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RAB11A aggravates PDGF-BB-stimulated proliferation, migration, and inflammation of airway smooth muscle cells via affecting the NF-κB and PI3K/AKT pathways

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Abstract
Background: Pediatric asthma is an usual disease and a kind of fearful health threat for children. Airway smooth muscle cells (ASMCs) with increased cell proliferation and migration abilities serve as important features in the progression of asthma. RAB11A has been shown to aggravate cancer progression and is closely associated with inflammation. Gene analysis discovered that RAB11A exhibited higher expression in asthmatic patients. However, the detailed regulatory function of RAB11A in asthma still needs further investigation.
Method: The mRNA and protein expressions of genes were examined through RT-qPCR and western blot. Cell proliferation was examined through MTT and BrdU assays. Cell apoptosis was tested through flow cytometry. The cell migration ability was detected through wound healing and transwell assays. The levels of TNF-α, IL-1β, IL-8, and IL-6 were measured through ELISA.
Result: In this study, the mRNA and protein expressions of RAB11A were increased with PDGF-BB treatment in a dose-dependent manner. Additionally, the silencing of RAB11A suppressed the proliferation ability of PDGF-BB-mediated ASMCs. Moreover, it was uncovered that the knockdown of RAB11A inhibited the migration ability of PDGF-BB-stimulated ASMCs. Besides, suppression of RAB11A relieved the inflammatory response in PDGF-BB-stimulated ASMCs. Lastly, inhibition of RAB11A retarded the NF-κB and PI3K/AKT pathways.
Conclusion: Our results revealed that RAB11A aggravated PDGF-BB-stimulated proliferation, migration, and inflammation of ASMCs through modulating NF-κB and PI3K/AKT signaling pathways. This finding implied that the RAB11A may be deemed as a novel and prospective biomarker for asthma treatment.

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Introduction

Childhood asthma is a common chronic disease, and its incidence rate has been augmented over the past few years.[1-3] The characteristics of asthma are bronchial hypersensitiveness, airway inflammation, and airway obstruction.[4] Basophils and type 2 innate lymphoid cells have been discovered to participate in the regulation of asthma.[5,6] ASMCs play critical roles in asthma progression. On the one hand, the increased ASMC proliferation and migration are conducive to thickness changes in airway smooth muscle and aggravate airway remodeling.[7] On the other hand, abnormal ASMCs can result in extracellular matrix production and deposition, as well as ASMC hypertrophy and hyperplasia, ultimately bringing about airway remodeling.[8] The abnormal proliferation of ASMCs is induced by frequent stimulation with contractile agonists, pro-inflammatory cytokines, and growth factors.[9,10] Among them, the PDGF-BB subtype of platelet-derived growth factor (PDGF) family is acquired from inflammatory cells and airway epithelial cells in asthma.[11] Researches have clarified that PDGF-BB drives the transformation of the contractile phenotype of ASMCs to the proliferative, migratory, and synthetic phenotypes (facilitating remodeling), therefore triggering the asthma model.[12,13]

RAB11A can control Rac activity, which belongs to the Rab family.[14] RAB11A has been notarized to participate in intercellular communication, and RAB11A is bound up with endosomes and is helpful to the assembly and function of spindle pole.[15,16] Lately, RAB11A disorders have been linked to diversified cancers' progression. For example, RAB11A is overexpressed in gastric cancer, and highly expressed RAB11A facilitates the activity of FAK/AKT pathway to strengthen cell proliferation and migration.[17] Furthermore, by stimulating the PI3K/AKT pathway, RAB11A enhances the expression of MMP protein to aggravate the hepatocellular carcinoma (HCC) cell migration, invasion, and other malignant phenotypes.[18] However, there are no reports on the regulation of RAB11A in asthma, and the minute mechanism is not clear. Gene analysis demonstrated that RAB11A exhibits higher expression in asthmatic patients,[19] suggesting that RAB11A may be closely related to the mechanism of asthma.

In this work, the objective is to probe the function and regulatory mechanism of RAB11A in asthma. Our results showed that RAB11A expressed higher in PDGF-BB-stimulated ASMCs and facilitated cell proliferation, migration, and inflammation through modulating NF-κB and PI3K/AKT signaling pathways. This work showed that our discovery may contribute to asthma treatment.

Materials and Methods

Cell lines and cell culture

The ASMCs (cat. no. 3400) were obtained from the Sciencell Research Laboratories (Carlsbad, CA, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) with 10% fetal bovine serum (FBS, Gibco, USA). These cells were kept in an incubator at 37°C with 5% CO_2.

The platelet-derived growth factor BB (PDGF-BB; 0, 5, 10, 20, and 40 ng/mL; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) was utilized to treat ASMCs, and 20 ng/mL PDGF-BB was adopted for constructing the cell model of asthma.

Transfection

The short hairpin RNA (shRNA) was designed to knockdown RAB11A (shRAB11A), and its negative control (shNC) was synthesized from GenePharma (Shanghai, China). The transfection of shNC/shRAB11A plasmids into ASMCs was made through Lipofectamine 3000 reagent (Invitrogen, USA).

RT-qPCR

The extraction of RNAs from ASMCs was performed with the TRIzol reagents (Invitrogen, Carlsbad, CA); then, they were reversely transcribed into cDNAs with the PrimeScript™ RT Master Mix kit (Takara, Dalian, China). qRT-PCR was performed using the SYBR Premix Ex Taq™ (Takara, Dalian, China). The quantification was normalized to GAPDH through the $2^{-\Delta\Delta Ct}$ method.

