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ORIGINAL ARTICLE

Regulation of KDM2B and Brg1 on Inflammatory Response of Nasal Mucosa in CRSwNP

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Abstract—Chronic nasal sinusitis with nasal polyps (CRSwNP) is a reversible nasal mucosal remodeling disease caused by persistent inflammation and structural changes in chronic nasal mucosa. Although there have been many studies on the inflammation of the nasal mucosa epithelium, the mechanism remains unclear. Our study found that H3K4me3 histone demethylase KDM2B (also known as JHDM1B) and transcriptional regulator Brg1 (also called SNF2-β or Smarca4) were significantly decreased in nasal mucosa of CRSwNP patients, and they were positively correlated. Brg1 and KDM2B co-localize in the epithelial cells of nasal mucosa. We used poly(I:C)-treated nasal mucosal epithelial cells (HNECs) to find that the expression of KDM2B and Brg1 was also decreased, and the main expression position transferred from the nucleus to the nuclear membrane. We used small interfering RNA to knock down the expression of KDM2B and Brg1 in nasal epithelial cells. It was interesting to find that the decreased expression of KDM2B and Brg1 produced similar effects to that of poly(I:C)-treated cells, which could promote inflammatory response of nasal mucosal epithelial cells. And Brg1 appears to play a role in KDM2B regulating gene promoters of IL-6 and TNF-α inflammatory. This study shows that KDM2B and Brg1 may have an inhibitory effect on the development of CRSwNP nasal mucosal epithelial inflammation. This study will provide a new perspective for gene targeting therapy of CRSwNPs.

KEY WORDS: CRSwNP; nasal epithelium; KDM2B; Brg1; inflammation.

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INTRODUCTION

Chronic nasal sinusitis (CRS) is a clinical nasal syndrome characterized by inflammation of the nasal mucosa and paranasal sinuses, as well as the persistence of symptoms [1]. The incidence of CRS is high and difficult to cure, which seriously affects the quality of life of patients. CRS is classified into chronic nasal sinusitis without nasal polyps (CRSsNP) and chronic nasal sinusitis with nasal polyps (CRSwNP) based on endoscopy results [1]. According to the degree of infiltration of eosinophils in the nasal mucosa, CRSwNP can be divided into eosinophils (EosCRSwNP) and non-eosinophilic CRSwNP (non-EosCRSwNP) [2, 3]. Some researchers have indicated that inflammation of CRSwNP is primarily caused by inappropriate or excessive immune responses to foreign factors, leading to persistent mucosal inflammation [4]. However, in most cases of CRSwNP, the etiology and pathogenesis remain unclear. Therefore, studying the pathogenesis and treatment strategies of CRSwNP is essential for improving the quality of life of patients with CRS.

Nasal mucosal epithelial cells play an active role in both innate and acquired immune responses by producing a variety of inflammatory cytokines [5]. Inflammatory factors secreted by nasal epithelial cells include TNF-α, IFNα/β, GM-CSF, eosinophil chemotactic factor, IL-6, IL-8, IL-13, GRO-α, MDC, TARC, MCP-4, BAFF, IL-25, IL-33, TSLP, *etc.* [6–9]. Nasal mucosal epithelial cells not only play an important role in regulating innate responses, they can regulate subsequent adaptive immune responses also. In addition, remodeling of nasal epithelial structure caused by abnormal proliferation and differentiation of epithelial cells is also an important cause of CRSwNP [10]. It is unclear that whether the epithelial cells inflammatory response is the underlying variation in the etiology and pathogenesis of CRSwNP, but it may be an area of active research that is interfering with epithelial cells cytokine expression for therapeutic CRSwNP [11, 12].

In recent years, the role of chromatin modifiers in the immune system has drawn increasing attention, and it plays an irreplaceable role in the temporal and spatial patterns of gene expression in the immune system [13]. Our previous study found that Brg1 is expressed in nasal epithelial cells and is significantly reduced in CRSwNP tissue, which has attracted our attention. Brg1 is one of the subunits of the BAF chromatin remodeling complex which is associated with the Swi/Snf complex [14, 15]. The SWI/SNF chromatin remodeling complex is recruited by the sequence-specific transcription factor to the promoter of the target gene, where they slide or expel the histone octamer in an ATP-dependent manner, thereby acting to affect gene expression [16]. Brg1 can bind to enhancers/promoters and participate in T cell activation and/or effector cell differentiation [17]. Brg1 is also required for Th2 differentiation and Th2 cytokine transcription [18]. Although many studies have shown that Brg1 can be involved in the regulation of immune response, its binding is related to gene activity, but the regulation of Brg1 on the expression of inflammatory factors in CRSwNP is unknown.

Our previous studies have found that Brg1 and demethylase KDM2B are significantly reduced in CRSwNPs, both of which can regulate the inflammatory response of cells, but the relationship between them and the effect on CRSwNPs is not clear. Typically, transcriptionally active chromatin is labeled with methylated histone H3 lysine 4 (H3K4), while transcriptional repression is synonymous with methylated H3K9 and H3K27 [19]. Studies have shown that methylation of H3K4 and H3K27 histones can alter the activity of brg1 [20]. Interestingly, we found that the expression of H3K36 [21, 22] and H3K4me3 [23, 24] demethylase KDM2b (also known as FBXL10 or JHDM1B) was also significantly reduced in CRSwNP tissues, positively correlated with the expression of Brg1, which has aroused our concern. KDM2b was the first paralog to identify the JmjC domain containing the histone demethylase FBXL11. In addition to the CxxC zinc finger that recognizes unmethylated CpG islands, it also contains a PHD domain, an F-box domain, and a leucine-rich repeat (LRR) [25, 26]. The JmjC domain is required for demethylation of the KDM2b target. KDM2b has been shown to play a key role in the self-renewal of solid tumors and cancer stem cells [27–30], but its role in remodeling of nasal epithelial cells and secretion of inflammatory factors is currently unclear.

