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MiR-328-3p promotes TGF-β1-induced proliferation, migration, and inflammation of airway smooth muscle cells by regulating the PTEN/Akt pathway

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KEYWORDS
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Abstract
Background: Recent studies have shown that the up-regulation of microRNA miR-328-3p expression increases seasonal allergy and asthma symptoms in children, but the specific mechanism remains unclear. Therefore, the aim of this study was to explore the role and mechanism of miR-328-3p in transforming growth factor (TGF)-β1-induced airway smooth muscle cells (ASMCs).
Methods: The effect of TGF-β1 on the expression of miR-328-3p in ASMCs was examined by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). Cells proliferation, migration, and inflammatory factors in TGF-β1-induced ASMCs were measured by cell counting kit-8 (CCK-8), transwell, and enzyme-linked immunosorbent assay (ELISA), respectively. Besides, TargetScan was used to predict phosphatase and tensin homolog (PTEN), the downstream target of miR-328-3p; double-luciferase reporter assay, western blot, and qRT-PCR were used to verify the targeting relationship between miR-328-3p and PTEN; western blot was also used to examine the effects of PTEN and miR-328-3p knockdown on the expression levels of PTEN, Akt, and p-Akt proteins.
Results: The expression of miR-328-3p was up-regulated in TGF-β1-induced ASMCs. Knockdown of miR-328-3p significantly inhibited proliferation, migration, and inflammation of ASMCs induced by TGF-β1 and decreased levels of tumor necrosis factor (TNF)-α and interleukin (IL)-1β. The dual-luciferase reporter assay results confirmed that PTEN was a target gene of miR-328-3p. Moreover, inhibition of PTEN expression reversed the inhibitory effect of low miR-328-3p expression on TGF-β1-induced ASMC's proliferation, migration, and inflammation. In comparison to the knockdown of miR-328-3p alone, the simultaneous knockdown of miR-328-3p with PTEN decreased PTEN protein expression levels and increased p-Akt/Akt ratio in TGF-β1-induced ASMCs.
Conclusion: Through regulating the expression of PTEN and the activity of Akt signaling pathway, miR-328-3p promotes TGF-β1-induced proliferation, migration, and inflammation of ASMCs.

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Introduction

Bronchial asthma is a common chronic airway inflammatory disease with intermittent and reversible attacks. Bronchial asthma affects approximately 300 million people worldwide and the prevalence increases year by year.[1] Asthma not only severely reduces the quality of life for sufferers but also places a severe financial and disease burden on society.[2] The pathogenesis of asthma is very complex and involves many factors such as environment, genetics, and infection.[2] The main clinical symptoms of asthma are bronchopulmonary hyperresponsiveness, airway remodeling, and airway inflammation.[3] Persistent chronic inflammation of the airways results in remodeling of the trachea, triggering histological changes in the airway architecture, including thickening of the airway basement membrane, smooth muscle hyperplasia, increased fibrosis, and ultimately impaired lung function.[4,5] Airway smooth muscle cells (ASMCs) are key effector cells of airway inflammation and airway remodeling.[6] ASMCs are involved in the process of airway inflammation by secreting a variety of cytokines, chemokines, and adhesion molecules.[7] In addition, ASMCs can facilitate airway remodeling by regulating vital cellular functions such as proliferation, migration, hypertrophy, and inflammation.[7] Upon stimulation, ASMCs can perform a transition from a proliferative phenotype to a contractile phenotype and vice versa, thereby inducing chronic airway inflammation and remodeling.[5] Currently, physicians have limited options for the clinical treatment of bronchial asthma, with only bronchodilators and anti-inflammatory drugs available. However, these drugs are not effective in inhibiting airway inflammation and delaying airway remodeling[8]; therefore, targeting ASMCs may provide an effective option for bronchial asthma treatment.

MicroRNAs (miRNAs), a class of non-coding RNAs of approximately 22 nucleotides in length, silence the expression of their target mRNAs through binding to 3′untranslated regions (3′-UTR) of the target mRNAs.[9] MiRNAs are widely distributed in various tissues in organisms. In addition to regulating the expression of target genes at the transcriptional and translational levels, miRNAs have been implicated in a variety of cellular physiological processes, including proliferation, migration, differentiation, metabolism, and apoptosis.[9] Several studies have uncovered a close relationship between the development of asthma and abnormal expression of miRNAs, including miR-620,[10] miR-145,[11] and miR-338,[12] among others. Specifically, miR-328-3p, located on chromosome 16q22.1, is capable of targeting and regulating multiple genes, as well as participating in cellular biological processes such as proliferation, migration, and apoptosis.[13,14] Shi et al. stated that miR-328-3p induced apoptosis of osteosarcoma cells via targeting matrix metalloproteinase 16 (MMP-16).[14] In addition, miR-328-3p inhibits the proliferation and metastasis of colorectal cancer by inhibiting the PI3K/Akt signaling pathway.[15] MiR-328-3p is also strongly associated with multiple levels of inflammatory factors. For example, miR-328-3p decreases tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) levels after targeting the PIM1 gene in HK-2 cells.[16] A recent study showed that miR-328-3p expression varies seasonally and is significantly associated with seasonal asthma symptoms and seasonal allergies in children. MiR-328-3p has a potentially deleterious effect on asthmatics.[17] Nevertheless, the role of miR-328-3p in bronchial asthma is unknown.

