Article

Discovery Medicine 2024; 36(181): 323–331
https://doi.org/10.24976/Discov.Med.202436181.30

SFRP5 Partially Inhibits the Proliferation and Migration of Airway Smooth Muscle Cells in Children with Asthma by Regulating the Wnt/β-Catenin Signaling Pathway

Yuyun Yuan[1,†], Honghua Zhu[2,†], Sihong Huang[1], Yantao Zhang[1], Yiyun Shen[1,*]

[1]Department of Pediatrics, Shanghai Baoshan Hospital of Integrated Traditional Chinese and Western Medicine, 201999 Shanghai, China
[2]Department of Medical Imaging, Shanghai Seventh People's Hospital, 200137 Shanghai, China
*Correspondence: bs0036@shutcm.edu.cn (Yiyun Shen)
[†]These authors contributed equally.
Published: 20 February 2024

Background: Childhood asthma is a chronic inflammatory disease of the respiratory tract characterized by bronchial inflammation, airway hyperresponsiveness, airflow disorder, and obstruction. Secreted frizzled-related protein 5 (SFRP5) may be associated with respiratory inflammatory diseases. This study investigated the effect of SFRP5 on human airway smooth muscle cells (HASMCs) to provide new ideas for treating asthma.
Methods: A total of 30 children with asthma and 30 children who had a physical examination at the same time were selected and divided into asthma and healthy groups. Serum SFRP5 levels were determined by enzyme-linked immunosorbent assay (ELISA) and real-time quantitative polymerase chain reaction (RT-qPCR). Lipofectamine 2000™ regent was used to transfect the *SFRP5* overexpression plasmid (pc-*SFRP5*) or corresponding negative control (pc-NC) into HASMCs. HASMCs were treated with 10 µg/L platelet-derived growth factor-BB (PDGF-BB), which is an inducer to mimic the asthma-like condition at the cellular level of childhood asthma. HASMCs were divided into control, PDGF-BB (PDGF-BB treatment), PDGF-BB+pc-NC (pc-NC transfection and PDGF-BB treatment), and PDGF-BB+pc-*SFRP5* (pc-*SFRP5* transfection and PDGF-BB treatment) groups. Cell proliferation was measured by 5-ethynyl-2′-deoxyuridine (EdU) and cell counting kit-8 (CCK-8) assay. Cell migration was detected by Transwell assay. The protein expression was detected by western blot.
Results: Serum SFRP5 expression in the asthmatic group was decreased versus the healthy group ($p < 0.0001$). Induction of PDGF-BB decreased SFRP5 expression in HASMCs ($p < 0.01$). SFRP5 expression in the pc-*SFRP5* group was increased ($p < 0.01$). The proliferation and migration of HASMCs increased after PDGF-BB treatment ($p < 0.001$, $p < 0.0001$), indicating that the asthma model was successfully inducted *in vitro*. Moreover, the expression of β-catenin, cellular-myelocytomatosis viral oncogene (c-Myc), and cyclinD1 proteins in HASMCs increased after PDGF-BB treatment ($p < 0.0001$). *SFRP5* overexpression partly inhibited PDGF-BB-induced proliferation, migration, and expressions of β-catenin, c-Myc, and cyclinD proteins in HASMCs ($p < 0.01$, $p < 0.001$, $p < 0.0001$).
Conclusions: Serum SFRP5 expression decreases in children with asthma. *SFRP5* overexpression partially inhibits PDGF-BB-induced HASMC proliferation and migration by regulating the wingless-type mouse mammary tumor virus (MMTV) integration site family (Wnt)/β-catenin pathway.

Keywords: children with asthma; SFRP5; HASMCs; proliferation; migration; Wnt/β-catenin

Introduction

Bronchial asthma is common in clinical practice and is often referred to as asthma [1]. Airway hyperresponsiveness, airway remodeling, and reversible respiratory restriction are the main pathological features of asthma [2]. Asthma is the most common chronic disease in pediatrics, characterized by recurrent symptoms, including chest tightness, shortness of breath, and cough [3]. Asthma often attacks or intensifies at night and in the early morning, which affects children's physical and mental health, life and study, and can extend to adulthood, becoming a lifelong disease for such children [4]. However, the exact pathogenesis of childhood asthma is still unclear, which may be related to genetic, immune, and neuromodulation factors [3]. Currently, the clinical treatment is mainly based on drugs, including glucocorticoids, β2 receptor agonists, theophyllines, etc. [5]. At present, the pathogenesis of asthma has not been clearly elucidated in clinical practice, and there is no effective drug to cure asthma completely. Therefore, the pathogenesis of childhood asthma should be further explored to provide a basis for finding new therapeutic targets and drugs.

Copyright: © 2024 The Author(s). Published by Discovery Medicine. This is an open access article under the CC BY 4.0 license.
Note: *Discovery Medicine* stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

DM DISCOVERY MEDICINE 2001

Secreted frizzled-related protein 5 (SFRP5), a member of the SFRP family, is a novel anti-inflammatory adipokine secreted by white adipose tissue [6]. SFRP5 has rich biological functions and is closely related to embryonic development, cell proliferation, inflammatory response, and glucose and lipid metabolism in the body [7]. SFRP5 is structurally similar to the wingless-type mouse mammary tumor virus (MMTV) integration site family (Wnt) signal-specific coiled protein (frizzled, Frz) receptor and can competitively inhibit Wnt protein activity [8]. The Wnt pathway is a widespread signaling pathway during development, which is involved in the growth of almost all tissues [9]. The Wnt pathway involves cell differentiation, migration, and proliferation, which affects tumor evolution, tissue homeostasis, and embryonic development [10]. Research has shown that the Wnt/β-catenin pathway relates to airway smooth muscle cells (ASMCs) differentiation, migration, and proliferation, and ASMCs have a vital role in airway inflammation and airway remodeling (an important pathological feature of asthma) [11]. SFRP5 can regulate cell differentiation and proliferation by inactivating the Wnt/β-catenin pathway and participating in embryonic development, inflammation and immune response, tumor formation, obesity, and insulin resistance [12]. Serum SFRP5 levels in children with asthma were significantly correlated with airway inflammation and acute attack severity [13,14]. At present, the further influence of SFRP5 on the pathogenesis of asthma is not clear, and this study will discuss this to provide a reference for asthma treatment.

