

# Brg1 Promotes Airway Mucus Hypersecretion via IL-13 and the JAK1/2-STAT6 Signaling Pathway in Asthma

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## Research Article

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# Abstract

**Background:** The chromatin remodeling factor Brg1 (Brahma-related gene 1) is an important nuclear protein that promotes the transcriptional activation or inhibition of target genes by regulating ATP hydrolysis to generate energy which rearranges the position of nucleosomes and the interaction of histone DNA. In this study, we explored the effect of *Brg1* on airway mucus hypersecretion in asthma.

**Methods:** Six-to-eight-week-old female wild-type C57BL/6 mice (wild-type, WT) and type II alveolar epithelial cells (AECII) specifically knockout *Brg1* mice (*Brg1*<sup>fl/fl</sup>) were selected as the experimental subjects. The asthma group was established with house dust mite (HDM), and the control group was treated with normal saline (n=10). Wright's staining was used to detect inflammatory cells in bronchoalveolar lavage fluid (BALF). Invasive lung function was used to assess the airway compliance. Hematoxylin and eosin and periodic acid-schiff staining were used to detect mucus secretion. The virus was used to knock down the *Brg1* gene in the bronchial epithelial cell line (16HBE) and stimulated with HDM. Immunohistochemistry was used to measure mucin glycoprotein 5AC (MUC5AC) protein expression in the airway epithelium and 16HBE cells. Western blotting was used to detect the expression of the MUC5AC and JAK1/2-STAT6 signaling pathways in mouse lung tissue and 16HBE. Co-immunoprecipitation (Co-IP) and Chromatin Immunoprecipitation (CHIP) were used to detect whether *Brg1* could regulate the JAK1/2-STAT6 signaling pathway.

**Results:** Specifically, knocking out the *Brg1* gene in AECII can reduce airway inflammation, airway compliance, and mucus hypersecretion in asthma. Knockdown of the *Brg1* gene can simultaneously reduce Interleukin-13 (IL-13) and the expression of MUC5AC protein in airway epithelial cells and the activation of the JAK1/2-STAT6 signaling pathway. The results of Co-IP and CHIP showed that *Brg1* could bind to the JAK1/2 promoter region, regulating the activity of the JAK1/2-STAT6 pathway affects airway mucus secretion in asthma.

**Conclusion:** *Brg1* gene knockout in airway epithelial cells can reduce asthmatic airway mucus hypersecretion and the expression of MUC5AC protein in airway epithelial cells partly by inhibiting the activation of the JAK1/2-STAT6 signaling pathway.

## 1. Background

Asthma is a common, serious, and chronic airway inflammatory disease in children. The clinical manifestations are mainly recurrent wheezing, shortness of breath, coughing, and chest tightness, and it is often accompanied by expiratory airflow limitation. According to current research, there are currently approximately 334 million asthma patients worldwide, and there are as many as 40 million asthma patients in China. In the past decade, the global asthma incidence has increased from 2.4% to 4.3% [1]. Due to the complexity of its etiology and pathogenesis, the clinical treatment of asthma is facing substantial challenges. Recent studies suggest that epigenetic regulation (heritable changes in gene expression that occur in the absence of alterations in DNA sequences) may in part mediate the complex

gene environment interactions that can lead to asthma [2]. Therefore, it is interesting to study the pathogenesis of asthma from the perspective of epigenetics. Brahma-related gene 1 (*Brg1*), a chromatin remodeling protein encoded by the SMARCA4 gene, is the core component of the mammalian switch/sucrose non-fermentable (SWI/SNF) class of chromatin remodeling complex, with ATPase activity. We previously found that *Brg1* plays an important role in the pathogenesis of asthma. High expression of *Brg1* can be detected in the peripheral blood of children with asthma [3]. Previous studies have suggested that knocking down *Brg1* in asthmatic mice can reduce chronic airway inflammation in asthma. Therefore, this study further explored the effect of *Brg1* on asthmatic airway mucus hypersecretion. Mucus hypersecretion is an important clinical manifestation of asthma. Consequently, further exploration of whether the *Brg1* gene can participate in the regulation of asthmatic airway mucus hypersecretion will provide a new direction for the treatment of asthma.

Mucus hypersecretion can be a critical contributor to asthma exacerbation and can be initiated by multiple intracellular signaling pathways [4]. MUC5AC is an important protein that encodes structurally related mucin glycoproteins, the principal macromolecules in airway mucus. MUC5AC overproduction is an additional disease feature of asthma. Signal transducer and activator of transcription 6 (STAT6) is necessary and sufficient for inducing the *MUC5AC* gene [5].

## 2. Methods

### 2.1. Animals

Female C57BL/6 mice (4-6 weeks old) were purchased from the Experimental Animal Center of Chongqing Medical University (Chongqing, China). AE2 and cubic cell-specific *Brg1*-knockout (SFTPC-Cre/*Brg1*<sup>fl/fl</sup>) mice were generated by hybridizing SFTPC-rtTA/(TetO)7 transgenic mice (The Jackson Laboratory, CA, USA) with *Brg1*<sup>loxP/loxP</sup> mice (kindly provided by Prof. Yujun Shi, West China hospital of Sichuan University). *Brg1* deletion in SFTPC-Cre/*Brg1*<sup>fl/fl</sup> mice was achieved by administering doxycycline (1 mg/mL) in drinking water containing 0.4% sucrose. The various control mice were *Brg1*<sup>fl/fl</sup> mice (no Cre) that received doxycycline. Experimental mice were housed under specific pathogen-free conditions and subjected to a 12 h/12 h dark/light cycle. The study was approved by the ethics committee of Chongqing Medical University.

### 2.2. Grouping and administration

Twenty female C57BL/6 mice were divided into control and asthma groups. Mice in the asthma group underwent house dust mite (HDM) challenge according to previous studies [6]. Briefly, mice received either 20 µg HDM (Greer, Los Angeles, CA, USA) dissolved in 30 µL normal saline (NS) (asthma group) or the same volume of NS (control group) by nasal inhalation on days 0, 14, 21, 23, 25, 27, and 29. A total of twenty SFTPC-Cre/*Brg1*<sup>fl/fl</sup> mice were divided into control/*Brg1*<sup>fl/fl</sup> and asthma/*Brg1*<sup>fl/fl</sup> groups. The modeling

method of the control and asthma groups of SFTPC-Cre/Brg<sup>fl/fl</sup> mice was the same as that of wild-type mice. All mice were sacrificed at 24 h after the last challenge and then euthanized by intraperitoneal injection of 4% pentobarbital (0.5 mg/kg).