The primer sequences were as follows:

RAB11A forward, 5′-TGCCTGTGTGTCAATGGGG-3′ and reverse, 5′-GCAGAGCTTCTACTGTACTGAGA-3′;

TNF-α forward, 5′-CTGCACTTTGGAGTGATCGG-3′ and reverse, 5′-GCTTGAGGGTTTGCTACAACAT-3′;

IL-1β forward, 5′-GGATATGGAGCAACAACTGG-3′ and reverse, 5′-ATGTACCAGTTGGGGAACTG-3′;

IL-8 forward, 5′-GACCACACTGCGCCAACAC-3′ and reverse, 5′-CTTCTCCACAACCCTCTGCAC-3′;

IL-6 forward, 5′-ACTCACCTCTTCAGAACGAATTG-3′ and reverse, 5′-CCATCTTTGGAAGGTTCAGGTTG-3′;

GAPDH forward, 5′-AACGTGTCAGTGGTGGACCTG-3′ and reverse, 5′-AGTGGGTGTCGCTGTTGAAGT-3′.

Western blot

The extraction of proteins from ASMCs was made with Radio-Immunoprecipitation Assay (RIPA) lysis buffer (Thermo Fisher Scientific, Inc., USA); then, they were segregated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and moved to polyvinylidene fluoride (PVDF) membranes (Beyotime, Shanghai, China). After being sealed, primary antibodies RAB11A (1 μg/mL; ab65200; Abcam, Shanghai, China), MMP9 (1:1000; ab76003), MMP2 (1:1000; ab92536), p-AKT (1:1000; ab38449), AKT (1:500; ab8805), p-mTOR (1:1000; ab109268), mTOR (1:1000; ab32028), p-NF-κB (1:1000; ab76302), NF-κB (0.5 μg/mL; ab16502), and β-actin (1 μg/ml; ab8226) were added to the membranes overnight at 4°C. Further, the appropriate secondary antibodies (1:2000; ab7090; Abcam) were mixed with the membranes for cultivation. β-actin was employed as the internal reference. Lastly, the chemiluminescence detection kit (Thermo Fisher Scientific, Inc., USA) was utilized to evaluate the bands.

MTT assay

ASMCs (1×10^3 cells/well) were placed in a 96-well plate. Post 24 h, a new medium with MTT solution (10 μL, 0.5 mg/mL, Solarbio, Beijing, China) was added. After 4 h, DMSO (100 μL, Sigma, USA) was added to each well. The absorbance at 490 nm was evaluated using a microplate reader (BioTek Instruments, Inc., USA).

BrdU assay

The BrdU-based Cell Proliferation ELISA (Roche Applied Science, Mannheim, Germany) was utilized to assess cell proliferation. In the control group, BrdU incorporation was set to 100%. The ratio was the absorbance of ASMCs in the other group/ASMCs in the control group.

Flow cytometry

The Annexin V-FITC/PI apoptosis kit (Nanjing KeyGen Biotech Co., Ltd., China) was employed to examine cell apoptosis. Post being harvested, annexin V-fluorescein isothiocyanate (Annexin V-FITC, 5 µL) and propidium iodide (PI, 5 µL) were added to ASMCs in the dark. The cell apoptosis was assessed through a flow cytometer (Thermo Fisher Scientific, Rockford, IL, USA). Cells were dyed with annexin V-FITC and PI to distinguish populations of early apoptotic (Q3), late apoptotic (Q2), and necrotic (Q1) cells. The percentage of apoptotic cells was calculated as the sum of the percentages of early apoptotic cells and late apoptotic cells.

Wound healing assay

ASMCs were added to a six-well plate until 90% confluence. Then, a wound was created through a pipette tip (200 µL). After being gently washed, ASMCs were further cultured for 12 h. The wound was assessed and imaged at 0 and 12 h under light microscopy.

Transwell assay

For migration assay, ASMCs in serum-free DMEM were put in the top chamber (8 µm pore size; Millipore, Billerica, MA, USA), and medium with 10% FBS was put in the bottom chamber. Post 24 h, the migrated cells were settled through methanol and dyed through crystal violet. The migrated cells were counted in five random optical fields under an inverted microscope (Nikon, Tokyo, Japan).

ELISA

ASMC culture supernatants were gathered, and the levels of TNF-α (ab181421), IL-1β (ab214025), IL-6 (ab178013), and IL-8 (ab46032) were assessed through the commercial ELISA kits (Abcam, Shanghai, China).

Statistical analysis

SPSS 20.0 software (IBM Corp. Armonk, NY, USA) was employed for statistical analysis. The data were presented as mean ± standard deviation (SD). All experiments were done in triplicate. The comparisons among groups (two or more) were made through the Student's t-test or one-way ANOVA. $p<0.05$ was deemed a significant difference.

Results

RAB11A exhibited higher expression in PDGF-BB-triggered ASMCs

As demonstrated in Figure 1A-B, the mRNA and protein expressions of RAB11A were elevated with PDGF-BB treatment (0, 5, 10, 20, and 40 ng/mL) in a dose-dependent manner (*$p<0.05$, ***$p<0.001$). RAB11A expression was markedly increased in PDGF-BB 20 and 40ng/mL treated cells; therefore, the 20 ng/mL PDGF-BB treatment was selected for subsequent experiments. These findings indicated that RAB11A exhibited higher expression in PDGF-BB-triggered ASMCs.

