

Exosomal miR-147-3p regulates the growth of mouse bronchial epithelial cells and asthma progression *via* targeting RUNX3: a pilot study

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Abstract. Exosomes are endogenous vesicles that transport microRNAs (miRNAs), thereby mediating intercellular communication. This study was assigned to expound the regulatory role of exosomal miR-147-3p in asthma progression. The ovalbumin-mediated murine model of asthma was established to obtain bronchoalveolar lavage fluid (BALF), and exosomes were isolated by ultrafiltration centrifugation. Differential expression of exosomal miRNA and mRNA was analyzed by RT-PCR. Dual-luciferase was utilized to validate potential binding between key miRNAs and target genes. Additionally, the impact of miRNA mimics and BALF-exosomes on growth of bronchial epithelial cells (BECs) was assessed, followed by RT-qPCR and Western blot. Ovalbumin exposure induced asthma phenotypes in mice. Notably, miR-147-3p and miR-146b-5p were markedly elevated in BALF exosomes from asthmatic mice, whereas miR-98-5p and RUNX3 were markedly decreased. Luciferase assay confirmed the direct binding of miR-147-3p to RUNX3 mRNA 3' UTR. Furthermore, PKH26-labeled exosomes were internalized by BECs. Asthmatic BALF-exosomes or miR-147-3p mimics reduced cell viability and migration while promoting apoptosis. This phenomenon could be reversed by miR-147-3p inhibition. miR-147-3p overexpression or asthmatic BALF-exosomes suppressed RUNX3 protein and mRNA expression. BALF-derived exosomal miR-147-3p modulated behaviors of BECs *via* targeting RUNX3, suggesting its potential as a therapeutic target and biomarker for asthma pathogenesis.

Key words: Asthma – Exosome – Bronchial epithelial cells – microRNA

Introduction

Asthma is a chronic inflammatory airway disease affecting over 300 million individuals globally, characterized by recurrent wheezing, airway hyper-responsiveness, and reversible airflow obstruction. Epidemiological studies indicate a ris-

ing prevalence, particularly in urbanized regions, imposing significant socioeconomic burdens due to frequent hospitalizations and diminished quality of life (Yuan et al. 2025). Traditional therapy options (e.g. long-acting β 2-agonists (LABA), inhaled corticosteroids (ICS), and leukotriene receptor antagonists (LTRA) primarily offer symptomatic relief but fail to address disease heterogeneity or reverse airway remodeling (Engelkes et al. 2015). For severe cases resistant to conventional therapies, monoclonal antibodies (mAbs) such as omalizumab, reslizumab, and mepolizumab have emerged as effective alternatives, targeting key inflammatory pathways to reduce exacerbation rates and improve lung function (Agache et al. 2020; Lyly et al. 2020; Kotoulas et

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al. 2022). However, approximately 5–10% of patients remain inadequately controlled (Sobieraj et al. 2018), highlighting the urgent need for novel molecular targets to modify disease progression.

Exosomes, small extracellular vesicles (30–150 nm) derived from diverse cell types, exert major roles in intercellular communication *via* cargoing microRNAs (miRNAs), proteins, and lipids. It has been reported that miRNAs can regulate post-transcriptional processes through mRNA degradation, influencing normal cellular processes. In respiratory diseases, exosomal miRNAs modulate inflammatory pathways and epithelial-mesenchymal interactions, positioning them as promising biomarkers and therapeutic agents (Li et al. 2019; Liu et al. 2024). Recent research identified specific exosomal miRNAs implicated in asthma pathogenesis. For instance, Tang et al. (2020) demonstrated that Chinese medicine-induced exosomal miRNAs, including miR-147-3p, miR-98-5p, and miR-10a-5p, alleviated asthma symptoms in murine models by targeting Wnt and MAPK inflammatory signals. Notably, miR-98 expression was elevated in bronchial biopsies of male asthma patients and inversely correlated with exacerbation frequency, while its serum level was markedly increased in children with different severity asthma compared to controls. Additionally, miR-147, induced by toll-like receptor signals (TLRs), can modulate macrophage-mediated inflammatory response and reduce inflammatory protein production, underscoring its potential anti-inflammatory role (Liu et al. 2009; Contreras and Rao 2012). Bioinformatic analyses have further advanced the miRNA biomarker identifying for asthma. (Cay et al. 2022) identified 30 miRNAs as candidate biomarkers, with miR-146b-5p being the only miRNA consistently upregulated in both lung tissue and serum from house dust mite-exposed murine models. Many dysregulated miRNAs are involved in asthma pathogenesis *via* Th2 signals. Among their target genes, runt-related transcription factor 3 (RUNX3) encodes a transcription factor crucial for maintaining the Th1/Th2 balance and regulating interleukin production, thereby modulating inflammatory responses and asthma progression (Yu et al. 2018). These findings underscore the significance of miRNAs and RUNX3 as key regulators in asthma pathogenesis and potential therapeutic targets.

Airway epithelial cells serve as the initial defense line and essential immunomodulatory interface in asthma. Epithelial dysfunction compromises barrier integrity and promotes the release of alarmins, such as thymic stromal lymphopoietin (TSLP) and IL-33, which sustain Th2-mediated inflammation (Heijink et al. 2020). Emerging evidence suggests that impaired epithelial repair and aberrant cell signaling contribute to airway hyperresponsiveness, mucus hypersecretion, and remodeling, thus exacerbating clinical symptoms (Varricchi et al. 2024). This study investigated the roles of

bronchoalveolar lavage fluid (BALF)-derived exosomal miRNAs in regulating growth of bronchial epithelial cells (BECs) and asthma progression. Through functional assays, we explored the miR-147/RUNX3 signaling pathway in epithelial cell dysfunction, providing insights into novel therapeutic strategies for asthma management.

Materials and Methods

Establishment of an asthma mouse model

Female BALB/c mice (6–8 weeks old) were derived from Shanghai Slake Laboratory Animal Co., Ltd. (Shanghai, China) and housed in pathogen-free conditions. All experimental procedures were approved by the Animal Care and Ethics Committee of Shanghai Baoshan District Hospital of Integrated Traditional Chinese and Western Medicine (approval no. PZSHUTCM210919010). All the mice were randomly assigned to two groups: control and model groups ($n = 6$ for each group). Mice in the model group received intraperitoneal injections of 0.1 ml allergen solution containing ovalbumin (OVA, 0.5 mg/ml) combined with aluminum hydroxide (2 mg/ml) on days 0, 7, 14, and 21. Subsequently, they were challenged with a 1% OVA solution *via* nasal drip (30 min/day) on days 28, 30 and 32. The control mice received the equal normal saline. BALF was collected 24 h after the final OVA challenge. The mice were anesthetized using isoflurane, tracheostomized, and cannulated for BALF collection *via* lung flushing with PBS. Mice were euthanized by CO₂ inhalation, and lung tissues were either fixed or stored at -80°C .