Transforming growth factor-β1 (TGF-β1) serves as an essential inflammatory factor. One study has reported that TGF-β1 contributes to pulmonary fibrosis in patients with severe asthma.[18] For another, TGF-β1 stimulates ASMCs to proliferate and migrate to the airway epithelial cell layer, as well as accelerates airway remodeling.[19] Given the significant role of TGF-β1 in the development of asthma, TGF-β1-induced ASMCs are frequently employed for in vitro studies of bronchial asthma.[10] In this study, ASMCs treated with 10 ng/mL of TGF-β1[10,20] were used to explore the role and mechanism of miR-328-3p in TGF-β1-induced proliferation, migration, and inflammation of ASMCs. First, with the help of real-time quantitative reverse transcription PCR (qRT-PCR), cell counting kit-8 (CCK-8), enzyme-linked immunosorbent assay (ELISA), and transwell assays, the role of miR-328-3p in TGF-β1-induced proliferation, migration, and inflammation of ASMCs was evaluated. The target gene of miR-328-3p was subsequently predicted using TargetScan (a prediction software for miRNA target genes) and further validated by dual-luciferase reporter assay. Finally, the mechanism of miR-328-3p in TGF-β1-induced ASMCs was further explored. More importantly, this study provides a scientific basis for the pathological mechanisms, treatment, and prevention of bronchial asthma.

Materials and Methods

Cell culture

The ASMCs were purchased from Shanghai Zhonghua Biotechnology Co., Ltd. Then the cells were cultured in a DMEM medium (Gibco, USA) containing 10% fetal bovine serum (FBS, Gibco, USA) and developed in an incubator at 37°C and 5% CO_2.

TGF-β1 induction and grouping

ASMCs in the logarithmic phase were starved for 24 hrs in a serum-free DMEM medium, followed by 2 hrs of incubation with TGF-β1 (PeproTech, USA) at 0, 1, 3, 5, and 10 ng/mL, respectively.[10,20] Subsequently, the expression level of miR-328-3p in the cell was detected by qRT-PCR.

The cells in the logarithmic phase were digested and resuspended before being seeded in 6-well plates at a density of 1.5×10^5 cells/well. The transfection was performed when the cell confluence reached 80–90%. Negative control inhibitor (Inh-NC) and miR-328-3p inhibitor (Inh-miR-328-3p), negative control mimics (NC mimics), and overexpressed miR-328-3p mimics (miR-328-3p mimics) and phosphatase and tensin homolog (PTEN) siRNA (si-PTEN) were designed and synthesized by The Beijing Genomics Institute. The above segments were transfected into ASMCs following the instructions of Lipofectamine TM 2000 (Thermo Fisher Scientific, USA). Five groups were set up: the Control group, the group induced by TGF-β1 (TGF-β1), the group transfected with negative inhibitor (TGF-β1 + Inh-NC), the group transfected with miR-328-3p (TGF-β1 + Inh-miR-328-3p),

and the group co-transfected with miR-328-3p inhibitor and PTEN siRNA (TGF-β1 + Inh-miR-328-3p + si-PTEN). The cells in the Control group were not treated at all; cells in the TGF-β1 group were incubated with 10 ng/mL of TGF-β1 for 2 hrs; cells in both TGF-β1 + Inh-miR-328-3p and TGF-β1 + Inh-miR-328-3p + si-PTEN groups were incubated with 10 ng/mL TGF-β1 for 2 hrs after the completion of transfection. The related cell experiments were subsequently performed.

Real-time quantitative reverse transcription PCR

Total RNA was extracted from the cells using Trizol reagent (Sigma, USA) and the concentration of RNA was determined using NanoDrop. With the instructions of the SuperScript VILO cDNA Synthesis Kit (Invitrogen, USA), RNA was reverse transcribed into cDNA. After that, relative miR-328-3p and PTEN expression levels were detected using Thermal Cycler Dice ® Real Time System, according to the operating instructions of SYBR ® Premix Ex Taq ™ II (Takara, Japan). GAPDH or U6 was used as an internal control, and the relative expression of the target gene was calculated using the $2^{-\Delta\Delta Ct}$ method. The sequence of primers used in this test is listed in Table 1.

