

MiR-491-5p Targets B4GalT5 to Alleviate Airway Inflammation and Remodeling in Asthma by Regulating Pulmonary Oxidative Stress

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Background: MicroRNA-491-5p (miR-491-5p) is a key regulator of cell proliferation and inflammation, but its role in asthma pathogenesis remains unclear. This study aimed to investigate the mechanistic involvement of miR-491-5p in airway remodeling and inflammation, focusing on its downstream target, B4GalT5, and oxidative stress pathways.

Methods: MicroRNA sequencing of airway smooth muscle (ASM) tissues from asthma patients revealed significant downregulation of miR-491-5p, and bioinformatic prediction combined with dual-luciferase reporter assays identified B4GalT5 as a direct downstream target. Clinical correlation analyses assessed the relationship between B4GalT5 expression, Ki-67 (a marker of cell proliferation), and percentage of airway wall area to total tracheal area (WA%). To explore the functional role of miR-491-5p, an ovalbumin (OVA)-induced asthma model was established in C57BL/6 mice, followed by intratracheal instillation of AAV-miR-491-5p to restore its expression in vivo. Airway inflammation and remodeling were evaluated using ELISA, and histological staining (HE, Masson and PAS staining). Oxidative stress markers, including reactive oxygen species (ROS), malondialdehyde (MDA), superoxide dismutase (SOD), and adenosine triphosphate (ATP), were also quantified. In vitro, airway smooth muscle cells (ASMCs) were stimulated with TNF- α to mimic the inflammatory microenvironment and then transfected with a miR-491-5p mimic to assess effects on cell proliferation, cytokine secretion, mitochondrial morphology, ROS production, and Ca²⁺ homeostasis.

Results: MiR-491-5p was significantly downregulated in ASM tissues from asthma patients. B4GalT5 was identified and validated as a direct target of miR-491-5p. Clinically, B4GalT5 expression was positively correlated with Ki-67 and WA%, suggesting a role in airway remodeling. In the OVA-induced asthma model, miR-491-5p overexpression markedly alleviated airway inflammation, mucus hypersecretion, collagen deposition, and structure remodeling, accompanied by reduced oxidative stress (decreased ROS and MDA, increased SOD activity and ATP levels). In TNF- α -stimulated ASMCs, miR-491-5p mimic suppressed excessive proliferation, cytokine release, mitochondrial dysfunction, ROS elevation and intracellular Ca²⁺ homeostasis. Mechanistic studies confirmed that miR-491-5p directly binds to the 3'-UTR of B4GalT5, inhibiting its expression and downstream oxidative stress pathways.

Conclusion: These findings demonstrate that miR-491-5p attenuates asthma-related airway remodeling and inflammation by targeting B4GalT5 and modulating oxidative stress, providing a novel target for therapeutic intervention.

Keywords: airway inflammation, airway remodeling, asthma, B4GalT5, miR-491-5p, oxidative stress

Introduction

Asthma is a common chronic inflammatory airway disease affecting over 300 million people worldwide, with increasing prevalence and significant clinical burden.¹ Persistent airway inflammation and structural remodeling, especially the thickening and hyperplasia of airway smooth muscle (ASM), are central to disease progression and treatment resistance.² Abnormal proliferation of ASM cells not only contributes to airway narrowing but also worsens airflow limitation and clinical outcomes.

Recent studies highlight mitochondrial oxidative stress as a key driver of ASM dysfunction in asthma. Excessive reactive oxygen species (ROS) disrupt mitochondrial homeostasis, leading to impaired ATP synthesis, aberrant calcium signaling, and sustained inflammation.^{3–5} These mitochondrial alterations reinforce a vicious cycle of “inflammation-oxidative stress” in airway smooth muscle cells (ASMCs) that exacerbates airway inflammation and remodeling, underscoring the urgent need for novel molecular targets to interrupt this process.

MicroRNAs (miRNAs) are key post-transcriptional regulators of gene expression and are implicated in the pathogenesis of numerous diseases. Among them, miR-491-5p has been reported to suppress cell proliferation, oxidative stress, and inflammation in cancer models.^{6–9} However, its role in chronic airway diseases such as asthma remains unexplored. This study aims to provide preliminary evidence regarding the potential role of miR-491-5p in the pathogenesis of asthma.

To further elucidate the mechanism by which miR-491-5p functions in asthma, we used bioinformatics analysis to identify β -1,4-galactosyltransferase 5 (B4GalT5), a β -1,4-galactosyltransferase involved in oxidative stress pathways, as a direct target of miR-491-5p.^{10,11} Although B4GalT5 has been primarily studied in the context of tumor progression and cardiac hypertrophy, where it interacts with UGCG to promote ROS production and pathological remodeling,¹² its role in asthma has not been explored. The involvement of B4GalT5 in airway inflammation and structural remodeling has yet to be elucidated.

In this study, we systematically investigated the expression patterns and functional roles of miR-491-5p and B4GalT5 in asthma-related airway pathology. We found that miR-491-5p was downregulated, while B4GalT5 was upregulated in ASM tissues of asthma patients. Functionally, miR-491-5p overexpression inhibited TNF- α -induced ASMC proliferation, inflammation, and mitochondrial oxidative stress, whereas B4GalT5 overexpression reversed these effects. These findings suggest that miR-491-5p may alleviate airway inflammation and remodeling in asthma by targeting B4GalT5, providing novel insights into the asthma-related molecular mechanisms and future treatment strategies.

Materials and Methods

Ethics Statement

The collection and use of human specimens in this study were approved by the Medical Ethics Committee of the People's Hospital of Zhengzhou University (Approval No.202407701). All procedures strictly adhered to the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants. The privacy and confidentiality of all subjects were strictly protected throughout the study.

Human Study

Inclusion criteria were as follows: (1) diagnosis of bronchial asthma based on the Global Initiative for Asthma (GINA) guidelines;¹³ (2) age between 18 and 65 years; (3) surgical specimens containing structurally intact segmental bronchi; (4) no acute asthma exacerbation within the past 4 weeks; (5) no systemic corticosteroid treatment within 4 weeks prior to surgery. Exclusion criteria included: (1) presence of other respiratory diseases besides asthma, such as chronic obstructive pulmonary disease (COPD), pulmonary hypertension, or lung cancer; (2) systemic inflammatory or autoimmune diseases such as systemic lupus erythematosus or rheumatoid arthritis; (3) severe cardiovascular, cerebrovascular, or other systemic diseases affecting lung function. Patients undergoing surgery for pulmonary nodules were used as the normal control group. All control subjects had normal pulmonary function and no clinical or histological evidence of airway inflammation. ASM tissues were collected from segmental or subsegmental bronchi in lung regions anatomically distant from the pulmonary nodules. These bronchi were histologically normal and clearly surrounded by smooth muscle layers, ensuring the reliability of the ASM samples as representative of healthy airways. Pulmonary function was assessed using the MasterScreen[®] spirometry system (Viasys Healthcare GmbH, Hoechberg, Germany).

Human Specimens

Segmental bronchial tissues resected during surgery were immediately transported on ice to the laboratory. After thorough rinsing with phosphate-buffered saline (PBS, Servicebio, Wuhan, China) to remove surface blood and mucus, segmental bronchi were dissected under a stereomicroscope. The bronchi were longitudinally opened, and the

mucosal and adventitial layers were removed, retaining only the central smooth muscle layer. Residual glandular and cartilaginous structures were carefully scraped off to isolate purified airway smooth muscle tissue. Remaining tissues were fixed in 4% paraformaldehyde and embedded in paraffin for subsequent histological staining and analysis.

HRCT Scanning

All participants underwent high-resolution computed tomography (HRCT, Philips, Netherlands) of the chest at the end of a quiet inspiratory phase. The scanning parameters were as follows: 1 mm slice thickness, 1 mm interslice gap, 120 kVp tube voltage, and 100–200 mA tube current. Subjects were scanned in the supine position from the lung apex to the base. Images were imported into the Carestream Health system for 3D reconstruction and analysis. To reduce inter-individual anatomical variation, we standardized our measurements by consistently selecting the apical segmental bronchus of the right lower lobe (RB10) in all subjects and performing all assessments at the same anatomical level across participants. Cross-sectional areas of the airway were manually or semi-automatically outlined to measure airway diameter (D) and luminal diameter (L) ([Supplementary Figure S1](#)). The percentage of wall area (WA%) was calculated using the following formula:

$$WA\% = \left[\pi(D/2)^2 - \pi(L/2)^2 \right] / \pi(D/2)^2 \times 100$$

Two radiologists independently measured the images in a double-blinded manner.

miRNA Sequencing and Analysis

After dissection of pure airway smooth muscle tissues from segmental bronchi of asthma patients (n=3) and control participants (n=3), total RNA was immediately extracted using Trizol reagent (Thermo Fisher, Waltham, MA, USA). The RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher), ensuring an OD260/280 ratio between 1.8 and 2.0. RNA integrity was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and only samples with an RNA integrity number (RIN) ≥ 7 were used for library construction. Qualified RNA samples were shipped on dry ice to Aiji Baike Biotechnology Co., Ltd. (Wuhan, Hubei, China) for small RNA library preparation and high-throughput sequencing. The library preparation included 3' and 5' adaptor ligation, reverse transcription, qRT-PCR amplification, and target fragment selection. Single-end sequencing was performed using the Illumina platform. Raw reads were subjected to quality control, including adaptor trimming and removal of low-quality reads, to obtain clean reads of 18–30 nt in length. Clean reads were mapped to the miRBase database (v22) to annotate known miRNAs. Differential expression analysis was conducted using DESeq2, with screening criteria of $|\log_2\text{FoldChange}| > 1$ and $P < 0.05$.