Materials and Methods

General Information

Thirty children with acute bronchial asthma attacks (asthma group) treated in Shanghai Baoshan Hospital of Integrated Traditional Chinese and Western Medicine from September 2019 to September 2022 were selected. In the same period, 30 healthy children who underwent physical examinations were included in the healthy group. Inclusion criteria: (1) Meeting diagnostic criteria of bronchial asthma [15]; (2) Patients have normal cognitive function and should cooperate with research; (3) Age $<$12 years old; (4) The clinical data were completed. Exclusion criteria: (1) Patients have other respiratory diseases or acute infectious disease; (2) Patients are complicated with hepatic and renal insufficiency; (3) Congenital cardiopulmonary insufficiency; (4) Patients are combined with other allergic diseases and in the attack period. There was no significant difference in gender and age between the two groups (Table 1) ($p > 0.05$). This study was approved by the Ethics Committee of Shanghai Baoshan Hospital of Integrated Traditional Chinese and Western Medicine (Approval number: 202314). All family members of enrolled children and healthy children signed informed consent. This study was conducted in accordance with the Declaration of Helsinki.

Table 1. Comparison of baseline data between asthmatic and healthy children.

Group	Age (year)	Gender (male/female)
Healthy (n = 30)	5.50 ± 1.83	12/18
Asthmatic (n = 30)	5.33 ± 1.79	16/14
t/χ^2	0.356	1.071
p	0.723	0.301

Serum SFRP5 Detection

5 mL blood samples were collected from healthy subjects on the day of physical examination and children with asthma during fasting examination in the morning. The collected venous blood was centrifuged for 10 min at 3000 r/min. Then, upper serum was taken and stored at –80 °C for later use. Serum SFRP5 levels were determined by an enzyme-linked immunosorbent assay (ELISA) kit (A099006, Fusheng, Shanghai, China) and real-time quantitative polymerase chain reaction (RT-qPCR).

Culture and Treatment of HASMCs

Human airway smooth muscle cells (HASMCs) were purchased from Shanghai Yaji Biotechnology Company Limited (YS2014C, Yaji Biological, Shanghai, China). The cells were tested for mycoplasma and authenticated by short tandem repeat (STR). *SFRP5* overexpressing plasmid (pc-*SFRP5*) and negative control (pc-NC) were purchased from Shanghai GenePharma Company (GenePharma, Shanghai, China). HASMCs were cultured in Dulbecco's modified Eagle's medium (DMEM) (12800017, Gibco, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS) (16000-044, Gibco, Carlsbad, CA, USA) at 37 °C and 5% CO_2. Cells were inoculated into a 6-well plate (2.5×10^5 cells/well) and divided into 2 groups: pc-NC and pc-*SFRP5*. Cell transfection was performed by Lipofectamine 2000™ reagent (11668-027, Invitrogen, Waltham, MA, USA). A childhood asthma *in vitro* model was constructed through stimulation of 10 ng/mL platelet-derived growth factor-BB (PDGF-BB) (4488-50, Biovision, San Francisco, CA, USA) on HASMCs for 24 h [16].

The coding sequence of *SFRP5*: ATGCGGGCGGC GGCGGCGGGGGGGGGCGTGCGGACGGCCGCGCT GGCGCTGCTGCTGGGGGCGCTGCACTGGGCGCCG GCGCGCTGCGAGGAGTACGACTACTATGGCTGGC AGGCCGAGCCGCTGCACGGCCGCTCCTACTCCAA GCCGCCGCAGTGCCTTGACATCCCTGCCGACCTG CCGCTCTGCCACACGGTGGGCTACAAGCGCATGC GGCTGCCCAACCTGCTGGAGCACGAGAGCCTGGC CGAAGTGAAGCAGCAGGCGAGCAGCTGGCTGCC GCTGCTGGCCAAGCGCTGCCACTCGGATACGCAG GTCTTCCTGTGCTCGCTCTTTGCGCCCGTCTGTCT CGACCGGCCCATCTACCCGTGCCGCTCGCTGTGC GAGGCCGTGCGCGCCGGCTGCGCGCCGCTCATGG AGGCCTACGGCTTCCCCTGGCCTGAGATGCTGCA CTGCCACAAGTTCCCCCTGGACAACGACCTCTGC

DM DISCOVERY MEDICINE 2001

ATCGCCGTGCAGTTCGGGCACCTGCCCGCCACCG CGCCTCCAGTGACCAAGATCTGCGCCCAGTGTGA GATGGAGCACAGTGCTGACGGCCTCATGGAGCA GATGTGCTCCAGTGACTTTGTGGTCAAAATGCGC ATCAAGGAGATCAAGATAGAGAATGGGGACCGG AAGCTGATTGGAGCCCAGAAAAAGAAGAAGCTG CTCAAGCCGGGCCCCCTGAAGCGCAAGGACACC AAGCGGCTGGTGCTGCACATGAAGAATGGCGCG GGCTGCCCCTGCCCACAGCTGGACAGCCTGGCGG GCAGCTTCCTGGTCATGGGCCGCAAAGTGGATGG ACAGCTGCTGCTCATGGCCGTCTACCGCTGGGAC AAGAAGAATAAGGAGATGAAGTTTGCAGTCAAA TTCATGTTCTCCTACCCCTGCTCCCTCTACTACCC TTTCTTCTACGGGGCGGCAGAGCCCCACTGA.