In the present study, our results demonstrated that the expression of Brg1 and KDM2B in CRSwNPs was significantly decreased. Knockout of Brg1 and KDM2B could affect the expression of inflammatory factors in nasal epithelial cells. KDM2B can affect the inflammatory response of nasal mucosal epithelial cells by directly acting on the promoter of the inflammatory factor gene. To further explore the regulatory effects of Brg1 and KDM2B on epithelial cell inflammation *in vitro*, we used poly(I:C), a double-stranded RNA analogue of respiratory viruses, such as respiratory syncytial virus, influenza A virus, and rhinovirus [31], as model stimulants. Poly(I:C) can induce inflammation signal transduction well, and some key receptors and related intracellular inflammation signal

transduction pathways have been clearly described [32]. In addition, the knockout of Brg1 may affect the recruitment of KDM2B to inflammatory factors. Therefore, we believe that Brg1 may assist KDM2B in inhibiting the inflammatory response of CRSwNPs. This study has determined that the expression of Brg1 and KDM2B can modulate the inflammatory response of CRSwNPs, and it can serve as a novel essential regulator of the inflammatory response. Perhaps, Brg1/KDM2B may serve as a new therapeutic target and clinical strategy for the regulation of inflammation-related diseases in the future.

MATERIALS AND METHODS

Study Population

Biopsy of patients with EosCRSwNPs ($n = 16$), non-EosCRSwNPs ($n = 16$), and healthy controls ($n = 22$) were obtained from the Department of Otorhinolaryngology, Second Hospital of Shandong University and Provincial Hospital Affiliated to Shandong University. Three months before the study, no patients received glucocorticoid or other antibody treatment. Mucosal biopsy of the inferior turbinate (IT) was obtained from 22 non-NP patients with nasal septal deviation. In our study, the criterion for the distinction between EosCRSwNPs and non-EosCRSwNPs was that CRSwNP was classified as eosinophils when the percentage of tissue eosinophils exceeded 10% of total infiltrating cells. All clinical samples (nasal polyps) obtained during surgery were divided into three parts: one was fixed in formalin for histological evaluation, and the other two were used to extract RNA and protein to detect the corresponding gene and protein expression. Table 1 details the specific information of the patient. The approval of this study came from the institutional review committee of the Chinese participating hospital (China, approval number: KYLL-2019(LW)002).

Exclusion criteria of patients included the following: (1) patients who complicated with nasal polyps, fungal rhinosinusitis, cystic fibrosis, and rhinitis or/and asthma; (2) history of glucocorticoid usage in the past 1 month; (3) patients who suffered from acute upper respiratory tract infection within 4 weeks. The diagnosis of allergic rhinitis is based on the diagnostic criteria of ARIA2008, which is based on GINA criteria.

Reagents

Poly(I:C) were purchased from InvivoGen (cat. no. tlrl-pic, InvivoGen). Rabbit polyclonal anti-KDM2B antibody (cat. no. 09-864, 1:3000 diluted for WB, 1:100 diluted for IF) was from Millipore, and rabbit polyclonal anti-GAPDH antibody (cat. no. AC027, 1:5000 diluted) was from ABclonal. Mouse polyclonal anti-Brg1 antibody (cat. no. 66561-1-lg, 1:2000 diluted for WB, 1:100 diluted for IF) was from Proteintech. Fluoroshield™ with DAPI was purchased from Sigma-Aldrich (cat. no. F6057).

Cell Culture and RNA Interference

Human nasal epithelial cells (HNECs) were purchased from Cellbio (cat. no. CBR130829, CellBio); it was grown and maintained in high-sugar medium (cat. no. 11965-092, Gibco) supplemented with 10% fetal bovine serum (FBS) (cat. no. 10099141, Invitrogen). Cells were plated at a density of 3×10^5 cells per 60 mm dish and cultured for 24 h.

siRNAs were obtained from GenePharma of Shanghai. The sequence of siRNAs is shown in Table S1.

HNECs cells were transfected with 30 nM siRNAs using jetPRIME® Transfection Agent (cat. no. 114-15, Polyplus) according to the manufacturer's instruction. In order to increase transfection efficiency, HNECs were transfected twice. After culture for 24 h, the second transfection was performed. Cells were cultured for 24 h and collected.

RNA Extraction and Quantitative Real-Time PCR

Total RNA of frozen nasal tissues or HNECs were extracted using TRIzol (cat. no. 15596026, Invitrogen). RNA reverse transcription was performed by RT reactions with PrimeScript™ RT reagent kit according to the manufacturer's protocol (cat. no. RR047A, Takara). And mRNA levels were detected by SYBR Green gene expression assays with TB Green™ Premix Ex Taq™ (Tli RNaseH Plus) (cat. no. RR420A, Takara). Quantitative real-time PCR (qPCR) was performed with qPCR primers; the sequence of primers is shown in Table S2.

Amplification and detection were run with an initial cycle of 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s, 60 °C for 20 s, then 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s. All PCR reactions were performed in triplicate. Negative control samples (without template) were processed in the same way as the experimental group. Relative gene expression was calculated using the comparative $2^{-\Delta\Delta Ct}$ method with the GAPDH as a reference. Each experiment was repeated three times.

Table 1. Patients' Characteristics

	Nasal mucosa: healthy control	EosCRSwNPs	Non-EosCRSwNPs
Sample sizes	22	16	16
Median age, years (IQR)	39.45 (15–54)	38.5(12–76)	45.9 (26–69)
Gender, male/female	14/8	10/6	9/7
Atopy, *n*/*N*	1/22	2/16	1/16
Asthma, *n*/*N*	0	1/16	0
Median CT score (IQR)	0	7.68 (5–12)***	7.50 (3–13)***
Median endoscopy score (IQR)	0	6.56 (3–9)***	6.12 (2–9)***
Eosinophilia‡	1/22	16/16	0

The level of significance (*P*) was obtained from the Student's *t* test. *P* value of < 0.05 was considered statistically significant. * means the *P* value is < 0.05, while symbol. ** means the *P* value is < 0.01. *** means the *P* value is < 0.001. Gender comparison was performed using the χ^2 test. ‡The percentage of eosinophils exceeding 10% was categorized as eosinophilia or neutrophilia
CT computed tomography, *IQR* interquartile range

Protein Isolation and Western Blot

Proteins of HNECs cells or frozen nasal tissues are extracted as previously described; briefly described, samples were homogenized in ice-cold RIPA lysis buffer (cat. no. P0013B, Beyotime) with 1 mM PMSF (cat. no. P0100, Solarbio) and 1× protease inhibitor cocktail (Roche). After homogenized on ice for 20 min, samples are centrifuged at 4 °C and the supernatant is collected. The protein samples were separated on a 12% SDS-PAGE gel and transferred to PVDF membrane (cat. no. IPVH00010, Millipore). The PVDF membranes were incubated with corresponding primary antibodies overnight at 4 °C, followed by incubation with the appropriate secondary antibodies for 1 h at room temperature; the signals were detected with the ECL system (Cell Signaling Technology).

