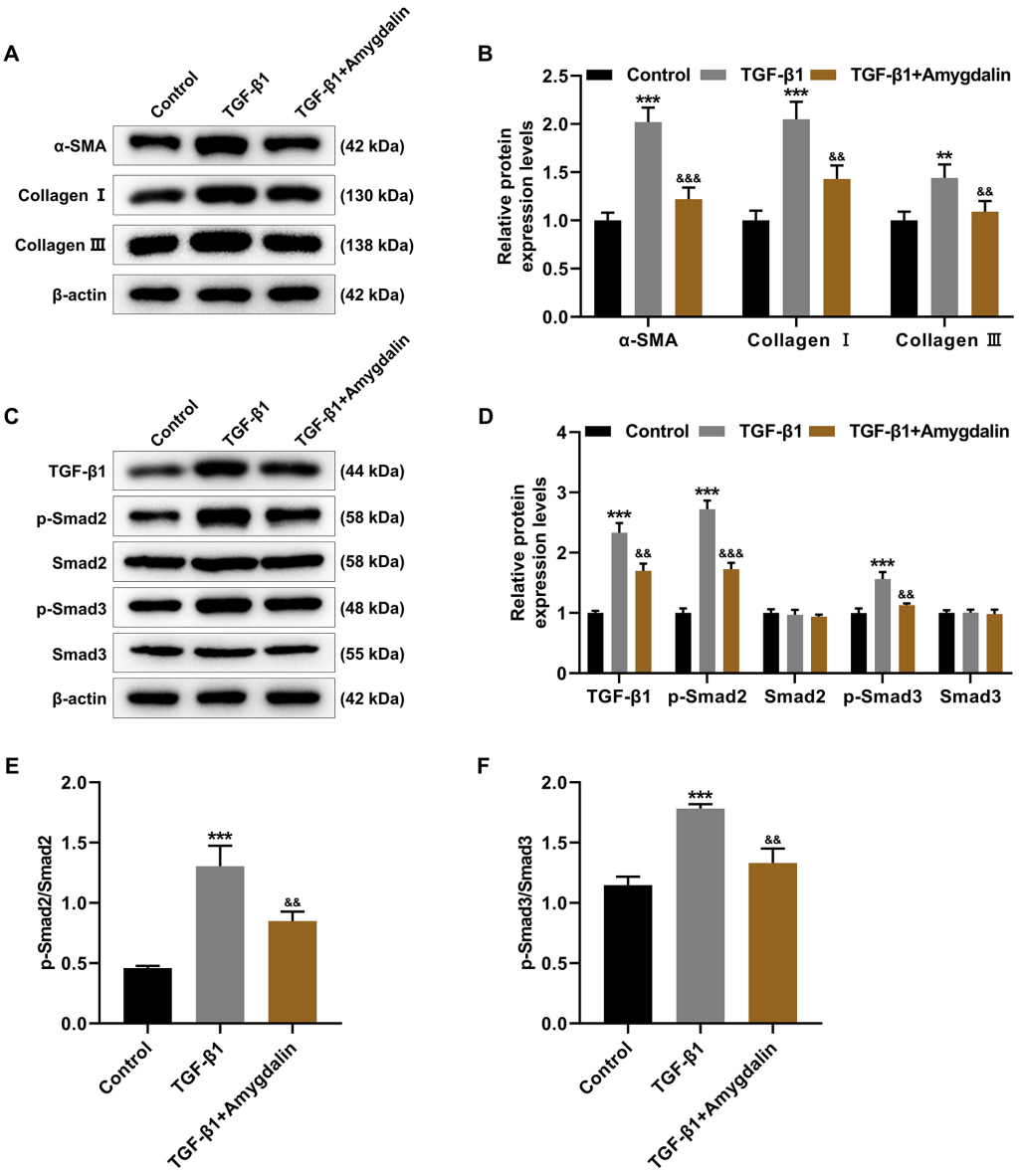

Fig. 4. Influences of Amygdalin on TGF-β1/Smads signaling pathway in ASMCs. (A,B) α-SMA, Collagen I and Collagen III protein levels in ASMCs (Western blot). (C,D) Levels of TGF-β1/Smads signaling pathway-related proteins in ASMCs (Western Blot). (E,F) Influences of Amygdalin on p-Smad2/Smad2 and p-Smad3/Smad3. ** $p < 0.01$, *** $p < 0.001$ vs Control; $^{\&\&}$ $p < 0.01$, $^{\&\&\&}$ $p < 0.001$ vs TGF-β1. n = 3.

dation. Smad7 has a negative regulatory effect on the biological activities of Smad2 and Smad3 [33].

Future research should focus on a more detailed exploration of the TGF-β1/Smad3 pathway to confirm the therapeutic potential of Amygdalin in asthma. Specifically, treating asthma mice with TGF-β1/Smad3 pathway agonists could help verify whether Amygdalin's therapeutic effects are mediated through this pathway. Additionally, while the TGF-β1/Smad3 pathway has been investigated, Amygdalin may also affect asthma through other target genes or pathways, warranting further investigation.

Conclusion

Amygdalin has been shown to inhibit the TGF-β1/Smad3 signaling pathway, effectively reducing OVA-induced injury in asthmatic mice. This finding highlights Amygdalin's potential as a novel therapeutic agent or target for the clinical treatment of asthma.

Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Author Contributions

JZ and YMW designed the research study; HXW performed the research; HXW collected and analyzed the data. All authors have been involved in drafting the manuscript and all authors have been involved in revising it critically for important intellectual content. All authors give final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

This study received ratification from the Ethics Committee of Zhejiang Center of Laboratory Animals (No. ZJCLA-IACUC-20010544). Every effort was made to minimize the suffering of the animals.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202537197.97.

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