### ***2.3. Measurement of airway hyper-responsiveness (AHR)***

AHR was determined using an invasive pulmonary function instrument (EMKA Technologies, Paris, France) within 24 h after the final HDM challenge. Mice were anesthetized with 2% pentobarbital sodium, intubated, and placed in whole-body plethysmography chambers. Airway resistance and compliance were measured every second. Baseline and changes in airway resistance were determined using NS and methacholine (3.125, 6.25, 12.5, 25, or 50 mg/mL) respectively, which were nebulized at a volume of 20 $\mu$ L<sup>[6]</sup>. Lung resistance at each concentration was calculated.

### ***2.4. Histological and immunohistochemical assessment of lung tissues***

The left lung tissues were fixed in 4% formalin buffer and embedded in paraffin. The paraffin blocks were then serially sectioned into 4- $\mu$ m-thick slices and subjected to immunohistochemical (IHC) staining. Antibodies against MUC5AC (1:300; Abcam, Massachusetts, USA) were used for IHC staining. Tissues or cells that were stained brown were recorded as being positive for MUC5AC. The intensity of IHC staining was quantified using Image-Pro Plus 6.0. Regarding the histochemical analysis, paraffin blocks were serially sectioned into 4- $\mu$ m-thick slices and subjected to hematoxylin/eosin (H&E) or periodic acid-schiff (PAS) (Leagene Biotechnology Co. Ltd, Beijing, China) staining. Three individual areas per section and lung tissues per group were evaluated for inflammatory cell infiltration.

### ***2.5. Broncho alveolar lavage fluid (BALF) analysis and cell counting***

The right lung of each animal was washed three times with 0.5 mL phosphate-buffered saline (PBS) in order to collect BALF. Approximately 1.3 mL of the instilled fluid was consistently recovered. All the cells in BALF were collected by centrifugation and treated with red blood lysis buffer, after which they were counted by microscopy using a cell counter. The remaining inflammatory cells were smeared on slides, dried, and stained using Wright-Giemsa stain (Jiancheng Techno Co, Nanjing, China), according to the manufacturer's instructions, to determine differential cell counts in accordance with conventional morphological criteria. At least 200 cells per slide were evaluated to determine the differential cell counts.

### ***2.6. Cell culture and Treatments***

Human bronchial epithelial cells (16HBE) were obtained from the American Type Culture Collection (ATCC, USA) and cultured in Dulbecco's phosphate-buffered saline medium (DMEM) containing 10% fetal bovine serum (FBS; Gibco, USA). The cells were cultured at 37°C in a 5% CO<sub>2</sub> atmosphere, and the growth status of the cells was evaluated under a microscope. Cells were grown to 85-90% confluence, passaged, and subjected to trypsin digestion. Short hairpin RNAs (shRNAs) against *Brg1* (*Brg1*-shRNA), as well as a negative control shRNA (NC-shRNA), were designed and cloned into pGMLV-SC5 RNAi backbone adenoviral vectors (Genomedtech, Shanghai, China). 16HBE cells were then infected with the *Brg1*-shRNA adenovirus vectors and screened for the stable expression of green fluorescent protein.

## ***2.7. Real-time RT-PCR***

Total RNA from 16HBE cells and lung tissues was purified, and cDNA synthesis was performed using a PrimeScript RT Reagent Kit (Takara, Otsu, Japan). PCR reactions were performed using Real MasterMix (SYBR Green, Tiangen, Beijing, China). The primer sequences of *Brg1* were forward 5-GACCAGCACTCCCAAGGTTAC-3 and reverse 5-CTGGCCCGGAAGACATCTG-3. The primer sequences for *MUC5AC* were forward 5-CAGCAGATCATCCGTCAGCAA-3 and reverse 5-ATCGCAGCGCAGAGTCACA-3. The primer sequences for *GAPDH* were forward 5-CAGCGACACCCACTCCTCCACCTT-3 and reverse 5-CATGAGGTCCACCACCTGTTGCT-3. The primer sequences for the  $\beta$ -*actin* were forward 5-CTCCATCCTGGCCTCGCTGT-3 and reverse 5-GCTGTACCTTCACCGTTC-3.

## ***2.8. Immunofluorescence detection***

The expression of MUC5AC in 16HBE cells was determined using immunofluorescence staining. Three groups of cells (16HBE, *Brg1*-sc, *Brg1*-sh) were made into cell slides, and the slides were removed 24 h later and fixed with 4% paraformaldehyde. The slides were stained with primary antibodies, rabbit anti-*Brg1* (1:200, Abcam, UK) for 12 h at 4°C. The sections were washed three times with PBS and stained for 30 min at room temperature with donkey anti-rabbit Cy5 secondary antibodies (1:500; Invitrogen, USA). Nuclei were counterstained with DAPI (4,6-diamidino-2-phenylindole) (Invitrogen, USA), and fluorescence of the samples was captured using a confocal laser scanning microscope (A1pR, Nikon, Tokyo, Japan).

## ***2.9. Western blot and Co-immunoprecipitation (Co-IP) assay***

Total protein from the right lung tissue and 16HBE cells was isolated using a total protein extraction kit (KeyGen BioTECH, China). Protein concentration was determined using the bicinchoninic acid (BCA) assay following the standard protocol. Protein samples (30 µg) were resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were then blocked and incubated overnight with primary antibodies and subsequently incubated with horseradish peroxidase-linked goat anti-rabbit IgG secondary antibody

(1:5000; Proteintech, China). The primary antibodies included mouse anti-*Brg1* (1:300; Abcam, USA), rabbit anti-MUC5AC (1:500, Proteintech, China), rabbit anti-phospho-JAK1/2 (1:500; CST, USA), rabbit anti-JAK1/2 (1:500, Proteintech, China), rabbit anti-phospho-STAT6 (1:500; CST, USA), rabbit anti-STAT6 (1:500, Proteintech, China), and rabbit anti- $\beta$ -actin (1:1000; Proteintech, China). Immunoreactivity was visualized using an enhanced chemiluminescence (ECL) kit (Thermo Fisher, USA). Band intensities were quantified using Quantity One software (version 4.6.2; Bio-Rad, Hercules, CA, USA) and normalized to  $\beta$ -actin. The Co-IP assay<sup>[7]</sup> was performed using Protein A/G agarose (Beyotime, China), following the manufacturer's instructions, and normal rabbit anti-IgG (Abcam, UK) was used as a control antibody. Harvested samples containing input (protein pretreated with nothing), IgG (protein pre-treated with A/G agarose and rabbit anti-IgG), anti-JAK1/2 (protein pre-treated with A/G agarose and rabbit anti-PI3K), and anti-*Brg1* (protein pre-treated with A/G agarose and rabbit anti-*Brg1*) were analyzed by western blotting.

