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Original Article

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Inhibition of transient receptor potential melastatin 8 alleviates airway inflammation and remodeling in a murine model of asthma with cold air stimulus

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Abstract

Cold air stimulus is an important environmental factor that exacerbates asthma. At the molecular level, the transient receptor potential melastatin 8 (TRPM8) plays a crucial part in cold detection. The roles of TRPM8 in airway inflammation and remodeling in a murine model of asthma with cold stimulus and the related molecular mechanism are largely unknown. In this study, C57BL/6 mice were randomly divided into four groups: phosphate-buffered saline control group (control), ovalbumin (OVA)-induced asthma group (OVA), OVA with cold air stimulus group (OVA+cold), and OVA+cold+shTRPM8 (TRPM8 short hairpin RNA) group. We showed that cold air stimulus-induced TRPM8 upregulation in the OVA+cold group. Moreover, TRPM8 knockdown significantly attenuated cold-induced inflammation and infiltration, decreased levels of immunoglobulin E, restored the Th1/Th2 balance, and reduced inflammatory cell accumulation and airway remodeling. Furthermore, we demonstrated that TRPM8 knockdown dramatically inhibited mitogen-activated protein kinase and nuclear factor-κB pathways. Collectively, these results revealed that cold air stimulus induced an airway inflammatory response and remodeling by increasing TRPM8 expression and that downregulation of TRPM8 alleviated these responses.

Key words: asthma, transient receptor potential melastatin 8 (TRPM8), airway inflammation, airway remodeling, cold air stimulus

Introduction

Bronchial asthma is a chronic obstructive disease characterized by inflammation and airway remodeling and is a serious public health problem, with increasing morbidity and mortality rates worldwide [1]. The prevalence of asthma has steadily increased from 0.3% to 17.0% in many countries, with a high prevalence (by 1.5% or more) in China [2]. Under the pathological conditions of asthma, inflammatory mediators, such as cytokines and chemokines, are released from these inflammatory cells (eosinophils, neutrophils, macrophages, and T lymphocytes) to induce sustained chronic airway inflammation, which eventually leads to bronchoconstriction and airway remodeling [3]. Therefore, it is important to explore the mechanisms of asthma and identify new strategies for the prevention and treatment of airway inflammation and remodeling.

Exposure to low temperatures often causes urticaria or asthma [4,5]. Cold air stimulus is a major environmental factor that aggravates chronic inflammatory airway diseases, such as chronic obstructive pulmonary disease and asthma [6,7]. Temperature-sensitive transient receptor potential channels (TS-TRPs) are thermally gated ion channels with variable calcium selectivity [8]. One

member of the TS-TRP family, transient receptor potential melastatin 8 (TRPM8), is a ligand-gated, non-selective cation channel that is activated by both cold and chemical stimuli *in vitro* [9]. The physiological role of TRPM8 in sensing cold was demonstrated in $TRPM8^{-/-}$ mice, who failed to sense cold [9,10]. Moreover, TRPM8 channels have been shown to be involved in cough and airway constriction associated with inhalation of cold air [11]. Sabnis *et al.* [12] provided important insights into the mechanisms controlling airway inflammation due to inhalation of cold air and suggested that TRPM8 variants may have a role in the pathophysiology of asthma. Moreover, TRPM8 protein and mRNA expression levels were significantly increased in patients with chronic obstructive pulmonary disease compared with that in healthy individuals, suggesting that TRPM8 may be involved in chronic airway inflammation [13]. Based on the above evidence, we hypothesized that TRPM8 may be involved in the pathogenesis of airway inflammation and remodeling in asthma.

In this study, we showed that cold air stimulus could induce airway inflammation and remodeling by increasing TRPM8 expression. Downregulation of TRPM8 alleviated airway inflammation and remodeling, and inhibited the mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) pathways in a murine model of asthma. These findings suggest that TRPM8 represents a promising therapeutic strategy for asthma.