RT-qPCR

Total RNA was extracted from cells by Trlzol reagent (15596026, Gibco, Carlsbad, CA, USA) and reversely transcribed into cDNA by reverse transcription kit (4374966, Invitrogen, Waltham, MA, USA). *SFRP5* expression was detected by RT-qPCR kit (E021005, Genepharma, Shanghai, China), with *β-actin* as the internal reference. The relative expression of *SFRP5* was calculated by $2^{-\Delta\Delta Ct}$. Primers were listed as follows:

SFRP5:
F: 5′-GTGCTGCACATGAAGAATGG-3′
R: 5′-GCAGGGGTAGGAGAACATGA-3′
β-actin:
F: 5′-CACCACACCTTCTACAATGAGC-3′
R: 5′-GTGATCTCCTTCTGCATCCTGT-3′

5-Ethynyl-2′-Deoxyuridine (EdU) Assay

EdU detection kit (C10371-3, RiboBio, Guangdong, China) was selected to detect cell proliferation. After 48 h transfection, HASMCs were stimulated with PDGF-BB for 24 h. 200 μL (50 $\mu mol \cdot L^{-1}$) EdU medium was added to each well. After incubation for 2 h, the medium was discarded, cells were fixed with 4% paraformaldehyde, and 1×Apollo staining solution (200 μL) was added to each well. After cultured at room temperature for 0.5 h, the nuclei were counterstained with 200 μL of 4′, 6-diamidino-2-phenylindole (DAPI, C1005, Beyotime, Shanghai, China) staining solution and observed under a laser confocal microscope (A1R+, Nikon, Tokyo, Japan). Positive cell rate = EdU-labeled cells number/DAPI-labeled cells number × 100%.

Cell Counting Kit-8 (CCK-8) Assay

HASMCs in the logarithmic growth phase were seeded in 96-well plates (1×10^4 cells/well). After cells were grouped and treated, the 96-well plate was incubated at 37 °C for 48 h. 10 μL CCK-8 solution (40203ES60, Yeasen, Shanghai, China) was added to each well. The 96-well plate was further incubated at 37 °C for 4 h, and the optical density (OD) value at 450 nm wavelength was measured in a plate reader (Multiskan GO, Thermo Scientific, Waltham, MA, USA).

Transwell Assay

When HASMCs had grown to 80% fusion, the cells were digested with pancreatic enzymes and suspended with serum-free DMEM. 2×10^5 cells were added to the upper chamber, and 600 μL DMEM of 10% FBS with PDGF-BB was added to the lower chamber. Transwell chamber (3422, Corning, Corning, NY, USA) was cultured for 24 h in an incubator. Cells that did not enter the membrane on the upper side of the membrane were wiped away. The cells under the membrane were fixed with methanol (646377, Sigma, St. Louis, MO, USA) and stained with crystal violet (M1415, Mreda, Beijing, China).

Western Blot

Cells in each group were collected. Cell lysate was added to extract total protein. Protein concentration was determined by a bicinchoninic acid (BCA) kit (P0010, Beyotime, Shanghai, China). The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, P0014A, Beyotime, Shanghai, China) and transferred to polyvinylidene fluoride (PVDF) membrane (IPVH00010, Millipore, Billerica, MA, USA). After being sealed at room temperature for 2 h, primary antibodies of SFRP5 (1:1000, ab230425, Abcam, Cambridge, UK), β-catenin (1:500, ab68183, Abcam, Cambridge, UK), cellular-myelocytomatosis viral oncogene (c-Myc) (1:500, ab51156, Abcam, Cambridge, UK), cyclinD1 (1:200, ab16663, Abcam, Cambridge, UK) and β-actin (1:2000, ab8227, Abcam, Cambridge, UK) were added at 4 °C overnight. After washing the next day, the second antibody (BHR101, Bersee, Beijing, China) was added and incubated for 1 h. Enhanced chemiluminescence (ECL) reagent (WP20005, Thermo Scientific, Waltham, MA, USA) was used for color development, and a gel imaging system was used to photograph. Image-J software (Version 1.41, National Institutes of Health, Bethesda, MD, USA) was used to analyze the gray value of each strip.

Statistical Analysis

Measurement data were represented by mean ± standard deviation ($\bar{x} \pm$ SD) and statistically analyzed using GraphPad Prism Software (version 6.0, GraphPad Software Company, San Diego, CA, USA). Students' t-tests were performed to compare the two groups. A one-way Analysis of Variance (ANOVA) followed by post-hoc Tukey's test was performed for multiple comparisons. Enumeration data were expressed as the number of cases. Age and gender were analyzed using SPSS v19.0 software (IBM Corp., Armonk, NY, USA) for the chi-square and T-test. $p < 0.05$ was deemed statistically significant.