Histopathological analysis and inflammatory cytokine detection

The collected lung tissues were fixed in 4% paraformaldehyde for 24 h, dehydrated through a graded ethanol series, embedded in paraffin, and sectioned into 4 μm thick slices. Then, the slices were stained with hematoxylin and eosin (H&E) following the standard protocols, and mounted with neutral gum. Histopathological images were acquired and observed under an Olympus IX70 microscope (Tokyo, Japan). In addition, the levels of IL-6 and TNF- α in BALF were quantified using murine ELISA kits (ELK Biotechnology, Wuhan, China) according to the manufacturer's instructions.

Isolation and characterization of BALF-derived exosomes

Exosomes were isolated from BALF using ultrafiltration centrifugation. BALF was sequentially centrifuged at 500 $\times g$ for 5 min, 2,000 $\times g$ for 30 min, and 10,000 $\times g$ for 30 min at 4 $^{\circ}\text{C}$. The supernatant was then ultracentrifuged at 120,000 $\times g$ for

70 min at 4°C. The resulting exosome pellet was resuspended and stored at -80°C.

The concentrations of isolated exosomes were determined using a BCA protein assay kit (Beyotime, Shanghai, China). Then, the morphology of the exosomes was examined by a transmission electron microscopy (TEM). Briefly, 5 µl of exosome suspensions were added to a formvar-coated copper grid, and incubated at room temperature for 10 min, washed, and stained with 10 µl of 2% phosphotungstic acid (pH 6.5) for negative staining for 2 min. Afterwards, the exosomes were observed using a JEOL JEM 1230 TEM (Tokyo, Japan) at 120 kV.

The particle size distribution of the isolated exosomes was evaluated *via* a nanoparticle tracking analysis (NTA). In brief, the exosome samples were resuspended in PBS, vortexed for uniformity, and analyzed using a NanoSight instrument (Malvern Instruments, UK). Additionally, the expression of the specific exosomes-related markers (CD9, CD81, TSG101) was detected using Western blot assay.

Western blot

Total protein concentrations from exosomes or cell lysates were detected using a BCA kit (Thermo Scientific, USA).

Equal amounts of protein were separated by the SDS-PAGE process and transferred onto PVDF membranes. Following blocking with 5% non-fat milk, the membranes were incubated with primary antibodies against CD9, CD81, TSG101, RUNX3, and GAPDH (Proteintech, USA) at optimized dilutions. After washing, the membranes were incubated with horseradish peroxidase-conjugated murine secondary antibodies (1:3000; Abcam) for 2 h. Finally, the protein bands were visualized *via* chemiluminescence, with GAPDH serving as the loading control.

Cell culture and transfection

Mouse BECs were obtained from Procell (Wuhan, China) and cultured in DMEM, supplemented with 5% fetal bovine serum (Gibco, UK) and 1% penicillin/streptomycin (100 IU/ml; Gibco, UK) at 37°C in a humidified 5% CO₂ atmosphere.

Cell transfection was performed using Lipofectamine 2000 (Invitrogen, USA) in line with the manufacturer's instructions. Briefly, miR-147-3p mimics, mimics negative control (NC), inhibitor NC, and miR-147-3p inhibitor were synthesized and provided by Shanghai Generay Biotechnology Co., Ltd. (Shanghai, China). The sequences

Table 1. The sequences of miR-147-3p mimics, mimics negative control (NC), inhibitor NC, and miR-147-3p inhibitor, as well as the sequences of all primes for RT-qPCR

Sequences (5'-3')	
<i>Cell transfection</i>	
miR-147-3p mimics-sense	GUGUGCGGAA AUGCUUCUGCUATT
miR-147-3p mimics-antisense	UAGCAGAAGCAU UCCGCACACTT
mimics NC-sense	UUCUCCGAACGUGUCACGUTT
mimics NC-antisense	ACGUGACACGUUCGGAGAATT
inhibitor NC	GGCCUCACCGGGUGUAAAUCAG
miR-147-3p inhibitor	UAGCAGAAGCAU UCCGCACAC
<i>RT-qPCR</i>	
mmu-miR-147-3p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGACTGGATACGACTAGCAG
mmu-miR-147-3p-F	GCGGTGTGCGGAAATGCTT
mmu-miR-98-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGACTGGATACGACAACAAT
mmu-miR-98-5p-F	GCGCGGTGAGGTAGTAAGTTGT
mmu-miR-146b-5p-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGACTGGATACGACAGCCTA
mmu-miR-146b-5p-F	GCGCGTGAGAACTGAATTCCA
Downstream universal primer	GTGCAGGGTCCGAGGT
U6-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGACTGGATACGACAAAATATG
U6-F	CTCGCTTCGGCAGCACA
U6-R	AACGCTTCACGAATTTGCGT
RUNX3-mF	GACTCCTTCCCCAACTATACACC
RUNX3-mR	CGCTGTTCTCGCCCATCTT
GAPDH-mF	GGTGAAGGTCGGTGTGAACG
GAPDH-mR	CTCGCTCCTGGAAGATGGTG

miR, microRNA; RUNX3, runt-related transcription factor 3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

of miR-147-3p mimics, mimics negative control (NC), inhibitor NC, and miR-147-3p inhibitor were shown in Table 1. The transfected cells were harvested after 24 h, and the cell transfection efficiency was assessed *via* determining the level of miR-147-3p using real-time quantitative PCR (RT-qPCR).

Co-culture of exosomes and mouse BECs

BALF-derived exosomes were labeled using the PKH67 Green Fluorescent Cell Linker Kit (Sigma-Aldrich, PKH-67GL-1KT) following the manufacturer's protocols. BECs were incubated with PKH67-labeled exosomes at 37°C for 24 h. The cellular uptake of exosomes was assessed using a Leica TCS SP8 confocal laser scanning microscope (Leica, Germany).

Assessment of cell viability, apoptosis, and migration

Cell viability was evaluated using the CCK8 kit (Beyotime, Shanghai, China). The BECs (1×10^4 cells/well) with different treatments were harvested, and the detection solution was added to each well and further co-cultured for 4 h. Absorbance was measured at 450 nm using a microplate reader.

The apoptosis of BECs with different treatments was carried out using the Annexin V-FITC/PI Kit (BD Biosciences, USA). Briefly, the BECs were harvested, washed, and incubated with 5 μ l Annexin V and 10 μ l propidium iodide for 15 min. Finally, the apoptotic cells were analyzed using a BD FACSCalibur flow cytometer (BD Biosciences, NJ, USA).