## **2.10. Chromatin Immunoprecipitation [CHIP]**

16HBE and *Brg1* knockdown 16HBE cells (*Brg1*-sh) were cultured for 48 h. One plate of cells (10 cm plate) was removed for formaldehyde cross-linking and ultrasonic disruption. We chose the CHIP kit from the American company Milipore, and all the experimental steps were performed in accordance with the kit's instructions. The concentration of *Brg1* antibody used in CHIP is 1:20, and the antibody was purchased from Zen-bio Biotechnology Co., Ltd., Chengdu, China. The ultrasonic fragmentation product was removed, and the ultrasonic effect was tested by electrophoresis. The next day, the immune complex was precipitated and washed. After de-crosslinking, DNA fragments were recovered. The final sample was dissolved in 30  $\mu$ L Elution buffer. Finally, the recovered DNA was subjected to q-PCR analysis. The primer sequences of *JAK1* were forward 5-GGCGTCGAAGCAAACCTGTTT-3 and reverse 5-TACCGTTGTGGGCTACGTTC-3. The primer sequences of *JAK2* were forward 5-GACAGACCTGAAGAGCAGCA-3 and reverse 5-CTGGCACCAGATCGTGTAGG-3.

## **2.11. Statistical analyses**

Data are expressed as the mean  $\pm$  standard error of the mean. GraphPad Prism software (version 5.0; GraphPad, San Diego, CA, USA) was used for the statistical analyses. Two-way analysis of variance was conducted to determine statistically significant differences in the tested variables among the different groups. If an overall test was significant, Tukey's test was used for specific comparisons between groups. Statistical significance was set at  $p < 0.05$ . All experiments were repeated at least thrice with consistent outcomes.

## **3. Results**

### **3.1. Knockout of *Brg1* reduced airway inflammation and airway ventilation resistance in asthmatic mice**

*Brg1* knockout mice and wild type mice asthma model was established using house dust mites (Fig.1A). The total number of airway inflammatory cells in the BALF of the *Brg1* knockout mouse asthma group was significantly lower than that of the wild-type asthma group, and the difference was statistically significant (Fig.1B). The number of eosinophils in the BALF of the *Brg1*<sup>fl/fl</sup>/asthma group was significantly lower than that in the WT/asthma group, and the difference was statistically significant (Fig.1C). There was no difference in the total inflammatory cells and eosinophils in BALF between the WT/Control group and *Brg1*<sup>fl/fl</sup>/Control groups (Fig.1B-C). HE staining results of mouse lung tissue showed that the infiltration of inflammatory cells around the bronchus of the *Brg1*<sup>fl/fl</sup>/asthma group was significantly less than that in the WT/asthma group (Fig.1D). The results of the invasive lung function test showed that the airway resistance of the *Brg1*<sup>fl/fl</sup>/asthma group was lower than that of the WT/asthma group. There was no difference in airway ventilation resistance between the WT/Control group and *Brg1*<sup>fl/fl</sup>/Control groups (Fig.1E).

### **3.2. Knockout of *Brg1* alleviated airway mucus secretion and MUC5AC protein expression in asthmatic mice**

PAS staining of mouse lung tissue showed that airway mucus secretion in the *Brg1*<sup>fl/fl</sup>/asthma group was significantly lower than that in the WT/asthma group (Fig.2A). Nucleic acid was extracted from lung tissue for qPCR detection. The results showed that the mRNA expression of MUC5AC in the lung tissue of the *Brg1*<sup>fl/fl</sup>/asthma group was lower than that in the WT/asthma group (Fig.2B). Immunohistochemical analysis of lung tissue also showed that the expression of MUC5AC protein in the airway of the *Brg1*<sup>fl/fl</sup>/asthma group was significantly reduced compared with that in the WT/asthma group (Fig.2C). We used ImageJ software to perform statistics on the immunohistochemical results of the MUC5AC protein. The results also showed that the airway expression of MUC5AC protein in the *Brg1*<sup>fl/fl</sup>/asthma group was lower than that in the WT/asthma group (Fig.2D). We collected mouse alveolar lavage fluid for ELISA experiments, and the results showed that the MUC5AC protein content in the BALF of the *Brg1*<sup>fl/fl</sup>/asthma group was lower than that of the WT/asthma group (Fig.2E). The above results suggest that knocking out the *Brg1* gene can significantly improve airway mucus hypersecretion in asthma.

### **3.3. Knockdown of *Brg1* reduced the expression of MUC5AC protein and inhibited the activation of the JAK1/2-STAT6 signaling pathway in asthma**

Proteins were extracted from mouse lung tissues for western blot analysis. The western blot results showed that there was no difference in the expression of MUC5AC protein in the lung tissues of the WT/Control group and *Brg1*<sup>fl/fl</sup>/Control group. The expression of MUC5AC protein in the lung tissue of the *Brg1*<sup>fl/fl</sup>/asthma group was significantly lower than that of the WT/asthma group (Fig.3A). The expression

of MUC5AC protein in lung tissue detected by Western blot was statistically analyzed (Fig.3B). Studies have shown that IL-13 is closely related to airway mucus hypersecretion during the asthma onset [8]. Therefore, we used ELISA experiments to detect the amount of IL-13 levels secretion in mouse BALF, and the results showed that IL-13 in the BALF of the *Brg1<sup>fl/fl</sup>*/asthma group were significantly lower than those in the WT/asthma group (Fig.3C). It has been reported in the literature that excessive activation of the JAK1/2-STAT6 pathway an important reason for the high secretion of airway mucus in asthma [9]. Western blotting revealed the activation of the JAK1/2-STAT6 signaling pathway in mouse lung tissue, and the results showed that the percentages of p-JAK1/2/JAK1/2 and p-STAT6/STAT6 in the *Brg1<sup>fl/fl</sup>*/asthma group were significantly lower than those in the WT/asthma group (Fig.3D). The percentages of p-JAK1/2/JAK1/2 and p-STAT6/STAT6 in the western blot results were statistically analyzed (Fig.3E-F). The above results suggested that knocking out of *Brg1* could inhibit the activation of the JAK1/2-STAT6 pathway in asthma, thereby reducing airway mucus hypersecretion.

