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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**KEYWORDS**
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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. The characteristics of asthma are bronchial hypersensitivity, airway inflammation, and airway obstruction. Basophils and type 2 innate lymphoid cells have been discovered to participate in the regulation of asthma. 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. 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. The abnormal proliferation of ASMCs is induced by frequent stimulation with contractile agonists, pro-inflammatory cytokines, and growth factors. Among them, the PDGF-BB subtype of platelet-derived growth factor (PDGF) family is acquired from inflammatory cells and airway epithelial cells in asthma. 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.

RAB11A can control Rac activity, which belongs to the Rab family. RAB11A has been noted to participate in intercellular communication, and RAB11A is bound up with endosomes and is helpful to the assembly and function of spindle pole. 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. 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. 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, 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% CO₂.

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-ΔΔCt method.

The primer sequences were as follows:

- Rab11A forward, 5′-TGGCTGTGCTCAATGTGGG-3′ and reverse, 5′-GCAGAGCTTCTACTGACTGAGA-3′;
- TNF-α forward, 5′-CTGGACCTTTGGAGTCTGGG-3′ and reverse, 5′-GCTTGGAGTTGTCTACAACTG-3′;
- IL-1β forward, 5′-GGATATGGAGCAACAACTGG-3′ and reverse, 5′-ATGTACAGTGGGGAACGTG-3′;
- IL-8 forward, 5′-GACCAACACTGCGCCAACAC-3′ and reverse, 5′-CTTCTCCACACACCTCTGCAC-3′;
- IL-6 forward, 5′-ACTCACCTTCTGAAAGCAATTG-3′ and reverse, 5′-CCACCTTGGGAAGTTCCAGTTG-3′;
- GAPDH forward, 5′-AACGGTGATCAGTGGGACCTG-3′ and reverse, 5′-AGTGGGTGTCGCTTTGAAG-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). The quantification was normalized to GAPDH through the 2-ΔΔCt method.

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.
RAB11A facilitates asthma progression

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).

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. 

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
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). In addition, the weakened cell apoptosis mediated by PDGF-BB treatment was reversed after RAB11A inhibition (Figure 4E) (**p<0.01, ***p<0.001 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<0.01, ***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.

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 of TNF-α, IL-1β, IL-8, and IL-6 induced by PDGF-BB treatment were weakened after RAB11A inhibition. *p < 0.05, **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.
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. 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. Furthermore, RAB11A facilitates the expression and recycling of VE-cadherin on the plasma membrane of endothelial cells, thus suppressing vascular leakage.

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. Moreover, scopoletin modulates the NF-κB signaling pathway to alleviate PDGF-BB-triggered proliferation and migration of ASMCs. LncRNA NKILA accelerates M2 macrophage polarization and represses the NF-κB pathway in asthmatic mice to relieve airway inflammation. In addition, berberine affects the NF-κB signaling pathway to ameliorate airway inflammation in the ovalbumin-triggered asthma rat model. 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. LncRNA RP5-857K21.7 suppresses PDGF-BB-triggered the ASMCs proliferation and migration through regulating miR-508-3p/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. Further, PDGF-BB has been substantiated to induce asthma cell models through enhancing cell proliferation and migration. Thus, the asthma cell model stimulated by PDGF-BB was first made. RAB11A has been confirmed to exacerbate cancers’ progression and exhibited higher expression in asthmatic patients. 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. 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. Furthermore, RAB11A facilitates the expression and recycling of VE-cadherin on the plasma membrane of endothelial cells, thus suppressing vascular leakage. In the present work, we discovered that suppression of RAB11A relieved the inflammatory response in PDGF-BB-stimulated ASMCs.

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AKT/mTOR axis. Additionally, galectin-1 inactivates the PI3K/Akt signaling pathway to retard PDGF-BB-mediated ASM growth. LncRNA-CASC7 weakens the PI3K/AKT signaling pathway to heighten corticosteroid sensitivity in severe asthma. Furthermore, triprolidine strengthens the G0/G1 cell cycle arrest and restrains the AKT/NF-κB/cyclinD1 signaling pathway to repress PDGF-stimulated ASM proliferation. 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