CCK-8 assay

After being inoculated into 96-well plates at a density of 3×10^3 cells/well, the cells were incubated with 0 or 10 ng/mL TGF-β1 for 2 hrs. The cell proliferation ability was detected according to the instructions of the CCK-8 test kit (Beyotime, China), and the absorbance at a wavelength of 450 nm was measured with a microplate reader (TECAN, Switzerland). Three replicate wells were set up for each group.

Transwell assay

Initially, Matrigel gel (BD, USA) was taken from −20°C to 4°C in a refrigerator overnight. Subsequently, Matrigel gel was diluted to the required concentration with serum-free DMEM medium at 4°C, and then added to a transwell chamber (Corning, USA) with a pore size of 8 μm. The cells in the logarithmic phase were dislodged with trypsin (Solarbio, China), resuspended in serum-free DMEM medium, and later inoculated into the upper chamber. Following the addition of DMEM medium containing 10% FBS into the lower chamber, the cells were cultured in an incubator at 37°C for 24 hrs. Afterward, the cells were fixed with methanol for 15 min and stained with 0.1% crystal violet for 20 min. The non-migrating cells in the chamber were wiped with a cotton swab prior to washing off the crystal violet staining solution with PBS. Finally, the cells were observed and photographed under a light microscope (Olympus, Japan).

Table 1 Primer sequences for qRT-PCR.

Genes	Primer sequences (5′ to 3′)
miR-328-3p	F: CCTCTCTGCCCTTCCG
	R: GAACATGTCTGCGTATCTC
PTEN	F: TGAGTTCCCTCAGCCGTTACCT
	R: GAGGTTTCCTCTGGTCCTGGTA
U6	F: CTCGCTTCGGCAGCACAT
	R: TTTGCGTGTCATCCTTGCG
GAPDH	F: GTCTCCTCTGACTTCAACAGCG
	R: ACCACCCTGTTGCTGTAGCCAA

Enzyme-linked immunosorbent assay

The cell culture medium was centrifuged at 12,000 rpm for 10 min at 4°C, and the supernatant was subsequently removed for testing. The levels of TNF-α and IL-1β in the supernatant were detected under the instructions of ELISA kits (mlbio, China).

Western blot

Total protein was extracted from cells on ice using RIPA lysate (Solarbio, China). The concentration of the extracted protein was determined by the Micro BCA Protein Assay Kit (Thermo, USA). At first, the protein samples (10/20 μg) were resolved by 10% or 12% SDS-PAGE. Later, the resulting protein was electrophoretically transferred onto a PVDF membrane (Bio-Rad, USA). Next, the PVDF membrane was blocked with 5% bovine serum albumin (BSA, Solarbio, China), followed by overnight incubation at 4°C with primary antibodies (PTEN, 22034-1-AP; p-Akt, 28731-1-AP; Akt, 10176-2-AP; β-actin, 20536-1-AP; Proteintech, USA). Upon washing with TBST (Solarbio, China), the membrane was supplemented with secondary antibodies (Proteintech, USA) and together incubated for 1 hr at ambient temperature. After that, ECL Plus Hypersensitive Luminescent Solution (PE0010, Solarbio, China) was evenly dropped onto the membrane. A FliorchemHD2 imaging system was used to scan the membrane for image acquisition and Image J was used to perform Grayscale value analysis. β-actin being an internal control protein, the relative expression of the protein was calculated.

Dual-luciferase reporter assay

Targetscan (http://www.targetscan.org/vert_72/) was employed to predict the target gene of miR-328-3p.[21] Transfection was performed when the ASMCs reached 80% confluency. Based on the instructions of Lipofectamine TM 2000 (Thermo Fisher Scientific, USA), the constructed PTEN wild-type (PTEN-WT) and mutant (PTEN-MUT) dual-luciferase reporter vectors were co-transfected into cells with miR-328-3p mimics or NC mimics and then cultured for 24 hrs. Afterward, the cells were lysed at ambient temperature for 20 min. Subsequent to extracting the supernatant after centrifugation, the luciferase activity of the supernatant was assessed using a luciferase reporter assay kit (Beyotime, China). Relative firefly luciferase activity was obtained, with Renilla luciferase activity being the internal control.[22]

Statistics and Analysis

SPSS 26.0 software was used to perform statistical analysis and Graphpad prism 9 software was used for mapping. All data were presented as mean ± standard deviation (SD). An independent T-test was used to compare the two groups, and a one-way analysis of variance was used to compare multiple groups. Correlation analysis was performed using Pearson correlation analysis. The value of $p < 0.05$ was deemed to be the criterion for judging a significant difference.

Results

MiR-328-3p is up-regulated in TGF-β1-induced ASMCs

To explore the role of miR-328-3p in TGF-β1-induced ASMCs, we adopted qRT-PCR to detect the expression levels of miR-328-3p in ASMCs treated with 0, 1, 3, 5, and 10 ng/mL TGF-β1. The results showed that miR-328-3p was significantly up-regulated in TGF-β1-induced ASMCs in a dose-dependent manner (Figure 1).