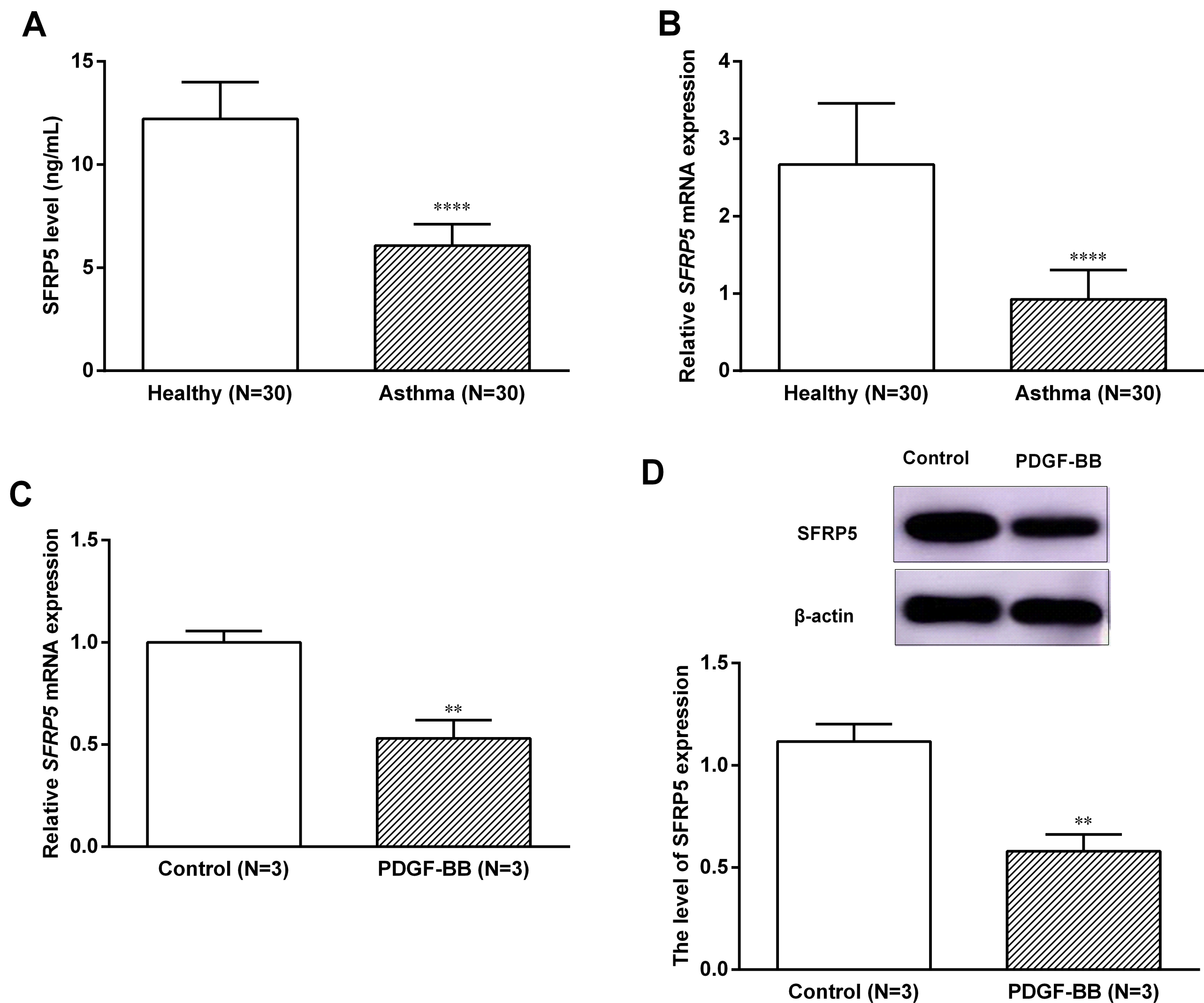

Fig. 1. SFRP5 expression in serum of children with asthma and HASMCs treated by PDGF-BB. (A,B) Serum SFRP5 expression levels in children with asthma (n = 30). (C,D) PDGF-BB inhibited the SFRP5 expression in HASMCs (n = 3). Data were presented as mean ± standard deviation. $^{**}p < 0.01$, $^{****}p < 0.0001$, compared to healthy or PDGF-BB group. SFRP5, secreted frizzled-related protein 5; HASMCs, human airway smooth muscle cells; NC, negative control; PDGF-BB, platelet-derived growth factor BB.

Results

SFRP5 Levels in Serum and PDGF-BB-Stimulated HASMCs

Serum SFRP5 levels in children with asthma decreased versus the healthy group (Fig. 1A,B) ($p < 0.0001$). After 24 h stimulation with 10 ng/mL PDGF-BB, SFRP5 expression decreased in HASMCs (Fig. 1C,D) ($p < 0.01$).

SFRP5 Overexpression Inhibited Proliferation of HASMCs Induced by PDGF-BB

Subsequently, cell proliferation and migration of HASMCs were detected after PDGF-BB treatment to identify whether the *in vitro* asthma model was successfully induced. pc-*SFRP5* was transfected into HASMCs to detect SFRP5 expression. SFRP5 expression in the pc-*SFRP5* group was up-regulated versus the pc-NC group (Fig. 2A,B) ($p < 0.01$). The effect of pc-*SFRP5* on the proliferation of HASMCs was detected by EdU and CCK-8 assays. It was found that PDGF-BB stimulation promoted cell proliferation versus the control group (Fig. 2C,D) ($p < 0.001$, $p < 0.0001$), suggesting the *in vitro* asthma model was successfully induced. Compared with the PDGF-BB+pc-NC group, the proliferation of HASMCs was inhibited in the PDGF-BB+pc-*SFRP5* group (Fig. 2C,D) ($p < 0.001$).

SFRP5 Overexpression Inhibited Migration of PDGF-BB-Induced HASMCs

Transwell measured cell migration and found that the cells migrated number increased in the PDGF-BB group versus the control group (Fig. 3) ($p < 0.0001$), suggesting the *in vitro* asthma model was successfully induced.