Animals and Treatments

Female C57BL/6 mice (20–22 g) were purchased from SPF Biotechnology Co., Ltd. (Beijing, China). Mice were housed under specific pathogen-free (SPF) conditions with a temperature of $22 \pm 2^\circ\text{C}$, relative humidity of 45–55%, and a 12-hour light/dark cycle. Mice were randomly assigned to four groups (n=6/group): (A) Control; (B) Asthma; (C) Asthma + AAV-NC-miR-491-5p; (D) Asthma + AAV-miR-491-5p. AAV-NC and AAV-miR-491-5p vectors (Fenghui Biotechnology Co., Ltd., Changsha, Hunan, China) were intratracheally instilled (50 μL per mouse) on days 0, 7, and 15. On days 0, 7, and 14, mice in groups B, C, and D were sensitized by intraperitoneal injection of 40 μg ovalbumin (OVA; Yuanye, Shanghai, China) mixed with 2 mg aluminum hydroxide in 0.2 mL saline. From day 21, these groups were challenged with 5% OVA aerosol by ultrasonic nebulization for 30 minutes per day for 8 consecutive days. Control mice received equal volumes of saline at all time points. No adverse effects were observed in any mice treated with AAV vectors, and all procedures were well tolerated. Mice were euthanized on day 30 with an intraperitoneal dose of 200 mg/kg sodium pentobarbital. The experimental protocol was approved by the Medical Ethics Committee of Zhengzhou University (Approval No. ZZU-LAC20240628[02]) and complied with the Helsinki Declaration and relevant guidelines for animal welfare.

Sample Collection

On day 30, mice were euthanized, and the trachea was exposed under sterile conditions. Bronchoalveolar lavage fluid (BALF) was collected by slowly instilling 1 mL sterile PBS (containing 1% protease inhibitor) in three aliquots of 0.3 mL, retaining each for 10 seconds before withdrawal. The recovery rate was >80%. The lavage fluid was centrifuged at $300 \times g$ for 10 minutes at 4°C, and the supernatant was stored at -80°C for subsequent analysis, while cell pellets were used for inflammatory cell counts. Approximately 0.8–1 mL of whole blood was drawn from the left ventricle using a 1 mL syringe, transferred into EDTA anticoagulation tubes, gently mixed, and stored at 4°C. Lungs were perfused with pre-cooled PBS via the pulmonary artery until the tissue turned white. The left lung was fixed in 4% paraformaldehyde, and the right lung was snap-frozen at -80°C.

Histologic Analysis of Lung Tissue

Lung tissues were fixed in 4% paraformaldehyde for 24 hours, then dehydrated, embedded in paraffin, and sectioned at 5 μ m for histological staining and morphological evaluation. Paraffin sections were deparaffinized, rehydrated, and stained with Hematoxylin and Eosin (HE; Baso, Guangzhou, China), Periodic Acid–Schiff (PAS; Solarbio, Beijing, China), and Masson trichrome stain (Solarbio) to assess inflammatory infiltration, goblet cell hyperplasia, mucus secretion, and collagen deposition. For immunohistochemistry, deparaffinized sections were subjected to antigen retrieval in citrate buffer (pH 6.0) at 121°C for 2 minutes. After PBS washing, sections were incubated overnight at 4°C with anti-B4GalT5 (Abmart, Shanghai, China) and anti-Ki-67 (Abclone, Wuhan, China) antibodies. After washing, HRP-conjugated anti-rabbit IgG (Thermo Fisher) was added and incubated at room temperature for 1 hour, followed by DAB chromogenic reaction (Solarbio), PBS rinse, hematoxylin counterstaining, and neutral resin mounting. Images were captured using a CX21 light microscope (Olympus, Tokyo, Japan), and quantitative scoring was performed. Inflammatory scoring (0–4) was based on HE staining: 0, no inflammation; 1, mild peribronchial infiltration; 2, moderate infiltration involving airways and vessels; 3, large clusters or layered distribution; 4, extensive infiltration with architectural changes.

PAS staining was scored from 0 to 6 based on goblet cell hyperplasia (0–3) and mucus production (0–3). Goblet cell score: 0, none; 1, 15–30% epithelial cells; 2, 30–50%; 3, >50%. Mucus production score: 0, none; 1, <1/3 airway circumference; 2, 1/3–2/3; 3, complete obstruction. Collagen deposition was assessed by measuring the percentage of blue collagen fiber area around the airway using ImageJ (v2.14.0). HE-stained images were used to quantify airway remodeling. Airways with 100–200 μ m luminal diameter, complete and nearly circular, were selected at 20 $\times$ magnification. For each mouse, three fields were randomly selected and averaged. As previously reported,¹⁴ the following parameters were measured using ImageJ: internal perimeter (Pi), wall area (Wai), smooth muscle area (WAm), and number of smooth muscle cells (N). Normalized indicators—Wai/Pi, WAm/Pi, Wai/WAm and N/Pi—were calculated to evaluate wall thickening and ASM remodeling. All experiments were performed in triplicate, and results were averaged for statistical analysis.

Cell Culture and Transfection

Airway smooth muscle cells were isolated from 6-week-old C57BL/6 mice and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin at 37°C in 5% CO₂. 293T cells were purchased from Fenghui Biotechnology Co., Ltd. and cultured in DMEM with 10% FBS. For TNF- α stimulation experiments, ASMCs were transfected with miR-491-5p mimic, miR-491-5p-NC, pcDNA3.1-B4GalT5, or pcDNA3.1-NC (Fenghui Biotechnology Co., Ltd.) using Lipofectamine 3000 (Thermo Fisher) for 24 hours, followed by treatment with 25 ng/ μ L TNF- α (Beyotime) for 24 hours. The cells were then collected for further analysis.

Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from cells or tissues using Trizol (Thermo Fisher) or the miRNA Purification Kit (Yeasten, Shanghai, China). Reverse transcription was performed using the 1st Strand cDNA Synthesis SuperMix (Yeasten) for mRNA and the miRNA 1st Strand cDNA Synthesis Kit for miRNA, according to the manufacturer's instructions, with 1 μ g of total RNA or miRNA. qRT-PCR was conducted using SYBR Green Master Mix (Yeasten), and specific primers

were used to amplify target genes and miRNAs. Each sample was analyzed in triplicate. Relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method, with GAPDH or U6 used as internal controls. Specific primer sequences are detailed in [Supplementary Table S1](#).

Western Blot

Total protein was extracted from cells and tissues using RIPA lysis buffer supplemented with 100 µg/mL PMSF and protease inhibitors. Protein concentrations were determined using the BCA Protein Assay Kit (Beyotime). Samples were mixed with 5× reducing loading buffer at a ratio of 4:1 and denatured at 100°C for 10 minutes. Equal amounts of protein (20–40 µg) were separated via SDS-PAGE and transferred onto PVDF membranes (Millipore, Bedford, MA, USA) using wet transfer. Membranes were blocked with 5% non-fat milk at room temperature for 1 hour and incubated overnight at 4°C with primary antibody against B4GalT5 (1:1000, Abmart, Shanghai, China). After washing with TBST, membranes were incubated with Goat anti-Rabbit secondary antibody (1:800, LI-COR, Lincoln, Nebraska, USA) in the dark at room temperature for 1 hour. Blots were visualized using the Li-COR ODYSSEY9120 imaging system. Band intensity was quantified using ImageJ software, and GAPDH (1:2000, Abmart) was used as the loading control.

Dual-Luciferase Reporter Assay

The wild-type (WT) and mutant (MUT) 3'-UTR fragments of B4GalT5 were synthesized and cloned into the psi-CHECK-2 vector, designated as B4GalT5-WT and B4GalT5-MUT, respectively. These constructs were co-transfected with miR-491-5p mimics or negative control (NC) into HEK293T cells using Lipofectamine 3000 (Thermo Fisher). After 48 hours of transfection, luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Gene Creat, Wuhan, Hubei, China).

ELISA

The levels of IL-6, IL-8, and IL-1β in cell culture supernatants and BALF were measured using ELISA kits (Coibo, Shanghai, China) according to the manufacturer's instructions. The detection sensitivity for IL-6, IL-8, and IL-1β was 0.1 pg/mL.

Cell Counting Kit (CCK)-8 Assay

Cell proliferation was assessed using the CCK-8 kit (Mlbio, Shanghai, China) following the manufacturer's instructions. Briefly, cells were seeded at 5×10^3 cells per well in a 96-well plate and incubated overnight. At the indicated time points (24, 48, and 72 hours), 10 µL of CCK-8 solution was added to each well and incubated at 37°C for 2 hours. Absorbance was measured at 450 nm using a microplate reader (BECKman Coulter, California, USA).

Measurement of Total Reactive Oxygen Species (ROS)

ASMCs were washed three times with PBS and incubated with 10 µM DCFH-DA (Uelandy, Suzhou, Jiangsu, China) at 37°C in the dark for 20–30 minutes. After incubation, cells were washed with PBS three times to remove excess probe. Green fluorescence (indicative of ROS levels) was observed and imaged using a fluorescence microscope (FITC channel, excitation 488 nm, emission 525 nm). For tissue detection, fresh lung tissues were sectioned into 8 µm frozen slices, washed with PBS, incubated with 10 µM DCFH-DA for 30 minutes, then washed and imaged. The mean gray value of fluorescence images was quantified using ImageJ software. Multiple fields per sample were analyzed for representative and accurate quantification.

Malondialdehyde (MDA) Assay

MDA levels in lung tissues were measured using the MDA Assay Kit (Beyotime). Lung tissues were finely minced and homogenized with physiological saline on ice. Homogenates were centrifuged at 12,000 rpm for 10 minutes at 4°C, and the supernatants were collected. According to the manufacturer's instructions, the supernatant was incubated with MDA reaction reagents at 37°C for 30 minutes. Absorbance was measured at 532 nm using a spectrophotometer or microplate reader, and MDA concentration was calculated based on a standard curve, expressed as µmol/g tissue.

