Brg1 Promotes Airway Mucus Hypersecretion in Asthma via STAT6 (Post-print)

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Abstract

Objective: To investigate the effect of the core catalytic subunit of the chromatin remodeling complex (Brg1) on airway mucus hypersecretion in asthmatic mice and its underlying mechanism. Methods: Female wild-type C57BL/6 mice and Brg1-/- mice (C57BL/6 mice with specific conditional knockdown of Brg1 in type II alveolar epithelial cells AEC2s) aged 6-8 weeks were randomly divided into four groups: normal control group, asthma group, Brg1 knockdown control group (Brg1-/-), and Brg1 knockdown asthma model group (Brg1-/-+asthma), with 10 mice per group. The asthma group and Brg1-/-+asthma group were challenged with chicken ovalbumin (OVA) to establish an allergic asthma model, while the control groups received normal saline. Specimens were collected, and ELISA was performed to detect the expression of mucin MUC5AC and IL-13 in mouse bronchoalveolar lavage fluid (BALF). Periodic acid-Schiff staining was used to assess airway goblet cell hyperplasia and mucus secretion, while q-PCR and immunohistochemistry were employed to detect and quantify airway mucin MUC5AC expression. Western blot analysis was conducted to determine the expression of STAT6 and p-STAT6 in lung tissues of each group. Results: Compared with the asthma group, the Brg1-/-+asthma group exhibited significantly reduced airway goblet cell hyperplasia and mucus secretion, markedly decreased expression of IL-13 and MUC5AC in BALF, substantially lower MUC5AC mRNA expression in lung tissue, and concurrently significant downregulation of STAT6 and phosphorylated STAT6 in lung tissue. Conclusion: Brg1 knockdown mice demonstrated attenuated airway mucus secretion compared with wild-type mice when the asthma model was established, possibly by modulating STAT6 to inhibit mucin MUC5AC expression and suppress airway mucus hypersecretion in bronchial asthma, suggesting that Brg1 promotes airway mucus hypersecretion in asthma.

Full Text

Preamble

Brahma-related gene 1 promotes airway mucus hypersecretion via STAT6 in asthmatic mice

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Abstract

Objective To investigate the effect of Brahma-related gene 1 (Brg1) on airway mucus hypersecretion in asthmatic mice and explore the underlying mechanism.

Methods Female C57BL/6 mice aged 6–8 weeks were randomized into four groups (n=10 per group): normal control, asthma, Brg1 knockdown control (Brg1 /), and Brg1 knockdown with asthma model (Brg1 / +asthma). Brg1 / mice with specific conditional knockdown of Brg1 in type II alveolar epithelial cells (AEC2s) were generated by crossing Brg1fl/fl transgenic mice with SP-C-rtTA/(tetO) -Cre mice, followed by doxycycline induction. The asthma and Brg1 / +asthma groups were sensitized with ovalbumin (OVA) to establish allergic asthma models, while control groups received normal saline. Specimens were collected for analysis. ELISA was used to detect mucin MUC5AC and IL-13 expression in bronchoalveolar lavage fluid (BALF). Goblet cell hyperplasia and mucus secretion were assessed by periodic acid-Schiff (PAS) staining. MUC5AC expression was measured by q-PCR and immunohistochemistry. Western blotting was performed to evaluate STAT6 and phosphorylated STAT6 (p-STAT6) expression in lung tissues.

Results Compared with wild-type asthmatic mice, Brg1 / +asthma mice exhibited significantly reduced goblet cell hyperplasia and mucus secretion, decreased IL-13 and MUC5AC levels in BALF, lower MUC5AC mRNA expression in lung tissue, and markedly downregulated STAT6 and p-STAT6 protein expression (all $P<0.05$).

Conclusion Knockdown of Brg1 in type II alveolar epithelial cells alleviates OVA-induced airway mucus hypersecretion in C57BL/6 mice, possibly by inhibiting STAT6 activation and subsequent MUC5AC expression. These findings

suggest that Brg1 promotes airway mucus hypersecretion in asthma and may represent a novel therapeutic target.

Keywords: asthma; Brahma-related gene 1; MUC5AC; STAT6; airway mucus hypersecretion

Introduction

Bronchial asthma is one of the most common chronic airway inflammatory diseases in childhood, with increasing morbidity and mortality imposing substantial psychological and economic burdens on affected families. Airway mucus hypersecretion represents a key pathological feature of asthma, where excessive mucus production obstructs airways, impairs lung function, increases susceptibility to pulmonary infections, and contributes to disease exacerbation and mortality.