Knockdown of miR-328-3p expression inhibits the proliferation and migration of ASMCs induced by TGF-β1

The development of airway asthma is greatly influenced by the proliferation and migration of ASMCs induced by inflammatory factors,[23] but the contribution of miR-328-3p to the proliferation and migration is unknown. In order to explore the effect of miR-328-3p on the proliferation and migration of ASMCs induced by TGF-β1, we knocked down miR-328-3p expression in ASMCs by transfection with Inh-miR-328-3p. QRT-PCR results showed that miR-328-3p expression in the TGF-β1 + Inh-miR-328-3p group was significantly reduced by 33.4% compared with the TGF-β1 + Inh-NC group after transfection with Inh-miR-328-3p ($p < 0.01$, Figure 2A), suggesting successful knockdown of miR-328-3p expression by transfection. As for CCK-8 and transwell results showed that TGF-β1-induced proliferation activity and migration of ASMC cells increased by 28.5 and 39.4%, respectively, compared with the Control group. However, cell proliferation activity and migration ability were reduced by 15.1 and 85% in the TGF-β1 + Inh-miR-328-3p group compared with the TGF-β1 + Inh-NC group, respectively (Figure 2B–D). Hence, it is logical to speculate that knockdown of miR-328-3p suppresses the proliferation and migration of ASMCs induced by TGF-β1.

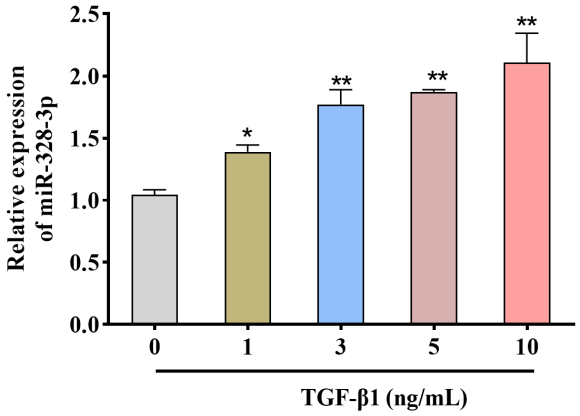

Figure 1 TGF-β1-induced up-regulation of miR-328-3p expression in ASMCs. qRT-PCR results of the expression levels of miR-328-3p in ASMCs treated with different concentrations of TGF-β1 (0, 1, 3, 5, and 10 ng/mL), *$p < 0.05$, **$p < 0.01$ vs. 0 ng/mL. TGF-β1, transforming growth factor-β1; ASMCs, airway smooth muscle cells; qRT-PCR, real-time quantitative reverse transcription PCR.

Knockdown of miR-328-3p suppresses the inflammation of ASMCs induced by TGF-β1

TGF-β1 can stimulate ASMCs to secrete inflammatory factors, thereby inducing airway inflammatory responses.[24] With the aim of investigating the effect of knockdown of miR-328-3p on inflammation of ASMCs induced by TGF-β1, the levels of inflammatory cytokines TNF-α and IL-1β in each group of cells were detected using ELISA kits. The results showed that the levels of cellular inflammatory cytokines TNF-α and IL-1β in the TGF-β1 group increased by 62.2 and 39.3%, respectively, compared with the Control group ($p < 0.01$). Knockdown of miR-328-3p was able to significantly reduce TNF-α and IL-1β levels in TGF-β1-induced ASMC cells by 28.5 and 22%, respectively ($p < 0.05$, Figure 3A–B), which highlighted the fact that knockdown of miR-328-3p can suppress inflammation of ASMCs induced by TGF-β1.

PTEN is a target gene of miR-328-3p

With the help of the TargetScan database, we predicted the downstream target gene of miR-328-3p and identified PTEN as the target gene of miR-328-3p (Figure 4A). Overexpression of miR-328-3p considerably decreased the luciferase activity of PTEN-WT but had no effect on the luciferase activity of PTEN-MUT, as revealed by dual-luciferase reporter assay results (Figure 4B). Correspondingly, a targeting relationship between miR-328-3p and PTEN was established. Besides, the expression of PTEN protein and mRNA in TGF-β1-induced ASMCs after the knockdown of miR-328-3p was examined by western blot and qRT-PCR. The results showed that after TGF-β1 stimulation, PTEN mRNA and protein expression in ASMC cells were significantly decreased compared with the Control group, by 59.6 and 51.5%, respectively ($p < 0.01$). However, PTEN mRNA and protein expression in ASMC cells in the TGF-β1 + Inh-miR-328-3p group were significantly up-regulated compared with the TGF-β1 + Inh-NC group, by 117.1 and 67.8%, respectively ($p < 0.01$, Figure 4C–E). In a nutshell, PTEN is a miR-328-3p target gene.