Superoxide Dismutase (SOD) Activity Assay

Supernatants from 10% lung tissue homogenates (prepared as described in the MDA assay) were used to measure SOD activity using the SOD Assay Kit (Beyotime). Reactions were carried out in 96-well plates, and absorbance was measured at 450 nm. SOD activity was calculated using a standard curve and expressed as U/mg protein.

Adenosine Triphosphate (ATP) Level Detection

Lung tissues were homogenized in pre-chilled lysis buffer on ice and centrifuged at 12,000 rpm for 10 minutes at 4°C to collect the supernatant. According to the ATP Assay Kit (Beyotime), 10 µL of the supernatant was mixed with 100 µL of ATP reaction solution containing a fluorescence substrate. Fluorescence intensity was measured at 485 nm using a fluorescence microplate reader. ATP levels were expressed as nmol/mg protein.

Transmission Electron Microscopy (TEM)

ASMCs were washed 2–3 times with pre-cooled PBS and fixed with 2.5% glutaraldehyde (Solarbio) at 4°C for 6 hours. Samples were washed three times with PBS (10 minutes each), dehydrated using graded ethanol solutions (50%, 70%, 80%, 90%, 95%, 100%, 10 minutes each), followed by acetone replacement. Samples were embedded in Epon812 (Solarbio) and polymerized at 60°C for 48 hours. Ultrathin sections (~70 nm) were cut, placed on copper grids, and stained with uranyl acetate (15 minutes) and lead citrate (10 minutes) (Solarbio). Sections were air-dried and observed under a transmission electron microscope to visualize mitochondrial morphology.

Quantitative analysis of mitochondrial morphology was performed using TEM images. Damaged mitochondria were defined as those exhibiting at least two of the following features: disrupted or lost cristae, mitochondrial swelling, vacuolization, or irregular shape. For each sample, 5–10 random fields were analyzed, and at least 50 mitochondria were evaluated per group. The percentage of damaged mitochondria was calculated as the ratio of damaged mitochondria to the total mitochondria. All quantifications were conducted in a blinded manner by two independent observers.

Intracellular Calcium Ion (Ca²⁺) Detection

After treatment, cells were washed twice with pre-warmed PBS and incubated with 5 µM Fluo-4 AM (Beyotime) dissolved in HBSS containing 0.02% Pluronic F-127 at 37°C for 30 minutes to allow intracellular loading. After incubation, cells were washed three times with PBS to remove excess dye. Green fluorescence (indicating intracellular calcium levels) was detected by flow cytometry (BD Biosciences, FACSCalibur) under 494 nm excitation. Fluorescence intensity was analyzed using FlowJo (v10.8.1), and mean fluorescence intensity was used to reflect intracellular calcium levels.

Statistical Analysis

All data represent the average of at least three separate experiments. Statistical analyses were performed using GraphPad Prism (v10.3.1). Data are presented as mean ± standard error of the mean (Mean ± SEM). Comparisons between two groups were conducted using unpaired Student's *t*-test, while comparisons among multiple groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. A *P*-value < 0.05 was considered statistically significant (**P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001).

Results

Downregulation of miR-491-5p and Upregulation of B4GalT5 in Airway Smooth Muscle of Asthma Patients

To investigate the role of miRNAs in asthma, we collected segmental bronchial tissues from three asthma patients and three control participants. The ASM was isolated and subjected to miRNA-Seq. A total of 22 differentially expressed miRNAs were identified, among which miR-491-5p was the only significantly downregulated miRNA. This unique expression pattern prompted us to focus on miR-491-5p for subsequent studies (Figure 1a). To systematically explore the regulatory network of miR-491-5p, we predicted its downstream target genes using three databases: TargetScan, miRDB, and miRTarBase. After merging the results and removing duplicates, eight candidate genes were identified (Figure 1b). Among them, B4GalT5

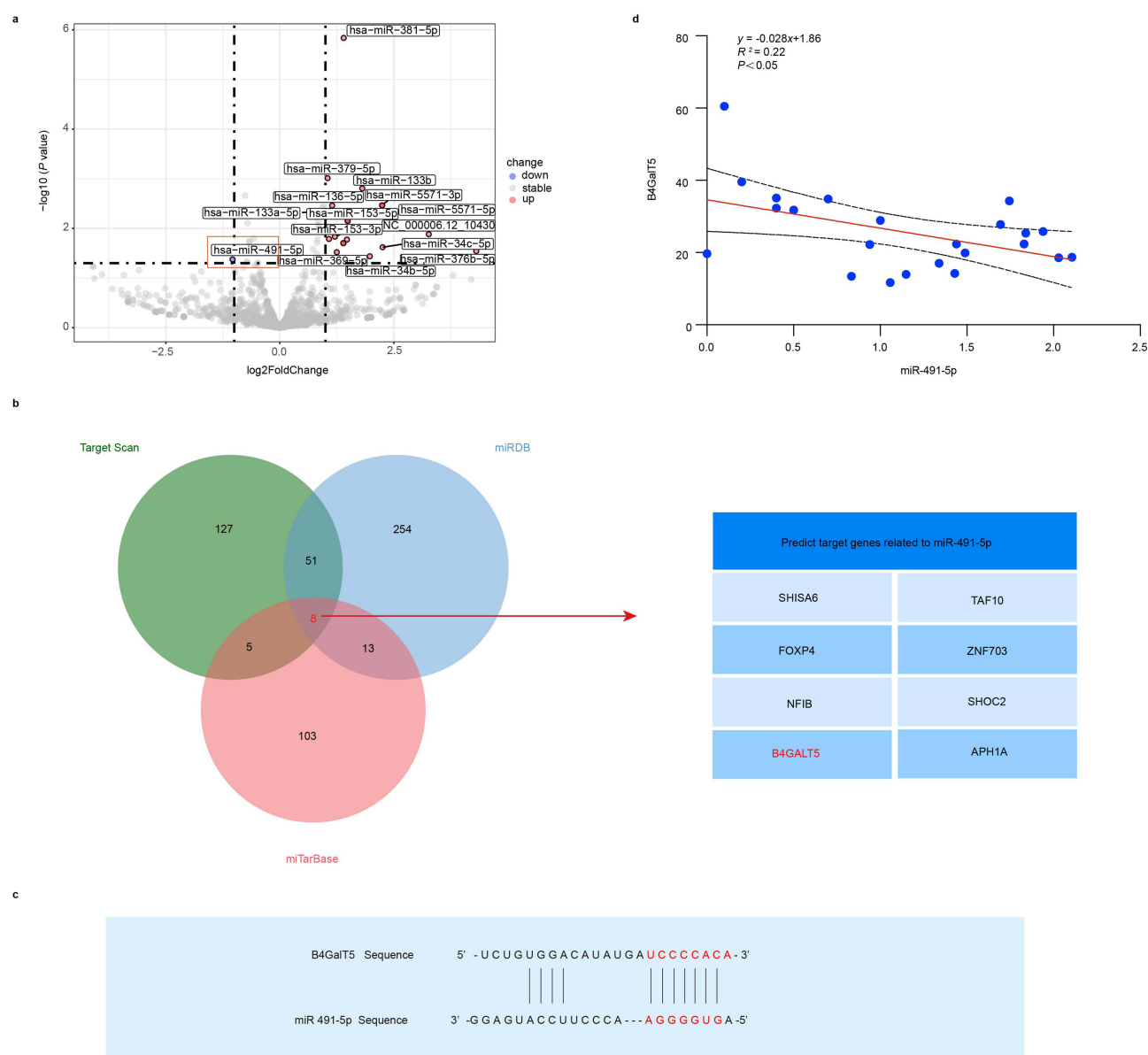


Figure 1 Screening and target prediction of miR-491-5p in asthma airway smooth muscle (a) Volcano plot showing differentially expressed miRNAs identified by miRNA-Seq of airway smooth muscle from asthma patients (n=3) versus control participants (n=3). miR-491-5p was the only miRNA significantly downregulated ($|\log_2\text{FoldChange}| > 1$, adjusted $P < 0.05$, highlighted in red frame). (b) A total of 8 predicted downstream genes were screened through the database. B4GalT5, marked in red, was selected for further study. (c) Predicted binding sequence between B4GalT5 mRNA 5'UTR and miR-491-5p. (d) Negative correlation between miR-491-5p and B4GalT5 expression in airway smooth muscle transcriptome data. Correlations were assessed using Spearman's rank correlation analysis ($R^2 = 0.22$, $P < 0.05$).

emerged as a key focus of this study due to its well-documented role in regulating oxidative stress and its scarce reporting in asthma research. Using the TargetScan Human 8.0 tool, we predicted a potential binding site between miR-491-5p and the 3'UTR of B4GalT5, revealing a sequence of seven consecutive complementary bases, indicating a potentially stable interaction (Figure 1c). To further validate this regulatory relationship, we analyzed miRNA and mRNA expression data of ASMCs from asthma patients using the GEO dataset GSE119580. The results showed a significant negative correlation between miR-491-5p and B4GalT5 expression (Figure 1d). Based on these findings, we further explored the expression patterns and functional implications of miR-491-5p and B4GalT5 in the ASM of asthma patients.