Emerging evidence implicates epigenetic mechanisms in asthma pathogenesis. Brahma-related gene 1 (Brg1), the core catalytic ATPase subunit of the SWI/SNF chromatin remodeling complex, utilizes ATP hydrolysis to reorganize chromatin structure and regulate gene expression. Previous studies have demonstrated that Brg1 plays crucial roles in epithelial cell proliferation and differentiation. Our prior work revealed elevated Brg1 mRNA expression in both asthmatic children and mouse models, and we verified that Brg1 disrupts airway epithelial integrity by inhibiting E-cadherin expression. Additionally, we observed that Brg1 knockdown mice exhibited significantly reduced airway hyperresponsiveness and inflammation when challenged with asthma induction, highlighting Brg1' s important role in asthma pathophysiology.

Since airway mucus hypersecretion has become recognized as an independent risk factor affecting asthma severity and prognosis, and the specific influence of Brg1 on mucus hypersecretion remains unreported, the present study builds upon our previous findings to further investigate Brg1' s role and potential mechanisms in asthmatic airway mucus hypersecretion, thereby providing novel therapeutic directions.

Methods

1.1 Experimental Animals

Specific pathogen-free (SPF) female C57BL/6 mice aged 6–8 weeks were obtained from the Animal Experimental Center of Chongqing Medical University. Brg1 / mice were generated on a C57BL/6 background with conditional knockdown of Brg1 specifically in type II alveolar epithelial cells (AEC2s). For gene knockout and genotyping, Brg1fl/fl transgenic mice were crossed with SP-C-rtTA/(tetO) -Cre mice. At approximately 7 days after birth, tail DNA was extracted for PCR genotyping. Homozygous mice were administered 2% doxycycline for 7 days to induce (tetO) -Cre recombinase activity, enabling targeted

deletion of Brg1 in AEC2s. Brg1 expression levels were confirmed by RT-PCR and Western blotting.

1.2 Reagents

Ovalbumin (OVA) and aluminum hydroxide gel were purchased from Sigma-Aldrich. ELISA kits for IL-13 and MUC5AC were obtained from NeoBioscience and Elabscience, respectively. The periodic acid-Schiff (PAS) staining kit was from Leagene Biotechnology. Rabbit two-step immunohistochemistry detection kit was from ZSGB-BIO. Rabbit anti-mouse STAT6 antibody was from Proteintech, and rabbit anti-mouse phosphorylated STAT6 antibody was from Abcam. BCA protein quantification kit was from Bioteke Corporation. PCR primers were synthesized by BGI Genomics. DNA Marker and SYBR Premix Ex Taq™ were from TaKaRa. High-purity total RNA rapid extraction kit was from Chongqing Karp Biotechnology.

1.3 OVA-Induced Asthma Model Construction

SPF-grade female mice aged 6–8 weeks were randomly divided into four groups: normal control, asthma, Brg1 / (knockdown control), and Brg1 / +asthma (knockdown with asthma model). The asthma and Brg1 / +asthma groups received intraperitoneal injections of 200 L sensitization solution containing 20 g OVA and 100 g aluminum hydroxide on days 0 and 14. From day 21, mice were challenged with 1.5% OVA solution (5 mL) via nebulization for 30 minutes daily for 7 consecutive days. Control and Brg1 / groups received equivalent volumes of normal saline instead of OVA for both sensitization and challenge, with all other procedures identical.

1.4 BALF Collection and ELISA Detection

Within 24 hours after the final nebulization, mice were anesthetized with 10% chloral hydrate. Following blood collection from the eyeball, mice were fixed on a foam board. A catheter was inserted and secured into the trachea. After exposing the thoracic cavity, the left pulmonary hilum was clamped with hemostats. The lungs were lavaged three times with ice-cold sterile PBS (0.5 mL each), yielding approximately 1.5 mL of recovered fluid. The lavage fluid was centrifuged at 2500 rpm for 5 minutes at 4°C, and the supernatant was collected, aliquoted, and stored at -80°C. IL-13 and MUC5AC levels were measured according to the ELISA kit instructions.