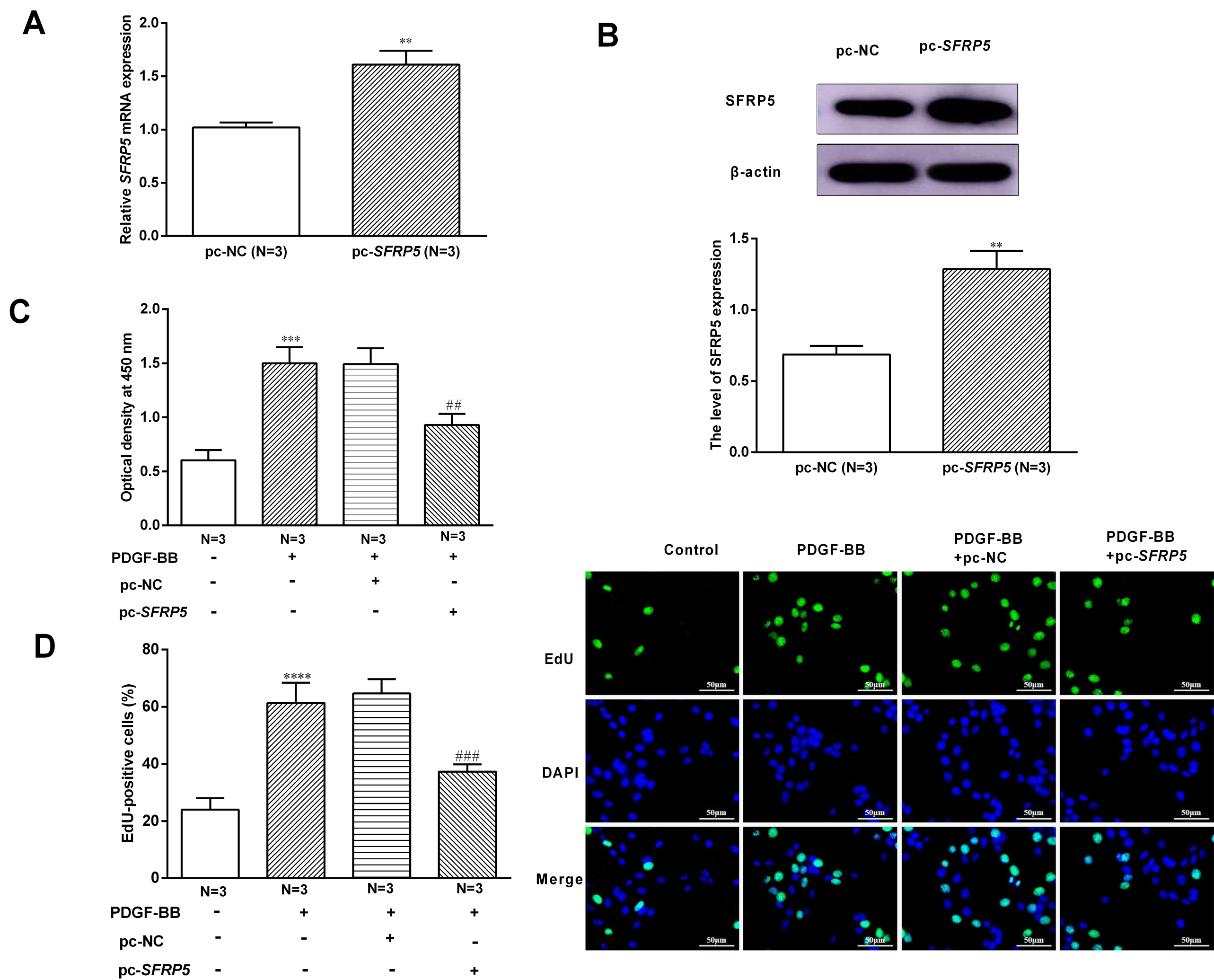

Fig. 2. Effects of *SFRP5* overexpression on proliferation of PDGF-BB-stimulated HASMCs. (A,B) The transfection efficiency of *SFRP5* overexpressing plasmid (pc-*SFRP5*) was detected by real-time quantitative polymerase chain reaction (RT-qPCR) and western blot assays (n = 3). (C,D) SFRP5 overexpression inhibited PDGF-BB-stimulated proliferation of HASMCs (green fluorescence indicates EdU stained cells, blue-fluorescence indicates DAPI stained cells) (n = 3) (Scale bar = 50 µm). Data were presented as mean ± standard deviation. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, compared to pc-NC or control group; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared to PDGF-BB+pc-NC group. EdU, 5-ethynyl-2′-deoxyuridine; DAPI, 4′, 6-diamidino-2-phenylindole.

Compared with the PDGF-BB+pc-NC group, the number of cell migrations in the PDGF-BB+pc-*SFRP5* group reduced (Fig. 3) ($p < 0.001$).

SFRP5 Overexpression Might Inhibit Wnt/β-Catenin Activation of HASMCs Induced by PDGF-BB

After HASMCs were stimulated with PDGF-BB, c-Myc, β-catenin, and cyclinD1 proteins expression notably increased (Fig. 4A) ($p < 0.0001$). After pc-*SFRP5* co-transfection of HASMCs induced by PDGF-BB, the level of the above proteins decreased (Fig. 4A) ($p < 0.0001$). This suggested that *SFRP5* overexpression might inhibit the Wnt/β-catenin signal in HASMCs.

To explore whether the Wnt/β-catenin pathway mediates the effect of SFRP5 on HASMCs proliferation and migration, a salvage experiment was performed using the specific activator of the Wnt/β-catenin pathway (LiCl). The c-Myc, β-catenin, and cyclinD1 protein expression significantly increased in the PDGF-BB+pc-SFRP5+LiCl group versus PDGF-BB+pc-SFRP5 group (Fig. 4A) ($p < 0.05$, $p < 0.01$, $p < 0.001$). LiCl could reverse the inhibition of HASMC proliferation induced by *SFRP5* up-regulation after PDGF-BB treatment (Fig. 4B,C) ($p < 0.05$). In addition, LiCl restored the decrease in the number of HASMC cell migrations caused by *SFRP5* upregulation after PDGF-BB treatment (Fig. 4D) ($p < 0.05$). These results suggest that the role of *SFRP5* in the progression of children with asthma could be mediated (at least partly) by the Wnt/β-catenin pathway.