Silencing of RAB11A weakened the proliferation ability of PDGF-BB-mediated ASMCs

At first, the knockdown capacity of shRAB11A was confirmed (Figure 2A and B) (***$p<0.001$ vs the control group; ###$p<0.001$ vs the PDGF-BB+shNC group). The increased cell proliferation stimulated by PDGF-BB treatment was attenuated after RAB11A suppression (Figure 2C-D) (***$p<0.001$

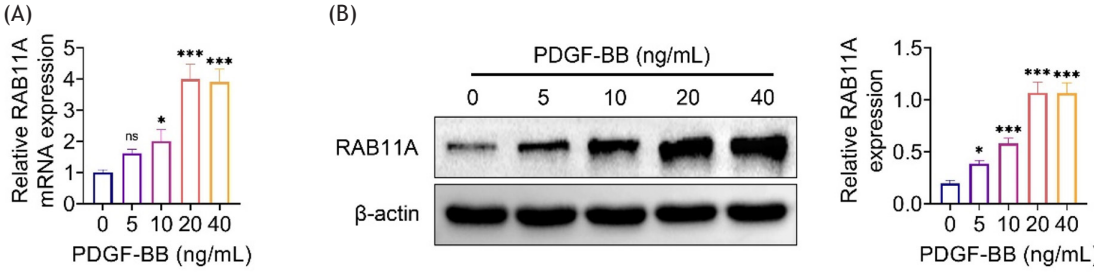

Figure 1 RAB11A was highly expressed in PDGF-BB-triggered ASMCs. (A) The increased mRNA expression of RAB11A was confirmed with different concentrations of PDGF-BB (0, 5, 10, 20, and 40 ng/mL) through RT-qPCR. (B) The increased protein expression of RAB11A was identified with different concentrations of PDGF-BB (0, 5, 10, 20, and 40 ng/mL) through western blot. *$p<0.05$, ***$p<0.001$.

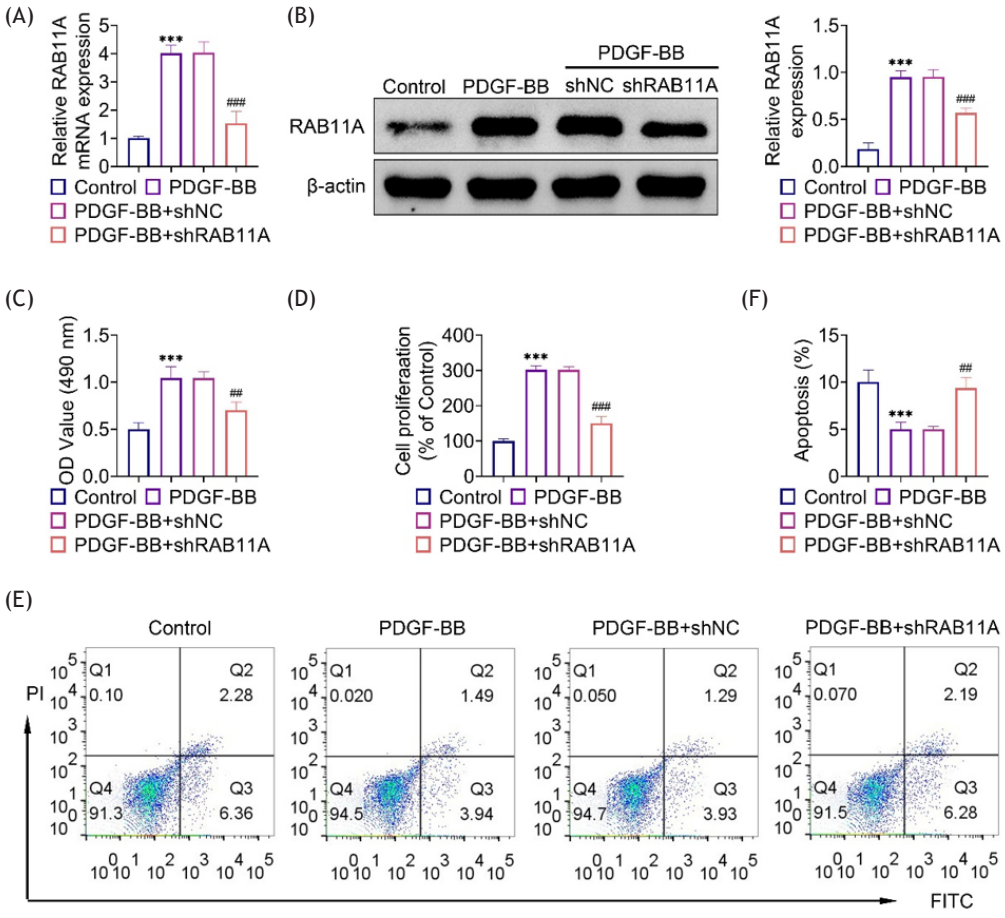

Figure 2 Silencing of RAB11A suppressed the proliferation ability of PDGF-BB-mediated ASMCs. Groups were divided into the Control, PDGF-BB, PDGF-BB+shNC, and PDGF-BB+shRAB11A group. (A, B) The mRNA and protein expressions of RAB11A were identified after RAB11A knockdown through RT-qPCR and western blot. The increased RAB11A mRNA and protein expressions stimulated by PDGF-BB treatment were relieved after RAB11A knockdown. (C, D) Cell proliferation was examined through MTT and BrdU assays. The enhanced cell proliferation mediated by PDGF-BB treatment was attenuated after RAB11A suppression. (E, F) Cell apoptosis was tested through flow cytometry. The reduced cell apoptosis mediated by PDGF-BB treatment was reversed after RAB11A inhibition. ***p<0.001 vs the control group; $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 vs the PDGF-BB+shNC group.