To assess the expression of miR-491-5p and B4GalT5 in ASM and their potential roles, we performed qRT-PCR analysis on ASM from asthma patients and control participants. As expected, miR-491-5p expression was significantly downregulated, while B4GalT5 mRNA levels were markedly upregulated in the asthma group compared to control participants (Figure 2a and b). To further confirm the protein expression of B4GalT5, we conducted WB analysis, which

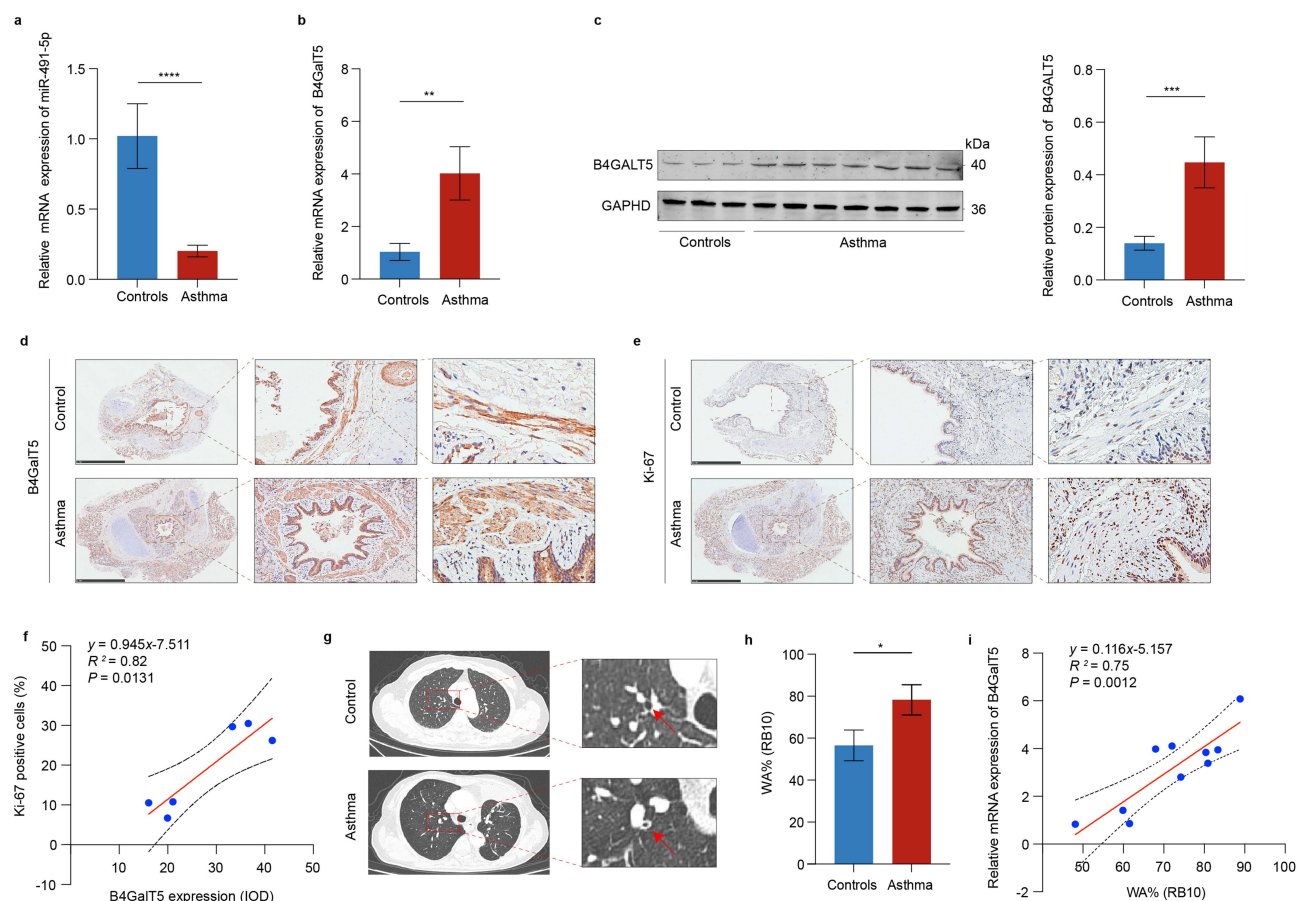


Figure 2 Altered expression of miR-491-5p and B4GalT5 associated with airway remodeling in asthma (a) The mRNA expression of miR-491-5p in airway smooth muscle were measured by qRT-PCR in both asthmatic patients (n=7) and control participants (n=3). (b) The mRNA expression of B4GalT5 in airway smooth muscle were measured by qRT-PCR in both asthmatic patients (n=7) and control participants (n=3). (c) Western blot analysis detected the protein expression of B4GalT5 in airway smooth muscle tissues from both asthmatic patients (n=7) and control participants (n=3). (d) The protein expression of B4GalT5 in airway smooth muscle were detected by IHC in both asthmatic patients (n=3) and control participants (n=3). Scale bar, 1 mm. (e) The protein expression of Ki-67 in airway smooth muscle were detected by IHC in both asthmatic patients (n=3) and control participants (n=3). Scale bar, 1 mm. (f) Correlation between B4GalT5 and Ki-67 expression levels in airway smooth muscle was assessed using Spearman analysis (Controls n=3; Asthma n=3). (g) Representative HRCT images of bronchial wall structure from control participants (n=3) and asthmatic patients (n=7). Red arrows point to the cross-section of the apex of the right lower lobe (RB10) bronchus, which was used to quantify WA%. (h) Measurement data of single-layer airway at the apex of the right lower lobe (RB10) (Controls n=3; Asthma n=7). (i) Correlation between B4GalT5 expression levels in airway smooth muscle and airway wall thickness in asthmatic patients was assessed using Spearman analysis. Data are presented as mean $\pm$ SEM and three or more independent experiments were performed. Group comparisons were performed using independent-samples t-test. Correlations were assessed using Spearman's rank correlation analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Abbreviations: qRT-PCR, quantitative reverse transcription polymerase chain reaction; IHC, immunohistochemistry; HRCT, high-resolution computed tomography.

consistently showed a significant increase in B4GalT5 protein levels in the ASM of asthma patients (Figure 2c). To investigate the potential role of B4GalT5 in ASM proliferation in asthma, we performed immunohistochemical staining to detect Ki-67 and B4GalT5 expression (Figure 2d and e). The result showed the expression level of B4GalT5 was positively correlated with the proportion of Ki-67-positive cells (Figure 2f). Since Ki-67 is a key marker of cell proliferation, its increased expression suggests that B4GalT5 may be involved in regulating ASM proliferation in asthma. Furthermore, we compared the airway wall area as a percentage of total airway area (WA%) between asthma patients and control participants, and found that WA% was significantly higher in the asthma group (Figure 2g and h). Additional analysis revealed a strong positive correlation between B4GalT5 expression and WA% (Figure 2i), supporting a link between B4GalT5 expression and structural airway remodeling, and providing further evidence for its potential role in ASM proliferation. Together, these results demonstrate that miR-491-5p is significantly downregulated, while B4GalT5 is significantly upregulated in the ASM of asthma patients. Moreover, miR-491-5p may participate in the regulation of ASM proliferation by negatively regulating B4GalT5.

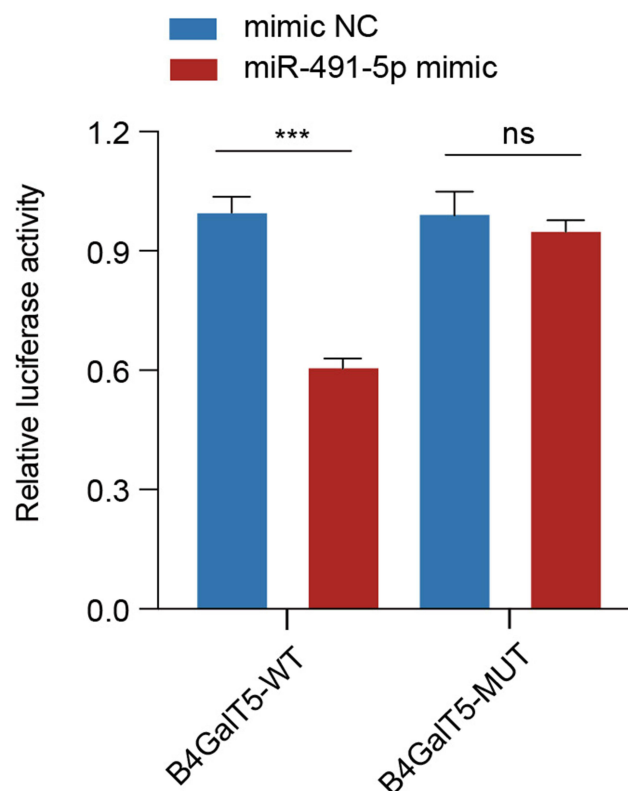


Figure 3 B4GalT5 were target of miR-491-5p Dual-luciferase reporter assay confirmed the interaction between miR-491-5p and B4GalT5. *** $P < 0.001$.
Abbreviation: ns, not significant.

B4GalT5 Is a Direct Target of miR-491-5p

To further confirm the targeting relationship between miR-491-5p and B4GalT5, as well as the predicted binding site, we performed a dual-luciferase reporter assay. The results showed that miR-491-5p significantly reduced the luciferase activity of the wild-type B4GalT5 3'UTR construct, whereas it had no effect on the mutant B4GalT5 3'UTR construct (Figure 3). These findings confirm that miR-491-5p directly binds to the 3'UTR of B4GalT5 mRNA, indicating a specific post-transcriptional regulatory relationship.