Materials and Methods

Experimental animals and grouping

Thirty-two female C57BL/6 mice (5–6 weeks of age; specific pathogen-free grade) were purchased from the Sipul-Bikai Experimental Animal Co., Ltd (Shanghai, China). Experimental mice were randomly assigned into the phosphate-buffered saline (PBS) group (control), ovalbumin (OVA) group (OVA), OVA plus cold air stimulus group (OVA+cold), and OVA plus cold air stimulus plus short hairpin RNA (shRNA) of TRPM8 lentivirus vector (LV-shTRPM8) group (OVA+cold+shTRPM8), with eight mice in each group. The experimental procedures were in accordance with the ethical standards of Medical Animal Research Ethics Committee at Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine.

Preparation of lentivirus containing shRNA targeting TRPM8

shRNA against TRPM8 and its corresponsive scrambled hairpin sequences were constructed and subcloned into the lentiviral vector U6-MCS-Ubi-EGFP (Genechem, Shanghai, China). Their sequences are as follows: shTRPM8: 5′-TCCCTTCGTTGTCTTCGCTTATC TCGAGATAAGCGAAGACAACGAAGGGTTTTTTC-3′; shNC: 5′-CC GGTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACAC GTTCGGAGAATTTTTG-3′. A lentiviral vector that expressed green fluorescent protein (GFP) alone was used as a control. The LV-shTRPM8 or LV-NC vectors were cotransfected with the packaging plasmids GV118 into HEK293T cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. After 48 h, virus particles were harvested.

Establishment of asthma model and experimental design

The murine asthma models were established by OVA treatment. On the 1st, 3rd, 5th, 7th, 9th, 11th, and 13th day, each mouse in the OVA, OVA+cold, and OVA+cold+shTRPM8 groups was sensitized by intraperitoneal (i.p.) injection of 300 μg of OVA (Sigma-Aldrich, St Louis, USA), emulsified in aluminum hydroxide solution (30 mg/ml in PBS) in a total volume of 0.2 ml. Each mouse in the control group was injected with 0.2 ml of PBS only. On Day 14, mice in the OVA+cold+shTRPM8 group were administered with LV-shTRPM8 by tail vein injection (i.v.). From Day 16, mice in the OVA+cold and OVA+cold+shTRPM8 groups were subject to 10 min of inhalation of cold air (4°C) using a mask twice a day until Day 29. From Day 21, the sensitized mice in the OVA, OVA+cold, and OVA+cold+shTRPM8 groups were kept in sealed glass containers and stimulated by intranasal (i.t.) administration of 50 μl saline containing 2% OVA with maximum inhalation for 25 min. This process was carried out once a day for 7 days. Mice in the control group were treated with saline only. Mice were sacrificed on Day 30 and lung tissues were collected for subsequent experiments.

Collection of bronchoalveolar lavage fluid

Bronchoalveolar lavage fluid (BALF) was collected after instillation of 1 ml sterile PBS through the trachea into the lung. The average fluid recovery rate was >90%. BALF was centrifuged at 800 *g* for 10 min at 4°C. The resulting cell pellets were subject to differential cell counts after Wright Giemsa staining. Total cells, eosinophils, and neutrophils were counted under a light microscope. The resulting supernatant was stored at −80°C for subsequent enzyme-linked immunosorbent assay (ELISA) [3].

Real-time quantitative reverse transcription polymerase chain reaction analysis

Total RNA was extracted from lung tissues using TRIzol reagent (Invitrogen, Carlsbad, USA). To detect target gene expression, a two-step SYBR Green II real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) procedure was carried out. Production of complementary DNA (cDNA) with oligo (dT) primers was performed according to the protocol provided for the Primer Script RT Reagent (TaKaRa, Dalian, China). Expression of *β-actin* was used as an internal control. The primer sequences were as follows: *TRPM8* forward 5′-GAGGAGCCGCAGAAATGGTA-3′ and reverse 5′-TCTGGGCATAACCACACTTG-3′; *β-actin* forward 5′-GCTG TATTCCCCTCCATCGT-3′ and reverse 5′-CATTGTAGAAGGTG TGGTGC-3′. The relative expression level of each gene was calculated and normalized using the $2^{-\Delta\Delta CT}$ method relative to *β-actin*. Each experiment was performed three times in biological triplicates.