Chromatin Immunoprecipitation Assay

Chromatin immunoprecipitation (ChIP) assay was performed using the ChIP Assay Kit (cat. no. 17-371, Millipore, USA) according to the manual instructions with slight modifications. Briefly, HNECs cells were crosslinked with 1% formaldehyde solution at room temperature for 10 min and quenched with 125 mm glycine. Cells were centrifuged for 3 min with 1500 rpm at 4 °C and washed twice in cold PBS. Cell suspension was centrifuged at 4 °C for 5 min at 1500 rpm. DNA fragments of 200–500 bp were obtained by ultrasound. The lysate was then immunoprecipitated with anti-KDM2B (1:1000), anti-Brg1 (1:1000), or IgG antibodies. Immunoprecipitated DNA fragments were analyzed by SYBR Green gene expression assays. The primers used in this study were shown in Table S3.

Aliquots of lysates containing 200 mg of nuclear protein were used for each immunoprecipitation reaction.

Flow Cytometry for Various Cytokines

HNECs (1×10^5 cells/well) were seeded in six-well plates and transfected as described above. Then cells were treated with poly(I:C) at a concentration of 50 μg/ml for 6 h after transfection for 24 h, then supernatant was collected. In order to detect the expression of cytokines in supernatant, we use LEGENDplexTM Human Th1/Th2 Cytokine Panel (cat. no. 740729, BioLegend) according to the manufacturer's instructions to quantify eight human cytokines in the cell culture supernatant by flow cytometry. For each sample, cytometric analyses were performed on a FACSCalibur (BD Biosciences). The main inflammatory cytokines tested included IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IFN-γ, and TNF-α.

Statistical Analysis

Data were shown as mean ± SD from at least three independently performed experiments. Comparisons of parameters between two groups were analyzed using unpaired Student's *t* test. Statistical significance was analyzed by a two-tailed paired *t* test by using GraphPad Prism Software 6.0 (GraphPad Software, La Jolla, CA). A *P* value < 0.05 was considered statistically significant. Each data point was expressed as mean (standard deviation).

