Original Article

MiR-140-3p Ameliorates The Inflammatory Response of Airway Smooth Muscle Cells by Targeting *HMGB1* to Regulate The *JAK2/STAT3* Signaling Pathway

Jun Meng, M.D.[1#], Yingxia Zou, M.D.[2#], Li Hou, M.D.[3#], Limin He, M.D.[4], Yuanjuan Liu, M.D.[4, 5], Menghan Cao, M.D.[4],

Chunjie Wang, M.D.[3*], Junying Du, M.D.[2*]

1. Maternity School, Yuhuangding Hospital, Yantai, Shandong Province, China
2. Children's Health Clinic, Yuhuangding Hospital, Yantai, Shandong Province, China
3. Department of Gynecology and Obstetrics, Yuhuangding Hospital, Yantai, Shandong Province, China
4. Department of Respiratory Medicine, Penglai Second People's Hospital, Penglai, Shandong Province, China
5. Department of Respiratory Medicine, The Second Hospital of Shandong University, Jinan, Shandong Province, China

Corresponding Addresses: Department of Gynecology and Obstetrics, Yuhuangding Hospital, Yantai, Shandong Province, China
Children's Health Clinic, Yuhuangding Hospital, Yantai, Shandong Province, China
Emails: ujju86586@163.com, xyuu60667@163.com

# These authors contributed equally to this work.

Abstract

Objective: The growth and migration of airway smooth muscle cells (ASMCs) are dysregulated in asthma. MicroRNAs (miRNAs) are associated with the pathogenesis of many diseases including asthma. Instead, the function of *miR-140-3p* in ASMCs' dysregulation in asthma remains inconclusive. This study aimed to explore the role and mechanism of *miR-140-3p* in ASMCs' dysregulation.

Materials and Methods: In this experimental study, ASMCs were stimulated with platelet-derived growth factor (PDGF)-BB to construct an asthma cell model *in vitro. MiR-140-3p* expression level in the plasma of 50 asthmatic patients and 50 healthy volunteers was measured with quantitative real-time polymerase chain reaction (qRT-PCR). Besides, the enzyme-linked immunosorbent assay (ELISA) was applied to detect the contents of interleukin (IL) -1β, IL-6, and tumor necrosis factor-α (TNF-α) in the cell culture supernatant of ASMCs. Additionally, CCK-8 and transwell assays were adopted to probe the multiplication and migration of ASMCs. In addition, the western blot was employed to examine *HMGB1, JAK2,* and *STAT3* protein expressions in ASMCs after *miR-140-3p* and *HMGB1* were selectively regulated.

Results: *miR-140-3p* expression was declined in asthmatic patients' plasma and ASMCs stimulated by PDGF-BB. Upregulating *miR-140-3p* suppressed the viability and migration of the cells and alleviated the inflammatory response while inhibiting *miR-140-3p* showed opposite effects. Additionally, *HMGB1* was testified as the target of *miR-140-3p*. HMGB1 overexpression could reverse the impact of miR-140-3p upregulation on the inflammatory response of ASMCs stimulated by PDGF-BB. *MiR-140-3p* could repress the activation of *JAK2/STAT3* via suppressing *HMGB1*.

Conclusion: In ASMCs, *miR-140-3p* can inhibit the *JAK2/STAT3* signaling pathway by targeting *HMGB1*, thus ameliorating airway inflammation and remodeling in the pathogenesis of asthma.

Keywords: Asthma, *HMGB1, JAK2/STAT3, miR-140-3p*

Introduction

Bronchial asthma is a prevalent chronic respiratory inflammatory disease, featuring airway inflammation, airway remodeling, and hyper responsiveness. It's been shown that various cells (eosinophils, T cells, neutrophils, mast cells, and airway epithelial cells, etc.) and inflammatory mediators are involved in its pathogenesis (1, 2). It is estimated that 5% of adults and 10% of children suffer from asthma (3, 4). The anti-inflammatory drugs and bronchodilators can effectively control airway inflammation and hyperresponsiveness respectively; however the current treatments are not enough to reverse airway remodeling. Reportedly, the abnormal proliferation and migration of airway smooth muscle cells (ASMCs) are pivotal in airway

remodeling (5). Specifically, platelet-derived growth factor BB (PDGF-BB) can promote the multiplication and migration of ASMCs, thus aggravating airway remodeling in asthma (6).

MicroRNAs (miRNAs) are single-stranded non-coding RNAs with about 19-22nt in length, which inhibit the translation process via binding with the 3´- untranslated region (3'-UTR) of mRNAs (7, 8). More and more evidence has shown that miRNAs are vital in modulating the phenotype of ASMCs in the pathogenesis of asthma (9). Up to now, many miRNAs including *miR-200a, miR-142*, and *miR-485* have been reported to be implicated in the regulation of multiplication and migration of ASMCs (10-12). In addition, *miR-140-3p* is declined in human bronchial smooth muscle cells which are

stimulated by interleukin (IL)-13 (13). Instead, how *miR-140-3p* modulates the dysfunction of ASMCs in asthma is indeterminate.