Furthermore, the cell migration was assessed using a Transwell system (JET BIOFIL, Guangzhou, China) with 8 μ m pore membranes. The BECs (1×10^5 cells/well) were seeded in the upper chamber, and treated with exosomes or different cell transfections for 48 h. Migrated cells on the lower surface were fixed and stained. The cell images were visualized using an Olympus IX70 inverted microscope (Olympus Corporation, Center Valley, PA) at a magnification of 200 $\times$.

RT-qPCR

Total RNA was extracted from the cells using TRIzol reagent (Invitrogen, USA), and cDNA synthesis was carried out using the PrimeScript™ RT Master Mix (TAKARA, Japan). All the primers were designed and provided by Sangon (Shanghai, Chia), as well as the sequences of all primers were presented in Table 1.

For miRNA quantification, the stem loop RT-PCR approach was employed, with U6 as the reference gene. Initially, a 10 μ l denaturing reaction mixture was prepared and

utilized for miRNA reverse transcription. The total volume for miRNA reverse transcription reaction was 20 μ l, comprising 10 μ l of the denaturing reaction mixture, 4 μ l of 5 $\times$ PrimeScript II buffer, 0.5 μ l of RNase inhibitor (40 U/ μ l), 1 μ l of PrimeScript II RTase (200 U/ μ l), and 4.5 μ l of RNase free dH₂O. The thermal cycling conditions for miRNA reverse transcription were set as 60 min at 42°C, followed by 5 min at 95°C. For qPCR, reverse transcriptase was used, with the reaction starting at 95°C for 5 min, followed by 40 cycles of 10 s at 95°C for and 30 s at 60°C.

For mRNA RUNX3 expression, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal standard. The thermal cycling protocol entailed incubating the reverse transcription mixture at 37°C for 60 min followed by 85°C for 5 s. For RT-qPCR, the reaction began with 3-min incubation at 95°C, followed by 40 cycles consisting of 10 s at 95°C and 30 s at 60°C. The expression levels of RUNX3, mmu-miR-147-3p, mmu-miR-98-5p, and mmu-miR-146b-5p were calculated using the $2^{-\Delta\Delta Ct}$ method

Dual-luciferase reporter gene assay

To validate potential interacting relationships between miR-147-3p and 3'untranslated region (3'UTR) of RUNX3, both wild type (WT) and mutant form (MUT) RUNX3 sequences were chemically synthesized, and cloned into the pGL3-Promoter vectors (pGL3-RUNX3-3'UTR-WT, and pGL3-RUNX3-3'UTR-MUT). The structure of pGL3-Promoter vectors was shown in Figure S1 (in Supplementary material). After that, the plasmids of pGL3-control (0.4 μ g), pGL3-RUNX3-3'UTR-WT (0.4 μ g), and pGL3-RUNX3-3'UTR-MUT (0.4 μ g) were co-transferred to HEK-293T cells with miR-147-3p mimics (100 nM) and NC mimics (100 nM) using Lipofectamine 2000 reagent (Invitrogen) following the protocols of the manufacturer. After transfected for 6 h, the medium was changed to complete medium, and after transfected for another 48 h, luciferase activity was measured using the detection kit (Promega, USA). Relative luciferase activities were calculated as the ratio of firefly Renilla luciferase signal.

Statistical analysis

Three independent replicates were performed for each experiment, and data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using GraphPad Prism v8.0. For multiple group comparison, one-way analysis of variance (ANOVA) with Tukey's *post hoc* test was employed. Differences between the two groups were analyzed using an unpaired t-test with Welch's correction. A *p*-value less than 0.05 was considered statistically significant.

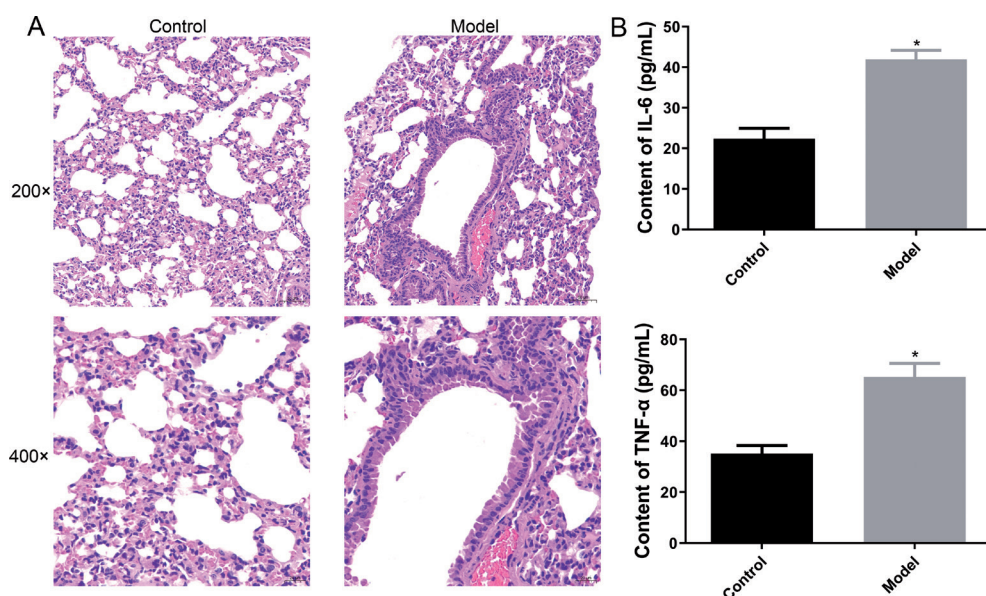


Figure 1. Establishment of an asthma mouse model. **A.** H&E staining of lung tissues at magnifications of 200× (upper) and 400× (lower). **B.** The levels of IL-6 and TNF-α in BALF measured by ELISA. * $p < 0.05$ compared to control.

Results

Identification of asthma mouse model

Histopathological analysis of lung tissues from asthmatic and control mice was conducted using H&E staining. Compared to control mice, asthmatic mice exhibited increased infiltration of inflammatory cells, including eosinophils and neutrophils, within the bronchial submucosa, bronchi, and perivascular regions (Fig. 1A). Additionally, these mice demonstrated typical basement membrane thickening, as well as abnormal epithelial cell features in the bronchial mucosa, consistent with the established asthma pathology (Soltzberg et al. 2011). Furthermore, the ELISA analysis displayed upregulated levels of IL-6 and TNF-α in asthmatic mice BALF samples compared to the control mice (Fig. 1B). These results indicated the asthmatic mice were successfully constructed.