Western blot analysis

Total protein was isolated from the lung tissues using radio-immunoprecipitation assay lysis buffer (Beyotime, Nanjing, China) and quantified using protein quantification reagents from Bio-Rad (Hercules, USA). Protein samples were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (Bio-Rad). After being blocked with 5% non-fat milk in TBS containing 0.05% Tween-20 (TBST) for 1 h, membranes were incubated with primary antibodies overnight at 4°C. Horseradish peroxidase (HRP)-conjugated anti-IgG (Proteintech, Wuhan, China) was used as the secondary antibody. Protein detection was performed using an enhanced chemiluminescence kit (AbFrontier, Seoul, Korea), and the blots were exposed to X-ray film. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the loading control. The following primary

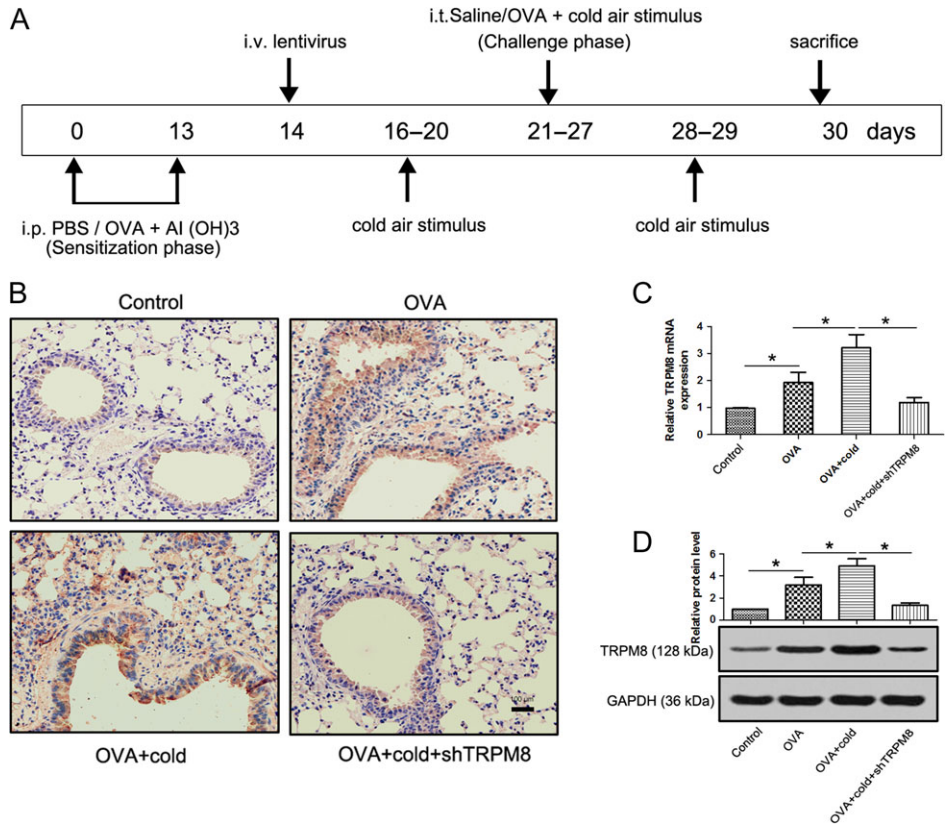

Figure 1. Expression of TRPM8 in a mouse model of asthma with cold air stimulus (A) Protocol for the asthmatic animal model and experimental design. (B) The expression of TRPM8 in lung tissue was detected using immunohistochemical staining. (C) *TRPM8* mRNA expression in the lung tissues was detected by qRT-PCR analysis. (D) The expression of TRPM8 protein in the lung tissues was measured by western blot analysis. The molecular weight of the detected protein was as expected for TRPM8. $*P < 0.05$.

antibodies were used: anti-TRPM8 antibody (Abcam, Cambridge, USA), and anti-MMP-2, anti-MMP-9, anti-p-ERK, anti-t-ERK, anti-p-JNK, anti-t-JNK, anti-p65, anti-Histone H1, and anti-GAPDH antibodies (Proteintech, Wuhan, China).