High mobility group box 1 (*HMGB1*), is known as a non-histone chromosome binding protein with a highly conserved structure (14). It is reported that *HMGB1* is an important inflammatory mediator, which is related to immune diseases, malignancies, and other diseases (15). *HMGB1* is also a pivotal regulator in airway inflammation and remodeling in asthma (16). The aim of this study was to investigate the regulatory effects of *miR-140-3p* on the proliferation, migration, and inflammatory response of ASMCs after PDGF-BB stimulation and to explore the interplay between *miR-140-3p* and *HMGB1*. This study revealed a new molecular mechanism in the process of asthma exacerbation and suggested a new theoretical foundation for its treatment.

Materials and Methods

Clinical samples

This experimental study, with the written informed consent of all patients, healthy volunteers, or guardians of participants, was endorsed by the Ethics Committee of Linyi Central Hospital (2017-0041). Experiments involving human tissue were performed according to the Declaration of Helsinki. Blood samples of 50 patients with acute asthma treated in Linyi Central Hospital from March 2018 to June 2019 were collected. Blood samples of 50 healthy volunteers were used as the negative control. The patients were selected by simple random sampling. Blood samples were collected by vacuum blood collection tubes containing ethylene diamine tetra acetic acid (EDTA) and centrifuged at 1600×g for 15 minutes at 4°C to obtain supernatant and at 16000×g for another 10 minutes at 4°C to separate plasma. Ultimately, the separated plasma was stored at -80°C for the subsequent analysis. 50 patients with acute exacerbation asthma, were included 32 males and 18 females, with a mean age of (35.8 ± 8.76) years. Inclusion criteria were A. Meeting the diagnostic criteria of the Global Initiative for Asthma (GINA) guidelines (2016) (17); B. Being in the acute exacerbation phase, C. Age ≥16 years old. Exclusion criteria: A. Any active severe infections, B. Any presence of malignant hematological diseases or tumors, and C. Recent treatment with corticosteroids, immunosuppressants, and immunomodulators. The enrolled controls were healthy individuals who underwent physical examination in our hospital during the same period, including 27 males and 23 females with a mean age of (36.7 ± 3.12) years. Inclusion criteria were: A. Normal lung function, B. No history of asthma or other allergic diseases, C. No infections within 4 weeks, and D. Age ≥16 years. Exclusion criteria: A. Those with autoimmune diseases, hematologic diseases, severe infections, and malignancies, B. Pregnant or lactating women, and C. A recent history of leukotriene receptor antagonists as well as glucocorticoid use.

Cell culture and cell transfection

In this experimental study, human ASMCs were available from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Subsequently, cells were cultivated in DMEM (Corning, Manassas, VA, USA) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin (Thermo Fisher Scientific, MA, USA) in 5% CO_2 at 37°C. When cells reached 90% confluence, subculture was carried out. ASMCs were treated with different concentration of PDGF-BB (0, 1, 10, 20, 40, 60 mg/mL, R&D Systems, Minneapolis, MN, USA) for different times (1-48 hours) to construct the *in vitro* model of asthma.

MiR-140-3p mimics (*miR-140-3p*), mimic negative control (miR-NC), *miR-140-3p* inhibitors (*miR-140-3p*-in), inhibitor negative control (miR-in), pcDNA3.0-*HMGB1* (*HMGB1*) and empty vector pcDNA3.0 were available from RiboBio (Guangzhou, China). Subsequently, ASMCs were transferred into a 24-well cell plate at $3×10^5$ cells/well, and cultured at 37°C in 5% CO_2 for 24 hours, and then the cells were transfected by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Quantitative real-time polymerase chain reaction

Total RNA was extracted from plasma and cells by TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was reverse transcribed into cDNA by TaqMan microRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) and PrimeScript RT Kit (Takara, Dalian, China). According to the manufacturer's protocol, qRT-PCR was conducted on ABI 7300 system (Applied Biosystems, Foster City, CA, USA) with an SYBR Green PCR Master Mix kit (Thermo Fisher Scientific, Carlsbad, CA, USA), with *U6* or *β-actin* as the endogenous control. Ultimately, the relative expressions were estimated by the $2^{-\Delta\Delta Ct}$ method. The primer sequences are detailed in Table 1.

Table 1: Primer sequences

Gene	Primer sequences (5´-3´)
miR-140-3p	F: GCGCGTACCACAGGGTAGAA
	R: AGTGCAGGGTCCGAGGTATT
U6	F: CTCGCTTCGGCAGCACATATACTA
	R: ACGAATTTGCGTGTCATCCTTGC
HMGB1	F: AGCTGCTAGCGCCTAGCGAT
	R: CCCGTCTGATAGCGCATTCGTGT
β-actin	F: CGTGCGTGACATTAAAGAG
	R: TTGCCGATAGTGATGACCT

Enzyme-linked immunosorbent assay

Cells and the medium were collected after 48 hours of continuous culture, and then the cells were under centrifuged at 1000×g for 10 minutes at 4˚C to collect the supernatant. ELISA kits (Shanghai Xitang Biotechnology Co, Ltd, Shanghai, China) were adopted to detect the contents of interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α), respectively, according to the manufacturer's instructions.

Cell proliferation assay

Cell counting kit 8 (CCK-8; Dojindo, Kumamoto, Japan) was adopted to estimate the proliferative capability of ASMCs. Cells were transferred into a 96-well plate (1×10^3 cells/well) and cultured at 37°C for 12 hours, and then 10 μL of CCK-8 solution was added to each well. After incubation of cells for 1 hour, the absorbance was detected at 450nm wavelength by a spectrophotometer reader (Bio-Rad, Hercules, CA, USA). With the same method, the absorbance of the cells was examined at the 24th, 48th, 72nd, and 96th hours, respectively. After that, the proliferation curve was plotted.