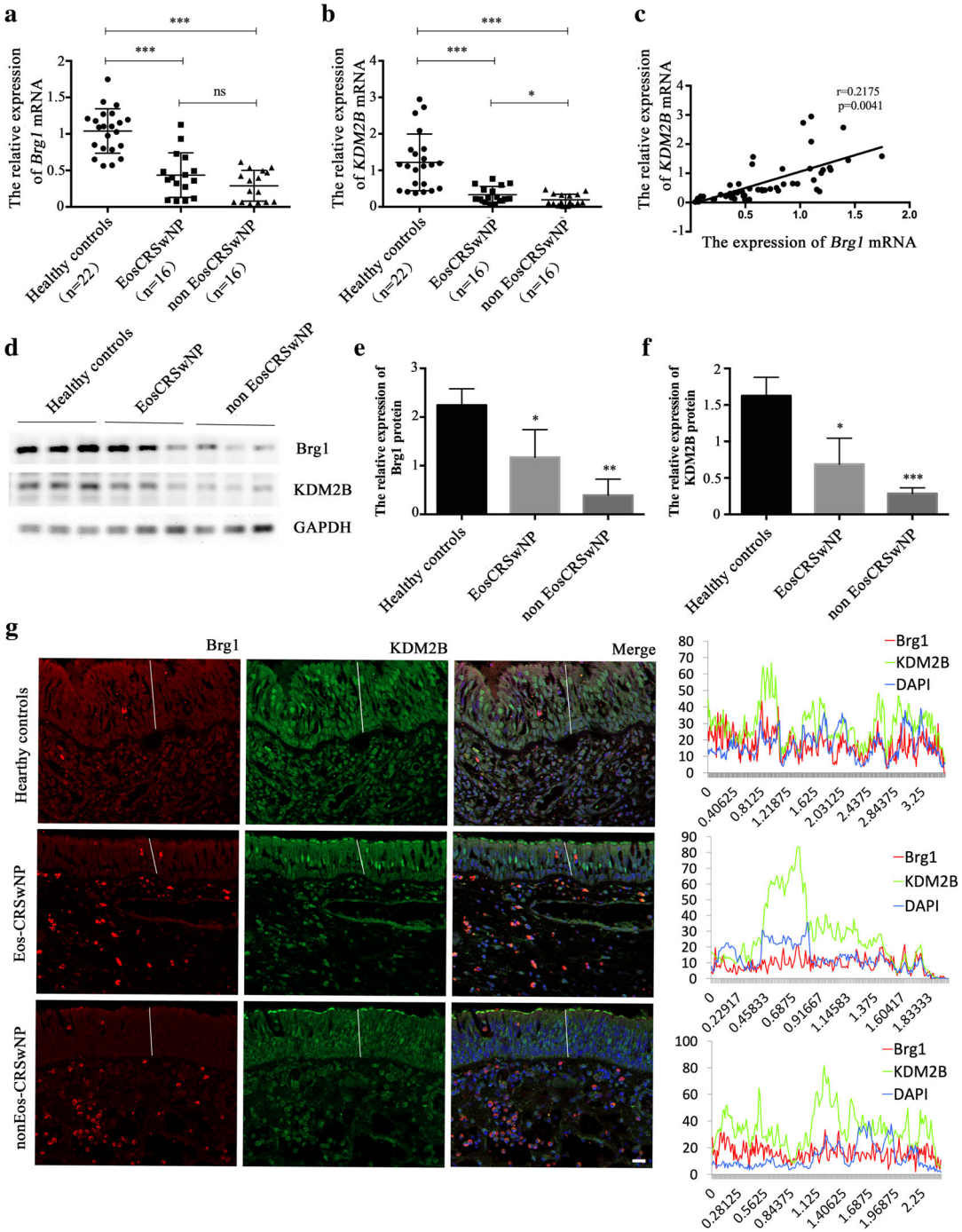


Fig. 1. Low expression of Brg1 and KDM2B in CRSwNPs nasal mucosa. **a**, **b** The relative mRNA levels of *Brg1* and *KDM2B* in the total nasal mucosal tissue of controlled, EosCRSwNP, and non-EosCRSwNP were detected by real-time quantitative PCR. **c** Significant positive correlations between *Brg1* and *KDM2B* mRNA expression were detected in all samples by real-time quantitative PCR ($n = 54$). **d** Western blotting was used to analyze the expression of Brg1 and KDM2B in the total nasal mucosal tissue protein of controlled, EosCRSwNP, and non-EosCRSwNP, where GAPDH served as a control. **e**, **f** The relative expression levels of Brg1 and KDM2B protein were assessed. **g** Expression patterns of Brg1 and KDM2B in nasal mucosal tissue of controlled, EosCRSwNP, and non-EosCRSwNP (×400 magnification; KDM2B stained in green; Brg1 stained in red; nucleus stained in blue; scale bar = 20 μm). The fluorescent intensity was quantified using ImageJ. The bar graphs and the table show quantification of the results, with each value represents the mean ± SD of three independent experiments. Statistical significance is shown using the Student's *t* test analysis; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

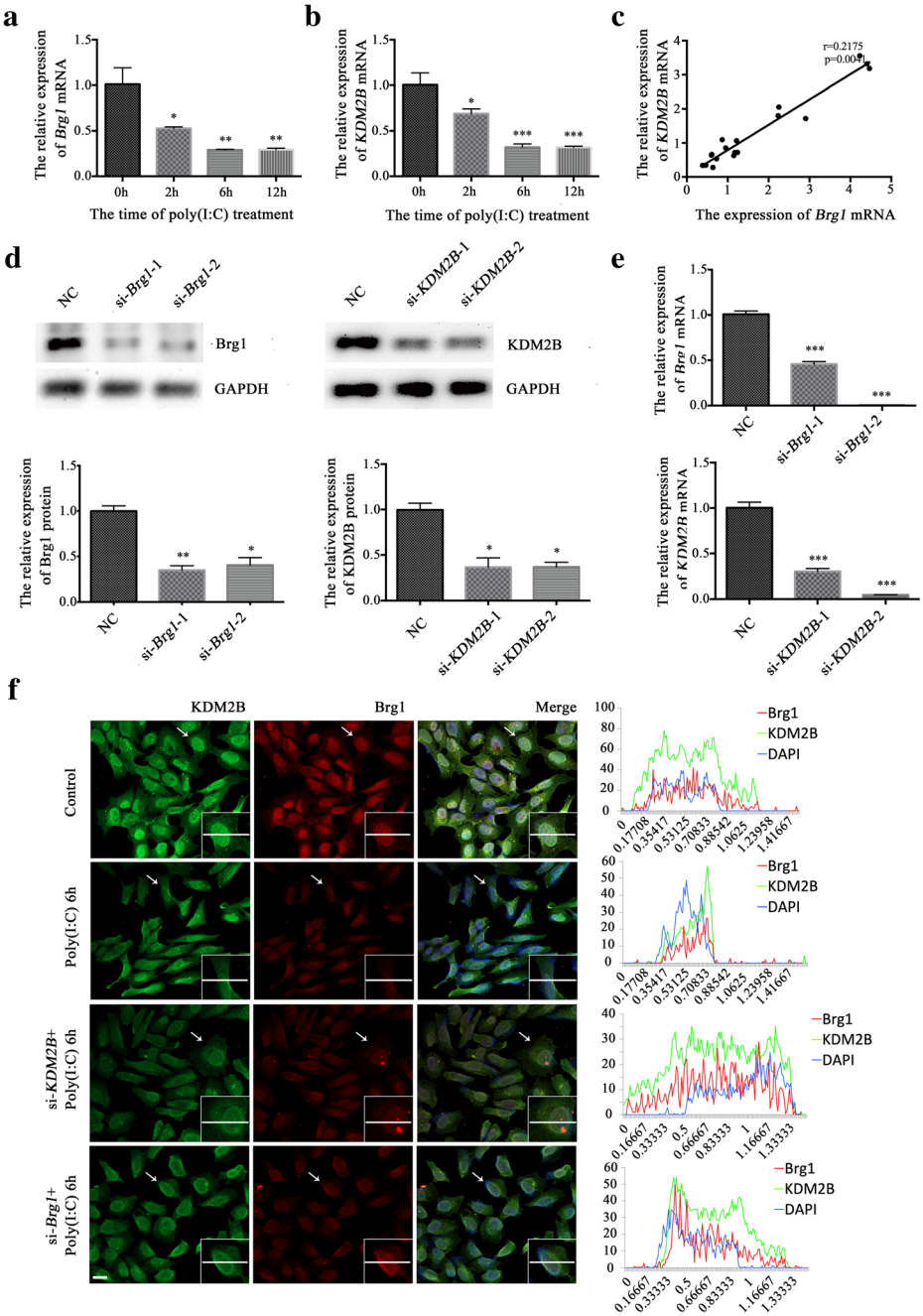


Fig. 2. Expression of Brg1 and KDM2B in nasal mucosal epithelial cells decreased with poly(I:C) treatment. **a**, **b** Poly(I:C) was added to the medium with low concentration of serum, and RNA were collected at different time points. The relative expression mRNA levels of *Brg1* and *KDM2B* were detected by qPCR. **c** Significant positive correlations between *Brg1* and *KDM2B* mRNA expression were detected by real-time quantitative PCR. **d** *Brg1* and *KDM2B* si-RNA were transfected into HNECs cells, and for efficient gene silencing, siRNAs were transfected twice, then the cells were harvested and lysed, and the supernatants were harvested for western blotting. Brg1 and KDM2B protein levels were detected, GAPDH as a control. The relative expression levels of protein were assessed. **e** After si-RNA were transfected, the relative mRNA levels of *Brg1* and *KDM2B* were detected by real-time quantitative PCR. **f** Immunofluorescence was used to detect the expression of Brg1 and KDM2B in HNECs after induction of poly(I:C), and the effect of si-Brg1 and si-KDM2B transfection on this process (×400 magnification; KDM2B stained in green; Brg1 stained in red; nucleus stained in blue; scale bar = 20 μm). The fluorescent intensity was quantified using ImageJ. The bar graphs and the table show quantification of the results, with each value represents the mean ± SD of three independent experiments. Statistical significance is shown using the Student's *t* test analysis; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