Characterization and uptake of BALF-derived exosomes

The BALF-derived exosomes were identified using NTA, TEM, and Western blot methods. TEM images revealed that exosomes from both asthmatic and control mice displayed a single-layer membrane structure and appeared as round or elliptical vesicles measuring 40–100 nm in diameter (Fig. 2A). NTA further validated these findings, with a peak exosome size of approximately 70 nm, consistent with previously reported data (Fig. 2B). Additionally, western blot assay confirmed the expression of exosomal markers (CD9, CD63, and TSG101) in exosome from the both two groups. These outcomes suggested that the exosomes were

successfully isolated from the asthmatic and control mice (Fig. 2C).

To assess the epithelial cells internalization, exosomes were labeled with PKH67 (green), and actin red was used to stain cytoskeleton (red). DAPI was employed to stain cell nucleus (blue). Following co-incubation for 24 h, green fluorescence (PKH67-labeled exosomes) was displayed in BECs, which indicated that BALF-derived exosomes from control and asthmatic mice could be taken up by BECs after co-culture (Fig. 2D).

Selection of the target miRNA and gene by RT-qPCR, and verification of their relationship

The miRNA levels in BALF-derived exosomes were evaluated by RT-qPCR. The RT-qPCR results demonstrated a significant up-regulation of mmu-miR-147-3p and mmu-miR-146b-5p in BALF-derived exosomes from the asthmatic mice compared to the control mice (Fig. 3A). Conversely, mmu-miR-98-5p and RUNX3 expression levels were markedly decreased in the asthmatic group in comparison with the control group (Fig. 3B). Among these, miR-147-3p was previously identified as a key regulator of inflammatory responses (Liu et al. 2009; Chen et al. 2017; Tang et al. 2020), and has been recognized as an asthma-related biomarker involved in inflammatory pathways during traditional Chinese medicine treatment for asthma. Based on its potential role in inflammation, miR-147-3p was selected for further investigation.

Further to determine whether exosomal miR-147-3p directly regulates RUNX3, a dual-luciferase assay was conducted. In the pGL3-RUNX3-3'UTR-WT plasmids, the relative luciferase activity after transfected with miR-147-3p

mimics was significantly reduced compared to that after transfected with NC mimics; whereas no significant change was observed between the NC mimics and miR-147-3p mimics in the pGL3-RUNX3-3'UTR-MUT plasmids (Fig. 3C). These results hinted that miR-147-3p could directly target RUNX3.

Effects of BALF-derived exosomes on the growth of murine BECs

In order to explore the effects of BALF-derived exosomes on the progression of asthma, the BALF-derived exosomes

from the control and asthmatic mice were used to treat with the BECs. CCK-8 results revealed that the BALF-derived exosomes from the asthmatic mice significantly reduced cell viability in a dose-dependent manner, whereas the BALF-derived exosomes from the control mice had no effect on the cell viability (Fig. 4A). Additionally, the exosome concentrations of 20 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$ exhibited significant inhibitory effects between the control and model groups (Figure 4A). Thus, 20 $\mu\text{g/ml}$ BALF-derived exosomes from the control and asthmatic mice were selected for subsequent experiments.

Thereafter, flow cytometry and transwell assays were used to determine cell apoptosis and migration. No significant

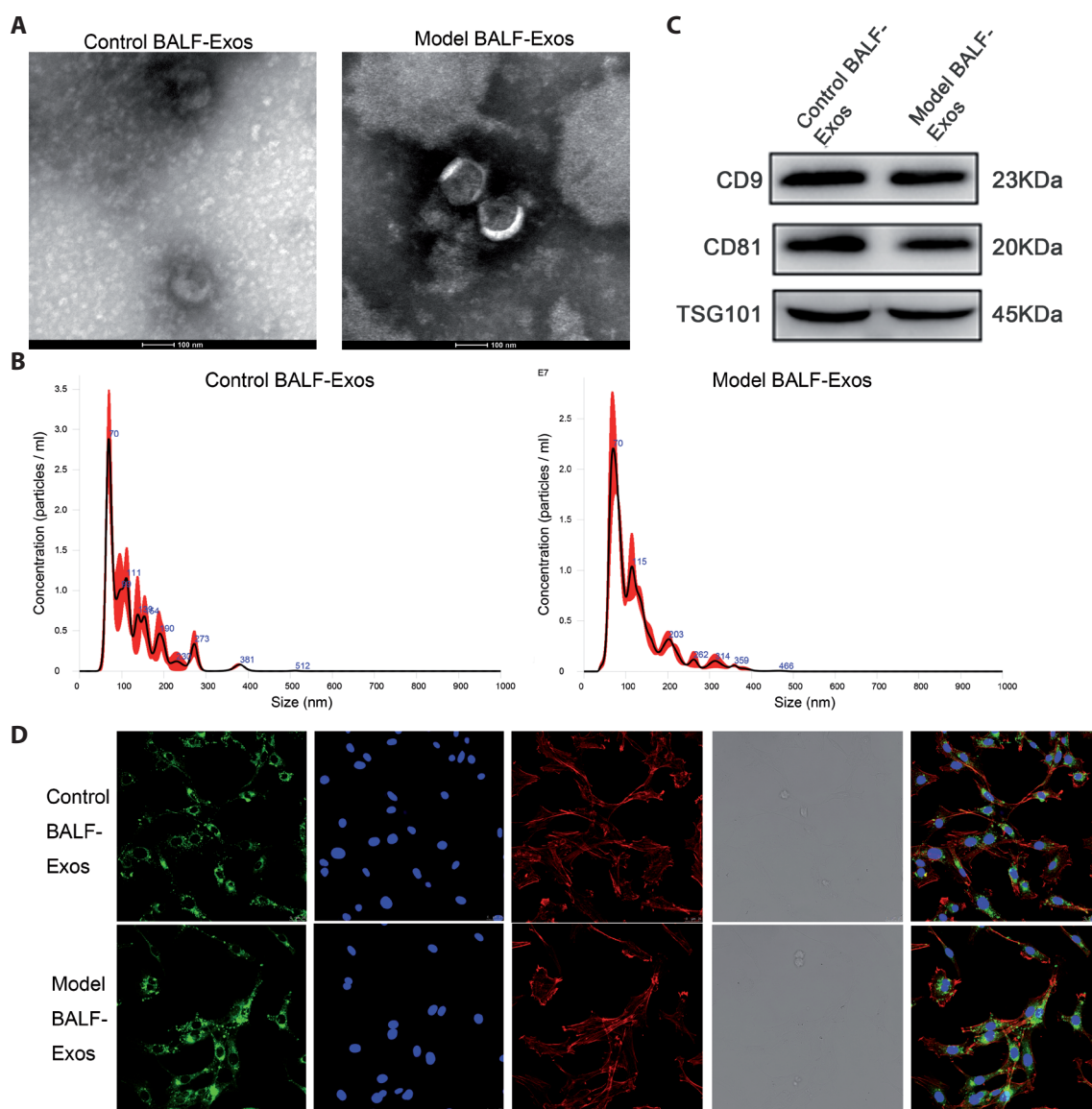


Figure 2. Identification and uptake of BALF-derived exosomes. **A.** TEM images of BALF-exosomes. **B.** NTA results of exosomes. **C.** Western blot assay for exosomes-specific markers (CD9, CD63, and TSG101). **D.** Confocal microscopy images showing uptake of PKH67-labeled exosomes by BECs (400 $\times$ magnification). DAPI-stained nuclei appear blue; actin filaments are red. (See online version for color figure.)