5-bromo-2´-deoxyuridine (BrdU) assay

Cell proliferation was also probed by a BrdU kit (Sigma-Aldrich, Louis, MO, USA). Briefly, the cells were transferred into 96-well plates and incubated with BrdU labeling reagent for 2 hours at 37°C. Next, the cells were incubated with FixDenat solution for 30 minutes at ambient temperature. Subsequently, the cells were incubated with the anti-BrdU antibody for 90 minutes in the dark. Thereafter the nucleus was stained with DAPI staining solution for 10 minutes. Subsequently, the ASMCs were washed with phosphate buffered saline (PBS, Beyotime Biotechnology, China) and observed under a fluorescence microscope.

Transwell assay

Cell migration was examined with the transwell chamber (24-well insert, 8-μm pore size, Corning Costar, Cambridge, MA, USA). Briefly, 1×10^5 cells resuspended in 200 μl of serum-free medium were transferred into the upper compartment, with the lower compartment filled with a medium containing 20% FBS. Twenty-four hours later, the ASMCs that failed to migrate were removed from the upper membrane surface with cotton swabs, and the migrating cells fixed in methanol were stained with 0.1% crystal violet. Cells were rinsed in tap water, dried, and then photographed under an optical microscope. Image J software was used for counting the cells.

Dual-luciferase reporter assay

Wild-type (WT) and mutant (MUT) sequences of *HMGB1* were subcloned into psi-CHECK2 luciferase reporter vector (Promega, Madison, WI, USA), and *HMGB1*-WT or *HMGB1*-MUT reporter vectors were constructed, respectively. Then luciferase reporter vector and *miR-140-3p* or miR-NC were co-transfected into ASMCs by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Forty-eight hours later, the luciferase activity was examined by a dual luciferase analysis system (Promega) as manufacturer's instructions.

Western blot

Total proteins in ASMCs were extracted with RIPA lysis buffer (Solarbio, Beijing, China), and their concentration was quantified by a BCA protein detection kit (Solarbio, Beijing, China). An equal amount of protein samples (20 μg per group) was separated by SDS-PAGE and transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, MA, USA), which was then blocked with 5% skimmed milk for 1 hour. The membrane was firstly incubated with the primary antibody at 4°C overnight, and then with the secondary antibody, goat anti-rabbit IgG H&L (Abcam, ab6721, 1: 3000) at 37°C for 1 hour. Ultimately, the bands were developed with the enhanced chemiluminescence reagent (Pierce Biotechnology, Rockford, IL, USA). The primary antibodies were: anti-*HMGB1* (1: 1000, ab79823), anti-p-*JAK2* (1: 1000, #8082), anti-p-*STAT3* (1: 1000, #9145), anti-*JKA2* (1: 1000, #3230), anti-*STAT3* (1: 1000, #12640) and anti-*β-actin* (1: 1000, ab6276). Among them, anti-*HMGB1* antibody and anti-*β-actin* antibody were bought from Abcam (Shanghai, China); anti-p-*JAK2* antibody, anti-p-*STAT3* antibody, anti-*JAK2* antibody, and anti-*STAT3* antibody were all purchased from Cell Signaling Technology (Cell Signaling Technology, Danvers, MA, USA).

Statistical analysis

SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA) was applied for statistical analysis, with data shown as mean ± standard deviation. Accordingly, the differences between the two groups were analyzed by student's t test. Besides, one-way ANOVA followed by Tukey post-hoc test was executed for comparing the data of more than two groups. $P<0.05$ indicated the statistical significance.

Results

miR-140-3p expression is declined in plasma of asthmatic patients and ASMCs stimulated by PDGF-BB

First, we evaluated *miR-140-3p* expression levels in the plasma of 50 asthmatic patients and 50 healthy volunteers by qRT-PCR and observed that *miR-140-3p* expressions in the plasma of asthmatic patients were remarkably

lower than that of healthy individuals (Fig.1A). qRT-PCR also suggested that PDGF-BB inhibited *miR-140-3p* expression in ASMCs dose- and time-dependently (Fig.1B, C). The half-inhibitory concentration (IC_{50}) value of PDGF-BB-induced *miR-140-3p* expression inhibition was about 22.37 ng/mL (Fig.1B). 24 hours of PDGF-BB treatment, were reduced *miR-140-3p* expression to less than 50% in ASMCs (Fig.1C). So, in the subsequent experiments, ASMCs were treated with 20 ng/mL PDGF-BB for 24 hours as the *in vitro* asthma model. It was found that compared with ASMCs in the control group, IL-1β, IL-6, and TNF-α levels in the asthma cell model were up-regulated (Fig.1D).
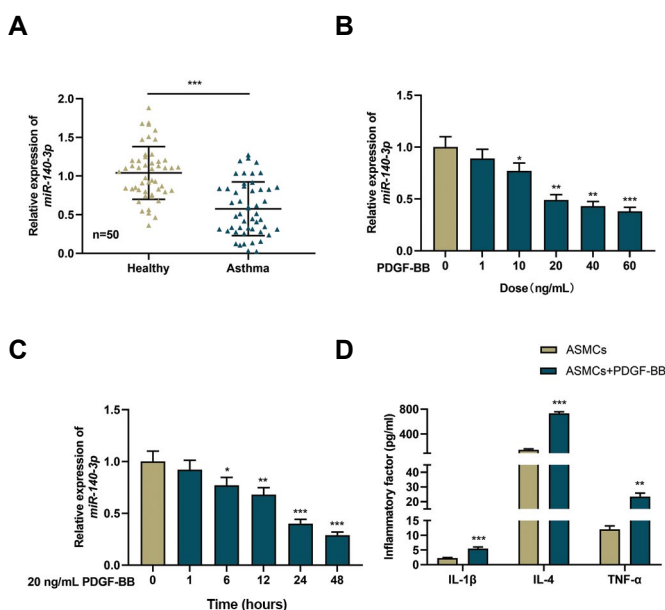