RESULTS

Low Expression of Brg1 and KDM2B in CRSwNPs Nasal Mucosa

In order to detect the expression of Brg1 and KDM2B in CRSwNP nasal mucosa, we selected the nasal mucosa obtained from surgery as the study object, 16 patients with eosinophilic chronic rhinitis-sinusitis (EosCRSwNPs), 16 patients with non-eosinophilic chronic rhinitis-sinusitis (non-EosCRSwNPs), and another 22 patients with nasal septum deviation as a healthy control. We detected the mRNA and protein expression of Brg1 and KDM2B in the nasal mucosa of EosCRSwNPs, non-EosCRSwNPs, and healthy controls by fluorescence real-time quantitative PCR (Fig. 1a, b) and Western blot (Fig. 1d~f), respectively. The results showed that the expression of Brg1 and KDM2B was significantly decreased in CRSwNP tissues compared with healthy controls, and the expression was significantly lower in non-EosCRSwNPs tissues, but the expression levels of Brg1 and KDM2B in EosCRSwNPs and non-EosCRSwNPs tissues were not significantly different (Fig. 1a~f). Interestingly, we found a positive correlation between KDM2B and Brg1 expression in nasal mucosa (Fig. 1c).

To clarify the expression pattern of Brg1 and KDM2B in the nasal mucosa epithelium, we performed immunofluorescence staining on tissue samples. The results showed that Brg1 was detected in the nasal epithelium like KDM2B, although Brg1 was not mainly expressed in the epithelium of the nasal mucosa, and the expression of Brg1 in the nasal epithelium was weaker than that of KDM2B (Fig. 1g). Moreover, the expression of Brg1 and KDM2B was attenuated in the CRswNP nasal mucosa epithelium. KDM2B is mainly localized to the nucleus of nasal epithelial cells. And in contrast to EosCRSwNP and non-EosCRSwNP, Brg1 and KDM2B are more likely to accumulate in the nucleus of nasal epithelial cells in healthy controls. We found a co-localization between the two by linear scanning of fluorescence intensity, but the results need to be further determined (Fig. 1g).

Expression of Brg1 and KDM2B in Nasal Mucosal Epithelial Cells Decreased with Poly(I:C) Treatment

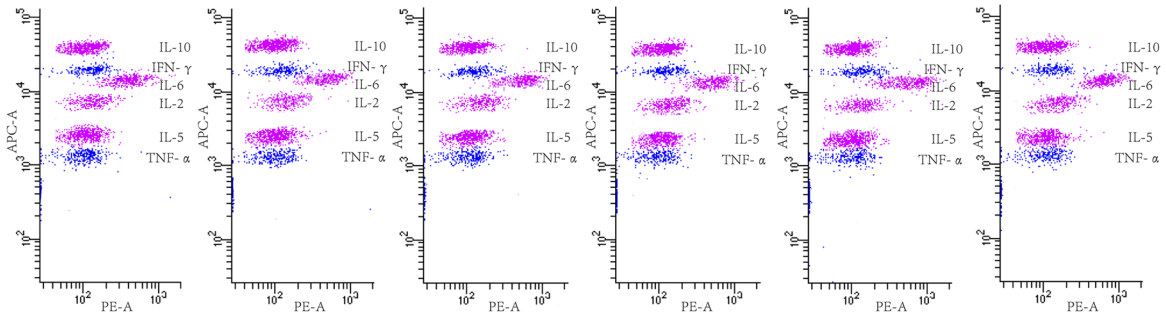
To examine the expression of Brg1 and KDM2B in nasal epithelial cells and the relationship between CRSwNP and their expression, we used poly(I:C) to stimulate nasal mucosal epithelial cell lines (HNECs). The expression of Brg1 and KDM2B in HNECs cells after poly(I:C) stimulation was detected by real-time quantitative PCR. The results showed that the expression of Brg1 and KDM2B decreased with the stimulation of poly(I:C) (Fig. 2a, b). And the expression levels of both are positively correlated (Fig. 2c), and we note that this is similar to the expression of Brg1 and KDM2B observed in our CRSwNP tissues.

To further investigate the relationship and function of Brg1 and KDM2B, we attempted to knock down the expression of Brg1 and KDM2B in HNECs cells. We transfected the si-RNA of Brg1 and KDM2B in HNECs cells, and detected the interference efficiency by Western blot and real-time quantitative PCR. We were pleased that the effective knockout of Brg1 and KDM2B was detected (Fig. 2d, e). We next examined the localization patterns of Brg1 and KDM2B in HNECs by immunofluorescence staining. We next examined the localization patterns of Brg1 and KDM2B in HNECs by immunofluorescence staining. The results show that Brg1 and KDM2B are co-localized in the nucleus of HNECs. However, when we treated cells with poly(I:C), although Brg1 and KDM2B were still co-localized, the co-localization position deviated from the nucleus, mainly co-localized to the nuclear membrane, and the expression of Brg1 and KDM2B was significantly decreased (Fig. 2f). The expression of Brg1 and KDM2B was disturbed by si-RNA, and the localization of Brg1 and KDM2B in HNECs cells showed a similar form to that of poly(I:C)-treated cells, which is very interesting (Fig. 2f). We further determined the fluorescence changes in cells after Brg1 and KDM2B knockdown or poly(I:C) treatment by linear scanning of fluorescence intensity (Fig. 2f), which made the results clearer.