### **3.4. Knockdown of *Brg1* reduced the expression of MUC5AC and inhibited the JAK1/2-STAT6 signaling pathway in 16HBE**

We used adenovirus to knock down the *Brg1* gene in bronchial epithelial cells (16HBE). To verify whether *Brg1* knockdown was successful in 16HBE, we extracted the cell protein and performed western blot experiments. The results showed that the *Brg1* protein expression of the cells in the *Brg1* knockdown group (*Brg1*-sh) was significantly lower than that of the virus empty control group (*Brg1*-sc), while the *Brg1*-sc group had no difference compared with the blank control group (16HBE) (Fig.4A-B). At the same time, we extracted cell nucleic acid for qPCR and found that *Brg1* mRNA levels in the *Brg1*-sh group were significantly lower than those in the *Brg1*-sc group, and the difference was statistically significant (Fig.4C). The results of the immunofluorescence staining of 16HBE showed that MUC5AC protein was expressed in the cells, and the fluorescence intensity of the *Brg1*-sh group was lower than that of the *Brg1*-sc group (Fig.4D). The expression of MUC5AC protein in the *Brg1*-sh was lower than that in the *Brg1*-sc group as determined by western blotting (Fig.4E). Concurrently, we also detected the changes of the JAK1/2-STAT6 pathway in 16HBE. The percentages of p-JAK1/2/JAK1/2 and p-STAT6/STAT6 in the *Brg1*-sh group were significantly lower than the *Brg1*-sc group, suggesting that *Brg1* knockdown can significantly inhibit the activation of the JAK1/2-STAT6 signaling pathway (Fig.4F). We found that the results of 16HBE cell proteins p-JAK1/2/JAK1/2 and p-STAT6/STAT6 were significantly lower in the *Brg1*-sh group than in the *Brg1*-sc group (Fig.4G-H). The above *in vitro* experimental results also suggest that knocking down *Brg1* in bronchial epithelial cells can simultaneously reduce the expression of MUC5AC and IL-13 protein and inhibit the activation of the JAK1/2-STAT6 pathway.

### **3.5. *Brg1* regulated the JAK1/2-STAT6 pathway partly by binding to the JAK1/2 gene promoter region**

Since *Brg1* gene is an epigenetic regulatory factor, it can participate in the transcriptional regulation of a variety of genes. Therefore, we used house dust mite extract to treat bronchial epithelial cells, and then collected the cells to detect the combination of *Brg1* and JAK1/2 using Co-IP. The results of Co-IP showed that HDM was used to stimulate 16HBE, and *Brg1* interaction with JAK1/2 protein in 16HBE (Fig.5A). The abnormally increased *Brg1* gene expression in asthma may be involved in the regulation of airway mucus hypersecretion in asthma. The results of the CHIP experiment showed that the *Brg1* gene can be detected in the airway epithelial cells to bind to the promoter region of the JAK1/2 gene (Fig.5B-C). Knockdown of the *Brg1* gene can inhibit asthmatic airway mucus hypersecretion and reduce MUC5AC and IL-13 secretion, possibly through inhibition of the activation of the JAK1/2-STAT6 pathway. The mechanism diagram of this study is shown in Fig.5D.

## 4. Discussion

In recent years, the incidence of asthma has gradually increased, with more than 300 million asthma patients worldwide. The pathological features of asthma mainly include airway epithelial injury, airway hyperresponsiveness, high mucus secretion and airway remodeling. Among them, the high secretion of airway mucus is an important factor that obstructs airway ventilation in asthma and affects the severity of the disease in children. Mucus hypersecretion is a pathophysiological feature of asthma and contributes to morbidity and mortality. Asthma is a disease closely related to genetic inheritance. In recent years, epigenetics has gained increasing attention in the pathogenesis of asthma. Studies have shown that there is epigenetic regulation of genes in many patients with asthma. *Brg1* is a chromatin remodeling protein. Our previous studies have found that the expression of *Brg1* gene is increased in asthmatic mice and children. Knockdown of the *Brg1* gene in mouse models can significantly reduce airway epithelial damage and airway inflammation in asthma. We investigated the involvement of *Brg1* in the regulation of *Brg1* in airway mucus hypersecretion in asthma, focusing on the role of *Brg1* in the JAK1/2-STAT6 pathway. In this study, *Brg1* knockout reduced airway mucus secretion in asthmatic mice. One study showed that *Brg1* regulated endothelial-derived IL-33 to promote ischemia-reperfusion-induced renal injury and fibrosis in mice<sup>[10]</sup>. This suggests that the *Brg1* plays an important role in the regulation of epithelial cell function. Previous studies have shown that overlapping contributions of multiple SWI/SNF subunit (*Brg1*) mutations in the translocation of the endometrium to distal sites, with loss of cell integrity being a common feature in SWI/SNF mutant (*Brg1*) endometrial epithelia<sup>[11]</sup>. Another study found that the decreased expression of KDM2B and *Brg1* produced similar effects to that of poly(I:C)-treated cells, which could promote the inflammatory response of nasal mucosal epithelial cells. *Brg1* may have an inhibitory effect on the development of nasal mucosal epithelial inflammation<sup>[12]</sup>. *Brg1* promotes the transcription of endothelial *Mrtfa* and *Mrtfb*, which elevates the expression of SRF and SRF target genes that establish embryonic capillary integrity. These data highlight the new and temporally specific role for *Brg1* in embryonic vasculature and provide novel information about epigenetic regulation of *Mrtf* expression and SRF signaling in developing blood vessels<sup>[13]</sup>. Research on the proliferation and migration of lung cancer cells found that *Brg1* regulates the transcription of *CCNB1* and *LTBP2* by changing histone modifications on the target promoter, recruiting KDM3A (a histone H3K9 demethylase)

to be removed from the target gene promoter Dimethyl H3K9, which activates transcription <sup>[14]</sup>. In a study on the of B cell activation process, it was found that activation of the JAK/STAT cytokine signaling pathway is particularly dependent on the regulation of the *Brg1* gene <sup>[15]</sup>. The above research results and the *Brg1* gene found in this study may be involved in the regulation of the JAK1/2-STAT6 signaling pathway. The abnormally high expression of *Brg1* gene in asthmatic mice may promote the activation of JAK1/2-STAT6 signaling pathway, thereby increasing the expression of MUC5AC protein and increasing mucus secretion in asthmatic mice.

Studies have shown that the JAK1/2-STAT6 signaling pathway is an important pathway that mediates the hypersecretion of airway mucus in asthma. Activation of the JAK1/2-STAT6 signaling pathway can be observed in OVA-induced asthma <sup>[16]</sup>. *Pseudomonas aeruginosa* and its virulence factor pyocyanin inhibit FOXA2 (a key transcriptional regulator of mucus homeostasis) by activating the antagonistic signaling pathways EGFR-AKT/ERK1/2 and IL-4/IL-13-STAT6-SPDEF), thereby exacerbating periodic pro-inflammatory reactions and excessive mucus secretion <sup>[17]</sup>. Srolo Bzhtang exhibited protective effects against cigarette smoke-induced airway inflammation and MUC5AC hypersecretion, which might be related to the downregulation of the IL-13/STAT6 signaling pathway <sup>[18]</sup>. In a study on gastrointestinal mucus, it was found that supplementation with gastric mucin (MUC5AC) significantly aggravated JAK/STAT signal transduction, indicating that the JAK/STAT signal pathway is involved in the regulation of MUC5AC protein <sup>[19]</sup>. Petra Dames et al. showed that IL-13 impairs ENaC-dependent sodium transport by activating the JAK1/2-STAT6 signaling pathway. These results improve our understanding of the mechanisms through which IL-13 functions as a key effector cytokine in ulcerative colitis, thereby contributing to the distinct pathology of this disease <sup>[20]</sup>. In addition to these classical pathways, IL-13 is also known to signal via MAP kinases. IL-13 actions via the heterodimeric type I receptor, as identified here in our present study using neutralizing antibodies, have been shown for different target genes and in different tissues to involve phosphorylation of JAK1/2 and subsequent phosphorylation and activation of the transcription factors STAT3 and STAT6 <sup>[20]</sup>. In this study, we also found that knocking out the *Brg1* gene can reduce the secretion of IL-13 in the airways of asthma. Therefore, it may reduce the activation of the JAK1/2-STAT6 pathway regulated by IL-13, thereby reducing the expression of MUC5AC mucus protein.