Fig.1: *miR-140-3p* is lowly expressed in plasma of asthmatic patients and ASMCs treated with PDGF-BB. **A.** The expression of *miR-140-3p* in plasma of asthmatic patients and healthy volunteers was detected by qRT-PCR. **B.** qRT-PCR was used to detect the relative expression of *miR-140-3p* after treating ASMCs with different concentrations (0, 1, 10, 20, 40, 60 mg/mL) of PDGF-BB for 24 hours. **C.** qRT-PCR was used to detect the relative expression of *miR-140-3p* after treating ASMCs with 20 ng/mL PDGF-BB for different time (1, 6, 12, 24, 48 hours). **D.** ELISA was used to detect the levels of IL-1β, IL-6 and TNF-α in the supernatant of ASMCs after treatment with 20 ng/mL PDGF-BB for 24 hours. Data are expressed as mean ± standard deviation (n=3). *; P<0.05, **; P<0.01, ***; P<0.001, ASMCs; Airway smooth muscle cells, qRT-PCR; Quantitative real-time polymerase chain reaction, PDGF-BB; Platelet-derived growth factor, ELISA; Enzyme-linked immunosorbent assay, IL-1β; Interleukin-1β, and TNF-α; tumor necrosis factor-α.

MiR-140-3p restrains the multiplication and migration of PDGF-BB-induced ASMCs

To study the biological function of *miR-140-3p* in the pathogenesis of asthma, we transfected miR-NC, *miR-140-3p* mimics, miR-in or *miR-140-3p* inhibitors into ASMCs, and successfully established the cell model of *miR-140-3p* overexpression or inhibition (Fig.2A). CCK-8, BrdU and transwell assays showed that overexpression of *miR-140-3p* significantly attenuated the promotion of PDGF-BB-induced proliferation and migration of

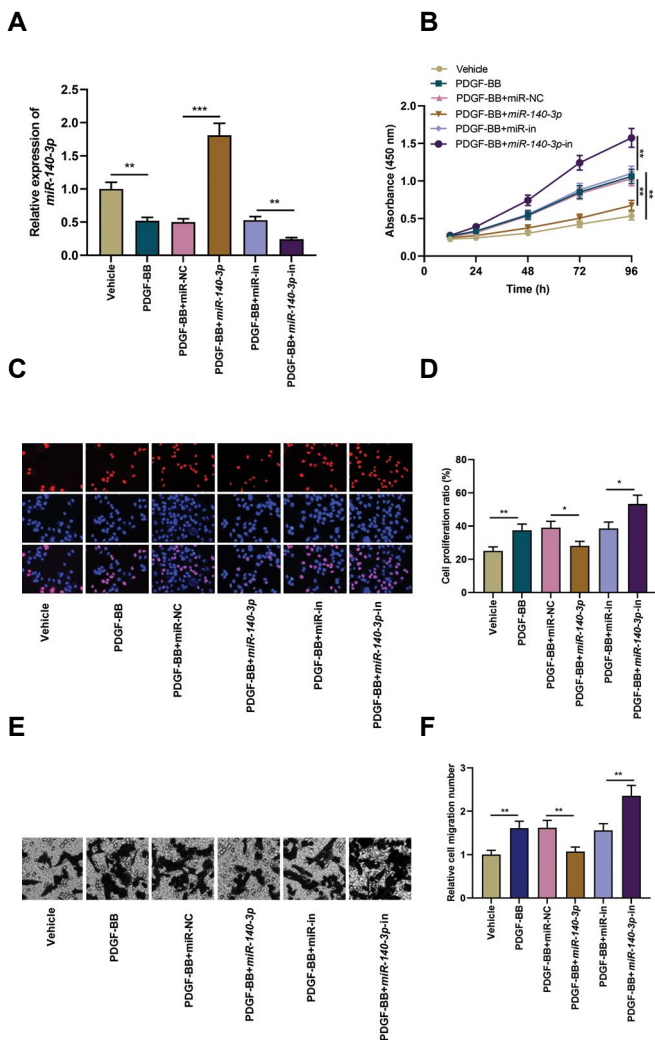ASMCs, while *miR-140-3p* inhibitor exerted the opposite effect (Fig.2B-F).