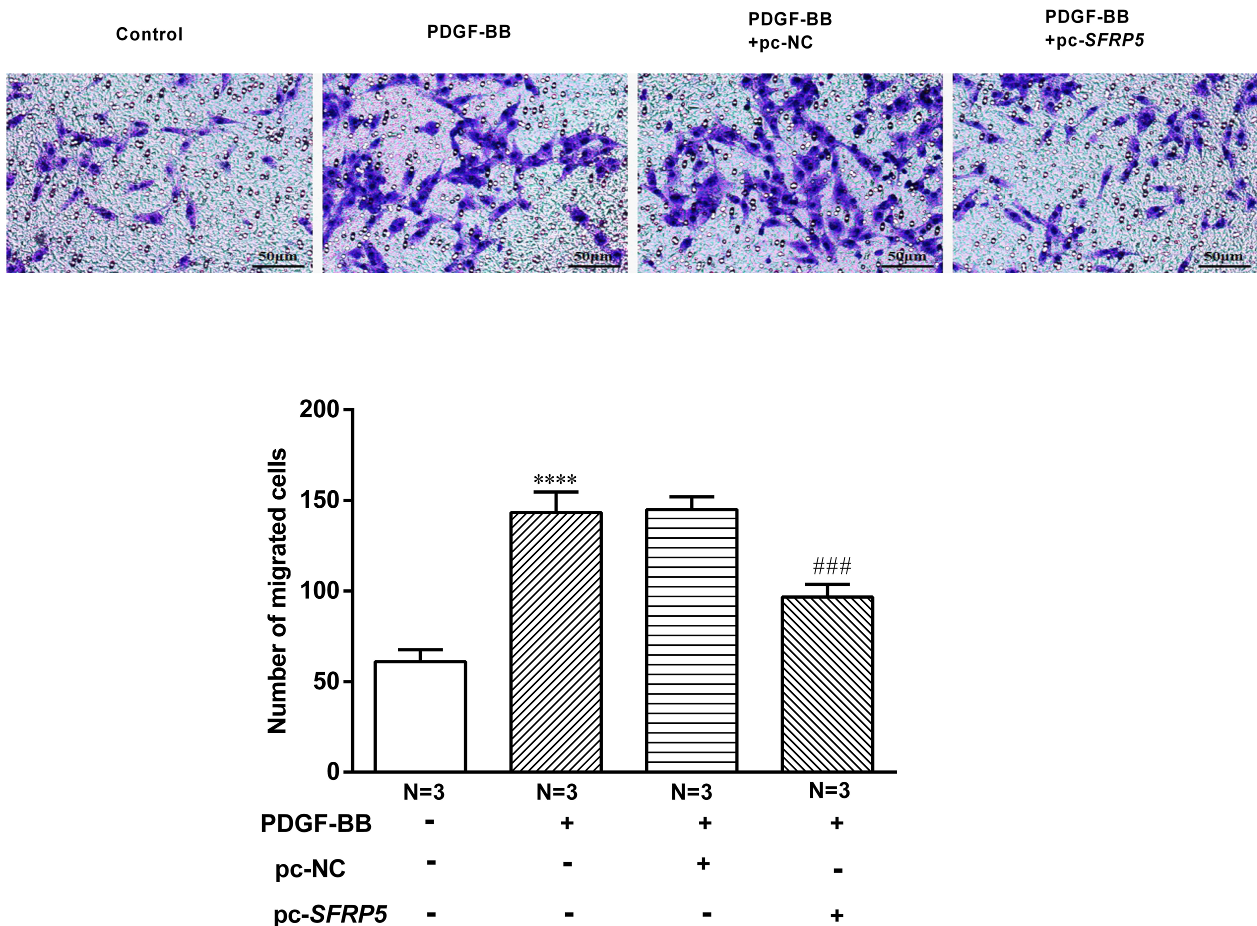

Fig. 3. *SFRP5* overexpression inhibited PDGF-BB-stimulated migration of HASMCs (n = 3) (Scale bar = 50 μm). Data were presented as mean ± standard deviation. $^{****}p < 0.0001$, compared with control group; $^{###}p < 0.001$, compared with PDGF-BB+pc-NC group.

Discussion

Asthma is a chronic respiratory tract involving many cell types and components, and its pathogenesis has not yet been clarified [17]. Repeated asthma attacks will aggravate the injury of airway endothelial tissue, and the pathological manifestation is excessive proliferation of smooth muscle cells, leading to airway stenosis, increasing airway hyperresponsiveness, and inducing airway remodeling [18]. The pathological characteristics of airway remodeling mainly showed smooth muscle cell hyperplasia, goblet cell hyperplasia, airway spasm reversibility decreased and gradually weakened to disappear, and lung function index decreased [19]. At present, the treatment of asthma lacks specific drugs, and mainly focuses on alleviating symptoms, but it is difficult to cure and has a high recurrence rate [20]. The prevention and treatment of asthma have also become a medical challenge that many researchers are committed to solving.

SFRP5 gradually decreases with asthma exacerbation in children and can be used as a molecular marker to evaluate the disease of children [21]. Similarly, serum SFRP5 levels were low in children with acute asthma attacks, and there was a significant relationship with the severity of the disease [13]. Herein, serum SFRP5 expression level was reduced in asthmatic children versus healthy children. The serum SFRP5 level is suggested to be low in children with asthma. The detection of serum SFRP5 may help evaluate the condition of asthmatic children to avoid the difficulty of disease assessment caused by the young age of children and poor compliance with lung function detection, and provide an objective basis for clinical diagnosis and treatment.

Airway smooth muscle cells (ASMC) dysfunction is one of the critical factors in asthma, and excessive ASMC proliferation can lead to airflow obstruction in asthma patients [22]. Abnormal proliferation and migration of ASMCs relate to asthma occurrence and development, and are important pathological changes in the course of asthma [23]. In addition, ASMCs may participate in the airway remodeling process by acquiring strong migration ability [24]. Huang *et al*. [14] reported that SFRP5 could block airway remodeling mediated by various inflammatory factors by inhibiting the Wnt signaling pathway, thereby alleviating disease and preventing disease progression. However,