1.5 PAS Staining

The left lung was immediately fixed in 4% neutral formaldehyde solution for 48 hours, followed by dehydration and paraffin embedding. Paraffin sections (4 m thickness) were prepared and stained using the PAS staining kit according to the manufacturer's protocol. PAS-positive substances (glycogen or polysaccharides) appeared red or purple-red, nuclei stained blue, and cytoplasm showed varying

shades of red. Images were captured from bronchioles of consistent diameter for quantitative analysis of airway mucus secretion.

1.6 Immunohistochemical Staining for MUC5AC in Lung Tissue

Paraffin-embedded blocks were sectioned at 4 m thickness. After dewaxing and hydration, sections were incubated overnight at 4°C with rabbit anti-mouse MUC5AC polyclonal antibody as the primary antibody. Goat anti-rabbit secondary antibody from the immunohistochemistry kit was applied, followed by DAB staining and hematoxylin counterstaining. For each mouse, five bronchioles with diameters of 500–1000 m were randomly selected. Image Pro-Plus 6.0 software was used to calculate the integrated optical density (IA) of positive signals, with MUC5AC-positive IA values representing protein expression levels.

1.7 Western Blot Detection of STAT6 and p-STAT6 Protein Expression

Lung tissues from each group were lysed with RIPA buffer, and total protein was extracted by centrifugation. Protein concentrations were determined using the BCA protein quantification kit. Twenty micrograms of total protein were separated by 10% SDS-PAGE and transferred to PVDF membranes (Millipore). After blocking, membranes were incubated overnight at 4°C with primary antibodies: rabbit anti-mouse STAT6 monoclonal antibody (1:500), rabbit anti-mouse p-STAT6 polyclonal antibody (1:500), and rabbit anti-mouse -actin monoclonal antibody (1:1000) as internal control. After washing, membranes were incubated with corresponding secondary antibodies (HRP-labeled goat anti-rabbit, 1:5000) for 1 hour at room temperature. Following TBST washes, ECL substrate was applied and chemiluminescence images were captured using a gel imaging system. Band intensities were quantified using Quantity One V4.62 software.

1.8 q-PCR Detection of MUC5AC mRNA Expression in Lung Tissue

Total RNA was extracted from lung tissues using the high-purity total RNA rapid extraction kit according to the manufacturer' s instructions. RNA concentrations were measured, and cDNA was synthesized by reverse transcription. Primers were: MUC5AC forward 5' -CAGCAGATCATCCGTCAGCAA-3' , reverse 5' -ATCGCAGCGCAGAGTCACA-3' ; GAPDH forward 5' -CAGCGACACCCACTCCTCCACCTT-3', reverse 5'-CATGAGGTCCACCACCCTGTTGCT-3' . PCR was performed in a 10 L reaction volume with the following conditions: 95°C pre-denaturation for 3 minutes; 39 cycles of 95°C denaturation for 5 seconds, 60°C annealing for 45 seconds; followed by melting curve analysis from 65°C to 95°C with 0.5°C increments, holding for 25 seconds at each step, and final extension at 72°C.

1.9 Statistical Analysis

Data were analyzed using GraphPad Prism 5.0 and SPSS 13.0 software. One-way ANOVA or Tukey's test was used for intergroup comparisons when variances were homogeneous; Kruskal-Wallis test was applied for heterogeneous variances. Two-way ANOVA was used for pairwise comparisons. Repeated measures data were analyzed using repeated measures ANOVA. $P<0.05$ was considered statistically significant.

Results

2.1 PAS Staining of Lung Tissues

PAS-positive substances appeared red or purple-red. Compared with the control group, the asthma group showed significant goblet cell hyperplasia in airway epithelium and excessive mucus secretion ($P<0.01$). In the Brg1 / +asthma group, both goblet cell numbers and mucus secretion were significantly reduced compared with the asthma group ($P<0.01$) [Figure 1: see original paper].

2.2 MUC5AC and IL-13 Levels in BALF Supernatant

ELISA analysis revealed that MUC5AC expression in BALF supernatant was significantly elevated in the asthma group (2.022 ± 0.2974) compared with the control group (1.508 ± 0.1445, $P<0.01$). The Brg1 / +asthma group (1.527 ± 0.1184) showed markedly reduced MUC5AC levels compared with the asthma group ($P<0.01$). IL-13 levels in BALF were also significantly increased in the asthma group (23.26 ± 5.512) versus the control group (9.322 ± 2.937, $P<0.01$), and were significantly decreased in the Brg1 / +asthma group (8.316 ± 7.715) compared with the asthma group ($P<0.01$) [Figure 2: see original paper].