Fig.2: The effects of *miR-140-3p* on proliferation and migration of ASMCs induced by PDGF-BB. **A.** miR-*140-3p* mimics or inhibitors were transfected into ASMCs stimulated by 20 ng/mL PDGF-BB, and the expression of *miR-140-3p* was detected by qRT-PCR. **B.** CCK-8 assay, **C, D.** BrdU assay, and **E, F.** Transwell assay were used to detect the proliferation and migration of ASMCs stimulated by 20 ng/mL PDGF-BB after transfection. Data are expressed as mean ± standard deviation (n=3) (scale bar: C: 250 μm, E: 100 μm). *; P<0.05, **; P<0.01, ***; P<0.001, ASMCs; Airway smooth muscle cells, PDGF-BB; Platelet-derived growth factor, qRT-PCR; Quantitative real-time polymerase chain reaction, CCK-8; Cell counting kit 8, and BrdU; 5-bromo-2'-deoxyuridine.

MiR-140-3p represses the inflammatory response of PDGF-BB-treated ASMCs

Next, we performed ELISA and found that compared with the PDGF-BB+miR-NC group, the contents of IL-1β, IL-6, and TNF-α in the supernatant of ASMCs were markedly decreased subsequent to the transfection of *miR-140-3p* mimics; as against PDGF-BB+miR-in group, *miR-140-3p* inhibition restrained the production of IL-1β, IL-6, and TNF-α of PDGF-BB-stimulated ASMCs (Fig.3A-C), suggesting that *miR-140-3p* may repress the inflammatory response in asthma.
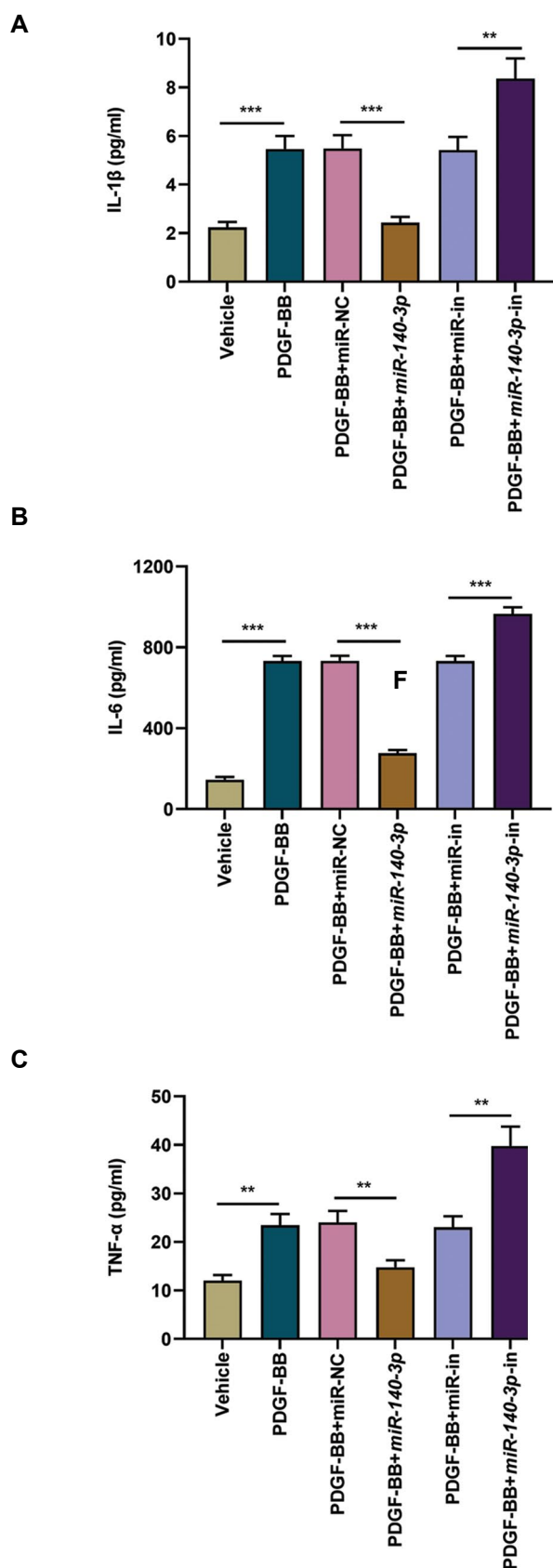
A