## 5. Conclusions

In summary, on the basis of the abnormally high expression of *Brg1* in asthma in a previous study, we further studied the effect of *Brg1* on the hypersecretion of airway mucus in asthma, and found that knocking out the *Brg1* gene in asthma models can reduced airway mucus secretion in mice and IL-13 and MUC5AC protein expression. We have verified from *in vivo* and *in vitro* experiments that knocking down the *Brg1* gene may inhibit the activation of the JAK1/2-STAT6 pathway, while the reduction of IL-13 secretion can also reduce the activation of the JAK1/2-STAT6 pathway and reduce the secretion of MUC5AC protein. Our research provides new ideas for reducing the mucus hypersecretion of asthma in the future and for the treatment of asthma.

## Abbreviations

Brg1:Brahma-related gene 1;

WT:wild-type;

AECIIs:alveolar epithelial type II cells;

Brg1<sup>fl/fl</sup>:type II alveolar epithelial cells (AECIIs) specifically knockout Brg1 mice; HDM:house dust mite;

BALF:bronchoalveolar lavage fluid;

16HBE:the bronchial epithelial cell line;

MUC5AC:mucin glycoprotein 5AC;

Co-IP:Co-immunoprecipitation;

CHIP:Chromatin Immunoprecipitation;

IL-13:Interleukin-13;

STAT6:Signal transducer and activator of transcription 6;

JAK1/2:Janus kinase 1/2;

SWI/SNF:the mammalian switch/sucrose non-fermentable;

AHR:airway hyper-responsiveness;

Q-PCR:Real-time RT-PCR;

IHC:immunohistochemical;

H&E:hematoxylin/eosin ;

PAS: periodic acid-schiff ;

## Declarations

### Ethics approval and consent to participate

The study was approved by the Ethics Committee of Chongqing Medical University.

### Consent for publication

Not applicable.

## Availability of data and materials

All data and materials are contained and described within the manuscript.

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

MZX designed and performed the experiments. JH and LLY analyzed and interpreted the data, and wrote the manuscript. GG contributed to the design of the study and the revision of the article. ZF contributed to the design of the study. WJZ and ZXL contributed to the conception and design of the study, the revision of the manuscript, and the final approval of the version to be published. All authors have read and approved the manuscript.

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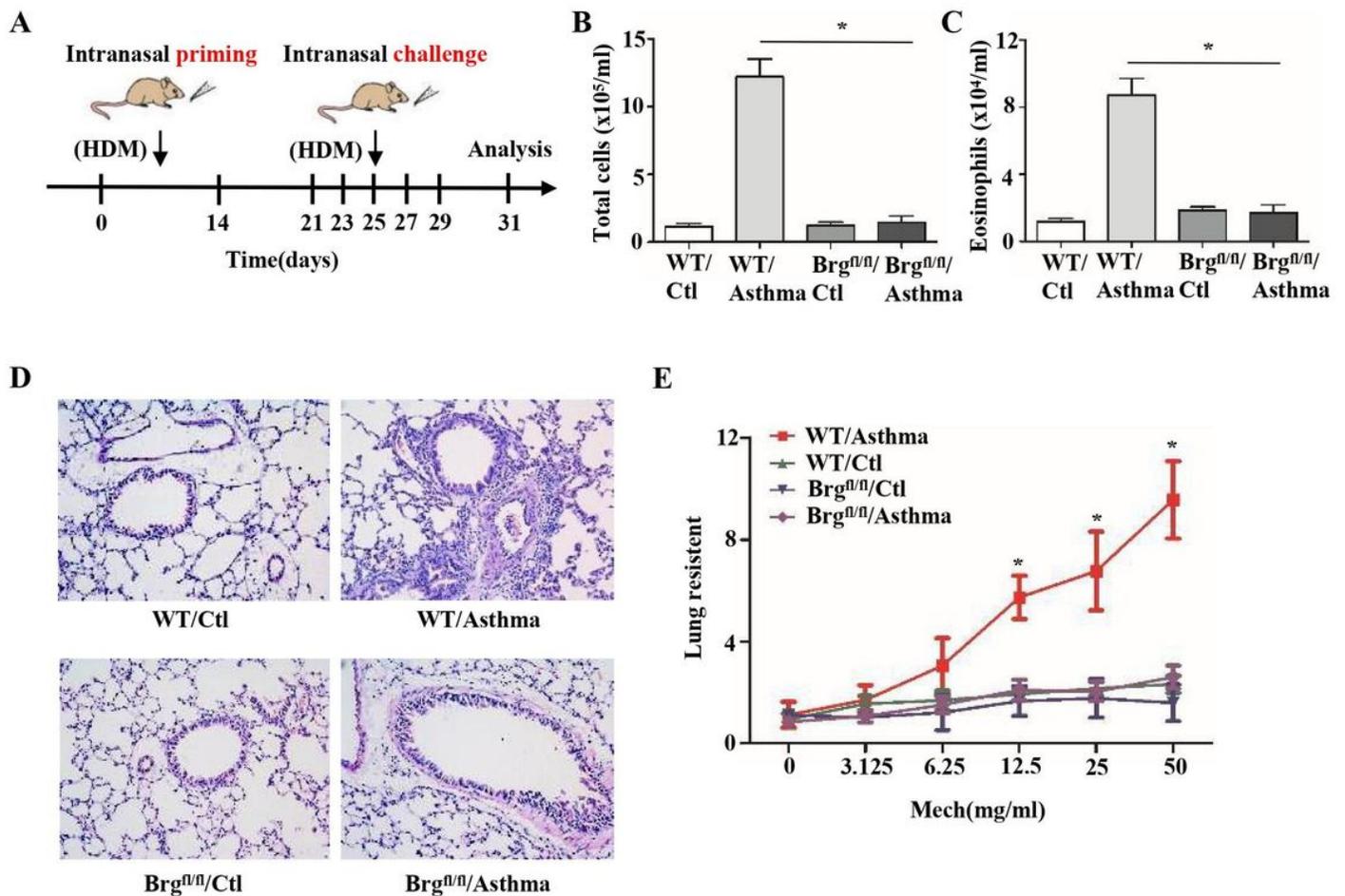
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## Figures