Overexpression of miR-491-5p Suppresses TNF- α -Induced Proliferation, Inflammation, and Mitochondrial Oxidative Stress in Airway Smooth Muscle Cells by Targeting B4GalT5

To investigate the role of miR-491-5p in the pathogenesis of asthma, ASMCs were treated with TNF- α to induce inflammatory responses and proliferation. Cells were divided into four groups: control, TNF- α , TNF- α + miR-491-5p mimic, and TNF- α + miR-491-5p NC. qRT-PCR and Western blot analyses revealed that miR-491-5p expression was significantly downregulated in the TNF- α group compared with the control group, while its target gene B4GalT5 was significantly upregulated at both mRNA and protein levels. Transfection with miR-491-5p mimic led to a significant increase in miR-491-5p expression, accompanied by a marked suppression of B4GalT5 expression at both the mRNA and protein levels, confirming successful transfection and target regulation (Figure 4a–c). Morphological observations and cell density quantification under a light microscope showed that, after 72 hours of TNF- α stimulation, ASMCs density was markedly increased, while miR-491-5p overexpression reduced this proliferative response (Figure 4d and e). Consistently, CCK-8 assays showed that TNF- α stimulation significantly promoted ASMCs viability, whereas miR-491-5p mimic treatment effectively attenuated TNF- α -induced proliferation (Figure 4f). ELISA analysis indicated that TNF- α

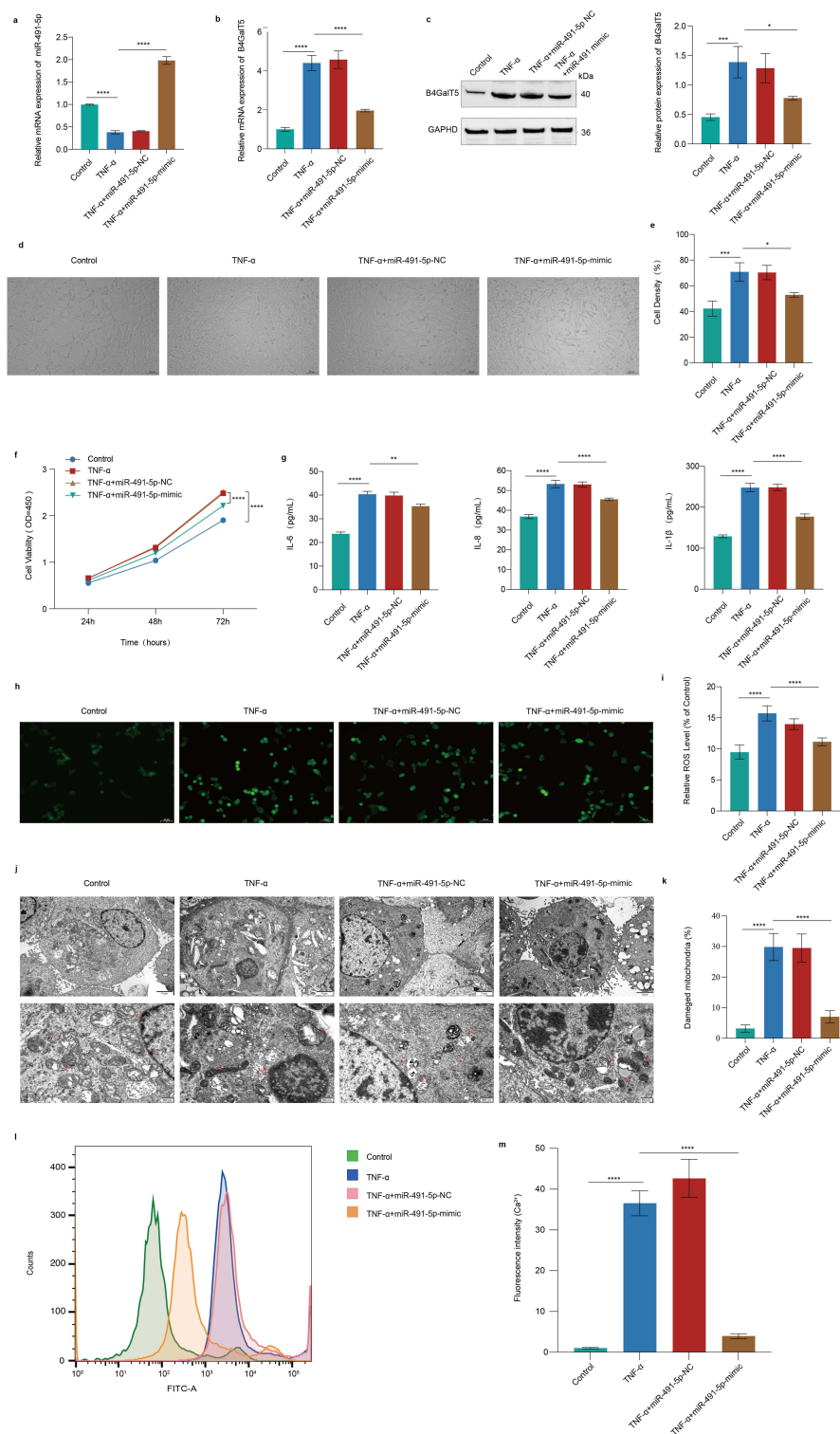


Figure 4 Effects of miR-491-5p on proliferation, inflammation, oxidative stress, and mitochondrial function in ASMCs (a) qRT-PCR verified the changes of miR-491-5p mRNA expression under TNF- α stimulation and after transfection of miR-491-5p mimic. (b) qRT-PCR verified the changes of B4GalT5 mRNA expression under TNF- α stimulation and after transfection of miR-491-5p mimic. (c) Western blot verified the changes of B4GalT5 protein expression under TNF- α stimulation and after transfection of miR-491-5p mimic. (d) The density and morphology of ASMCs. Scale bar, 100 μ m. (e) Quantitative analysis of ASMCs cell density. (f) CCK-8 detected proliferation of ASMCs at indicated time (24, 48 or 72h). (g) ELISA assay examined the IL-6, IL-8 and IL-1 β level in ASMCs. (h) DCFH-DA dye was used to detect the expression of ROS in ASMCs. Scale bar, 100 μ m. (i) ROS quantification. (j-k) Representative transmission electron microscopy images and quantitative analysis of mitochondrial morphology (arrowhead represents mitochondria). Scale bars: 2 μ m (low magnification), 500 nm (high magnification). (l) Flow cytometry was performed to measure the mean fluorescence intensity of intracellular Ca²⁺ in ASMCs. (m) Ca²⁺ fluorescence intensity. Data are presented as means $\pm$ SEM and three or more independent experiments were performed. Significance was calculated by one-way ANOVA followed by Tukey's post-hoc test. * P <0.05, ** P <0.01, *** P <0.001, **** P <0.0001.

Abbreviations: qRT-PCR, quantitative reverse transcription polymerase chain reaction; ASMCs, airway smooth muscle cells; ROS, reactive oxygen species.

stimulation markedly increased the levels of pro-inflammatory cytokines IL-6, IL-8, and IL-1 β , while miR-491-5p overexpression significantly reduced their expression (Figure 4g).

Furthermore, intracellular ROS levels were measured using the DCFH-DA fluorescent probe. DCFH-DA itself is non-fluorescent and becomes fluorescent DCF upon oxidation by ROS inside cells. Quantitative analysis of DCF fluorescence using ImageJ revealed that ROS levels were significantly elevated in the TNF- α group compared with the control group, while miR-491-5p overexpression significantly attenuated ROS accumulation (Figure 4h and i). Given that mitochondria are a major target of ROS-induced cellular damage, we further examined mitochondrial ultrastructure using transmission electron microscopy. In the control group, mitochondria displayed typical elongated or branched morphology with well-organized and intact structures. In contrast, TNF- α treatment significantly increased the proportion of damaged mitochondria and induced characteristic morphological alterations including swelling and vacuolization. Notably, overexpression of miR-491-5p markedly alleviated TNF- α -stimulated mitochondrial damage (Figure 4j and k). Considering that mitochondria are the main intracellular reservoirs of calcium and that calcium homeostasis is closely associated with mitochondrial oxidative stress, we further investigated whether miR-491-5p regulates intracellular calcium levels. Fluorescence imaging with a calcium-sensitive dye showed that TNF- α significantly elevated intracellular Ca²⁺ levels in ASMCs, whereas miR-491-5p overexpression significantly restored calcium homeostasis (Figure 4l and m).

Overexpression of B4GalT5 Reverses the Inhibitory Effects of miR-491-5p on TNF- α -Induced Airway Smooth Muscle Cells Proliferation, Inflammation and Mitochondrial Oxidative Stress

To further elucidate whether the functional regulation of ASMCs by miR-491-5p is mediated through B4GalT5, we first transfected ASMCs with plasmids overexpressing B4GalT5 or control vector. The overexpression efficiency of B4GalT5 was verified by both qRT-PCR and Western blot, which showed significantly increased B4GalT5 expression at the mRNA and protein levels in the pcDNA3.1-B4GalT5 group compared with the vector control (Figure 5a and b). These results confirm the effective transfection and functional overexpression of B4GalT5 in ASMCs. Subsequently, cells were divided into two groups for rescue experiments: TNF- α + miR-491-5p mimic + pcDNA3.1-vector group and TNF- α + miR-491-5p mimic + pcDNA3.1-B4GalT5 group. After 72 hours of treatment, cell morphology was examined under a light microscope, and cell density was quantified. The results showed that overexpression of B4GalT5 significantly reversed the inhibitory effect of miR-491-5p on TNF- α -induced ASMCs proliferation (Figure 5c and d). CCK-8 assays further confirmed that B4GalT5 overexpression significantly reversed the inhibitory effect of miR-491-5p on cell proliferation (Figure 5e). ELISA results indicated that the levels of inflammatory cytokines IL-6, IL-8, and IL-1 β were significantly elevated in the TNF- α + miR-491-5p mimic + pcDNA3.1-B4GalT5 group compared to the TNF- α + miR-491-5p mimic + pcDNA3.1-vector group, suggesting that B4GalT5 can counteract the inhibitory effects of miR-491-5p on cytokine production in TNF- α -stimulated ASMCs (Figure 5f).