Brg1 and KDM2B Can Affect Inflammatory Cytokine Expression

To investigate whether expression of Brg1 and KDM2B affects inflammatory cytokine production, we examined changes in various inflammatory cytokines in HNECs cells by flow cytometry. The results showed that compared with the control cells, poly(I:C) mainly caused up-regulation of IL-6, IL-2, TNF-α, and IFN-r inflammatory factors, while IL-5 and IL-10 did not change significantly. In addition, IL-4 and IL-13 were not detected due to too low expression levels, and the results were not shown (Fig. 3a). Subsequently, we found that knockdown of Brg1 and KDM2B produced a similar change in IL-6 as that caused by poly(I:C). Treatment of cells with poly(I:C) after knockdown of Brg1 and KDM2B enhanced IL-6 up-regulation. The results showed that knockdown of Brg1 and KDM2B both affected the expression of IL-6, but TNF-α was mainly affected by the expression of KDM2B. Brg1 and KDM2B also had a certain effect on the

a



	IL-5	IL-10	IL-6	IL-2	TNF- α	IFN- γ
NC	1.89±0.12	1.05±0.07	47.34±0.70	<1.94	4.36±0.37	<1.95
NC+poly(I:C)	1.82±0.12	1.11±0.10	66.96±9.60	2.99±0.44	6.76±0.84	2.71±0.10
si-Brg1	1.97±0.15	0.95±0.04	77.28±4.26	3.88±0.01	4,36±0.37	2.21±0.10
si-Brg1+ poly(I:C)	1.99±0.13	1.08±0.10	73.52±0.46	2.32±0.01	3.31±0.68	2.39±0.19
si-KDM2B	1.67±0.13	1.00±0.05	84.38±1.38	2.83±0.57	3.86±1.46	<1.95
si-KDM2B+ poly(I:C)	1.85±0.15	1.16±0.10	90.07±0.84	3.56±0.33	5.93±0.61	2.80±0.19

b

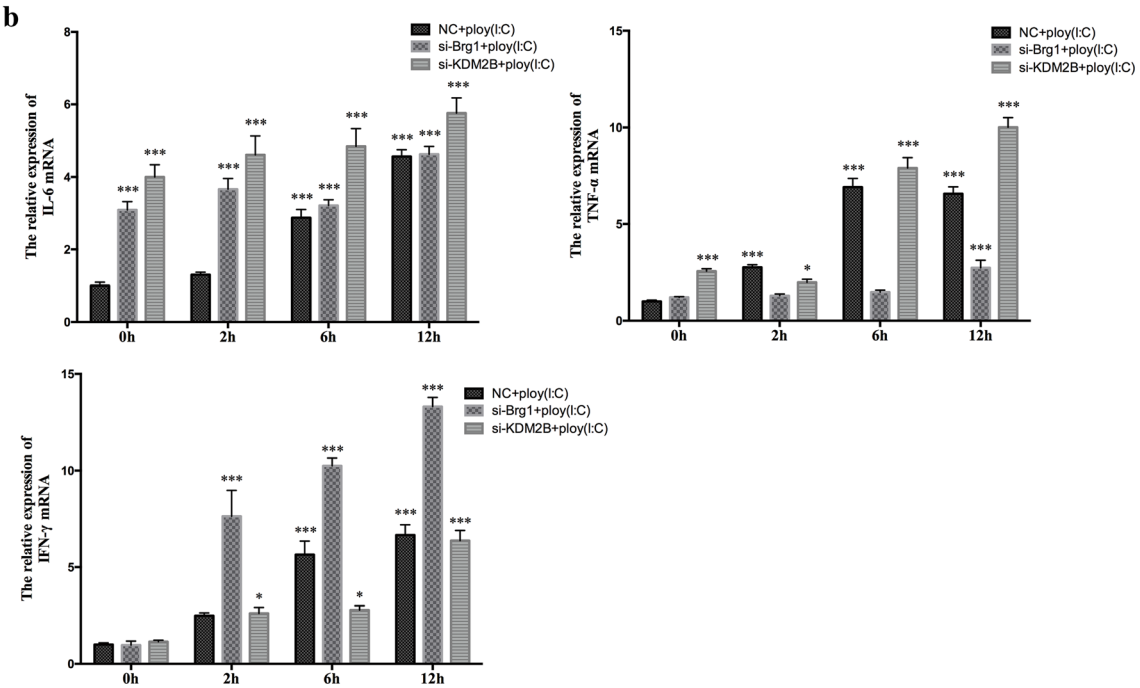


Fig. 3. Brg1 and KDM2B regulate inflammatory cytokine expression. **a** HNECs cells were transfected with si-RNA of Brg1 and KDM2B and treated with poly(I:C) for 6 h. The cell culture supernatant is then collected and the levels of various inflammatory cytokines in the supernatant are measured by flow cytometry. **b** The relative mRNA levels of IL-6, IFN-γ, and TNF-α in HNECs cells were detected by real-time quantitative PCR. The bar graphs and the table show quantification of the results, with each value represents the mean ± SD of three independent experiments. Statistical significance is shown using the Student's *t* test analysis; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

expression of IL-2. In contrast, IFN-r was mainly affected by the expression of Brg1 (Fig. 3a). And IFN-r did not show significant up-regulation changes similar to IL-6. In order to further determine the above experimental results, we used real-time quantitative PCR to detect the expression of IL-6, IL-2, TNF-α, and IFN-r inflammatory cytokine mRNA in HNECs cells. The results showed that knockdown of Brg1 and KDM2B both affected the expression of IL-6, but TNF-α was mainly affected by the expression of KDM2B. In contrast, IFN-r was mainly affected by the expression of Brg1 (Fig. 3b). In addition, we did not observe significant changes in IL-2 mRNA and the results were not shown. We hypothesized that IL-2 expression regulation may be different from IL-6, TNF-α and IFN-r, but we have not discussed it in detail here.