MiR-328-3p promotes the proliferation, migration, and inflammation of ASMCs induced by TGF-β1 via PTEN/Akt pathway

Furthermore, to explore the mechanism and role of PTEN in the proliferation, migration, and inflammation of ASMCs

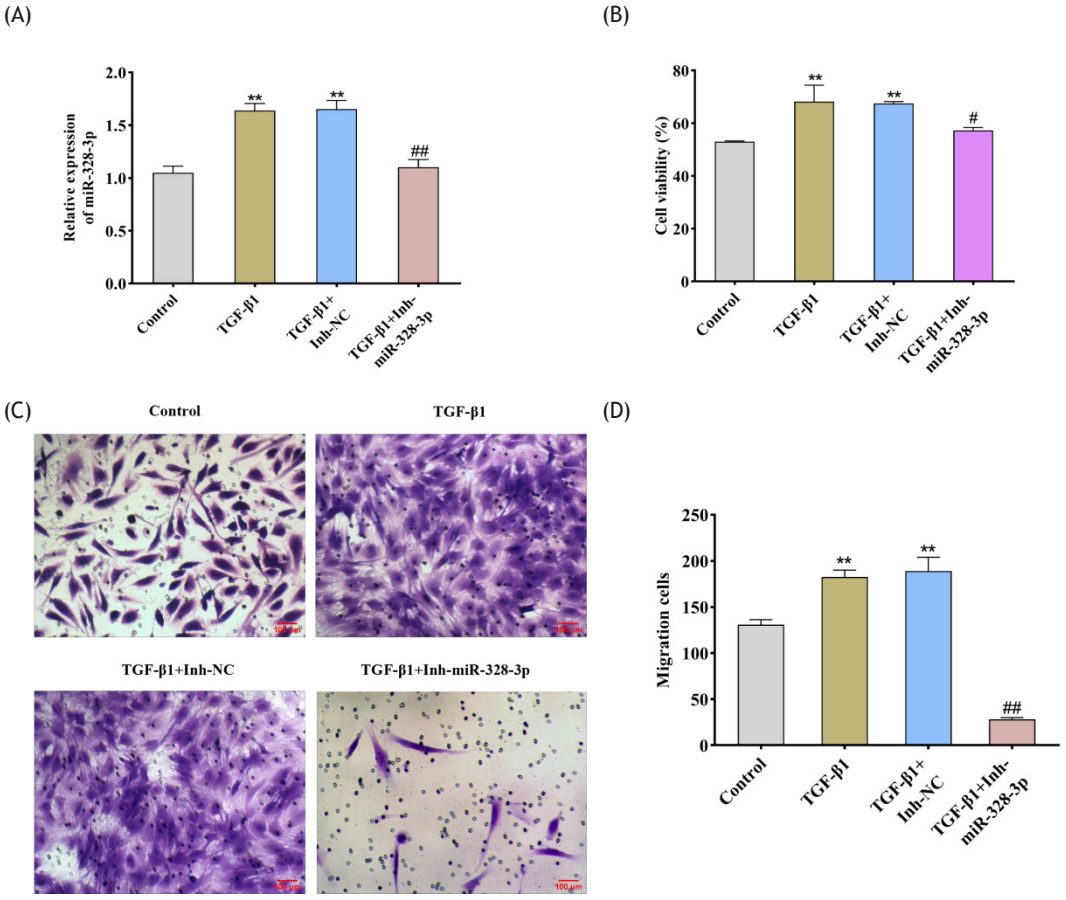

Figure 2 Knockdown of miR-328-3p inhibits the proliferation and migration of ASMCs induced by TGF-β1. (A) qRT-PCR results for expression levels of miR-328-3p in ASMCs and in transfected ASMCs. (B) CCK-8 results of the effect of knockdown of miR-328-3p on proliferation ability of TGF-β1-induced ASMCs. (C,D) Transwell assay results of the effect of knockdown of miR-328-3p on migration ability of TGF-β1-induced ASMCs, **p < 0.01 vs. Control; #p < 0.05, ##p < 0.01 vs. TGF-β1+Inh-NC. TGF-β1, transforming growth factor-β1; ASMCs, airway smooth muscle cells; qRT-PCR, real-time quantitative reverse transcription PCR; CCK-8, cell counting kit-8.