**Figure 1**



**Figure 1**

***Knockout of the Brg1 gene in asthmatic mice can reduce airway inflammation and airway ventilation resistance.*** (A) Protocol for inducing asthmatic mice using HDM. (B) Total number of inflammatory cells in the alveolar lavage fluid of WT and Brg1<sup>fl/fl</sup> mice. (C) The total number of eosinophils in the alveolar lavage fluid of WT and Brg1<sup>fl/fl</sup> mice. (D) Paraffin sections of WT and Brg1<sup>fl/fl</sup> mice lung tissue for HE staining. The magnification of the picture is 100X. (E) Invasive lung function test for airway ventilation resistance in WT and Brg1<sup>fl/fl</sup> mice. (n=6 per group; \*P < 0.05 WT asthma group compared with Brg1<sup>fl/fl</sup> asthma group).

Figure 2

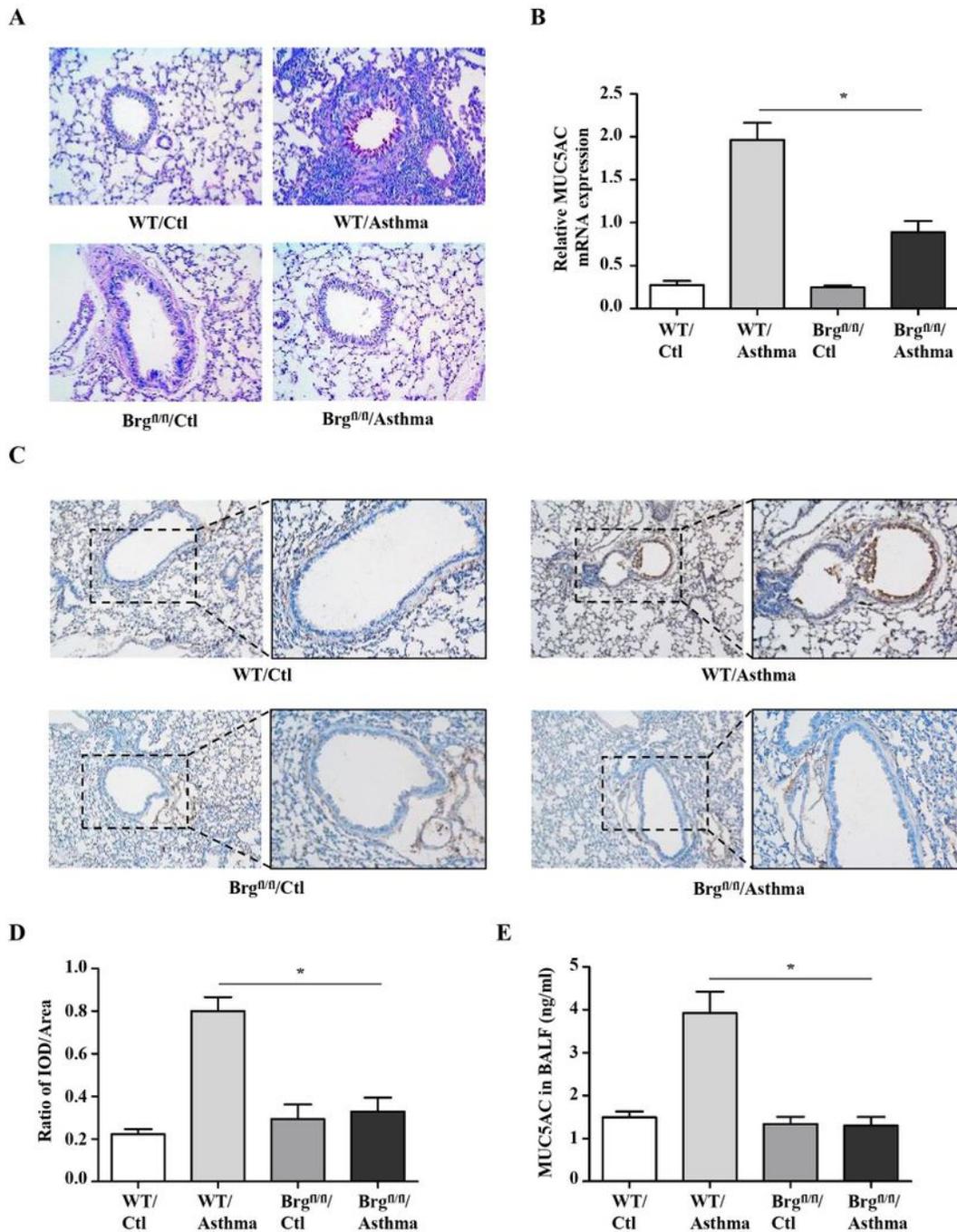
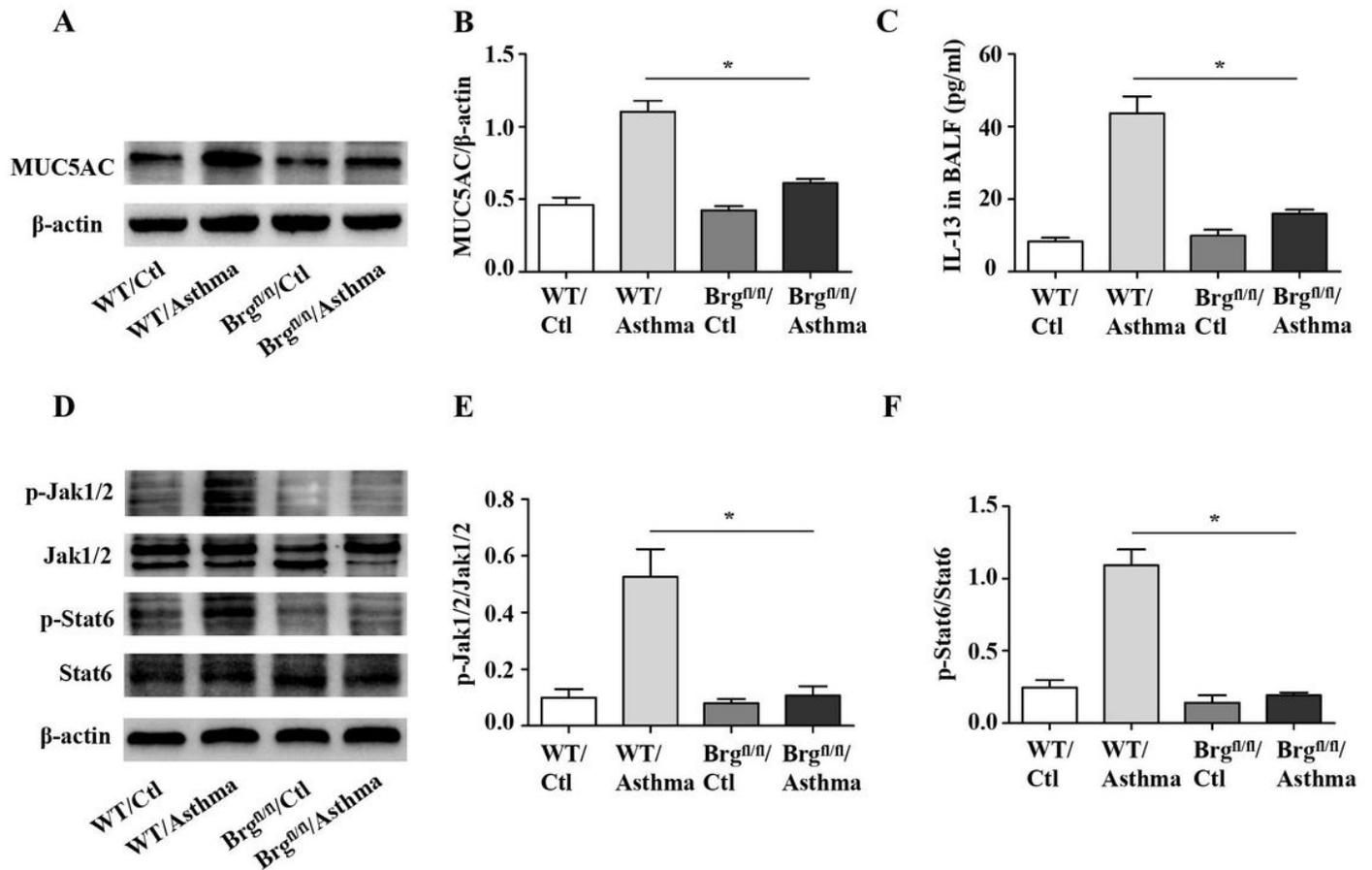