To further explore the impact of B4GalT5 on mitochondrial oxidative stress, we measured intracellular ROS levels and observed mitochondrial ultrastructural changes. DCFH-DA fluorescence probe assays showed that ROS levels were significantly elevated in the TNF- α + miR-491-5p mimic + pcDNA3.1-B4GalT5 group compared to the TNF- α + miR-491-5p mimic + pcDNA3.1-vector group (Figure 5g and h), indicating that miR-491-5p inhibits TNF- α -induced oxidative stress, at least in part, by targeting B4GalT5. TEM further revealed that in the TNF- α + miR-491-5p mimic + pcDNA3.1-B4GalT5 group, mitochondria exhibited significant pathological changes, including matrix swelling and increased cristae spacing. Quantitative analysis showed a markedly increased proportion of damaged mitochondria in this group, indicating that excessive ROS accumulation induced by B4GalT5 overexpression leads to mitochondrial oxidative stress damage, thereby reversing the protective effects of miR-491-5p on mitochondrial morphology and function (Figure 5i and j). Furthermore, intracellular calcium levels were assessed using a calcium-sensitive fluorescent probe. The results showed a significant increase in Ca²⁺ fluorescence intensity in the TNF- α + miR-491-5p mimic + pcDNA3.1-B4GalT5 group compared to the TNF- α + miR-491-5p mimic + pcDNA3.1-vector group (Figure 5k and l), indicating that overexpression of B4GalT5 disrupts intracellular Ca²⁺ homeostasis. These findings suggest that B4GalT5 may exacerbate mitochondrial dysfunction by disturbing calcium homeostasis.

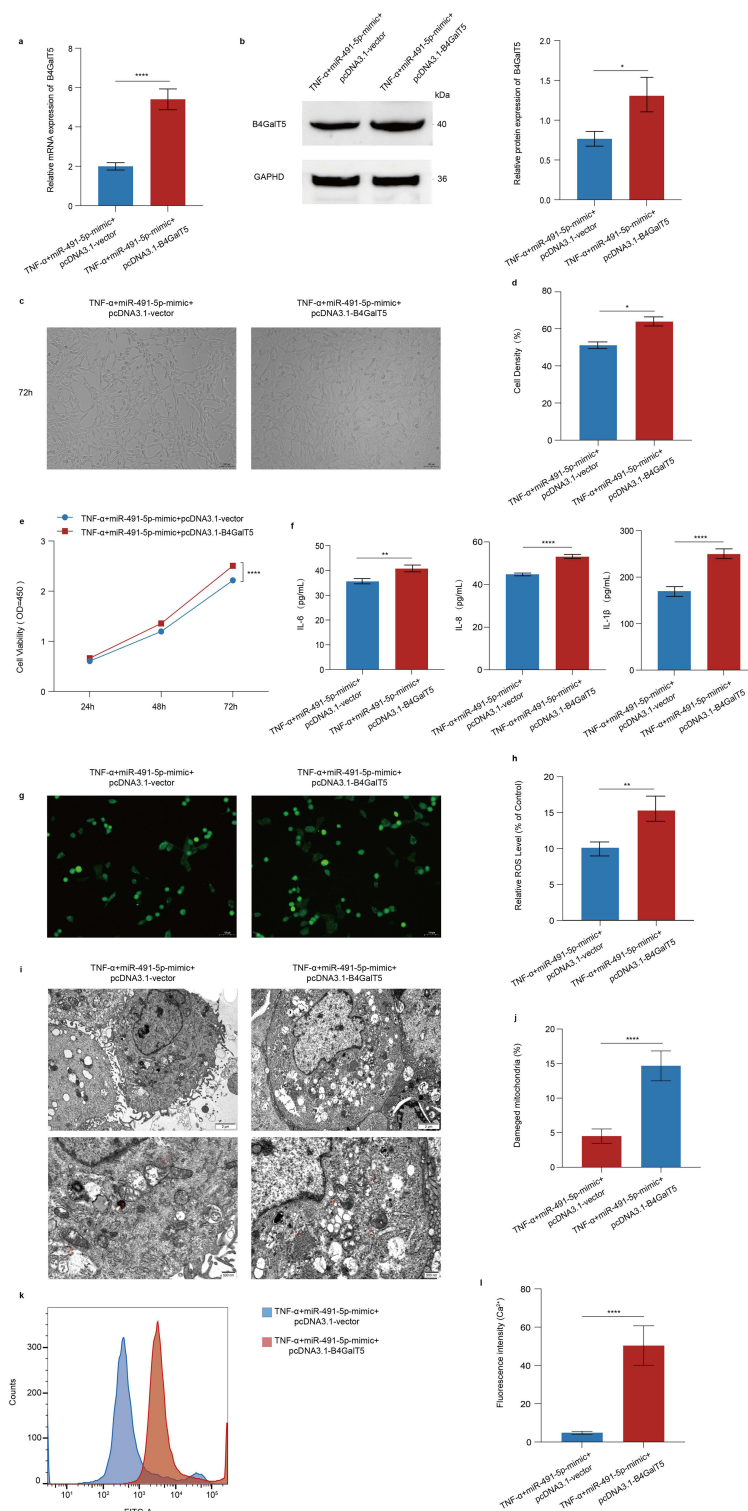


Figure 5 B4GalT5 overexpression reverses the protective effects of miR-491-5p on proliferation, inflammation, and oxidative stress in ASMCs (a) qRT-PCR verified the changes of B4GalT5 mRNA expression in ASMCs following co-transfection with miR-491-5p mimic and pcDNA3.1-B4GalT5 under TNF- α stimulation. (b) Western blot validated the rescue of B4GalT5 protein expression in ASMCs transfected with miR-491-5p mimic following TNF- α stimulation. (c) The density and morphology of ASMCs. Scale bar, 100 μ m. (d) Quantitative analysis of ASMCs cell density. (e) CCK-8 detected proliferation of ASMCs at indicated time (24, 48 or 72h). (f) ELISA assay examined the IL-6, IL-8 and IL-1 β level in ASMCs. (g) DCFH-DA dye was used to detect the expression of ROS in ASMCs. Scale bar, 100 μ m. (h) ROS quantification. (i-j) Representative transmission electron microscopy images and quantitative analysis of mitochondrial morphology (arrowhead represents mitochondria). Scale bars: 2 μ m (low magnification), 500 nm (high magnification). (k) Flow cytometry was performed to measure the mean fluorescence intensity of intracellular Ca^{2+} in ASMCs. (l) Ca^{2+} fluorescence intensity. Data are presented as means $\pm$ SEM and three or more independent experiments were performed. Group comparisons were performed using independent-samples *t*-test. **p*<0.05, ***p*<0.01, ****p*<0.0001.

Abbreviations: qRT-PCR, quantitative reverse transcription polymerase chain reaction; ASMCs, airway smooth muscle cells; ROS, reactive oxygen species.

Overexpression of miR-491-5p Alleviates Airway Remodeling, Pulmonary Inflammation, and Mitochondrial Oxidative Stress in Asthmatic Mice

To investigate the regulatory role of miR-491-5p in airway remodeling, pulmonary inflammation, and mitochondrial oxidative stress *in vivo*, we established an ovalbumin (OVA)-induced asthma mouse model. 24 nude mice were randomly distributed across four groups: control, asthma, asthma + AAV-miR-491-5p, and asthma + AAV-NC-miR-491-5p. A typical allergic asthma mouse model was induced by OVA stimulation,¹⁵ and miR-491-5p-AAV was used for intervention treatment (Figure 6a). qRT-PCR results showed that, compared to the control group, miR-491-5p expression was significantly reduced in the airway smooth muscle tissue of the OVA group, while B4GalT5 expression was significantly increased. Importantly, in the asthma + AAV-miR-491-5p group, miR-491-5p expression was significantly upregulated compared to the asthma group, indicating successful *in vivo* delivery of miR-491-5p via AAV. Correspondingly, B4GalT5 expression was significantly downregulated in this group (Figure 6b and c). Western blot further confirmed that, compared to the control group, B4GalT5 protein levels were significantly elevated in the airway smooth muscle of the OVA group, and miR-491-5p-AAV intervention significantly reversed this phenomenon (Figure 6d). These results indicate that miR-491-5p can similarly target and inhibit B4GalT5 expression *in vivo*. We then measured the inner perimeter (Pi), internal area (Wai), smooth muscle area (WAm), and the number of bronchial smooth muscle cells (N). The results showed that, compared to the control group, the asthma group exhibited increased N/Pi, Wai/Pi, and WAm/Pi levels, while the Wai/WAm ratio decreased. However, miR-491-5p-AAV intervention significantly reversed these changes in the asthma mice (Figure 6e–h). These results indicate that miR-491-5p significantly improves airway remodeling in asthma mice.

Next, we examined the pathological changes in lung tissue using HE, Masson, and PAS staining. HE staining revealed increased inflammatory infiltration around the bronchi, airway wall thickening, and epithelial damage in the asthma group, while miR-491-5p-AAV intervention significantly alleviated these pathological changes. PAS staining showed a significant increase in mucin secretion and obvious goblet cell hyperplasia in the airway epithelium of the OVA group, while miR-491-5p-AAV intervention significantly suppressed excessive mucin secretion and goblet cell hyperplasia. Masson staining results confirmed increased collagen deposition and significant airway fibrosis around the airway epithelium in the asthma group, while in miR-491-5p asthma mice, collagen deposition and airway fibrosis were alleviated (Figure 6i). Furthermore, HE inflammation scores, PAS scores, and Masson collagen deposition area also confirmed that miR-491-5p significantly improved airway inflammation, excessive mucin secretion, and fibrotic pathological changes in the asthma mice (Figure 6j–l).