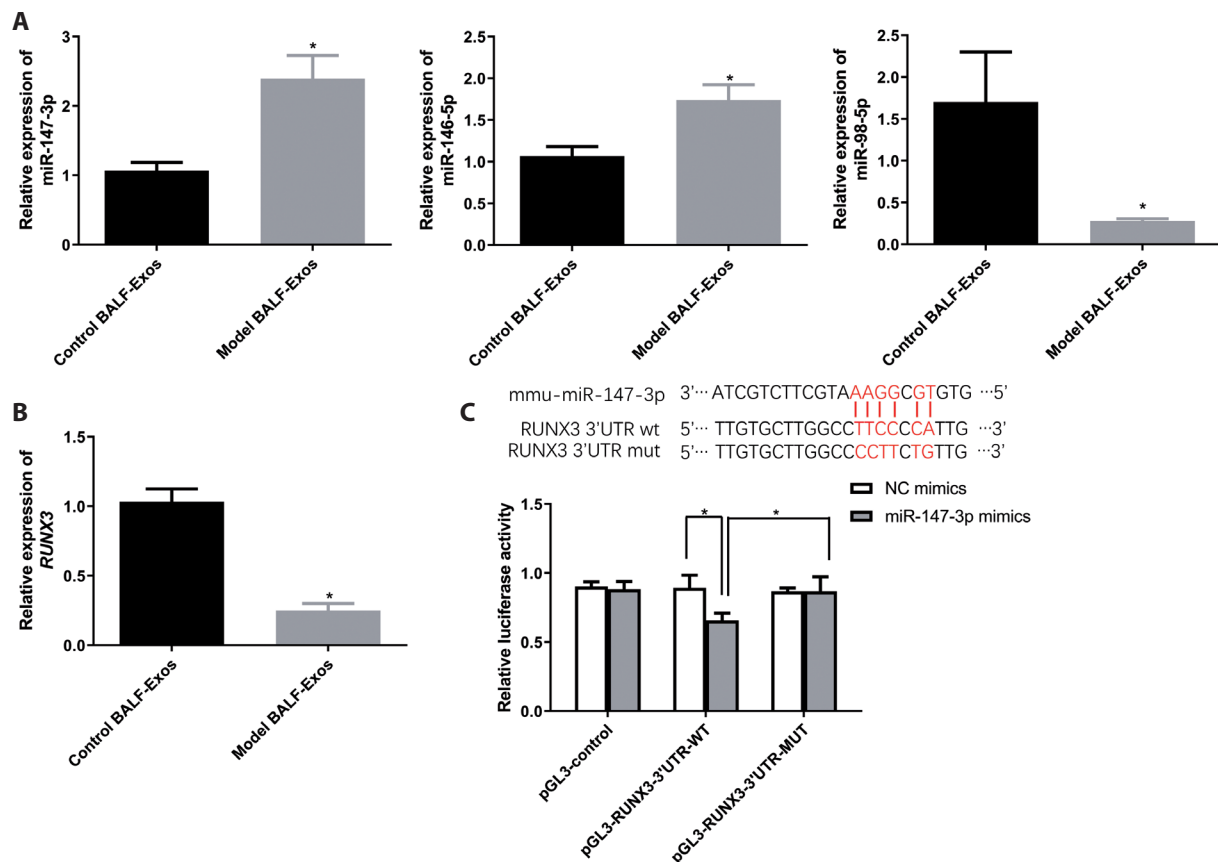


Figure 3. Selection of the target miRNA and gene by RT-qPCR, and verification of their relationship. qPCR analysis of exosomal miRNAs (A) and RUNX3 (B). * $p < 0.05$. C. miR-147-3p could directly bind to RUNX3 using a dual-luciferase reporter gene assay.

differences were found in cell apoptosis rate and cell migration number between the blank and control BALF-derived exosomes (Fig. 4B,C). However, compared with the blank cells, the treatment of BALF-derived exosomes from the asthmatic mice significantly increased the apoptosis rate of BECs, while evidently reduced the cell migration number of BECs ($p < 0.05$; Fig. 4B,C).

Additionally, RT-qPCR and Western blot were employed to measure the levels of miR-147-3p and RUNX3. As shown in Figure 4D, BECs co-cultured with asthmatic BALF-derived exosomes exhibited significantly increased level of miR-147-3p; while an evidently reduction of RUNX3 expression. Furthermore, Western blot analysis also confirmed the down-regulated expression trend RUNX3 in the BECs treated with asthmatic BALF-derived exosomes (Fig. 4E).

The roles of exosomal miR-147-3p in the regulation of murine BEC functions

Further to investigate the regulatory roles of exosomal miR-147-3p in the growth of BECs, the BECs were transfected

with miR-147-3p mimics, miR-147-3p inhibitor, and their corresponding NC (inhibitor NC and mimics NC), and then the cell transfection efficiency was validated by RT-qPCR. It was obvious that there was no significant difference in miR-147-3p levels among the blank, inhibitor NC and mimics NC groups (Fig. 5A). Compared with the blank cells, the miR-147-3p level was significantly declined in the cells transfected with miR-147-3p inhibitor; whereas was markedly elevated in the BECs transfected with miR-147-3p mimics (Fig. 5A). These indicated that the BECs with miR-147-3p inhibition or overexpression were successfully established, and could be used for follow-up study.