Brg1 Assists KDM2B in Directly Inhibiting Inflammatory Cytokine Transcription

To understand how Brg1 and KDM2B affect the expression of inflammatory cytokines, we performed ChIP experiments. It was found in the experimental results that poly(I:C) treatment enhanced the occupancy rate of KDM2B in IL-6 and TNF-α inflammatory gene promoters in HNECs cells, except for IFN-γ (Fig. 4a, c, e). It is noteworthy that Brg1 appears to play a role in KDM2B regulating of the gene promoters of IL-6 and TNF-α inflammatory in HNECs cells, as Brg1 knockdown attenuates KDM2B occupancy of inflammatory gene promoters, indicating that Brg1 may play a role in the promoter region where KDM2B is recruited to pro-inflammatory mediators. Interestingly, we also observed that Brg1 can also directly act on the promoter regions of IL-6 and IFN-γ, but the knockdown of KDM2B has little effect on the occupancy of Brg1 in inflammatory gene promoters (Fig. 4b, d, f). So we believe that Brg1 assists KDM2B to directly inhibit inflammatory cytokine transcription, but this effect is not mutual.

DISCUSSION

Brg1 is a catalytic subunit of BAF complex, and mammalian chromatin remodeling is a BAF chromatin remodeling complex related to yeast Swi/Snf complex [14]. Regulation of gene expression is primarily dependent on sequence-specific transcription factors, and transcription factors must act together with enzymes that regulate chromatin structure. These enzymes can be divided into ATP-dependent chromatin remodeling and histone modifying enzymes [33, 34]. Many studies have shown that Brg1 can participate in the regulation of the immune system by regulating transcription factors. Brg1 usually binds gene enhancers/promoters according to the status of cell activation and/or effector lineage differentiation, and the binding patterns of different target genes are different [17]. Some data suggest that Brg1 has the ability to regulate the remodeling activity of some target genes in early thymocytes [35], and Brg1 can increase the transcriptional activation of EBF (early B cytokine) and Pax5 transcription factors to promote BCR signal transduction [36]. However, the role of Brg1 in the inflammatory response of chronic rhinitis and sinusitis is still unclear.

In our study, Brg1 may be involved in the regulation of inflammatory factor gene expression in chronic rhinitis and sinusitis together with demethylase KDM2B. KDM2B is a key driver of epigenetic program; it can regulate gene expression by regulating the transcription of target genes. KDM2B can silence gene expression by interacting with abnormal PRC1 complexes containing cyclic 1b E3 ubiquitin ligase and other proteins, such as SKP1 and PCGF1/NSPC1 (BMI1 paralog) [25, 37–39]. In our study, the expression of Brg1 and KDM2B in chronic rhinitis and sinusitis with nasal polyps was significantly decreased, and both of them were localized in nasal epithelial cells, which attracted our attention (Fig. 1). However, the role and function of KDM2B in chronic rhinitis and sinusitis are not clearly defined, as Brg1.

Nasal epithelial cells are the first barrier of the nasal immune system to the external environment. The remodeling of nasal epithelial cells and abnormal damage of the immune system are important manifestations of chronic rhinitis and sinusitis. Therefore, the study on the structural development of nasal mucosal epithelial cells and the regulation of immune system is an important research content of chronic rhinitis and sinusitis. In order to study the role and function of KDM2B and Brg1 in nasal epithelial cells, we observed the localization pattern of Brg1 and KDM2B in nasal epithelium and cells by immunofluorescence staining (Fig. 1g, Fig. 2f). The results showed that Brg1 and KDM2B were co-localized mainly in the nucleus of epithelial cells. Many studies have shown that Brg1 and KDM2B are related to cell proliferation [22, 40]; we used si-RNA to interfere with the expression of Brg1 and KDM2B in nasal epithelial cell lines. We examined the effects of Brg1 and KDM2B knockdown on the proliferation of epithelial cells (The results were not shown). The results showed that Brg1 and KDM2B had little effect on the proliferation of nasal epithelial cells.

In order to further study the function of Brg1 and KDM2B in chronic rhinitis and sinusitis, poly(I:C) was used to stimulate nasal epithelial cells, and the expression of Brg1 and KDM2B and the secretion of inflammatory