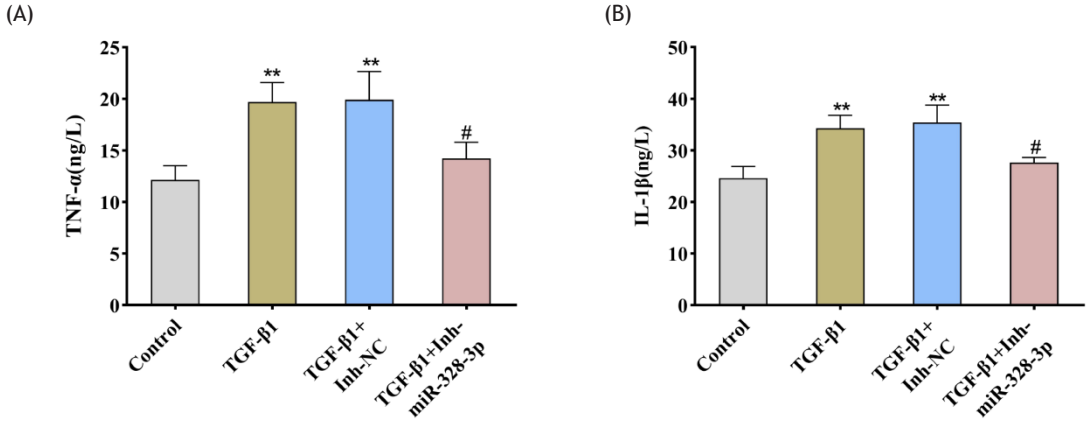

Figure 3 Knockdown of miR-328-3p suppresses inflammation of ASMCs induced by TGF-β1. (A,B) ELISA results of the effect of knockdown of miR-328-3p on levels of TNF-α (A) and IL-1β (B) in TGF-β1-induced ASMCs, **p < 0.01 vs. Control; #p < 0.05 vs. TGF-β1+Inh-NC. TGF-β1, transforming growth factor-β1; ASMCs, airway smooth muscle cells; ELISA, enzyme-linked immunosorbent assay; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β.

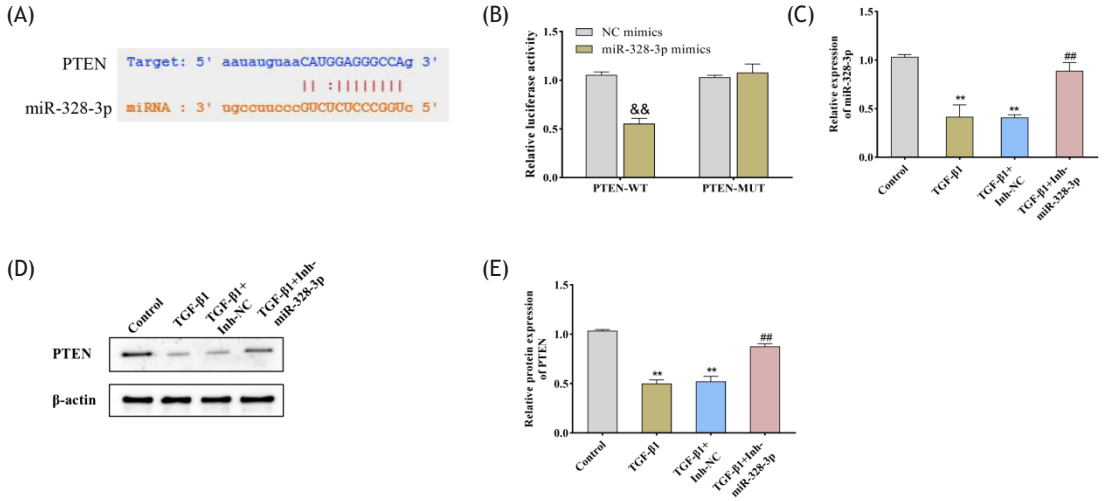

Figure 4 PTEN is a target gene of miR-328-3p. (A) TargetScan results for the prediction of complementary target sequences for miR-328-3p and PTEN. (B) Dual-luciferase reporter assay results of the targeting relationship between PTEN and miR-328-3p. (C) qRT-PCR results for mRNA expression levels of PTEN in the ASMCs of each group. (D,E) Western blot results of the relative protein expression level of PTEN in ASMCs of each group, ##p < 0.01 vs. NC mimics, **p < 0.01 vs. Control; ##p < 0.01 vs. TGF-β1+Inh-NC. PTEN, phosphatase and tensin homolog; qRT-PCR, real-time quantitative reverse transcription PCR; ASMCs, airway smooth muscle cells.