vs the control group; $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 vs the PDGF-BB+shNC group). In addition, the weakened cell apoptosis mediated by PDGF-BB treatment was reversed after RAB11A inhibition (Figure 2E-F) (***p<0.001 vs the control group; $^{\#\#}p$<0.01 vs the PDGF-BB+shNC group). Taken together, the silencing of RAB11A suppressed the PDGF-BB-mediated ASMC proliferation.

Knockdown of RAB11A inhibited the migration ability of PDGF-BB-triggered ASMCs

Further experiments revealed that the enhanced cell migration ability induced by PDGF-BB treatment could be offset after silencing RAB11A (Figure 3A and B) (**p<0.01, ***p<0.001 vs the control group; $^{\#}p$<0.05, $^{\#\#\#}p$<0.001 vs the PDGF-BB+shNC group). Moreover, the upregulated MMP9 and MMP2 levels mediated by PDGF-BB treatment were relieved by repressing RAB11A (Figure 3C) (***p<0.001 vs the control group; $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 vs the PDGF-BB+shNC group). In summary, knockdown of RAB11A inhibited the PDGF-BB-stimulated ASMC migration.

Suppression of RAB11A relieved the inflammatory response in PDGF-BB-mediated ASMCs

The increased mRNA expression of TNF-α, IL-1β, IL-8, and IL-6 triggered by PDGF-BB treatment was rescued after RAB11A knockdown (Figure 4A) (***p<0.001 vs the control group; $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 vs the PDGF-BB+shNC group). Additionally, through ELISA, it was discovered that the enhanced levels TNF-α, IL-1β, IL-8, and IL-6 induced by PDGF-BB treatment were weakened after RAB11A inhibition (Figure 4B) (*p<0.05, ***p<0.001 vs the control group; $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 vs the PDGF-BB+shNC group). In short, suppression of RAB11A relieves the inflammatory response in PDGF-BB-stimulated ASMCs.

Inhibition of RAB11A retarded the NF-κB and PI3K/AKT pathways

At the end, the NF-κB and PI3K/AKT pathway-related proteins were examined through western blot. It was demonstrated that the strengthened protein expressions of