Determination of cytokine production

The levels of IL-4, IL-5, IL-12, and IFN-γ in the supernatant of BALF and the levels of MMP-2 and MMP-9 in lung tissues were detected using the corresponding ELISA kits (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions.

Immunohistochemistry

Tissue specimens were fixed with 10% neutral-buffered formalin (Jiancheng Bioengineering Institute, Nanjing, China), dehydrated, and embedded in paraffin. The sections were then deparaffinized and rehydrated. Hydrogen peroxide was applied to block endogenous peroxide activity for 10 min. After antigen retrieval using a microwave, the sections were treated with 1% bovine serum albumin to block non-specific binding. The sections were then incubated with primary anti-mouse TRPM8 (Abcam) in a humidified chamber overnight at 4°C. After wash with PBS, tissue sections were treated with biotinylated secondary antibody (Zymed, San Francisco, USA), followed by further incubation with streptavidin-HRP complex. After rinsing, diaminobenzidine (DAB; Abcam) was used as a chromogen, and the sections were counterstained with hematoxylin. Samples incubated with PBS only served as negative controls. Slides were examined under a light microscope and photographed on a Nikon ECLIPSE E600 microscope (Nikon Instruments, Melville, USA).

Hematoxylin and eosin staining

Paraffin-embedded lung sections were stained with hematoxylin and eosin (H&E) for inflammatory cell infiltration. The lung tissue samples in all groups were placed in 10% formalin for 24 h. After ethanol dehydration, cleaning, dewaxing, and embedding, tissues were sectioned into 4-μm-thick paraffin slices, which were then processed by H&E staining using standard techniques to evaluate histological changes.

Statistical analysis

SPSS software (version 13.0; SPSS, Chicago, USA) was used for statistical analysis. All data were expressed as the mean ± SD of at least three determinations and multiple group comparisons were performed using a one-way analysis of variance (ANOVA). Student's *t*-test (two-tailed) was used for comparisons between groups. Differences with *P*-values of <0.05 were considered significant.

Results

TRPM8 expression in lung tissues of asthma model mice treated with cold air stimulus

Following the sensitization and challenge protocol (**Fig. 1A**), a well-established murine model of OVA-induced asthma with cold air