Figure 2

**Airway mucus secretion and MUC5AC protein expression are significantly reduced in asthmatic mice with *Brg1* gene knockout.** (A) Paraffin sections of mouse lung tissue were stained with PAS glycogen. The purple substance represents mucus secretion. (B) qPCR detection of MUC5AC gene mRNA level in mouse lung tissue. (C) Immunohistochemical staining was used to detect the expression of MUC5AC protein in mouse lung tissue, and the brown area represents positive expression. The pictures are 100X and 200X

respectively. (D) Use Image-Pro Plus 6.0 software to count the expression of MUC5AC in lung tissues stained by immunohistochemistry. (E) Detection of MUC5AC protein expression in mouse BALF by ELISA. (n=6 per group; \*P < 0.05 WT asthma group compared with the Brg1<sup>fl/fl</sup> asthma group).

**Figure 3**



**Figure 3**

**Knockout of the Brg1 gene in mouse lung tissue can reduce IL-13 and the activation of the JAK1/2-STAT6 pathways.** (A) Western blot detection of MUC5AC protein expression in mouse lung tissue. (B) Statistical analysis of the intensity of MUC5AC is normalized to β-actin. (C) Detection of IL-13 in mouse BALF by ELISA. (D) Western blot detection of JAK1/2-STAT6 signal pathway activation in mouse lung tissue. (E) Statistical analysis of the percentage of p-JAK1/2 to total JAK1/2 protein. (F) Statistical analysis of the percentage of p-STAT6 to total STAT6 protein. (n=6 per group; \*P < 0.05 WT asthma group compared with Brg1<sup>fl/fl</sup> asthma group).