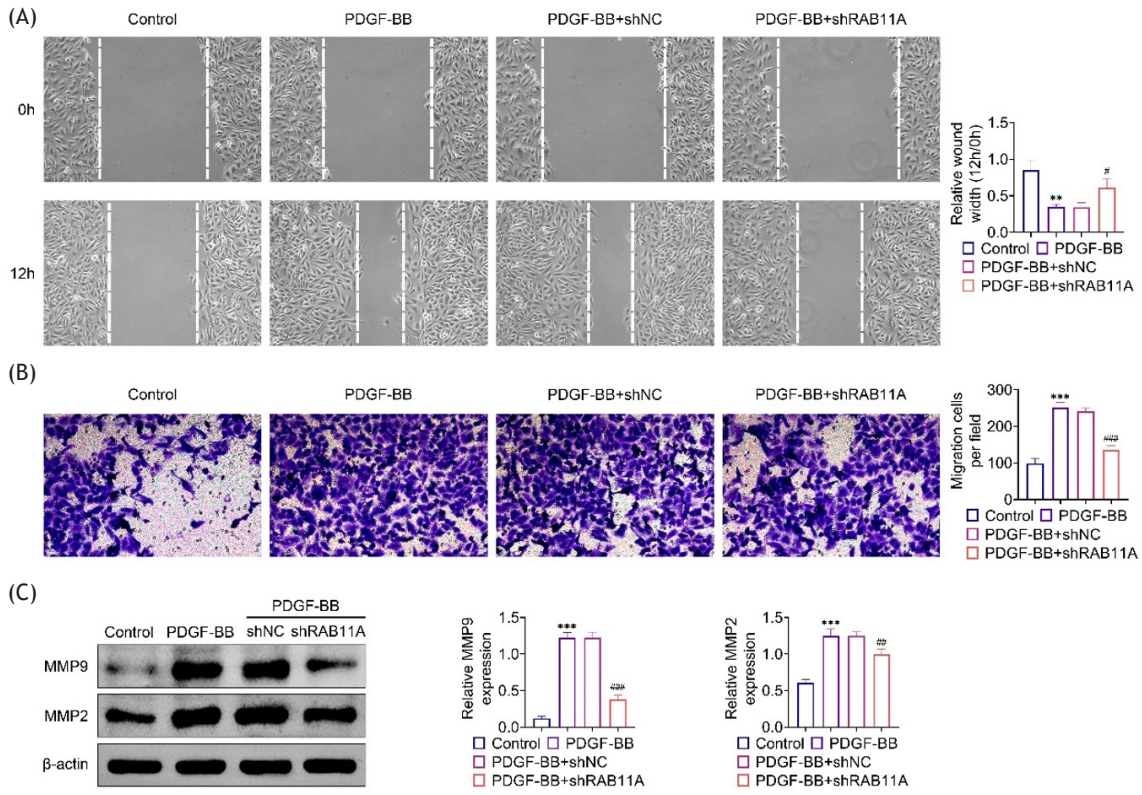

Figure 3 Knockdown of RAB11A inhibited the migration ability of PDGF-BB-stimulated ASMCs. Groups were divided into the Control, PDGF-BB, PDGF-BB+shNC, and PDGF-BB+shRAB11A group. (A, B) The cell migration ability was detected through wound healing and transwell assays. The increased cell migration ability induced by PDGF-BB treatment could be offset after silencing RAB11A. (C) The protein expression of MMP9 and MMP2 was examined through western blot. The upregulated MMP9 and MMP2 levels mediated by PDGF-BB treatment were relieved by repressing RAB11A. ** $p<0.01$, *** $p<0.001$ vs the control group; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ vs the PDGF-BB+shNC group.

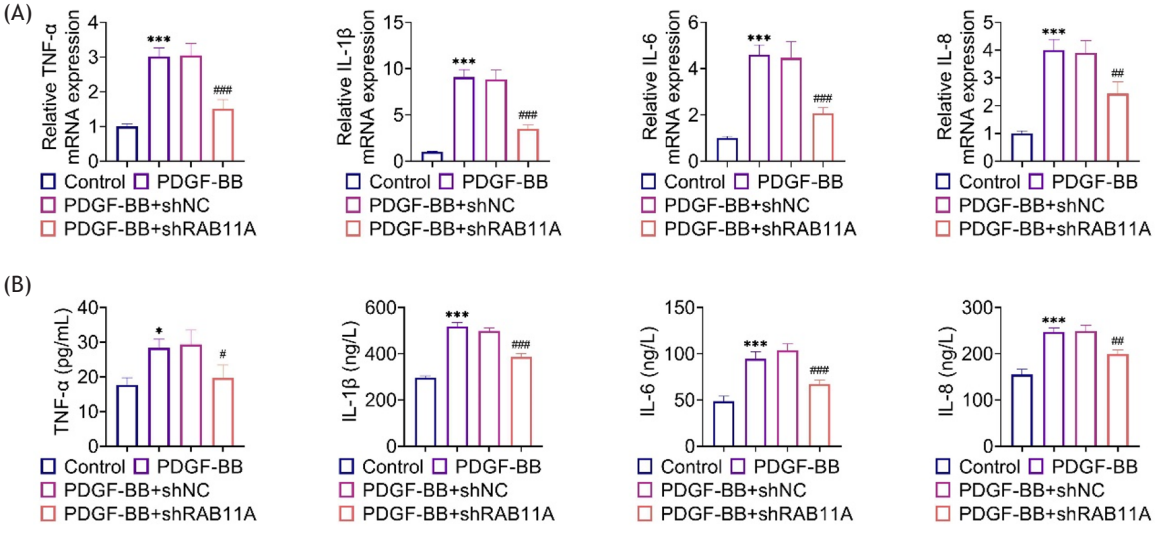

Figure 4 Suppression of RAB11A relieved the inflammatory response in PDGF-BB-stimulated ASMCs. Groups were divided into the Control, PDGF-BB, PDGF-BB+shNC, and PDGF-BB+shRAB11A group. (A) The mRNA expression of TNF-α, IL-1β, IL-8, and IL-6 was examined through RT-qPCR. The increased mRNA expression of TNF-α, IL-1β, IL-8, and IL-6 triggered by PDGF-BB treatment was rescued after RAB11A knockdown. (B) The levels of TNF-α, IL-1β, IL-8, and IL-6 were measured through ELISA. The enhanced levels TNF-α, IL-1β, IL-8, and IL-6 induced by PDGF-BB treatment were weakened after RAB11A inhibition. * $p<0.05$, *** $p<0.001$ vs the control group; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ vs the PDGF-BB+shNC group.