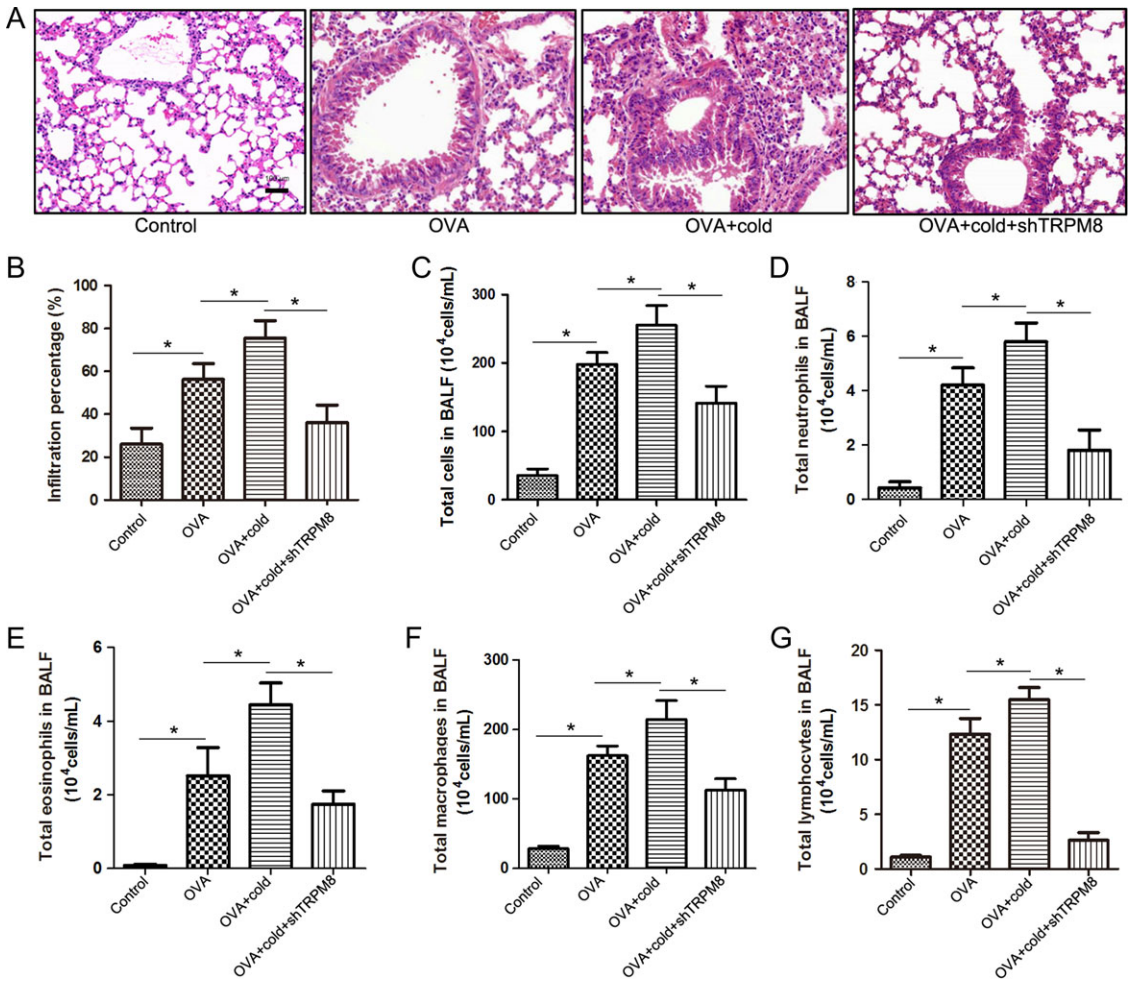

Figure 2. Knockdown of TRPM8 decreases the infiltration of inflammatory cells in a murine model of asthma with cold air stimulus (A) Histological examination of lung tissue. (B) Quantitative analysis of histological sections. (C) Total cell counts were determined in the BALF. (D–G) Total numbers of inflammatory cells, including neutrophils, eosinophils, macrophages, and lymphocytes, were analyzed in the BALF. $*P < 0.05$.

stimulus was used to evaluate the therapeutic effects of TRPM8. We first measured TRPM8 expression in the lung tissues of control, OVA, OVA+cold, and OVA+cold+shTRPM8 groups using immunohistochemistry (IHC) staining. Our results showed that TRPM8 expression in the lung tissues was obviously increased in OVA group than in control group, and was higher in the OVA+cold group than in the OVA group, and was lower in the OVA+cold+shTRPM8 group than in the OVA+cold group (**Fig. 1B**). Moreover, qRT-PCR and western blot analysis confirmed that cold air stimulus induced TRPM8 overexpression, whereas silencing of TRPM8 with shRNA significantly inhibited TRPM8 expression (**Fig. 1C,D**). These results showed that TRPM8 expression was induced by cold air stimulus in a murine model of asthma and TRPM8 silencing effectively downregulated the expression of TRPM8.

Knockdown of TRPM8 decreased inflammatory cell recruitment in a murine model of asthma with cold air stimulus

In pathological findings from lung tissues, inflammatory infiltration in the OVA and OVA+cold groups was much more severe than that in the control and OVA+cold+shTRPM8 groups, indicating that knockdown of TRPM8 significantly reduced the extent of inflammation and cellular infiltration under cold air stimulus (**Fig. 2A,B**). To analyze the effects of shTRPM8 on the degree of inflammatory infiltration, the cell numbers (total cells, neutrophils, eosinophils, macrophages, and lymphocytes) from BALF were analyzed. It was found that the total cell number in BALF was significantly enhanced in the OVA+cold group compared with that in the control and OVA groups, whereas an obvious decrease was observed in the OVA +cold+shTRPM8 group (**Fig. 2C**). The total number of inflammatory cells, including neutrophils, eosinophils, macrophages, and lymphocytes, was increased in the BALF from mice in the OVA+cold group compared with that in the control and OVA groups, and shTRPM8 markedly reduced the numbers of all inflammatory cells (**Fig. 2D–G**). These data suggested that TRPM8 modulated the recruitment of inflammatory cells in the airway.