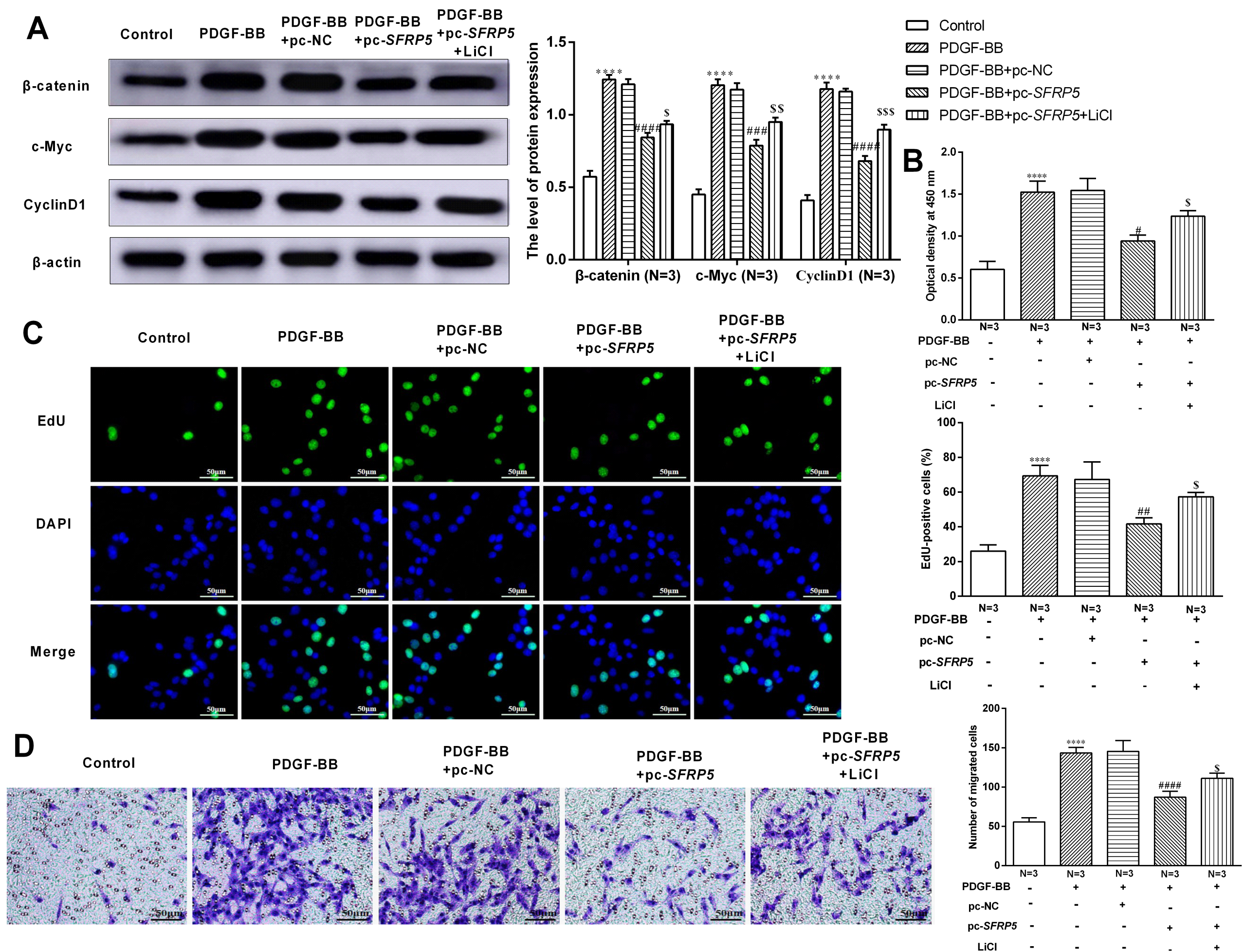

Fig. 4. *SFRP5* overexpression inhibited PDGF-BB-stimulated proliferation and migration of HASMCs partly by the wingless-type mouse mammary tumor virus (MMTV) integration site family (Wnt)/β-catenin pathway. (A) *SFRP5* overexpression inhibited cellular-myelocytomatosis viral oncogene (c-Myc), β-catenin, and cyclinD1 protein expressions in PDGF-BB-stimulated HASMCs (n = 3). (B,C) Cell proliferation was detected by CCK-8 (n = 3) and EdU (green fluorescence indicates EdU stained cells, blue-fluorescence indicates DAPI stained cells) assays (n = 3) (Scale bar = 50 µm). (D) Cell migration was detected by Transwell assay (n = 3) (Scale bar = 50 µm). Data were presented as mean $\pm$ standard deviation. $^{****}p < 0.0001$, compared to control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$, compared with PDGF-BB+pc-NC group; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$, compared to PDGF-BB+pc-*SFRP5* group. CCK-8, cell counting kit-8.

there are few studies on the effects of SFRP5 on HASMC proliferation and migration. In this study, PDGF-BB induced HASMCs to establish an *in vitro* model of childhood asthma. On this basis, SFRP5 expression in HASMCs decreased after PDGF-BB treatment. Further studies showed that overexpression of *SFRP5* reduced HASMC proliferation and migration after PDGF-BB treatment. It is suggested that SFRP5 might play a specific role in airway remodeling.

The Wnt/β-catenin pathway associated with asthma can regulate the proliferation, migration, and differentiation of ASMC [25]. In this experiment, c-Myc, β-catenin, and cyclinD1 protein expression increased after PDGF-BB stimulation of HASMCs, and the increase of the above proteins was inhibited after *SFRP5* overexpression. Our experiment also found that PDGF-BB induced the proliferation and migration of HASMCs partly by activating the Wnt/β-catenin pathway of HASMCs. Thus, SFRP5 could hamper HASMC proliferation and migration partly by inhibiting the Wnt/β-catenin pathway, thus playing a role in airway remodeling. However, which Wnt proteins play an important role in this process is still unknown and will be the focus of our subsequent research.

Conclusions

In summary, SFRP5 was under-expressed in serum and *in vitro* asthmatic cell models of children with asthma. Overexpressed *SFRP5* could inhibit HASMC proliferation and migration to some extent by the Wnt/β-catenin path-

DM
DISCOVERY MEDICINE
2001

way. However, this study is only a preliminary exploration of the possible role of *SFRP5* in asthma disease and its possible mechanisms. The pathogenesis of asthma and the action mechanism of *SFRP5* are complex, so further studies are still needed.

Availability of Data and Materials

All data generated or analyzed during this study are included in the published article. The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Author Contributions

YYS designed the research study. YYY and HHZ performed the research. SHH and YTZ collected and analyzed the data. YYY and HHZ 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 strictly adhered to the guidelines of Shanghai Baoshan Hospital of Integrated Traditional Chinese and Western Medicine, and this study was approved by the Ethics Committee of Shanghai Baoshan Hospital of Integrated Traditional Chinese and Western Medicine (Approval number: 202314).