ELISA results showed that compared with the control group, levels of IL-6, IL-8, and IL-1 β in the BALF were significantly elevated in the OVA group, while miR-491-5p-AAV intervention markedly reduced these inflammatory cytokines (Figure 7a). In addition, miR-491-5p-AAV effectively decreased the elevated levels of OVA-specific IgE in the serum of OVA-challenged mice, suggesting that miR-491-5p can significantly alleviate OVA-induced pulmonary inflammation (Figure 7b).

Finally, we measured the levels of oxidative stress and lipid peroxidation markers in the lung. The results showed that, compared to the control group, ROS fluorescence intensity and MDA levels were significantly increased in the lung tissue of the OVA group, whereas miR-491-5p-AAV intervention significantly reduced ROS fluorescence intensity and MDA levels, and quantitative analysis further confirmed this (Figure 7c and d). In addition, SOD activity in the lung tissue of the OVA group was significantly lower than that of the control group, and miR-491-5p-AAV treatment partially reversed this trend (Figure 7e). To assess the impact of miR-491-5p on mitochondrial energy metabolism, we measured ATP levels in lung tissue. Interestingly, compared to the control group, ATP levels were significantly reduced in the OVA group, and treatment with miR-491-5p-AAV partially restored ATP levels (Figure 7f).

Discussion

MicroRNAs function as upstream regulators in gene networks, can target multiple genes and pathways to form regulatory networks involved in the pathogenesis of various diseases, including asthma.¹⁶ This study reveals the key regulatory role of miR-491-5p in the pathogenesis of asthma and its underlying molecular mechanisms. Our findings show that miR-491-5p is

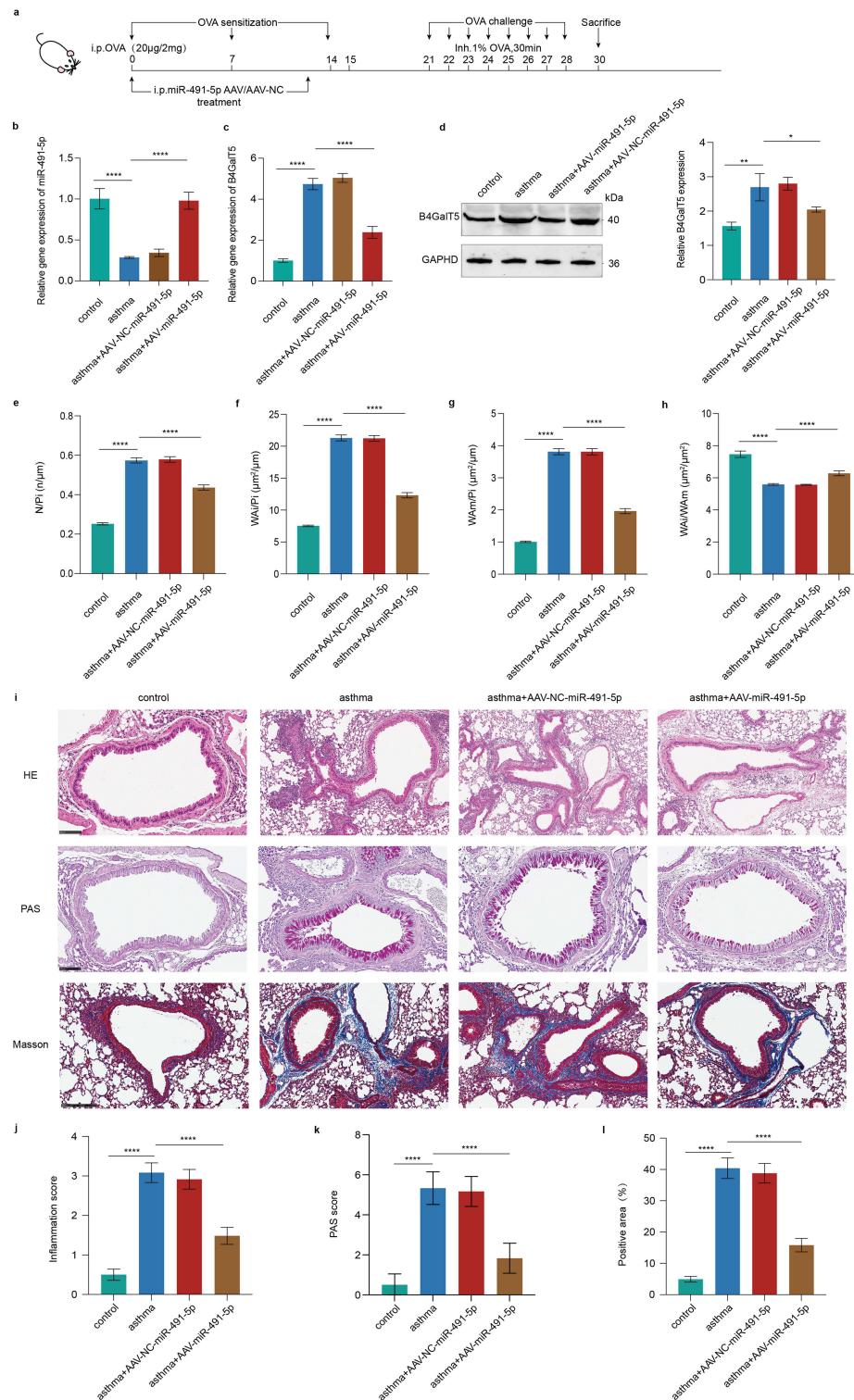


Figure 6 miR-491-5p attenuates airway inflammation and remodeling in OVA-induced asthma mouse model **(a)** Schematic diagram of OVA-induced asthma model establishment and AAV-miR-491-5p treatment. **(b)** qRT-PCR verified the changes of miR-491-5p mRNA expression in asthma model mice and with or without AAV-miR-491-5p treatment. **(c)** qRT-PCR verified the changes of B4GalT5 mRNA expression in asthma model mice and with or without AAV-miR-491-5p treatment. **(d)** Western blot validated the B4GalT5 protein expression in asthma model mice and with or without AAV-miR-491-5p treatment. **(e–h)** N/Pi, WAI/Pi, WAm/Pi and WAI/WAm level was calculated. **(i)** Lung tissue sections were stained by HE, PAS and Masson. Scale bar, 100 μm. **(j–l)** HE staining inflammation score, PAS glycogen staining score and Masson collagen fibrillar staining score. Data are presented as means ± SEM and three or more independent experiments were performed. Significance was calculated by one-way ANOVA followed by Tukey's post-hoc test. **P*<0.05, ***P*<0.01, ****P*<0.0001. *n* = 6 mice/group.

Abbreviations: qRT-PCR, quantitative reverse transcription polymerase chain reaction; HE, hematoxylin and eosin; PAS, periodic acid–Schiff; Pi, inner perimeter of the bronchial wall; WAI, inner area of the bronchial wall; WAm, airway smooth muscle area; N, the number of bronchial smooth muscle cells.