Knockdown of TRPM8 relieved airway inflammatory and remodeling in the murine model of asthma with cold air stimulus

We further detected the levels of OVA-specific immunoglobulin E (IgE) in the serum and found that IgE levels were higher in the OVA+cold

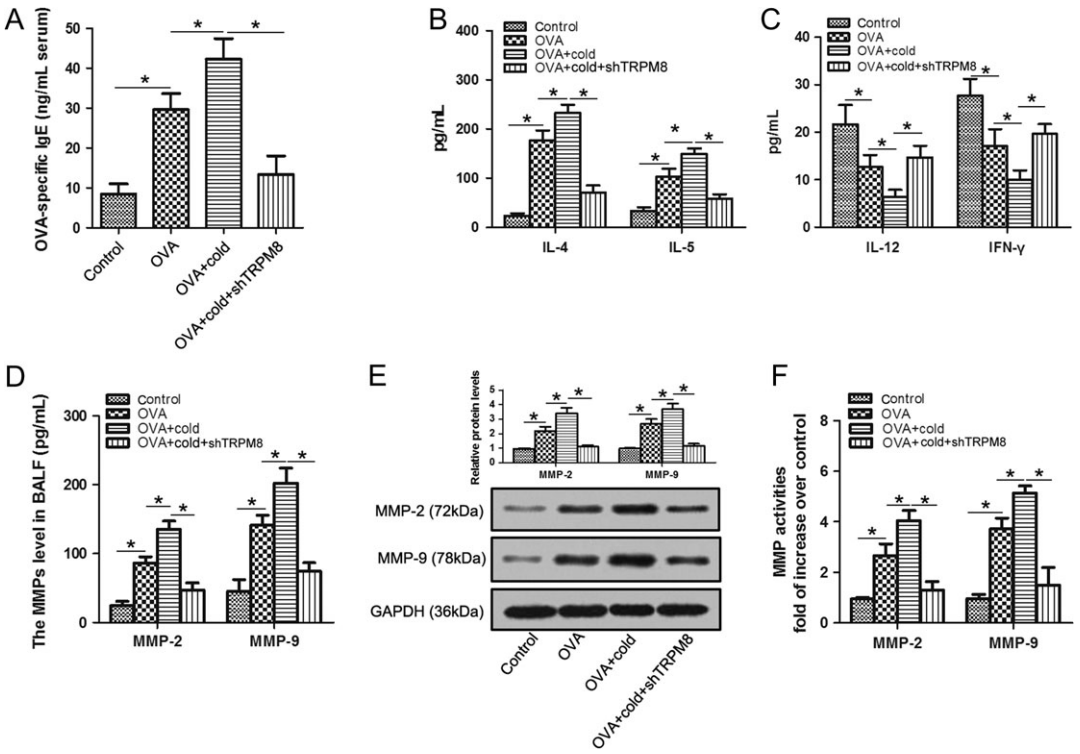

Figure 3. Effects of shTRPM8 on the expressions of IgE, IL-4, IL-5, IL-12, IFN-γ, MMP-2, and MMP-9 in OVA-sensitized mice (A) The level of OVA-specific IgE was measured in serum. (B) The concentrations of IL-4 and IL-5 were analyzed in the BALF by ELISA. (C) The concentrations of IL-12 and INF-γ were analyzed in the BALF by ELISA. (D) The levels of MMP-2 and MMP-9 in the BALF were measured by ELISA. (E) MMP-2 and MMP-9 protein expression in lung tissues was detected by western blot analysis. (F) The activities of MMP-2 and MMP-9 were analyzed by Biotrak TM activity assays. The molecular weights of the detected proteins were as expected for these proteins. $*P < 0.05$.