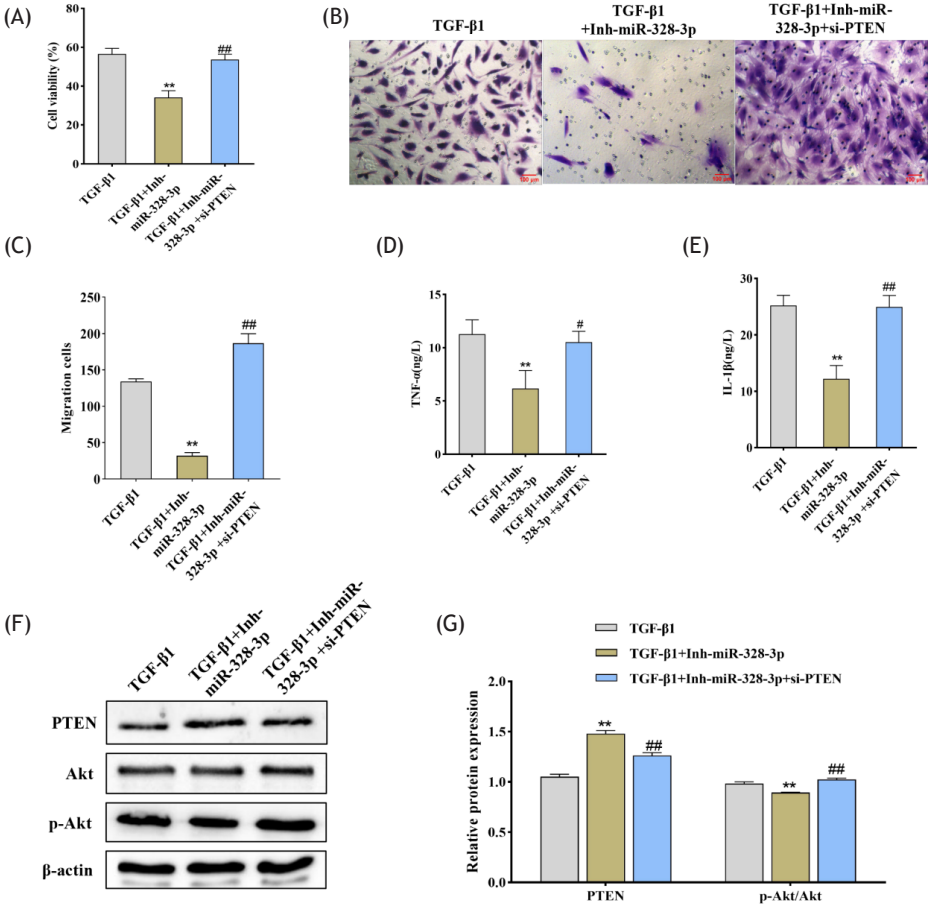

Figure 5 MiR-328-3p promotes the proliferation, migration, and inflammation of ASMCs induced by TGF-β1 through PTEN/Akt pathway. (A) CCK-8 results on the proliferation capacity of ASMCs in each group; (B,C) Transwell assay results of the migration ability of ASMCs in each group; (D,E) ELISA results of TNF-α (D) and IL-1β (E) levels in ASMCs in each group; (F,G) Western blot results of PTEN, Akt and p-Akt protein expression levels in ASMCs in each group, **p < 0.01 vs. TGF-β1; ##p < 0.01 vs. TGF-β1+Inh-miR-328-3p. TGF-β1, transforming growth factor-β1; ASMCs, airway smooth muscle cells; CCK-8, cell-counting kit-8; ELISA, enzyme-linked immunosorbent assay; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; PTEN, phosphatase and tensin homolog.

mediated by miR-328-3p, miR-328-3p and PTEN were simultaneously knocked down in ASMCs and the transfected ASMCs were subsequently treated with TGF-β1. The results showed that cell proliferation and migration were significantly decreased by 39.5 and 76.1% ($p < 0.01$), and the levels of TNF-α and IL-1β were significantly decreased by 45.4 and 51.6% ($p < 0.01$) in the TGF-β1 + Inh-miR-328-3p group compared with the TGF-β1 group, respectively. Compared with the TGF-β1 + Inh-miR-328-3p group, the TGF-β1 + Inh-miR-328-3p + si-PTEN group had significantly increased cell proliferation and migration by 57 and 483.4% ($p < 0.01$, Figure 5A–C), and significantly up-regulated TNF-α and IL-1β levels by 70.7 and 104.7%, respectively (Figure 5D,E). The outcomes implicate that miR-328-3p targets PTEN to regulate the proliferation, migration, and inflammation of TGF-β1-induced ASMCs.

PTEN can participate in cellular life activities such as cell proliferation, apoptosis, and migration by regulating downstream Akt signaling.[25] Therefore, the expression of proteins involved in the PTEN/Akt signaling pathway was identified in our study using western blotting. The results showed that PTEN protein level was significantly increased by 35.5%, while the p-Akt/Akt ratio was significantly decreased by 14.6% in the TGF-β1 + Inh-miR-328-3p group compared with the TGF-β1 group. PTEN protein expression in ASMC cells in the TGF-β1 + Inh-miR-328-3p + si-PTEN group was significantly decreased by 10.9% compared with the TGF-β1 + Inh-miR-328-3p group, while the p-Akt/Akt ratio was significantly increased by 15.7% ($p < 0.01$, Figure 5F,G). Overall, miR-328-3p promotes the proliferation, migration, and inflammation of TGF-β1-induced ASMCs through the PTEN/Akt pathway.