MiR-140-3p targets HMGB1

We then predicted the target genes of *miR-140-3p* with the StarBase database and observed that there was a complementary binding site between *miR-140-3p* and *HMGB1* mRNA 3´-UTR (Fig.4A). Dual-luciferase reporter gene assay showed that transfection of *miR-140-3p* mimics impaired *HMGB1*-WT activity, but that of *HMGB1*-MUT was not significantly affected (Fig.4B). qRT-PCR and western blot showed that in PDGF-BB-treated ASMCs, *miR-140-3p* up-regulation restrained *HMGB1* mRNA and protein expression, while *miR-140-3p* down-regulation had the opposite effect (Fig. 4C, D). Collectively, *HMGB1* was the downstream target of *miR-140-3p* in ASMCs.
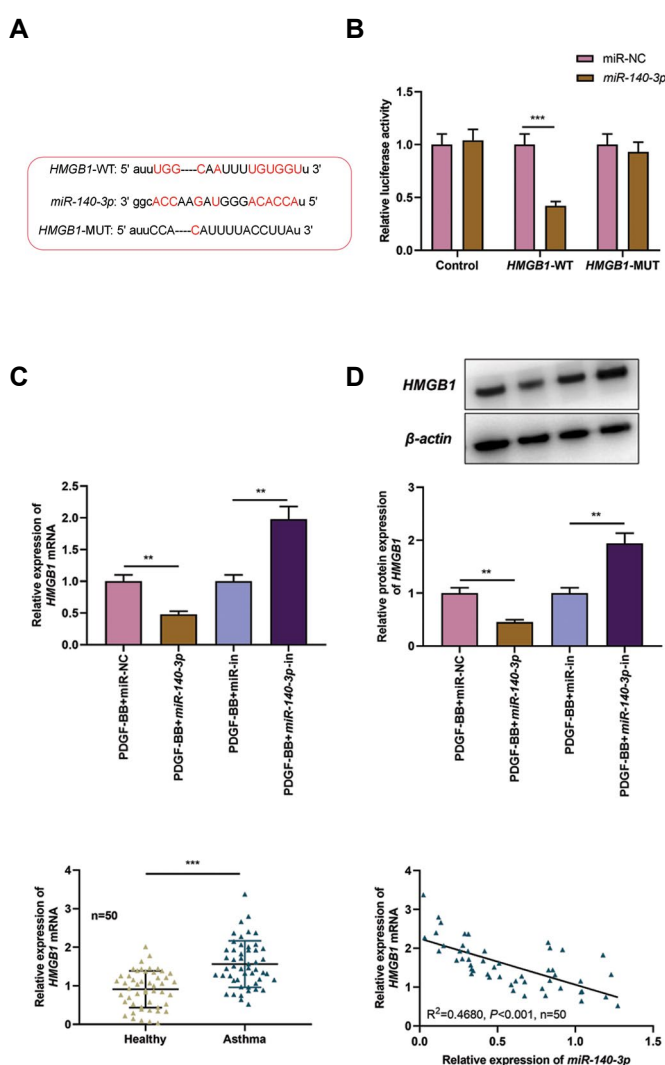
B

C

Fig.3: *miR-140-3p* inhibits the inflammatory reaction of ASMCs induced by PDGF-BB. ELISA was used to detect the levels of **A.** IL-1β, **B.** IL-6, and **C.** TNF-α in ASMC supernatants stimulated by 20 ng/mL PDGF-BB after transfection of *miR-140-3p* mimics or inhibitors. Data are expressed as mean ± standard deviation (n=3). **; P<0.01, ***; P<0.001, ASMCs; Airway smooth muscle cells, PDGF-BB; Platelet-derived growth factor, ELISA; Enzyme-linked immunosorbent assay, IL-1β; Interleukin-1β, and TNF-α; Tumor necrosis factor-α.

Fig.4: *HMGB1* is the downstream target of *miR-140-3p*. **A.** The binding sequence between *miR-140-3p* and *HMGB1* 3'-UTR. **B.** Dual-luciferase reporter gene experiment was used to verify the targeting relationship between *miR-140-3p* and *HMGB1*. **C.** qRT-PCR and **D.** Western blot were used to detect *HMGB1* mRNA and protein expression in ASMCs transfected with *miR-140-3p* mimics or inhibitors and stimulated by 20 ng/mL PDGF-BB. Data are expressed as mean ± standard deviation (n=3). **; P<0.01, ***; P<0.001, *HMGB1*; High mobility group box 1, qRT-PCR; Quantitative real-time polymerase chain reaction, ASMCs; Airway smooth muscle cells, and PDGF-BB; Platelet-derived growth factor.

HMGB1 **counteracts the impact of** ***miR-140-3p*** **in asthmatic ASMCs**

To study the function of the *miR-140-3p/HMGB1* axis in regulating the dysfunction of ASMCs, we transfected *miR-140-3p* mimics, *HMGB1* overexpression plasmid or co-transfected *miR-140-3p* mimics and *HMGB1* overexpression plasmids in ASMCs, respectively. qRT-PCR and western blot showed that *HMGB1* overexpression enhanced *HMGB1* mRNA and protein expressions in ASMCs stimulated by PDGF-BB and attenuated the impact of *miR-140-3p* overexpression on *HMGB1* (Fig.5A, B). CCK-8, BrdU, and transwell assays indicated that *HMGB1* overexpression markedly accelerated the multiplication and migration of PDGF-BB-treated ASMCs, and greatly counteracted *miR-*