group than in the control and OVA groups but were reduced by shTRPM8 (**Fig. 3A**). The imbalance of Th1 and Th2 cytokines is responsible for the pathogenesis of asthma [14]. Th2-related cytokines, including IL-4 and IL-5, and Th1-related cytokines, including IFN-γ and IL-12, have been shown to regulate IgE-mediated allergy and asthma. Therefore, these cytokines were quantified in the BALF of OVA+cold air stimulus-induced asthmatic mice by ELISA. As expected, the levels of IL-4 and IL-5 in BALF were increased, whereas the levels of IL-12 and INF-γ were greatly reduced in the OVA+cold group compared with those in the control and OVA groups. shTRPM8 transfection significantly reduced the levels of IL-4 and IL-5 and increased the levels of IL-12 and INF-γ in the BALF of mice induced by OVA+cold air stimulus (**Fig. 3B,C**). These data revealed that knockdown of TRPM8 effectively downregulated the Th2-immune response and upregulated the Th1-immune response in mice with OVA+cold air stimulus-induced allergic asthma.

The MMP family regulates airway inflammation in asthma; in particular, MMP-9 and MMP-2 can reflect the status of airway remodeling [15,16]. Thus, we evaluated the levels of MMP-2 and MMP-9 in the BALF. Our findings showed that MMP-2 and MMP-9 levels were obviously increased in the OVA+cold group compared with those in the control and OVA groups, and were reduced in the OVA+cold+shTRPM8 group (**Fig. 3D**). We also measured MMP-2 and MMP-9 expressions in the lung tissue, and our findings were consistent with the results in the BALF (**Fig. 3E**). Moreover, we demonstrated that the activities of MMP-2 and MMP-9 were enhanced in the OVA+cold group compared with those in the control and OVA groups, and this enhancement was impaired by shTRPM8 transfection in the OVA+cold+shTRPM8 group (**Fig. 3F**). All these data revealed that downregulation of TRPM8 dramatically inhibited MMP-2 and MMP-9 levels induced by cold air stimulus in the OVA-induced asthma model, suggesting that TRPM8 may contribute to airway remodeling in asthma.

Knockdown of TRPM8 suppressed MAPK and NF-κB pathway activation in a murine model of asthma with cold air stimulus

Members of the MAPK family control the synthesis and secretion of pro-inflammatory mediators during inflammatory responses [17]. NF-κB inhibition also suppresses inflammation and relieves asthma in a mouse model of bronchial asthma [18]. In order to determine whether shTRPM8 inhibits airway inflammation and remodeling through the regulation of the MAPK and NF-κB pathways, the levels of phospho-c-Jun N-terminal kinase (p-JNK), total-JNK (t-JNK), phospho-extracellular signal-regulated kinase (p-ERK), and total-ERK (t-ERK) in the lung tissues of four groups were measured. As shown in **Fig. 4A,B**, the expression levels of t-JNK and t-ERK were not statistically different among all groups. However, p-JNK and p-ERK levels were increased in the OVA+cold group compared with those in the control and OVA groups, but were decreased in the OVA+cold+shTRPM8 group. We also detected the level of p65 subunit of NF-κB in the nucleus of lung tissues from the four groups and found that p65 expression was higher in the OVA+cold group than in the control and OVA groups, but was decreased by shTRPM8 transfection (**Fig. 4C**). These results indicated that