Figure 4

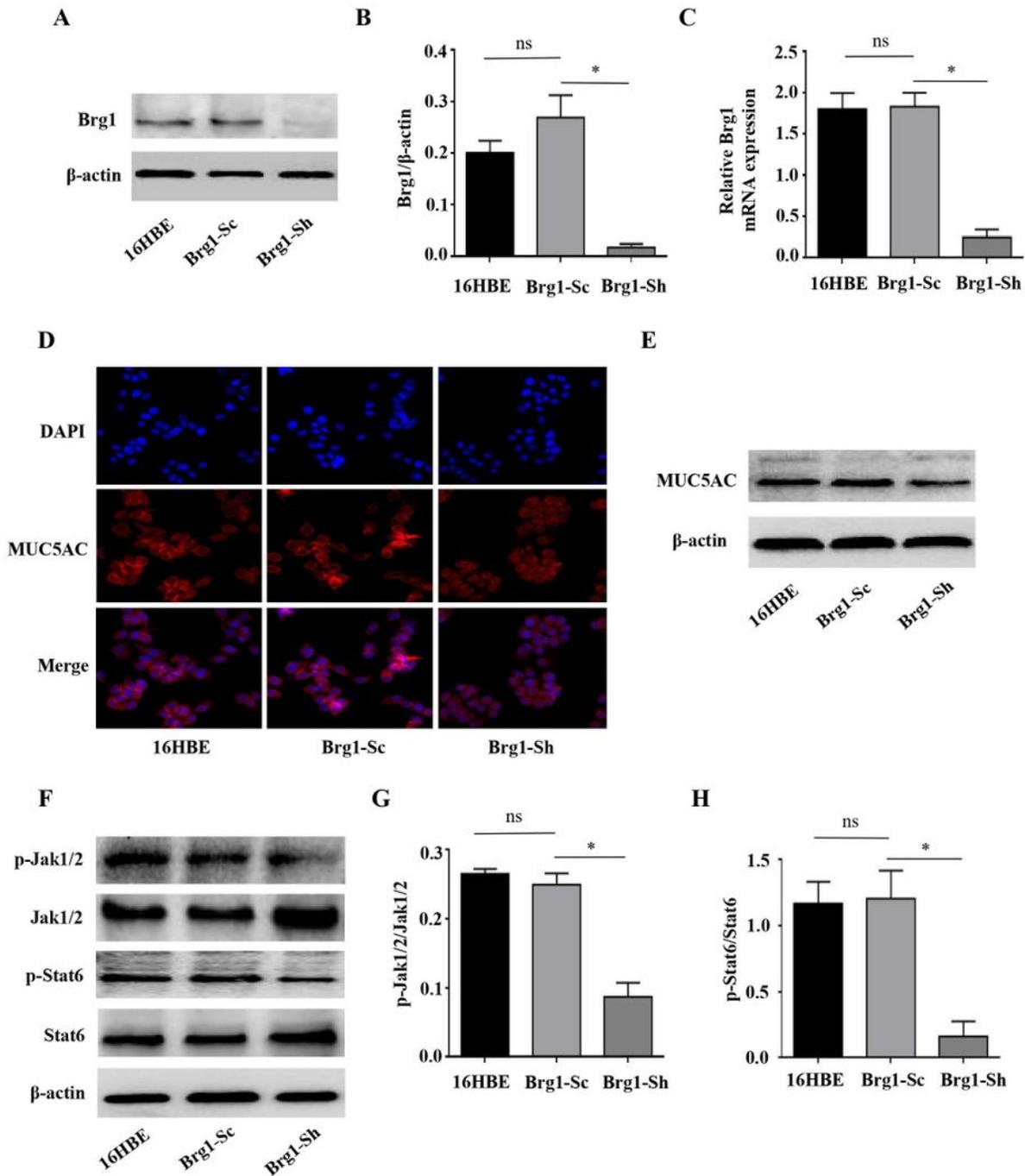
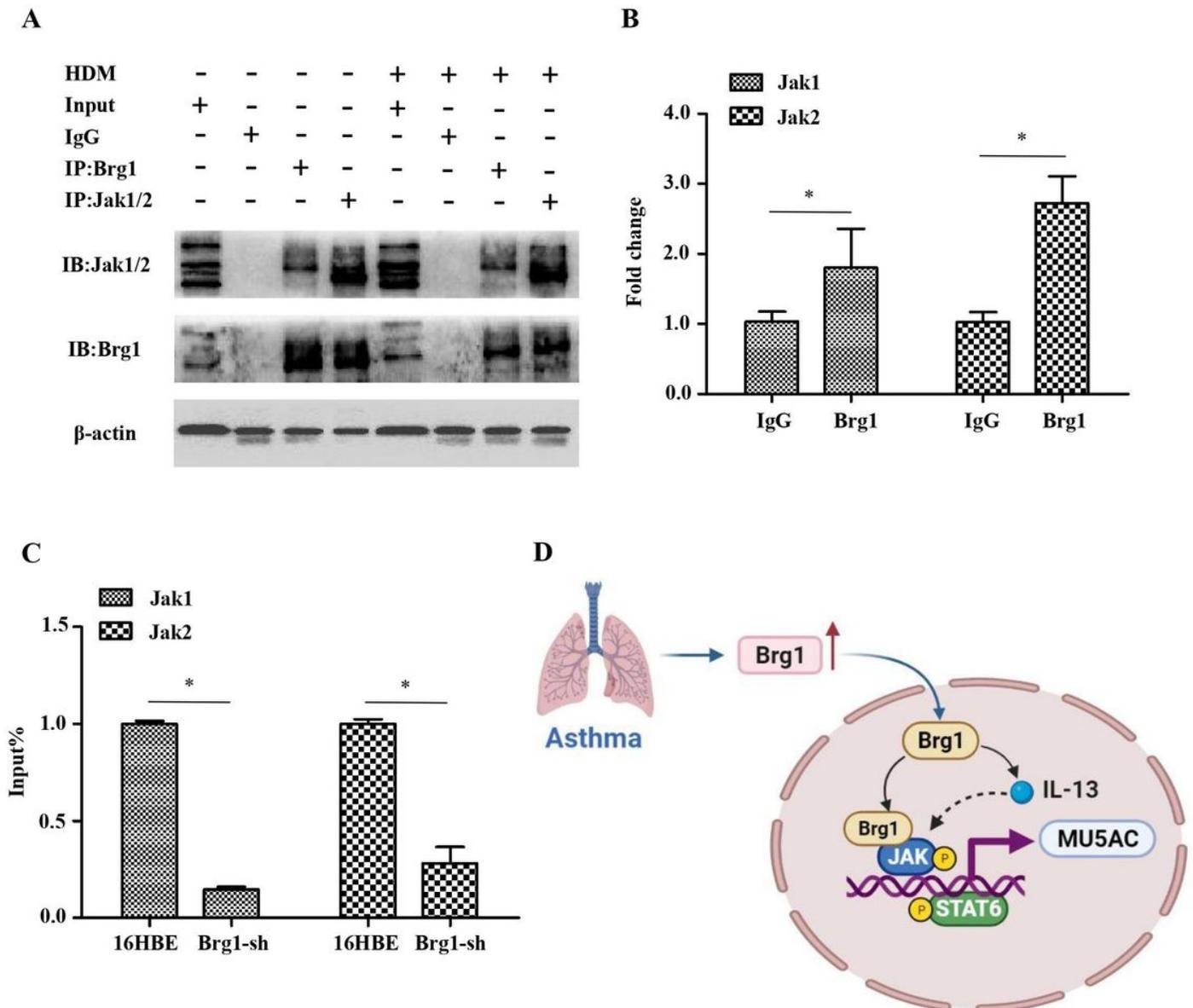


Figure 4

**Knockdown of the *Brg1* gene in 16HBE cells can reduce MUC5AC protein expression and JAK1/2-STAT6 signaling pathway activation.** (A) Western blot verification of *Brg1* protein knockdown in 16HBE cells. (B) Statistical analysis of the intensity of MUC5AC normalized to  $\beta$ -actin. (C) qPCR detection of the *Brg1* gene mRNA level in 16HBE cells. (D) Immunofluorescence detects the expression of MUC5AC protein in 16HBE cells after knocking down the *Brg1* gene. (E) Western blot was used to detect the expression of

MUC5AC protein in 16HBE cells after knocking down the *Brg1* gene. (F) Western blot was used to detect the activation of the JAK1/2-STAT6 signaling pathway in 16HBE cells after knocking down *Brg1*. (G) Statistical analysis of the percentage of p-JAK1/2 to total JAK1/2 protein. (H) Statistical analysis of the percentage of p-STAT6 to total STAT6 protein. (n=5 per group; \*P < 0.05 16HBE group compared with the *Brg1*-sh group).

**Figure 5**



**Figure 5**

***Brg1 can bind to the JAK1/2 gene transcription region, and may be a mechanism regulating the activation of the JAK1/2-STAT6 signaling pathway.*** (A) Co-immunoprecipitation found that *Brg1* and JAK1/2 protein bound to each other in 16HBE cells. (B) CHIP-qPCR verifies the specificity of *Brg1* antibody for CHIP experiment. (C) CHIP-qPCR experiment found that *Brg1* in 16HBE cells can bind to the JAK1 or JAK2 gene promoter region. (D) The mechanism model diagram of this research. (n=3 per group; \*P<0.05 16HBE group compared with the *Brg1*-sh group).