Acknowledgment

Not applicable.

Funding

The financial of this research was supported by Medical Key Specialty of Baoshan District Municipal Health Commission in Shanghai (No. BSZK-2023-BZ08).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Sufianov A, Bessonova M, Begliarzade S, Kudriashov V, Danilov A, Ilyasova T, *et al*. Studies on the role of non-coding RNAs in controlling the activity of T cells in asthma. Non-coding RNA Research. 2023; 8: 211–217.
[2] Bridgeman MB, Wilken LA. Essential Role of Pharmacists in Asthma Care and Management. Journal of Pharmacy Practice. 2021; 34: 149–162.
[3] Stern J, Pier J, Litonjua AA. Asthma epidemiology and risk factors. Seminars in Immunopathology. 2020; 42: 5–15.
[4] Conrad LA, Cabana MD, Rastogi D. Defining pediatric asthma: phenotypes to endotypes and beyond. Pediatric Research. 2021; 90: 45–51.
[5] Alwarith J, Kahleova H, Crosby L, Brooks A, Brandon L, Levin SM, *et al*. The role of nutrition in asthma prevention and treatment. Nutrition Reviews. 2020; 78: 928–938.
[6] Teliewubai J, Ji H, Lu Y, Bai B, Yu S, Chi C, *et al*. SFRP5 serves a beneficial role in arterial aging by inhibiting the proliferation, migration and inflammation of smooth muscle cells. Molecular Medicine Reports. 2018; 18: 4682–4690.
[7] Yang G, Zheng D, Yang G, Liu Y. The relationship between secreted frizzled-related protein 5 and abnormal postpartum glucose metabolism in patients with gestational diabetes mellitus. Tianjin Medical Journal. 2022; 50: 1182–1186.
[8] Xu Q, Lü Z, Wang X, Zhu Q, Wu H. Secreted frizzled-related protein 5 suppresses aggressive phenotype and reverses docetaxel resistance in prostate cancer. Journal of Investigative Medicine: the Official Publication of the American Federation for Clinical Research. 2019; 67: 1009–1017.
[9] Russell JO, Monga SP. Wnt/β-Catenin Signaling in Liver Development, Homeostasis, and Pathobiology. Annual Review of Pathology. 2018; 13: 351–378.
[10] Colozza G, Koo BK. Wnt/β-catenin signaling: Structure, assembly and endocytosis of the signalosome. Development, Growth & Differentiation. 2021; 63: 199–218.
[11] Hussain M, Xu C, Lu M, Wu X, Tang L, Wu X. Wnt/β-catenin signaling links embryonic lung development and asthmatic airway remodeling. Biochimica et Biophysica Acta. Molecular Basis of Disease. 2017; 1863: 3226–3242.
[12] Liu LB, Chen XD, Zhou XY, Zhu Q. The Wnt antagonist and secreted frizzled-related protein 5: implications on lipid metabolism, inflammation, and type 2 diabetes mellitus. Bioscience Reports. 2018; 38: BSR20180011.
[13] Xu X, Yuan B, Ji F. The expression and correlation analysis of serum SFRP5, IL-13 and MPO in children with acute asthma attack. Translational Medicine Journal. 2021; 10: 358–361.
[14] Huang F, Li J, Diao Z, Lu H. Study on the correlation between serum SFRP5 Level and airway inflammation as well as pulmonary function in patients with bronchial asthma. Chinese Medical Record. 2021; 22: 102–105.
[15] Board E. Guideline for the diagnosis and optimal management of asthma in children (2016). Chinese journal of pediatrics. 2016; 54: 167–181.
[16] Pang X, Qiao J. Galectin-1 inhibits PDGF-BB-induced proliferation and migration of airway smooth muscle cells through the inactivation of PI3K/Akt signaling pathway. Bioscience Reports. 2020; 40: BSR20193899.
[17] Yuan JY, Wang XY, Tong ZY, Dong YC, Zhao JY, Zhang Y, *et al*. Promising Therapeutic Functions of Bone Marrow Mesenchymal Stem Cells Derived-Exosome in Asthma. Canadian Respiratory Journal. 2022; 2022: 1485719.
[18] Xiong DJP, Martin JG, Lauzon AM. Airway smooth muscle function in asthma. Frontiers in Physiology. 2022; 13: 993406.
[19] Banno A, Reddy AT, Lakshmi SP, Reddy RC. Bidirectional interaction of airway epithelial remodeling and inflammation in asthma. Clinical Science. 2020; 134: 1063–1079.
[20] In 't Veen JCCM. Treatment strategy in asthma: is the aim symptom reduction or controlling exacerbations? Nederlands Tijdschrift Voor Geneeskunde. 2018; 162: D2987.
[21] Rao M, Wu X, Lu Z. Relationship between serum SFRP5, CXCR4, 8-iso PG levels and airway inflammation and airway remodeling in children with bronchial asthma. Anhui Medical and Pharmaceutical Journal. 2022; 26: 2231–2236.
[22] Wang Y, Dai Q, Ru L. The changes and clinical significance of

serum Rac1 levels in children with bronchial asthma. Chinese Journal of General Practice. 2023; 21: 736–739.

[23] Wang KCW, Donovan GM, James AL, Noble PB. Asthma: Pharmacological degradation of the airway smooth muscle layer. The International Journal of Biochemistry & Cell Biology. 2020; 126: 105818.

[24] Salter B, Pray C, Radford K, Martin JG, Nair P. Regulation of human airway smooth muscle cell migration and relevance to asthma. Respiratory Research. 2017; 18: 156.

[25] Zhu Y, Guo Y, Qiao Y, Han J. Verbenalin inhibits airway inflammation and remodeling by modulating Wnt/B-catenin signal pathway in asthma rats. The Journal of Practical Medicine. 2018; 34: 2880–2883.