The CCK-8 results showed miR-147-3p overexpression significantly reduced the cell viability, similar to the treatment of asthmatic BALF-derived exosomes and inhibitor NC, compared with the mimics NC group (Fig. 5B). In contrast, miR-147-3p inhibition significantly enhanced the viability of BECs induced by the asthmatic BALF-derived exosomes (Fig. 5B). The flow cytometry analysis revealed that miR-147-3p overexpression or treatment with asthmatic BALF-exosomes significantly increased apoptosis in BECs

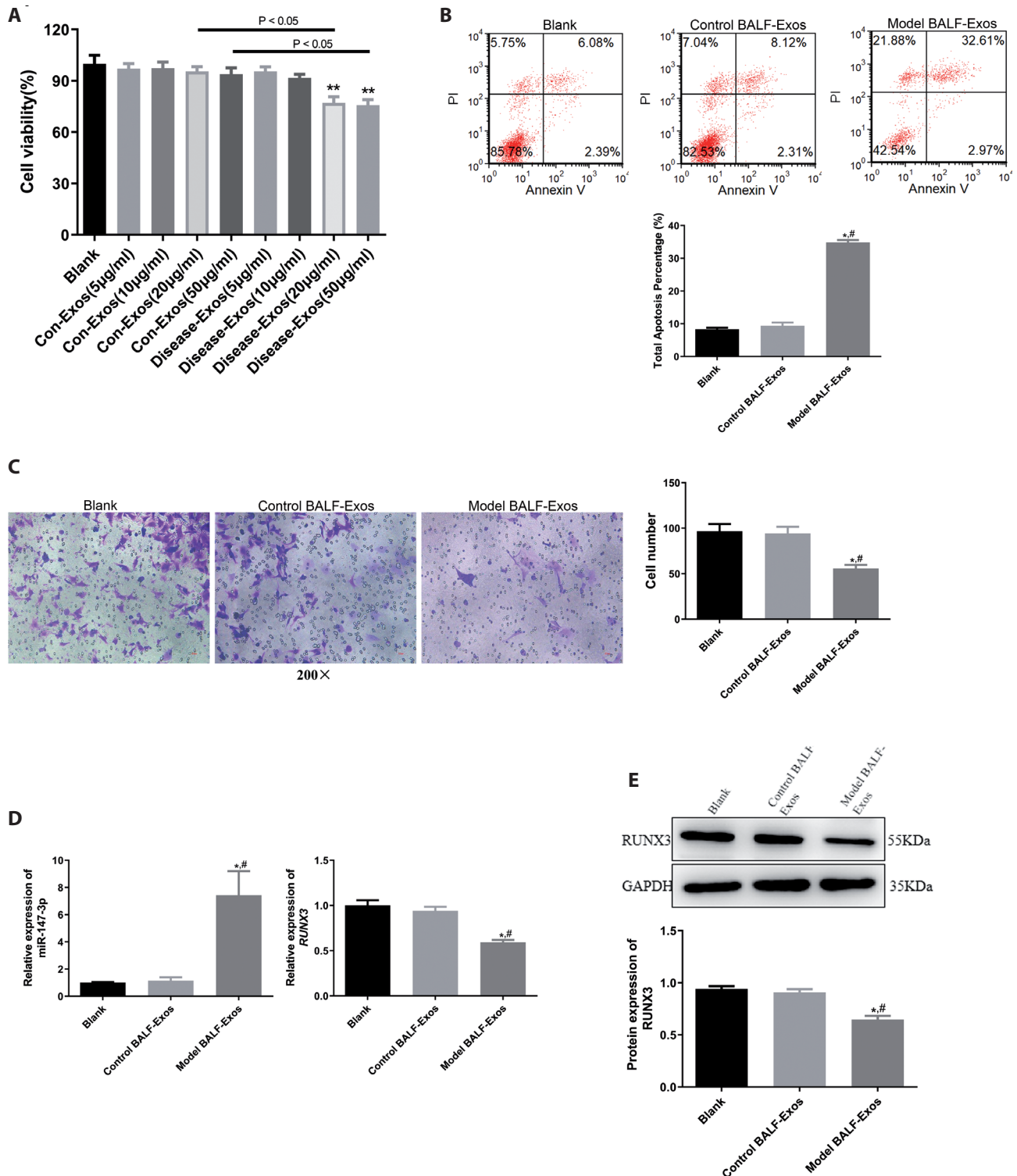


Figure 4. Effects of BALF-derived exosomes on the growth of murine bronchial epithelial cells (BECs) **A**. Murine bronchial epithelial cells were incubated with different concentrations of exosomes (5, 10, 20, 50 µg/ml) for 48 h, and cellular viability was detected by CCK-8 kit. Bronchial epithelial cells were incubated with BALF-exosomes from asthmatic model and control groups for 48 h. Apoptotic cell percentages were analyzed by flow cytometry (**B**), and cell migration was evaluated using Transwell assay (**C**). BECs were co-cultured with BALF-derived exosomes from asthmatic and control groups for 48 h. miR-147-3p and RUNX3 expression were analyzed by RT-qPCR (**D**) and Western blotting (**E**). $p < 0.05$ vs. blank; $p < 0.05$ vs. control exosome group.

in comparison with the mimics NC group, whereas miR-147-3p inhibition reduced apoptosis induced by asthmatic BALF-exosomes (Fig. 5C). For cell migration, compared with the mimics NC group, a marked decrease of cell number in BECs was observed in the BECs with miR-147-3p mimics or asthmatic BALF-exosome treatment, but suppression of

miR-147-3p could evidently reverse this effect caused by the asthmatic BALF-exosomes (Fig. 5D).

In addition, Western blot showed that compared with the mimics NC group, the RUNX3 expression was significantly downregulated in the BECs transfected with miR-147-3p mimics or in the BECs treated with asthmatic BALF-ex-