140-3p overexpression's impacts on the growth and migration of PDGF-BB-stimulated ASMCs (Fig.5C-E). ELISA showed that compared with the PDGF-BB group, the contents of IL-1β, IL-6, and TNF-α in the PDGF-BB+*HMGB1* group were up-regulated significantly; compared with those in the PDGF-BB+*miR-140-3p* group, these inflammatory factors in the PDGF-BB+*miR-140-3p*+*HMGB1* group were also significantly increased (Fig.5F). Besides, Western blot showed that up-regulation of *miR-140-3p* repressed p-*JAK2* and p-*STAT3* expression levels in ASMCs, and *HMGB*1 overexpression significantly raised p-*JAK2* and p-*STAT3* expression levels, counteracting *miR-140-3p* overexpression's impact on p-*JAK2* and p-*STAT3* (Fig.5G).
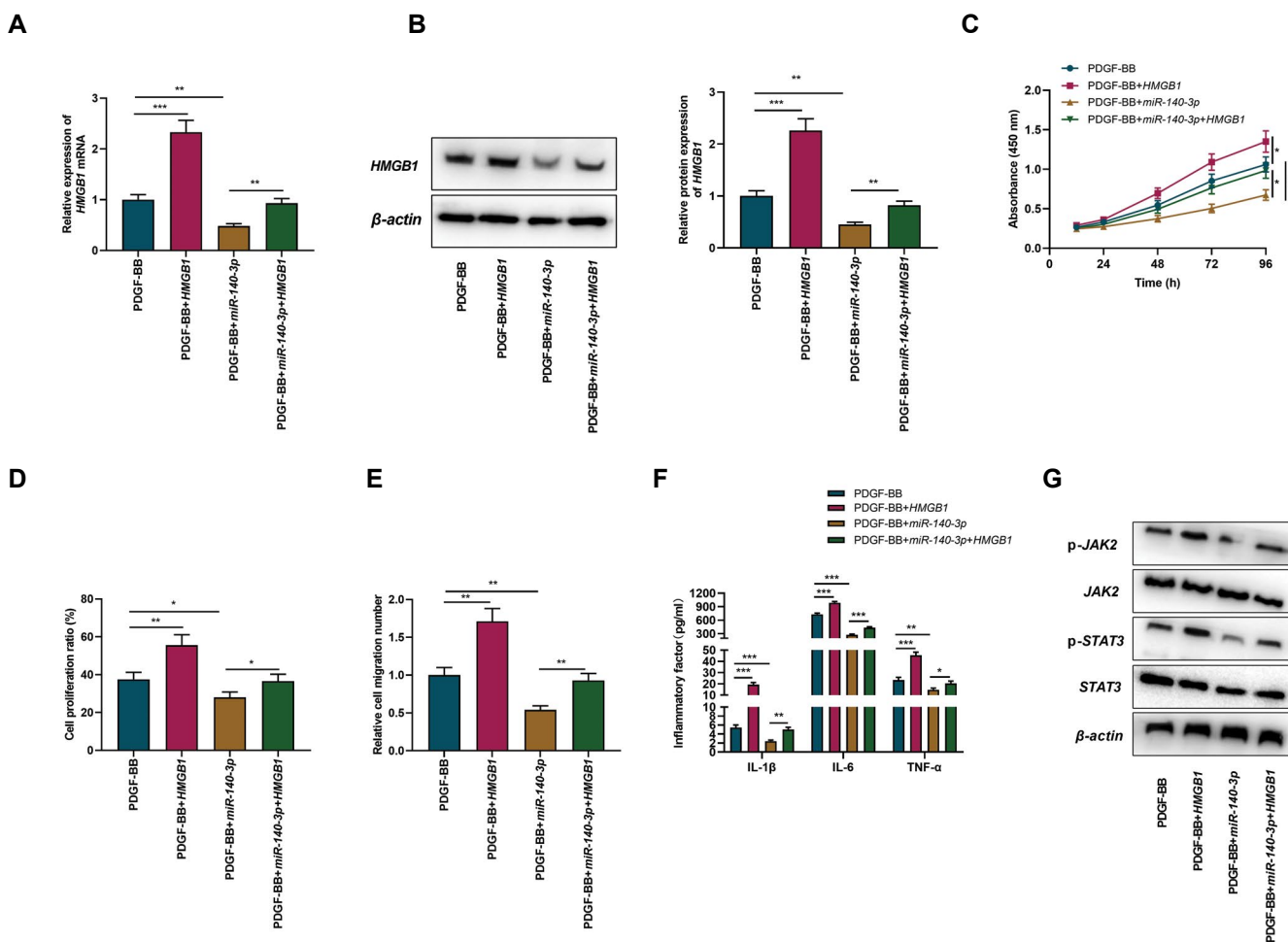

Fig.5: *miR-140-3p* targets *HMGB1* through *JAK2/STAT3* signaling pathway and inhibits the pathogenesis of asthma. **A.** qRT-PCR and **B.** Western blot were used to detect HMGB1 mRNA and protein expression in ASMCs stimulated with 20 ng/mL PDGF-BB after transfection with miR-140-3p mimics, HMGB1 overexpression plasmid or miR-140-3p mimics +HMGB1 overexpression. **C.** CCK-8 assay, **D.** BrdU assay, **E.** Transwell assay were used to detect the proliferation and migration of ASMCs stimulated by 20 ng/mL PDGF-BB after transfection. **F.** ELISA was used to detect the contents of IL-1β, IL-6, and TNF-α in the supernatant of ASMCs stimulated with 20 ng/mL PDGF-BB after transfection. **G.** Western blot assay was used to detect the protein expression of p-JAK2, p-STAT3, JAK2, and STAT3 in ASMCs stimulated by 20 ng/mL PDGF-BB after transfection. Data are expressed as mean ± standard deviation (n=3). *; P<0.05, **; P<0.01, ***; P<0.001, *HMGB1*; High mobility group box 1, *JAK2*; Janus Kinase 2, *STAT3*; Signal transducer and activator of transcription 3, qRT-PCR; Quantitative real-time polymerase chain reaction, ASMCs; Airway smooth muscle cells, PDGF-BB; Platelet-derived growth factor, CCK-8; Cell counting kit 8, and BrdU; 5-bromo-2'-deoxyuridine, ELISA; Enzyme-linked immunosorbent assay, IL-1β; Interleukin-1β, and TNF-α; Tumor necrosis factor-α.