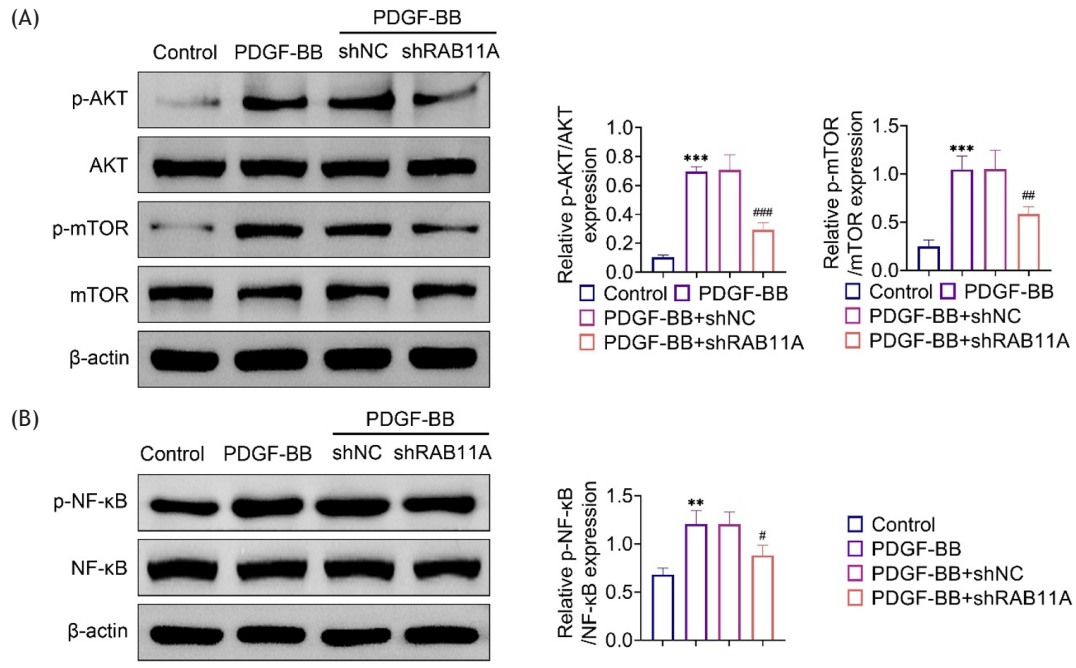

Figure 5 Inhibition of RAB11A retarded the NF-κB and PI3K/AKT pathways. (A, B) The protein expressions of p-AKT, AKT, p-mTOR, mTOR, p-NF-κB, and NF-κB were tested in the Control, PDGF-BB, PDGF-BB+shNC, and PDGF-BB+shRAB11A groups through western blot. The strengthened protein expressions of p-AKT/AKT, p-mTOR/mTOR, and p-NF-κB/NF-κB mediated by PDGF-BB treatment were receded after RAB11A knockdown. **p<0.01, ***p<0.001 vs the control group; #p<0.05, ##p<0.01, ###p<0.001 vs the PDGF-BB+shNC group.

p-AKT/AKT, p-mTOR/mTOR, and p-NF-κB/NF-κB mediated by PDGF-BB treatment were receded after RAB11A knockdown (Figure 5A-B) (**p<0.01, ***p<0.001 vs the control group; #p<0.05, ##p<0.01, ###p<0.001 vs the PDGF-BB+shNC group). These results suggested that inhibition of RAB11A retarded the NF-κB and PI3K/AKT pathways.

Discussion

In this study, it was shown for the first time that the mRNA and protein expressions of RAB11A were elevated with PDGF-BB treatment in a dose-dependent manner. Additionally, the silencing of RAB11A suppressed the cell proliferation, migration, and inflammatory response in PDGF-BB-stimulated triggered ASMCs. Lastly, inhibition of RAB11A retarded the activated NF-κB and PI3K/AKT pathways mediated by PDGF-BB treatment.

The hyperactive ASMCs are the main features of asthma.[20,21] Further, PDGF-BB has been substantiated to induce asthma cell models through enhancing cell proliferation and migration.[22] Thus, the asthma cell model stimulated by PDGF-BB was first made. RAB11A has been confirmed to exacerbate cancers' progression.[17,18] and exhibited higher expression in asthmatic patients.[19] However, its function in asthma remains vague. In this study, it was shown that the mRNA and protein expressions of RAB11A were elevated with PDGF-BB treatment in a dose-dependent manner. Additionally, silencing of RAB11A suppressed the proliferation and migration capacity of PDGF-BB-mediated ASMCs.

Meanwhile, RAB11A expression has been investigated to be closely related to the inflammatory response. In mice subjected to an endotoxin attack, intratracheal infusion of RAB11A-knockdown macrophages cut down neutrophils and relieved inflammatory lung injury.[23] Additionally, RAB11A controls the biochemical association of YAP with a variety of adhesion-linked components, including α-catenin, β-catenin, and the tumor suppressor Merlin, for rapid repair after tissue injury.[24] Furthermore, RAB11A facilitates the expression and recycling of VE-cadherin on the plasma membrane of endothelial cells, thus suppressing vascular leakage.[25] In the present work, we discovered that suppression of RAB11A relieved the inflammatory response in PDGF-BB-stimulated ASMCs.

The NF-κB pathway has been proven to be a valid regulatory pathway in asthma. For instance, Shikonin retards the ERK-NF-κB pathway to attenuate allergic airway remodeling.[26] Moreover, scopoletin modulates the NF-κB signaling pathway to alleviate PDGF-BB-triggered proliferation and migration of ASMCs.[27] LncRNA NKILA accelerates M2 macrophage polarization and represses the NF-κB pathway in asthmatic mice to relieve airway inflammation.[28] In addition, berberine affects the NF-κB signaling pathway to ameliorate airway inflammation in the ovalbumin-triggered asthma rat model.[29] The PI3K/AKT pathway also plays critical roles in the regulation of asthma. For example, miR-200a targets FOXC1 in ovalbumin-mediated asthmatic mice to modulate the PI3K/AKT pathway and influences the ASMC proliferation and airway remodeling.[30] LncRNA RP5-857K21.7 suppresses PDGF-BB-triggered the ASMCs proliferation and migration through regulating miR-508-3p/PI3K/

AKT/mTOR axis.[31] Additionally, galectin-1 inactivates the PI3K/Akt signaling pathway to retard PDGF-BB-mediated ASMC growth.[32] LncRNA-CASC7 weakens the PI3K/AKT signaling pathway to heighten corticosteroid sensitivity in severe asthma.[33] Furthermore, triptolide strengthens the G0/G1 cell cycle arrest and restrains the AKT/NF-κB/cyclinD1 signaling pathway to repress PDGF-stimulated ASMC proliferation.[34] Importantly, in this study, we discovered that inhibition of RAB11A retarded the NF-κB and PI3K/AKT pathways.