Discussion

Bronchial asthma, a common chronic inflammatory airway disease, has become a serious public health issue as its incidence increases year by year.[1] Notably, ASMCs play a crucial role in the pathogenesis of asthma.[7,8] On the other hand, recent studies have revealed a strong correlation between miRNAs and the pathogenesis of a myriad of asthma.[10–12] In our study, ASMCs induced by TGF-β1 showed concentration-dependent upregulation of miR-328-3p. In findings of further exploration, miR-328-3p promoted the proliferation, migration, and inflammation of ASMCs induced by TGF-β1 via targeting PTEN/Akt signaling.

The anomalous proliferation and migration of ASMCs thicken the airway walls, reduce the airway lumen, and promote airway remodeling in asthmatics.[26] In addition, ASMCs secrete inflammatory factors and chemokines when triggered by inflammatory mediators, which further aggravate tracheal inflammation and remodeling.[7] However, TGF-β1 can induce activities such as survival, proliferation, and synthesis of extracellular matrix proteins in ASMCs, all of which are closely related to the tracheal injury.[19] Our study discovered that the proliferation and migration of ASMCs were significantly encouraged after TGF-β1 stimulation, which is similar to the pathological process of remodeling in ASMCs.[27] Likewise, the inflammation of ASMCs was promoted by TGF-β1 stimulation, which is consistent with previous studies.[24] In addition, low expression of miR-328-3p was found to suppress the proliferation, migration, and inflammation of ASMCs induced by TGF-β1. In terms of the findings of Gao et al, miR-217 was concentration-dependently down-regulated in TGF-β1-induced ASMCs, and high expression of miR-217 inhibited the proliferation and migration of TGF-β1-induced ASMCs by targeting ZEB1.[28] Interestingly, the two miRNAs exhibit opposite biological functions in the cell, which may be related to the complex mechanism of action of the miRNAs.

PTEN, a lipid phosphatase, is essential in multiple signal transduction pathways.[29] PTEN has been demonstrated to be involved in the regulation of a variety of cellular biological functions, including cell survival, proliferation, and migration.[30] On the other hand, PTEN is also a key regulator of pathological mechanisms in asthma.[29] Interpretatively, PTEN expression was reduced in the lung tissue of rats with asthma, and significantly reduced in tracheitis and bronchial hyperresponsiveness.[31] Zhao et al. claimed that PTEN is involved in regulating the proliferation and migration of ASMCs, as well as affecting airway remodeling.[32] In addition, earlier studies have described that PTEN maintains cell cycle arrest in the G1 phase by inhibiting the formation of PIP3 from PIP2 and facilitating the conversion of PIP3 to PIP2.[33] With the conduction of TargetScan database prediction, dual-luciferase reporter assay, and expression assay, our results showed that miR-328-3p targeted and regulated the PTEN gene, and inhibition of PTEN expression could reverse the inhibitory effect of low miR-328-3p expression on the proliferation, migration, and inflammation of TGF-β1-induced ASMCs. All in all, miR-328-3p promotes the proliferation, migration, and inflammation of TGF-β1-induced ASMCs by targeting PTEN.

PTEN plays a role in cell activity by modulating PIP3 signaling and inhibiting the activation of Akt signaling.[25] Akt is a key factor in various cellular biological processes such as cell proliferation, growth, survival, migration, and invasion.[34] In our findings, PTEN protein expression in the TGF-β1 + InhmiR-328-3p + si-PTEN group was significantly decreased, while the p-Akt/Akt ratio was obviously increased compared with the TGF-β1 + InhmiR-328-3p group. Collectively, miR-328-3p is involved in the proliferation, migration, and inflammation of TGF-β1-induced ASMCs by targeting PTEN/Akt signaling. However, the study does have some limitations. First, relevant animal experiments are required to verify the role and mechanism of miR-328-3p in bronchial asthma. Second, more follow-up experiments are needed to explore how miR-328-3p targets PTEN/Akt signaling to promote proliferation, migration, and inflammation in ASMCs.

Taken together, this study revealed that miR-328-3p is highly expressed in TGF-β1-induced ASMCs. More significantly, miR-328-3p promotes the proliferation, migration, and inflammation of TGF-β1-induced ASMCs via regulating the expression of its downstream target gene PTEN as well as the activity of Akt signaling pathway. Briefly, this study provides a scientific basis for the pathological mechanisms, treatment, and prevention of bronchial asthma.

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Competing Interests

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

Authors Contribution

Jie Gao designed the study and carried them out. Xiao-lei Wu supervised the data collection and analyzed and interpreted the data. Jie Gao and Xiao-lei Wu wrote the manuscript. All authors have read and approved the manuscript.

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