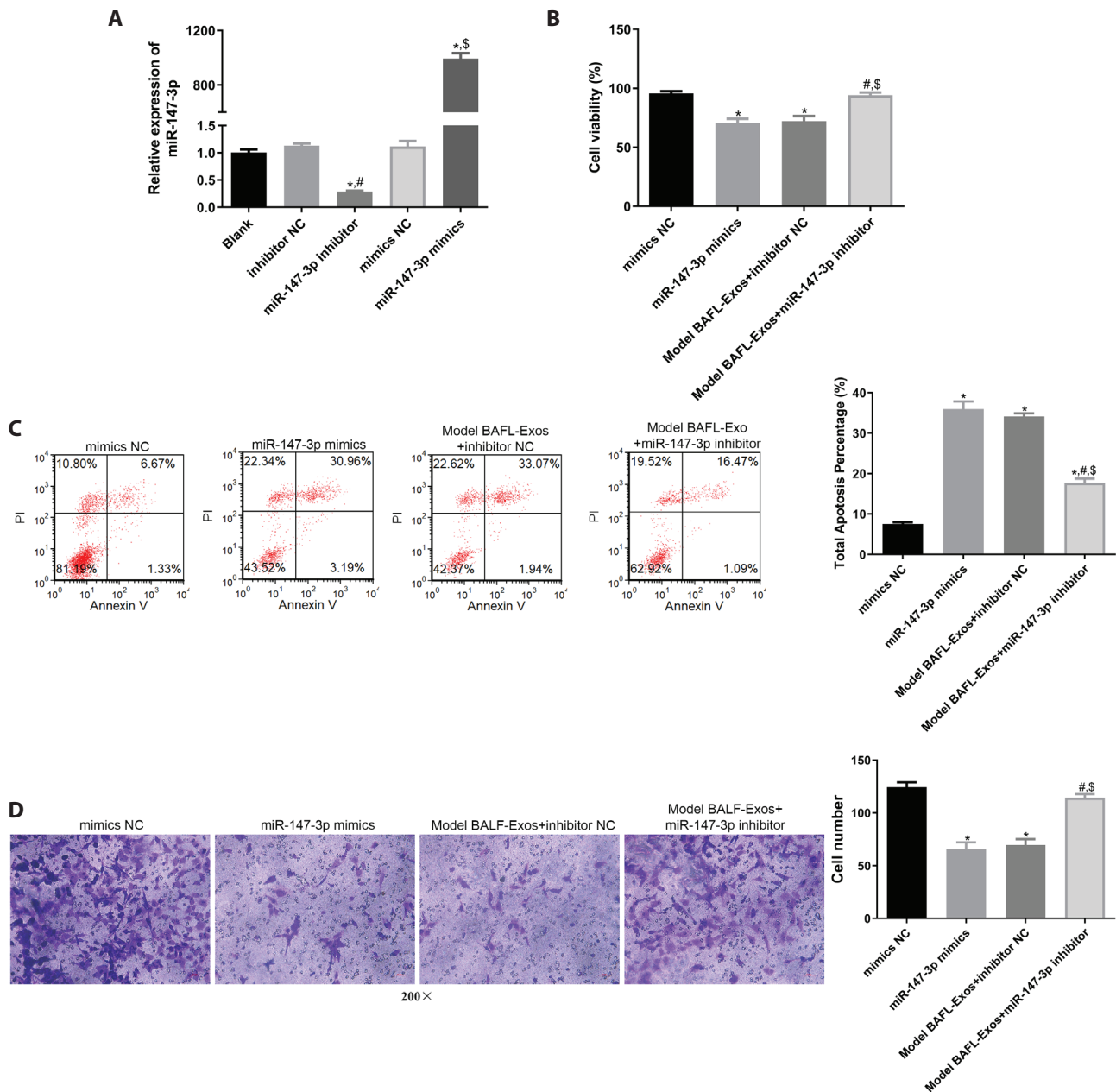


Figure 5. Exosomal miR-147-3p could suppress cell growth, apoptosis, and migration of murine bronchial epithelial cells. **A.** The transfection efficiency assessed by determining miR-147-3p level using RT-qPCR. * $p < 0.05$ vs. blank, # $p < 0.05$ vs. mimics NC, \$ $p < 0.05$ vs. inhibitor NC. Bronchial epithelial cells were transfected with miRNA mimics or exosomes for 48 h and CCK-8 assay detected cell viability (**B**), flow cytometry assay for apoptotic cells assessing (**C**), transwell assay for cellular migration (**D**). * $p < 0.05$ vs. mimics NC; # $p < 0.05$ vs. miR-147-3p mimics; \$ $p < 0.05$ vs. inhibitor NC+model exosomes.

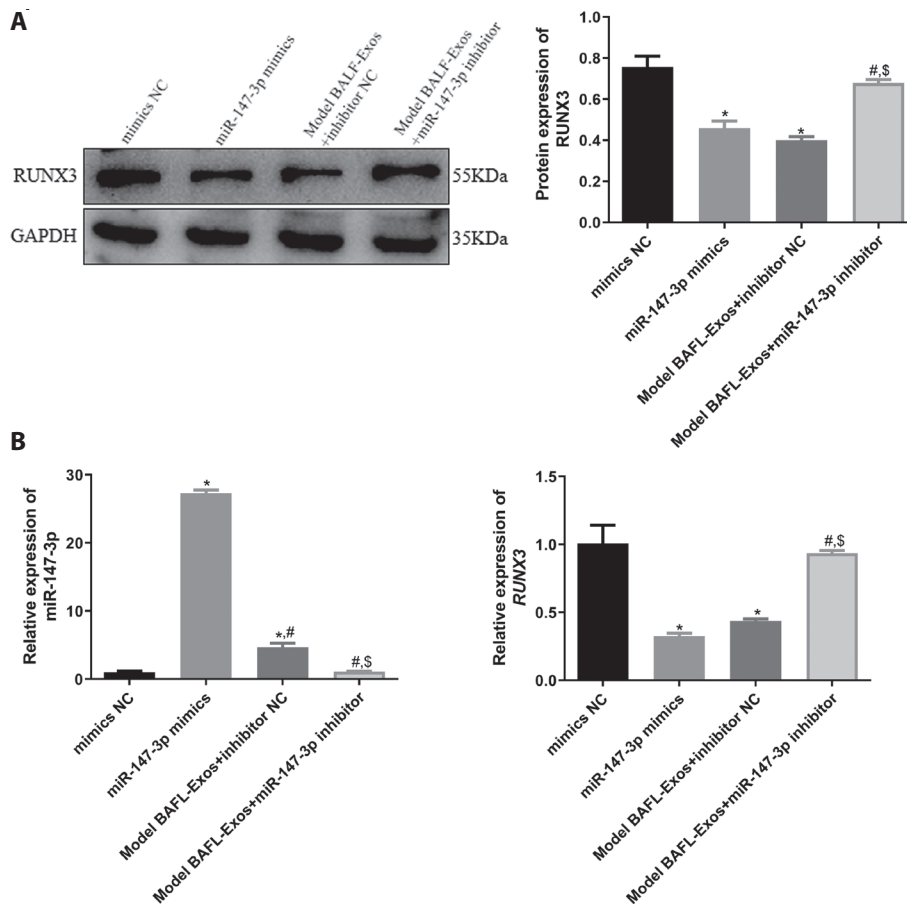


Figure 6. Exosomal miR-147-3p could inhibit RUNX3. The miRNA mimics or exosomes were incubated with murine bronchial epithelial cells for 48 h. The relative levels of RUNX3 protein (A) and miR-147-3p and RUNX2 mRNA levels (B) determined by Western blot and RT-qPCR. * $p < 0.05$ vs. mimics NC; # $p < 0.05$ vs. miR-147-3p mimics; \$ $p < 0.05$ vs. inhibitor NC+model exosomes.

osomes (Fig. 6A). However, silencing miR-147-3p restored the RUNX3 expression to a similar level of the mimics NC group (Fig. 6A). For miR-147-3p, its level was significantly elevated in the BECs transfected with miR-147-3p mimics or in the BECs treated with asthmatic BALF-exosomes compared with the mimics NC group; while was markedly decreased after miR-147-3p suppression in the asthmatic BALF-exosomes-treated BECs (Fig. 6B). The tendency of RUNX3 mRNA expression in the different groups tested by RT-qCPR was consistent with that of protein expression detected by Western blot (Fig. 6B).