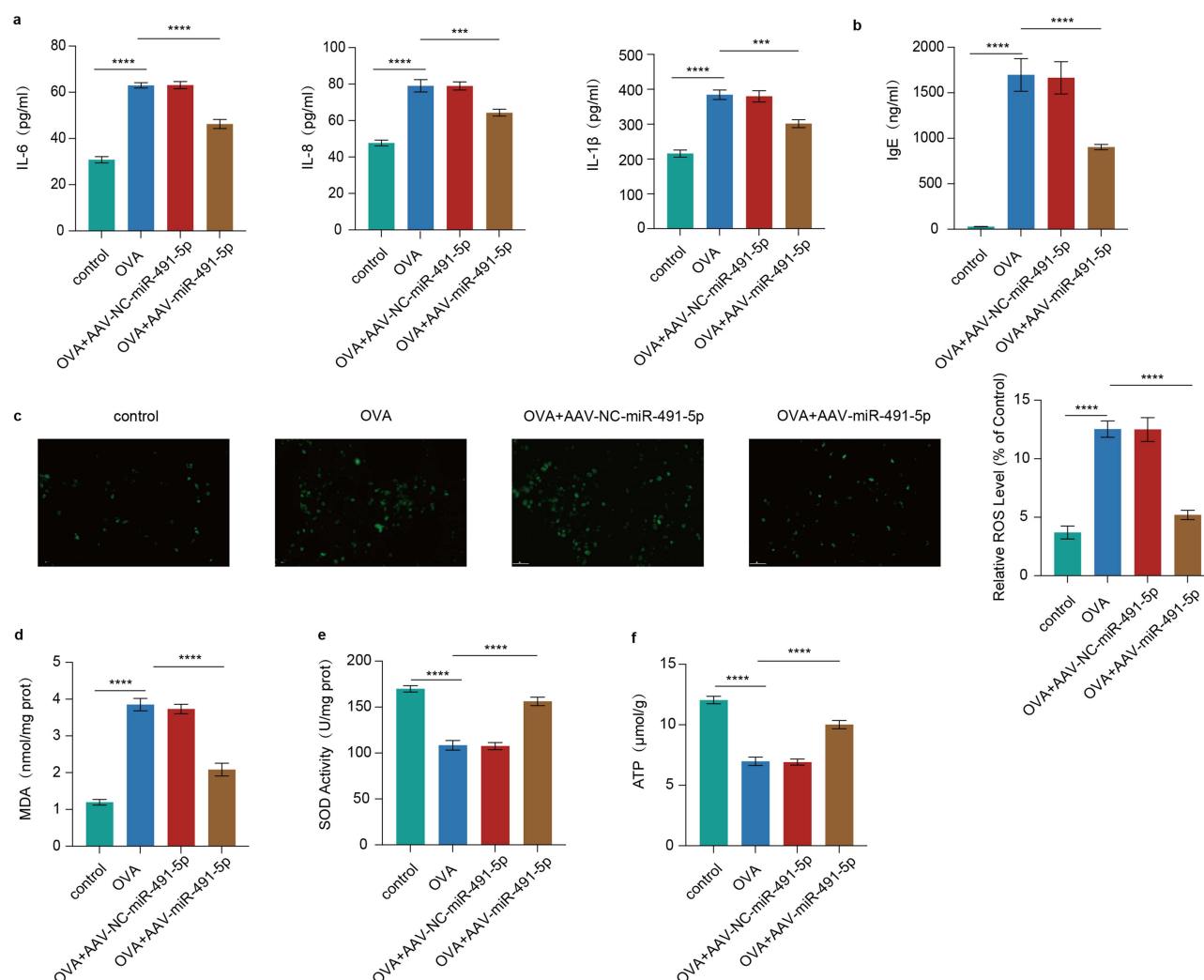


Figure 7 miR-491-5p attenuates pulmonary inflammation and oxidative stress in OVA-induced asthma mouse model (a) ELISA assay examined the IL-6, IL-8 and IL-1 β level in BALF. (b) ELISA assay examined the IgE antibody level in serum. (c) Detection of ROS production in lung tissue by DCFH-DA fluorescence assay. Scale bar, 50 μ m. (d–f) Determination of MDA, SOD and ATP in the lung tissue. Data are presented as means $\pm$ SEM and three or more independent experiments were performed. Significance was calculated by one-way ANOVA followed by Tukey's post-hoc test. *** P <0.001, **** P <0.0001. n = 6 mice/group.

Abbreviations: BALF, bronchoalveolar lavage fluid; IgE, immunoglobulin E; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; ATP, adenosine triphosphate.

significantly downregulated in the ASM of asthma patients, and its expression level is significantly negatively correlated with B4GalT5. Importantly, through a systematic *in vivo* and *in vitro* validation, we confirmed that miR-491-5p directly targets and negatively regulates B4GalT5 expression, and further elucidated the “miR-491-5p/B4GalT5” regulatory axis, which exacerbates asthma airway inflammation and remodeling through mediating oxidative stress. This core finding is further supported by clinical data: the expression of B4GalT5 is not only significantly positively correlated with the cell proliferation marker Ki67 but also with the WA%, which provides clinical evidence for the crucial role of this regulatory pathway in ASM proliferation and remodeling. This discovery offers new theoretical insights into the pathogenesis of asthma and lays an experimental foundation for the development of asthma therapeutic strategies targeting miR-491-5p.

The pathological progression of asthma is primarily driven by chronic airway inflammation and structural remodeling, with these two processes mutually reinforcing each other in a vicious cycle. In terms of inflammation, continuous release of pro-inflammatory and chemotactic factors leads to epithelial damage, increased mucus secretion, and immune cell infiltration, further inducing airway hyperresponsiveness.¹⁷ Recurrent inflammatory stimuli trigger airway remodeling, including abnormal proliferation of ASMCs, thickening of the basement membrane, and deposition of extracellular matrix, ultimately leading to irreversible airway narrowing and progressive decline in lung function.¹⁸ Notably, mitochondrial oxidative stress serves as

a key link between airway inflammation and remodeling, exacerbating this pathological process through multiple mechanisms. Specifically, mitochondrial dysfunction leads to excessive ROS production, which, on one hand, activates the NF- κ B and NLRP3 inflammasome pathways to promote the cascade release of pro-inflammatory cytokines such as IL-6, IL-1 β , and TNF- α , amplifying the inflammatory response;^{5,19} on the other hand, ROS interferes with Ca²⁺ homeostasis, inducing Ca²⁺ overload in ASMCs, which not only enhances their contractility but also significantly promotes their proliferation and phenotypic transformation.²⁰ This vicious cycle of mitochondrial dysfunction-oxidative stress-chronic inflammation-tissue remodeling forms the core pathological basis of the persistent progression of asthma, and also provides an important pathophysiological background for the miR-491-5p/B4GalT5 regulatory axis discovered in this study.

In this study, we identified through transcriptome sequencing that miR-491-5p is the only significantly downregulated miRNA in the airway smooth muscle tissue of asthma patients. While previous studies have reported that miR-491-5p regulates cell proliferation and differentiation in cancers such as gastric cancer and breast cancer,^{6,21} its role in respiratory diseases has not been explored. Notably, miR-491-5p downregulation in oxygen-glucose deprivation-treated brain microvascular endothelial cells was found to mitigate oxidative stress damage, reduce ROS levels, improve cell viability, and promote angiogenesis.²² This finding parallels our results in ASMCs. Our *in vitro* experiments confirmed that upregulation of miR-491-5p significantly alleviates mitochondrial damage in ASMCs, reduces ROS generation, inhibits abnormal Ca²⁺ release, and thus relieves ASMC proliferation and inflammation. To further investigate this, we used AAV-miR-491-5p for intranasal delivery to mice, and found that AAV-miR-491-5p reversed the decrease in miR-491-5p expression in the asthma model mice, significantly improving oxidative stress in the lung tissue and effectively alleviating airway inflammation and remodeling. These findings provide direct evidence for miR-491-5p as a therapeutic target for airway remodeling in asthma.

To elucidate the intrinsic mechanism by which miR-491-5p regulates airway inflammation and remodeling in asthma, we employed bioinformatics analysis and experimental validation to identify B4GalT5 as a key target gene in miR-491-5p-mediated regulation of oxidative stress. B4GalT5, a β -1,4-galactosyltransferase, plays a pivotal role in modifying mitochondrial surface channel proteins through abnormal glycosylation, thereby triggering oxidative stress, promoting ROS production, and exacerbating mitochondrial dysfunction. Studies have shown that B4GalT5 mediates mitochondrial oxidative stress to promote myocardial hypertrophy in cardiomyocytes.¹² We provide evidence supporting that in ASMCs, overexpression of B4GalT5 can reverse miR-491-5p-mediated oxidative damage, alleviating ASMC proliferation and inflammation, and supporting the therapeutic potential of miR-491-5p agonists in treating asthma airway remodeling.

The significance of this study lies in the fact that, despite the involvement of numerous miRNAs in the pathological process of asthma, miR-491-5p exerts a unique and critical regulatory role in asthma airway smooth muscle. By integrating clinical sample analysis, molecular mechanism exploration, and animal experiments, this study not only establishes the potential of miR-491-5p as a reliable asthma biomarker but also reveals that miR-491-5p directly targets and inhibits B4GalT5 transcription, significantly reducing mitochondrial oxidative stress damage, and ultimately providing multiple protective effects on ASMCs' abnormal proliferation, inflammation, and phenotypic transformation. These findings suggest potential clinical applications, as miR-491-5p expression levels in airway tissues may serve as a valuable diagnostic biomarker for monitoring asthma progression and preventing airway remodeling. Furthermore, the targeted delivery of miR-491-5p mimics or B4GalT5 inhibitors to airway smooth muscle cells through nanoparticle systems could offer a novel precision medicine strategy for managing refractory asthma. However, these translational prospects should be interpreted with consideration of the study's limitations. First, the number of clinical samples used for miRNA sequencing was small ($n=3$), which may limit the generalizability of the results and warrants validation in larger cohorts. Second, while we identified B4GalT5 as a direct target of miR-491-5p, we did not perform direct inhibition of B4GalT5 to verify whether its knockdown would phenocopy the effects of miR-491-5p overexpression. Third, we did not explore in depth the role of mitochondrial oxidative stress in the miR-491-5p/B4GalT5-mediated regulatory axis. Further studies are required to address these important questions and to fully elucidate the underlying mechanisms.

Conclusions

Our study confirms that miR-491-5p regulates B4GalT5 by binding to its 3'-UTR, which alters oxidative stress levels in ASMCs, thereby leading to airway inflammation and remodeling. This study sheds light on the miR-491-5p/B4GalT5

axis as a key target in regulating airway remodeling in asthma, providing a theoretical foundation for understanding the pathogenesis of asthma and future development of innovative therapies.

Abbreviations

B4GalT5, β -1,4-galactosyltransferase; ASM, Airway smooth muscle; WA%, Percentage of airway wall area to total tracheal area; OVA, Ovalbumin; qRT-PCR, Quantitative real-time PCR; ROS, Reactive oxygen species; MDA, Malondialdehyde; SOD, Superoxide dismutase; ATP, Adenosine triphosphate; ASMCs, Airway smooth muscle cells; GINA, Global initiative for asthma; COPD, Chronic obstructive pulmonary disease; PBS, Phosphate-buffered saline; HRCT, High-resolution computed tomography; RIN, RNA integrity number; SPF, Specific pathogen-free; BALF, Bronchoalveolar lavage fluid; Pi, Internal perimeter; WAI, Wall area; WAm, Smooth muscle area; N, Number of smooth muscle cells; DMEM, Dulbecco's modified eagle medium; FBS, Fetal bovine serum; WT, Wild-type; MUT, Mutant; NC, Negative control; CCK-8, Cell counting kit 8; TEM, Transmission electron microscopy.

Data Sharing Statement

The sequencing data generated in this study are currently under controlled access and will be made publicly available upon publication. The publicly available dataset used for correlation analysis was obtained from the Gene Expression Omnibus (GEO) database under accession number GSE119580.

Ethic Approval

The collection and use of human specimens in this study were approved by the Medical Ethics Committee of the People's Hospital of Zhengzhou University (Approval No. 202407701). All procedures strictly adhered to the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants. The privacy and confidentiality of all subjects were strictly protected throughout the study. All protocols related to animal experimental was approved by the Medical Ethics Committee of Zhengzhou University (Approval No. ZZU-LAC20240628[02]) and complied with the Helsinki Declaration and relevant guidelines for animal welfare.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests.

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