To sum up, RAB11A aggravated PDGF-BB-stimulated proliferation, migration, and inflammation of ASMCs through modulating NF-κB and PI3K/AKT pathways. This discovery suggested that RAB11A may be a useful target for asthma treatment. However, there are still many limitations to our study, which only explores the effects of RAB11A on cell proliferation, migration, and inflammation in asthma. In the future, more experiments associated with RAB11A are needed to affirm its other effects in asthma.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Competing Interests

The authors state that there are no conflicts of interest to disclose.

Contribution of Authors

Ying Gong and Yunhai Hu designed the study, Jianqiong Huang supervised the data collection and analyzed interpreted the data, and Haibo Wang prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

Reference

1. Boulet LP, O'Byrne PM. Asthma and exercise-induced bronchoconstriction in athletes. N Engl J Med. 2015;372(7):641–48. https://doi.org/10.1056/NEJMra1407552
2. de Benedictis FM, Attanasi M. Asthma in childhood. Eur Respir Rev. 2016;25(139):41–47. https://doi.org/10.1183/16000617.0082-2015
3. Zhang S, Shao Q, Jia L, Zhou F. ANXA3 regulates HIF1α-induced NLRP3 inflammasome activity and promotes LPS-induced inflammatory response in bronchial epithelial cells. Signa Vitae. 2021;17(3):206–13. https://doi.org/10.22514/sv.2021.078
4. Redhu NS, Shan L, Movassagh H, Gounni AS. Thymic stromal lymphopoietin induces migration in human airway smooth muscle cells. Sci Rep. 2013;3:2301. https://doi.org/10.1038/srep02301
5. Poddighe D, Mathias CB, Brambilla I, Marseglia GL, Oettgen HC. Importance of basophils in eosinophilic asthma: the murine counterpart. J Biol Regul Homeost Agents. 2018;32(2):335–39.
6. Salter BM, Aw M, Sehmi R. The role of type 2 innate lymphoid cells in eosinophilic asthma. J Leuko Biol. 2019;106(4):889–901. https://doi.org/10.1002/JLB.3MR1217-497R
7. Bentley JK, Hershenson MB. Airway smooth muscle growth in asthma: proliferation, hypertrophy, and migration. Proc Am Thorac Soc. 2008;5(1):89–96. https://doi.org/10.1513/pats.200705-063VS
8. Tsai MK, Lin YC, Huang MY, Lee MS, Kuo CH, Kuo PL, et al. The effects of asthma medications on reactive oxygen species production in human monocytes. J Asthma. 2018;55(4):345–53. https://doi.org/10.1080/02770903.2017.1339798
9. Yang Q, Shi W. Rho/ROCK-MYOCD in regulating airway smooth muscle growth and remodeling. Am J Physiol Lung Cell Mol Physiol. 2021;321(1):L1–L5. https://doi.org/10.1152/ajplung.00034.2021
10. Seidel P, Roth M. Anti-inflammatory dimethylfumarate: a potential new therapy for asthma? Mediators Inflamm. 2013;2013:875403. https://doi.org/10.1155/2013/875403
11. Kardas G, Daszyńska-Kardas A, Marynowski M, Brząkalska O, Kuna P, Panek M. Role of platelet-derived growth factor (PDGF) in asthma as an immunoregulatory factor mediating airway remodeling and possible pharmacological target. Front Pharmacol. 2020;11:47. https://doi.org/10.3389/fphar.2020.00047
12. Ohno I, Nitta Y, Yamauchi K, Hoshi H, Honma M, Woolley K, et al. Eosinophils as a potential source of platelet-derived growth factor B-chain (PDGF-B) in nasal polyposis and bronchial asthma. Am J Respir Cell Mol Biol. 1995;13(6):639–47. https://doi.org/10.1165/ajrcmb.13.6.7576701
13. Shimizu S, Gabazza EC, Hayashi T, Ido M, Adachi Y, Suzuki K. Thrombin stimulates the expression of PDGF in lung epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2000;279(3):L503–10. https://doi.org/10.1152/ajplung.2000.279.3.L503
14. Kessler D, Gruen GC, Heider D, Morgner J, Reis H, Schmid KW, et al. The action of small GTPases Rab11 and Rab25 in vesicle trafficking during cell migration. Cell Physiol Biochem. 2012;29(5–6):647–56. https://doi.org/10.1159/000295249
15. Ramel D, Wang X, Laflamme C, Montell DJ, Emery G. Rab11 regulates cell-cell communication during collective cell movements. Nat Cell Biol. 2013;15(3):317–24. https://doi.org/10.1038/ncb2681
16. Hehnly H, Doxsey S. Rab11 endosomes contribute to mitotic spindle organization and orientation. Dev Cell. 2014;28(5):497–507. https://doi.org/10.1016/j.devcel.2014.01.014
17. Du J, Fu L, Hao J, Lin X, Dong Q. Rab11a Is Overexpressed in gastric cancer and regulates FAK/AKT signaling. J Oncol. 2020;2020:3494396. https://doi.org/10.1155/2020/3494396
18. Zhang ZY, Lu M, Liu ZK, Li H, Yong YL, Zhang RY, et al. Rab11a regulates MMP2 expression by activating the PI3K/AKT pathway in human hepatocellular carcinoma cells. Pathol Res Pract. 2020;216(9):153046. https://doi.org/10.1016/j.prp.2020.153046
19. Bigler J, Boedigheimer M, Schofield JPR, Skipp PJ, Corfield J, Rowe A, et al. A Severe asthma disease signature from gene expression profiling of peripheral blood from U-BIOPRED cohorts. Am J Respir Crit Care Med. 2017;195(10):1311–20. https://doi.org/10.1164/rccm.201604-0866OC
20. Zhao C, Wu AY, Yu X, Gu Y, Lu Y, Song X, et al. Microdomain elements of airway smooth muscle in calcium regulation and cell proliferation. J Physiol Pharmacol. 2018;69(2):151–63. https://doi.org/10.26402/jpp.2018.2.01.
21. Makinde T, Murphy RF, Agrawal DK. The regulatory role of TGF-beta in airway remodeling in asthma. Immunol Cell Biol. 2007;85(5):348–56. https://doi.org/10.1038/sj.icb.7100044
22. Wang H, Yao H, Yi B, Kazama K, Liu Y, Deshpande D, et al. MicroRNA-638 inhibits human airway smooth muscle cell proliferation and migration through targeting cyclin D1 and NOR1. J Cell Physiol. 2018;234(1):369–81. https://doi.org/10.1002/jcp.26930
23. Jiang C, Liu Z, Hu R, Bo L, Minshall RD, Malik AB, et al. Inactivation of Rab11a GTPase in macrophages facilitates phagocytosis of apoptotic neutrophils. J Immunol.