2.3 MUC5AC Expression in Airway Epithelium

MUC5AC immunohistochemical staining produced yellow or brownish-yellow granules in the cytoplasm and cell membrane of airway epithelial cells. Immunohistochemical analysis showed significantly higher MUC5AC expression in the asthma group (44.62 ± 11.76) compared with the control group (12.29 ± 3.336, $P<0.01$). The Brg1 / +asthma group (22.71 ± 3.510) exhibited significantly reduced MUC5AC expression compared with the asthma group ($P<0.01$) [Figure 3: see original paper].

2.4 MUC5AC mRNA Expression in Lung Tissue

q-PCR analysis demonstrated that MUC5AC mRNA expression was significantly increased in the asthma group compared with the control group ($P<0.01$). The Brg1 / +asthma group showed significantly reduced MUC5AC mRNA expression compared with the asthma group ($P<0.01$) [Figure 4: see original paper].

2.5 STAT6 and p-STAT6 Expression in Lung Tissue

Western blot analysis revealed that both STAT6 and p-STAT6 protein levels were significantly elevated in the asthma group compared with the control group ($P<0.01$). The Brg1 / +asthma group exhibited markedly downregulated STAT6 and p-STAT6 expression compared with the asthma group ($P<0.01$) [Figure 5: see original paper].

Discussion

Asthma pathogenesis is closely associated with genetic and environmental factors, with epigenetic regulation serving as a crucial mediator between these influences. Epigenetics investigates heritable changes in gene expression without alterations in DNA sequence, including DNA methylation, histone acetylation, and chromatin remodeling. Brg1, located on chromosome 19p13, is a highly conserved member of the human SWI/SNF chromatin remodeling complex. As a phosphorylated nuclear protein, Brg1 primarily regulates gene expression by ATP-dependent disruption of histone-DNA contacts and alteration of chromatin structure.

The present study demonstrates that OVA sensitization in Brg1 knockdown mice results in reduced goblet cell numbers and decreased mucus secretion in airway epithelium, with statistically significant differences. Airway mucus primarily consists of mucins secreted by goblet cells. Previous reports indicate that asthmatic patients have three times more goblet cells than healthy individuals, with goblet cell metaplasia and MUC5AC upregulation representing characteristic features of asthmatic mucus hypersecretion. Our experiments revealed that both BALF mucin MUC5AC levels and lung tissue MUC5AC expression were significantly decreased following Brg1 knockdown, collectively indicating that Brg1 plays an important role in asthmatic airway mucus hypersecretion.

The mechanisms underlying mucus hypersecretion in asthma are complex and involve multiple signaling pathways, among which the IL-13/STAT6 pathway is the most extensively studied. IL-13, a pleiotropic cytokine primarily secreted by Th2 cells, induces goblet cell metaplasia and mucus hypersecretion. Studies have shown that IL-13 inhibitors or IL-13 knockout significantly reduce goblet cells and MUC5AC expression. In our study, Brg1 knockdown mice exhibited significantly decreased IL-13 levels in BALF after asthma model establishment.

Signal transducer and activator of transcription 6 (STAT6) is widely involved in regulating various cellular activities and serves as a crucial component in intracellular signal transduction. STAT6 is closely associated with asthma pathogenesis, with reports confirming abundant STAT6 expression in bronchial epithelial cells of asthmatic patients. STAT6 promotes Th2 immune responses and B cell differentiation. IL-13 functions as an upstream stimulatory factor for the STAT6 signaling pathway. Research indicates that IL-13 binding to its receptors (IL-4R or IL-13R 1) induces tyrosine phosphorylation, which recruits and phosphorylates STAT6. Activated STAT6 then translocates to the nucleus

and binds to specific sites in the MUC5AC promoter region, thereby activating MUC5AC transcription. Our study detected significantly reduced STAT6 and p-STAT6 expression in lung tissues of Brg1 knockdown mice, accompanied by decreased MUC5AC mRNA transcription.

In summary, Brg1 knockdown prior to OVA sensitization reduces goblet cell numbers, mucus secretion, and MUC5AC mRNA and protein expression in asthmatic mice. Additionally, IL-13, STAT6, and p-STAT6 expression were significantly decreased in the Brg1 knockout asthma group. These findings suggest that Brg1 may influence asthmatic mucus hypersecretion through modulation of the STAT6 signaling pathway, providing new targets and directions for future research and treatment of mucus hypersecretion-predominant asthma, though the specific mechanisms require further investigation.

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