Discussion

Airway epithelial cells serve as the primary defense against inflammatory stimuli and antigens, playing an important role in bridging innate immunity and allergic disease through the activating of epithelial TLRs (Laulajainen-Hongisto et al. 2020). Beyond their defensive function, these cells act as inflammatory regulators, directing dendritic cells toward a Th2 response and producing various

inflammatory/anti-inflammatory mediators. In asthma, epithelial homeostasis is disrupted, leading to impaired innate defenses, persistent type 2 inflammation, barrier dysfunction, and tissue remodeling (Russell et al. 2024). Abnormalities in epithelial repair mechanisms, particularly those involving cell growth and proliferation, are central to airway remodeling. Upon injury, the bronchial epithelium releases various growth factors, such as epidermal growth factor (EGF) and transforming growth factor- β (TGF- β), which stimulate fibroblasts and myofibroblasts, resulting in excessive extracellular matrix (ECM) deposition and airway wall thickening (Heijink et al. 2020). In asthmatic airways, this repair process is aberrant and characterized by increased basal cell markers, heightened proliferation, and reduced differentiation. In our study, we used an OVA-induced asthma mouse model to explore the influences of exosomal miRNAs on BECs behavior. *In vitro*, data demonstrated that BALF-derived exosomes from asthma mice influence BECs behavior, with miR-147-3p, 146b-5p, and 98-5p identified as dysregulated.

Emerging evidence highlights the involvement of exosomal miRNAs in asthma by modulating inflammation, airway

hyperresponsiveness, and remodeling. Certain miRNAs, like miR-34a, miR-92b, miR-223, and miR-142a, promote asthma progression by regulating Th2 polarization, dendritic cell maturation, and allergic airway inflammation (Pua et al. 2019; Bartel et al. 2020). Conversely, anti-inflammatory miRNAs, including miR-182-5p, mitigate asthma by reducing oxidative stress and epithelial inflammation (Wang et al. 2022; Liu et al. 2024). Our results demonstrated miR-147-3p is upregulated in asthma BALF, and may contribute to epithelial dysfunction. BALF-derived exosomes or miR-147-3p treatment in BECs significantly reduced cell viability and migration while increasing apoptosis, an effect reversed by miR-147-3p inhibition. Moreover, molecular assays verified miR-147-3p was upregulated, whereas RUNX3 was downregulated in BALF-exosome-treated BECs. These findings suggested that the miR-147-3p/RUNX3 axis played a role in asthma pathogenesis, warranting further investigation into its potential as a therapeutic target.

Dual-luciferase revealed a direct binding relationship between miR-147-3p and RUNX3, supporting the regulatory mechanisms of miRNAs in post-transcriptional gene expression. The transcriptional factor RUNX3 is essential for T-cell differentiation, enhancing the naïve CD4⁺ T cells progressed to Th1 cells while inhibiting Th2-driven IL-4 production. This regulation maintains the Th1/Th2 balance and modulates airway inflammation (Fainaru et al. 2004). RUNX3 deficiency was involved in spontaneous eosinophilic airway inflammation, airway remodeling, and mucus hypersecretion in murine models, highlighting its protective function against asthma (Yu et al. 2018). Notably, genetic studies have linked the RUNX3 locus to increased asthma susceptibility, underscoring its relevance in disease pathogenesis (Fainaru et al. 2005). Differential DNA methylation of RUNX3 in peripheral blood mononuclear cells has been associated with variations in T-cell maturation, IL-4-driven Th2 immunity, and oxidative stress, contributing to asthma severity (Liao et al. 2024).

Within airway epithelial cells, RUNX3 plays a critical role in responding to environmental stimuli and regulating apoptosis (Gan et al. 2015). It also influences epithelial-to-mesenchymal transition (EMT), a process central to airway remodeling and fibrosis in chronic pulmonary diseases (Yang et al. 2017). Low RUNX3 level promote EMT, while high level suppresses TGF- β -mediated EMT. EMT is a reversible process in which epithelial cells lose their features and adopt mesenchymal phenotype, facilitating migration and tissue repair. Under normal conditions, EMT supports physiological processes such as growth, development, and wound healing. However, in chronic inflammatory airway diseases, persistent exposure to inflammatory cytokines like EGF and TGF- β promotes excessive EMT, leading to pathological fibrosis and airway remodeling (Wesley et al. 2007; Zhang et al. 2025). Our results indicated that BALF-derived

exosomal miR-147-3p downregulates RUNX3 in BECs, resulting in increased apoptosis and impaired migration. Overexpression or inhibition of miR-147-3p significantly altered RUNX3 expression at both mRNA and protein levels. Collectively, these results suggest BALF-derived exosomal miR-147-3p contributes to bronchial epithelial apoptosis and EMT dysregulation *via* RUNX3 suppression, thereby facilitating asthma progression.

Recent studies have explored RUNX3 modulation as a potential therapeutic strategy for asthma. Androgen and estrogen have been shown to regulate the Th1/Th2 balance by modulating RUNX3 expression in BECs, suggesting a role for hormone therapy in asthma management (He et al. 2024). Additionally, Pim1 kinase inhibition enhances RUNX3 expression, reducing airway inflammation and mucus hypersecretion (Fang et al. 2024). Furthermore, miR-19a inhibition has been found to mitigate Th2-driven airway inflammation by targeting RUNX3 (He et al. 2023). These findings collectively establish RUNX3 as a key regulator in asthma pathogenesis and highlight its potential as a therapeutic target.

Several limitations should be acknowledged. First, additional murine models of asthma are required to further validate the regulatory mechanism between miR-147-3p and RUNX3. Second, as RUNX3 may be regulated by multiple miRNAs in asthma (Fan et al. 2016; He et al. 2023), a more comprehensive analysis of the miRNA-RUNX3 regulatory network is necessary to fully elucidate its complexity. Third, the precise mechanisms by which RUNX3 expression in BECs influences intercellular communication with T cells remain unclear, warranting further investigation. Finally, future research with expanded sample sizes is essential to confirm the reproducibility of our observations and strengthen the clinical relevance of the conclusions.

Conclusion

In conclusion, this study revealed that exosomal miR-147-3p, derived from asthma BALF, inhibits BEC growth and migration while promoting apoptosis through RUNX3 downregulation. Our findings suggest that miR-147-3p and its target gene RUNX3 might be useful biomarkers and potential therapy targets for asthma, providing novel insights into disease pathogenesis and intervention strategies.

Ethics approval. All experimental procedures were approved by the Animal Care and Ethics Committee of Shanghai Baoshan District Hospital of Integrated Traditional Chinese and Western Medicine (approval No. PZSHUTCM210919010).

Availability of data and material. The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of interests. The authors have no conflicts of interest to declare that are relevant to the content of this article.

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