2017; 198(4): 1660–72. https://doi.org/10.4049/jimmunol.1601495
24. Goswami S, Balasubramanian I, D'Agostino L, Bandyopadhyay S, Patel R, Avasthi S, et al. RAB11A-mediated YAP localization to adherens and tight junctions is essential for colonic epithelial integrity. The J Biol Chem. 2021;297(1):100848. https://doi.org/10.1016/j.jbc.2021.100848
25. Yan Z, Wang ZG, Segev N, Hu S, Minshall RD, Dull RO, et al. Rab11a mediates vascular endothelial-cadherin recycling and controls endothelial barrier function. Arterioscler Thromb Vasc Biol. 2016;36(2):339–49. https://doi.org/10.1161/ATVBAHA.115.306549
26. Wang TY, Zhou QL, Li M, Shang YX. Shikonin alleviates allergic airway remodeling by inhibiting the ERK-NF-κB signaling pathway. Int Immunopharmacol. 2017;48:169–79. https://doi.org/10.1016/j.intimp.2017.05.011
27. Fan Z, Tang D, Wu Q, Huang Q, Song J, Long Q. Scopoletin inhibits PDGF-BB-induced proliferation and migration of airway smooth muscle cells by regulating NF-κB signaling pathway. Allergol Immunopathol. 2022;50(1):92–98. https://doi.org/10.15586/aei.v50i1.517
28. Li Q, Lu L, Li X, Lu S. Long non-coding RNA NKILA alleviates airway inflammation in asthmatic mice by promoting M2 macrophage polarization and inhibiting the NF-κB pathway. Biochem Biophys Res Commun. 2021;571:46–52. https://doi.org/10.1016/j.bbrc.2021.07.023
29. Li Z, Zheng J, Zhang N, Li C. Berberine improves airway inflammation and inhibits NF-κB signaling pathway in an ovalbumin-induced rat model of asthma. J Asthma. 2016;53(10):999–1005. https://doi.org/10.1080/02770903.2016.1180530
30. Liu Y, Miao Y, Gao X, Wang YY, Wang H, Zheng YW, et al. MicroRNA-200a affects the proliferation of airway smooth muscle cells and airway remodeling by targeting FOXC1 via the PI3K/AKT signaling pathway in ovalbumin-induced asthmatic mice. Cell Physiol Biochem. 2018;50(6):2365–89. https://doi.org/10.1159/000495097
31. Wang X, Xu L, Yu Y, Fu Y. LncRNA RP5-857K21.7 inhibits PDGF-BB-induced proliferation and migration of airway smooth muscle cells through the miR-508-3p/PI3K/AKT/mTOR axis. Autoimmunity. 2022;55(1):65–73. https://doi.org/10.1080/08916934.2021.1998895
32. 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. Biosci Rep. 2020;40(6): BSR20193899. https://doi.org/10.1042/BSR20193899
33. Liu JH, Li C, Zhang CH, Zhang ZH. LncRNA-CASC7 enhances corticosteroid sensitivity via inhibiting the PI3K/AKT signaling pathway by targeting miR-21 in severe asthma. Pulmonology. 2020;26(1):18–26. https://doi.org/10.1016/j.pulmoe.2019.07.001
34. He S, Chen M, Lin X, Lv Z, Liang R, Huang L. Triptolide inhibits PDGF-induced proliferation of ASMCs through G0/G1 cell cycle arrest and suppression of the AKT/NF-κB/cyclinD1 signaling pathway. Eur J Pharmacol. 2020;867:172811. https://doi.org/10.1016/j.ejphar.2019.172811