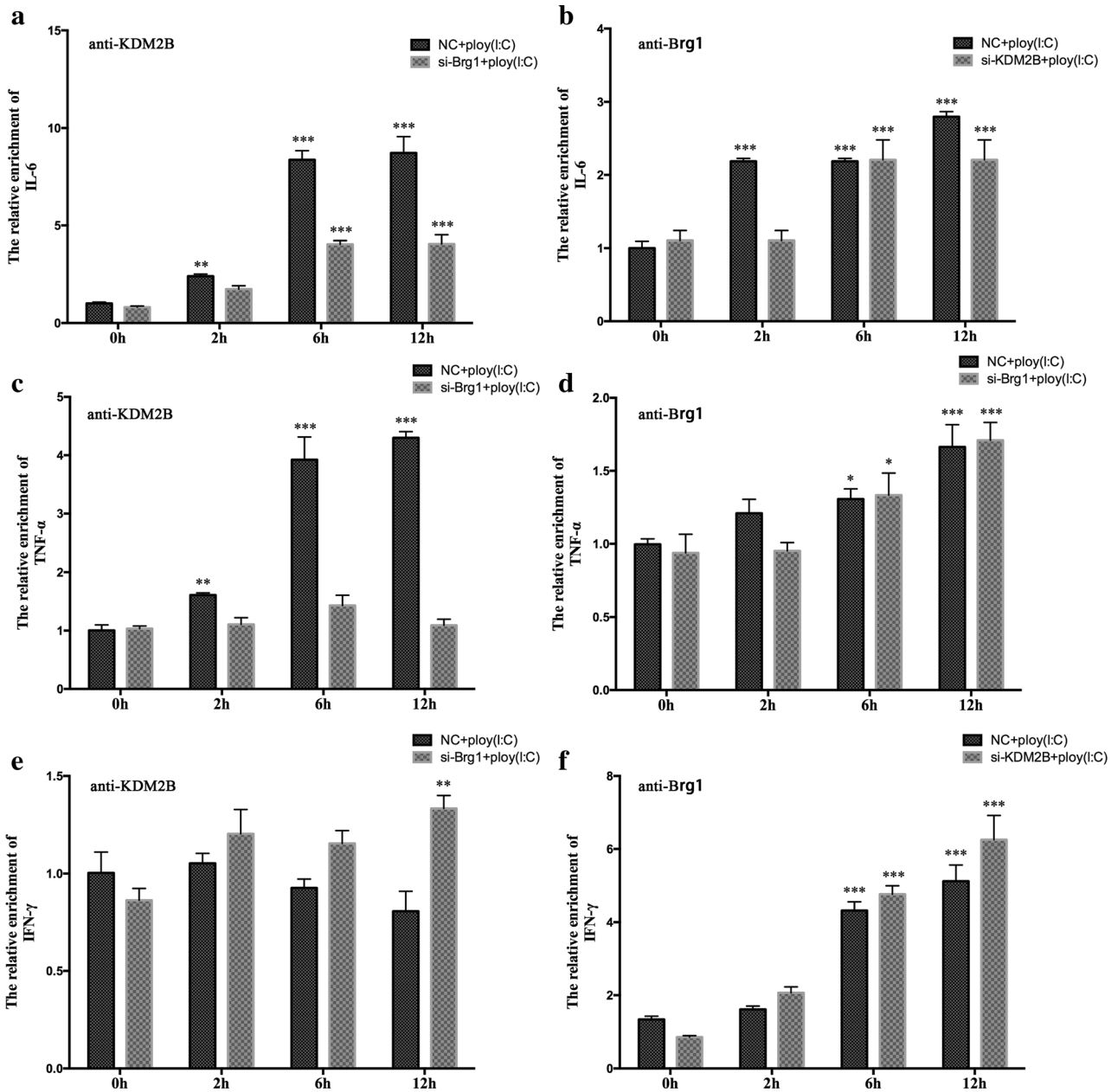


Fig. 4. Brg1 assists KDM2B in directly inhibiting inflammatory cytokine transcription. **a** HNECs cells were transfected with si-RNA of Brg1 followed by treatment with poly(I:C) for 2, 6, and 12 h. ChIP assay was performed with anti-KDM2B. qPCR analysis was done to evaluate the occupancy of KDM2B at the IL6 promoter in cells. **b** HNECs cells were transfected with si-RNA of KDM2B followed by treatment with poly(I:C) for 2, 6, and 12 h. ChIP assay was performed with anti-Brg1. qPCR analysis was done to check the occupancy of Brg1 at the IL6 promoter in cells. **c** HNECs cells were transfected with si-RNA of Brg1 followed by treatment with poly(I:C) for 2, 6, and 12 h. ChIP assay was performed with anti-KDM2B. qPCR analysis was done to evaluate the occupancy of KDM2B at the TNF-α promoter in cells. **d** HNECs cells were transfected with si-RNA of KDM2B followed by treatment with poly(I:C) for 2, 6, and 12 h. ChIP assay was performed with anti-Brg1. qPCR analysis was done to check the occupancy of Brg1 at the TNF-α promoter in cells. **e** HNECs cells were transfected with si-RNA of Brg1 followed by treatment with poly(I:C) for 2, 6, and 12 h. ChIP assay was performed with anti-KDM2B. qPCR analysis was done to evaluate the occupancy of KDM2B at the IFN-γ promoter in cells. **f** HNECs cells were transfected with si-RNA of KDM2B followed by treatment with poly(I:C) for 2, 6, and 12 h. ChIP assay was performed with anti-Brg1. qPCR analysis was done to evaluate the occupancy of Brg1 at the IFN-γ promoter in cells.

factors were detected (Figs. 2a~e and 3). The results showed that poly(I:C) stimulation could induce the expression of Brg1 and KDM2B in cells to decrease, and the location of Brg1 and KDM2B was deviated from the nucleus. We used si-RNA to interfere with the expression of Brg1 and KDM2B in nasal epithelial cell lines, and found that the knockdown of Brg1 and KDM2B could promote the expression of inflammatory factors, which was consistent with the effect of poly(I:C) stimulating cells. Therefore, we believe that Brg1 may cooperate with KDM2B to inhibit the expression of inflammatory factors in chronic rhinitis and sinusitis. In addition, our study also showed that Brg1 knockdown reduced the occupancy of KDM2B to IL-6 and TNF-α promoters, and reduced the transcriptional inhibition of IL-6 and TNF-α induced by KDM2B (Fig. 4a, c, e). On the contrary, the knockdown of KDM2B had little effect on the occupancy of Brg1 to the promoter of inflammation gene (Fig. 4b, d, f). Therefore, we believe that Brg1 may assist KDM2B in recruiting into the promoter region of pro-inflammatory mediators and assist KDM2B in the transcriptional inhibition of inflammatory factors. In this study, we defined the important roles of Brg1 and KDM2B in the pathogenesis of chronic rhinitis and sinusitis.

Accurate biomarkers are important for the treatment of chronic rhinitis and sinusitis. Identification of genes and proteins that affect the development of inflammatory response in chronic rhinitis and sinusitis is important for targeting chronic rhinitis and sinusitis. Our results suggest that Brg1 and KDM2B may be involved in the regulation of chronic rhinitis and sinusitis by negatively regulating the expression of inflammatory factors. Therefore, Brg1 and KDM2B can be used as new biomarkers for predicting targeted drugs for chronic sinusitis, which is of great significance for the research and treatment of chronic sinusitis. However, we acknowledge that we have not thoroughly studied the specific action sites of Brg1 and KDM2B on inflammatory genes, which is the limitation of this study.

CONCLUSION

Our study shows that the expression of Brg1 and KDM2B in CRSwNPs is significantly decreased. Brg1 may assist KDM2B to inhibit the expression of cytokines, thus affecting the inflammatory process of CRSwNPs. Brg1 and KDM2B play an irreplaceable role in the whole inflammatory process of CRSwNPs. This study will provide a new perspective for gene targeting therapy of CRSwNPs.

AUTHORS' CONTRIBUTION

XM and LCC did conception and design; LCC, SC, ZX, KF, ZMQ, ZM, CXJ, and WXX conducted analysis and interpretation; XM and LCC drafted the manuscript for important intellectual content; the version submitted for publication was finally approved by XM, LCC, and SC.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. All authors declared that no conflict of interest exists.

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