Discussion

The abnormal growth and migration of ASMCs are crucial in the pathogenesis of respiratory diseases, like asthma (18). Many stimuli, such as growth factors, contraction agonists, inflammatory cytokines, and extracellular matrix proteins, have been reported to induce the multiplication and migration of ASMCs (19). PDGF-BB-induced growth and migration of ASMCs have been used to study the dysfunction of ASMCs in asthma *in vitro* (5). Here we observed that the stimulation of PDGF-BB markedly promoted the dysfunction of ASMCs, and when ASMCs were stimulated by PDGF-BB, the secretion of inflammatory cytokines was also significantly promoted, which is coherent with the previous studies (5, 20).

More and more evidence has shown that miRNAs exert crucial functions in regulating cell proliferation, migration, differentiation, and apoptosis, and are closely relevant to the progression of many human diseases, such as tumors, cardiovascular diseases, and asthma (21, 22). Importantly, it is reported that some miRNAs modulate the proliferation and migration of ASMCs (5, 23, 24). For example, *miR-638* expression is declined in PDGF-BB-induced ASMCs, and it suppresses the excessive growth and migration of ASMCs via pointing to *Cyclin D1* and *NOR1* (20); *miR-590-5p* represses the proliferation of ASMCs induced by PDGF via inhibiting *STAT3* (23); *miR-375* is down-regulated in ASMCs treated with PDGF, and it can block the proliferation and migration of ASMCs by targeting the *JAK2/STAT3* signaling (24). In this work, we demonstrated that the circulating *miR-140-3p* was down-regulated in the plasma of asthmatic patients. Furthermore, *miR-140-3p* expression was reduced in PDGF-BB-stimulated ASMCs, consistent with what was found in a previous study (25). Reportedly, *miR-140-3p* expression is impaired in ASMCs treated with TNF-α, and *miR-140-3p* can block the activation of *p38 MAPK* in ASMCs and inhibit the up-regulation of *CD38* induced by TNF-α (26). In addition, *miR-140-3p* inhibits PDGF-BB-induced ASMCs proliferation and promotes apoptosis by targeting *C-Myb* and *BCL-2* (25). Similarly, the present study confirmed that *miR-140-3*p up-regulation inhibited PDGF-BB-induced proliferation of ASMCs. Additionally, *miR-140-3p* up-regulation restrained the migration of ASMCs and the secretion of inflammatory cytokines induced by PDGF-BB; on the contrary, inhibition of *miR-140-3p* facilitated the growth, migration, and inflammation of ASMCs. Collectively, *miR-140-3p* could modulate the dysfunction of ASMCs in asthma.

To expound on the mechanism by which *miR-140-3p* regulates the proliferation and migration of ASMCs, we predicted the downstream targets of *miR-140-3p*. Interestingly, *HMGB1* was predicted as a target for *miR-140-3p*. Our results indicated that *miR-140-3p* could negatively modulate *HMGB1* expression in ASMCs. *HMGB1* can serve as a modulator in airway inflammation (27). Reportedly, resveratrol inhibits airway inflammation and remodeling in asthma via blocking the *HMGB1/*

TLR4/NF-κB pathway (28); cucurbitacin E can alleviate the injury and inflammation of bronchial epithelial cells induced by lipopolysaccharide via suppressing *HMGB1/TLR4/NF-κB* signaling (29). In a mouse model, *HMGB1* and *TLR4* depletion ameliorate asthma induced by diisononyl phthalate (DINP) (30). These studies imply that targeting *HMGB1* can probably alleviate the symptoms of asthma. In this study, we discovered that *HMGB1* overexpression promoted the viability, migration, and the activation of *JAK/STAT3* pathway of ASMCs stimulated by PDGF-BB. In addition, the effect of transfection of *miR-140-3p* mimics on the growth, migration, and inflammation of PDGF-BB-induced ASMCs could be counteracted by *HMGB1* overexpression. As reported, the abnormal activation of the *JAK/STAT3* pathway aggravates inflammatory diseases (31, 32). These findings suggest that, in the pathogenesis of asthma, the biological function of *miR-140-3p* in ASMCs is partly mediated by *HMGB1*.

Conclusion

miR-140-3p expression is suppressed in asthmatic patients' plasma and ASMCs stimulated by PDGF-BB. *miR-140-3p* inhibits *JAK/STAT3* signaling activation via targeting *HMGB1*, thus blocking the growth, migration, and inflammatory response of PDGF-BB-stimulated ASMCs. Our findings highlight that the *miR-140-3p/HMGB1* axis is pivotal in regulating the pathogenesis of asthma and imply that targeting the *miR-140-3p/HMGB1* axis is a promising strategy to treat asthma.

Acknowledgments

Authors' Contributions

Y.L., C.W., J.D.; Designed the study and experiments. J.M., Y.Z., L.Ho., Y.L., M.C.; Collected clinical samples and performed the experiments. J.M., Y.Z., L.Ho., Y.L., L.He.; Conducted the data analysis. J.M., Y.Z., L.Ho., Y.L.; Drafted the manuscript. Y.L., J.M.; Reviewed and revised the manuscript. All authors read and approved the final manuscript.

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