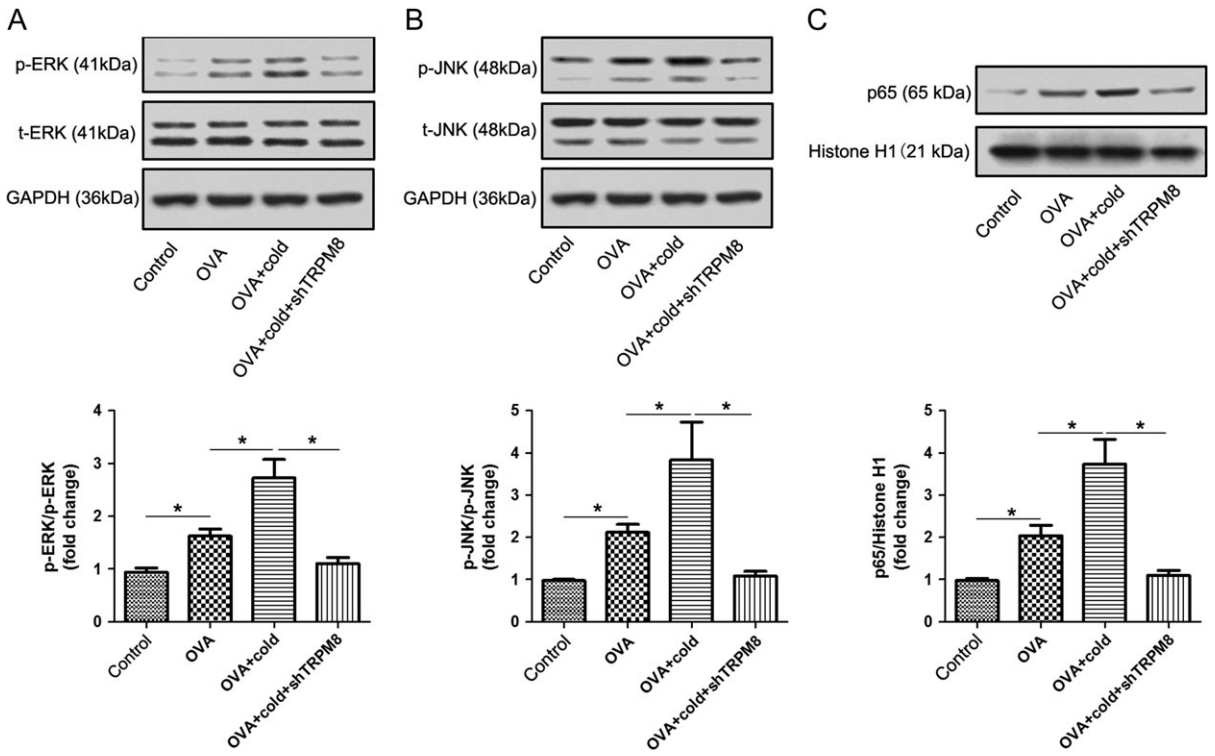

Figure 4. TRPM8 knockdown inhibits MAPK and NF-κB pathway activation in a murine model of asthma (A,B) MAPK-related factors p-ERK, t-ERK, p-JNK, and t-JNK were detected by western blot analysis. (C) NF-κB p65 expression in the nucleus was measured by western blot analysis in the control, OVA, OVA+cold, and OVA+cold+shTRPM8 groups. The molecular weights of the detected proteins were as expected for these proteins. $*P < 0.05$.

knockdown of TRPM8 markedly suppressed MAPK and NF-κB pathway activation in a murine model of asthma with cold air stimulus.

Discussion

In this study, we first confirmed that TRPM8 expression was markedly increased by cold air stimulus in our OVA-induced asthma model. Compared with the control and OVA groups, airway inflammation, inflammatory cells, IgE, Th2-related cytokines (IL-4 and IL-5), and MMP-2 and MMP-9 activity and expression were enhanced in the OVA+cold group, whereas the Th1-related cytokines (IFN-γ and IL-12) were greatly reduced in the OVA+cold group, and all were attenuated by TRPM8 knockdown. Moreover, we demonstrated that inhibition of TRPM8 suppressed MAPK and NF-κB pathways. These data supported previous findings and indicated that TRPM8 may be involved in the pathogenesis of asthma, including airway inflammation and remodeling [19,20].

TRPM8 is one of the temperature-sensing TRP channels (thermo TRP channels) activated by cold temperatures of <25°C [11]. Airway vagal afferent nerves express TRPM8 receptors, and activation of TRPM8 receptors by cold excites these airway autonomic nerves, pulmonary arterial smooth muscle cells, and lung epithelial cells [21]. Activation of cold-sensitive channels TRPM8 and TRPA1 inhibits the proliferative airway smooth muscle cell phenotype [20]. Toluene diisocyanate exposure induces airway inflammation of bronchial epithelial cells via the activation of TRPM8 [19]. Activation of the TRPM8 variant in lung epithelial cells by cold air leads to increased expression of several cytokine and chemokine genes [12]. These findings suggest that TRPM8 receptors may be involved in the airway inflammatory response induced by cold air. Furthermore, a previous genome-wide association study indicated that TRPM8 had significant associations with the phenotype of toluene diisocyanate-induced occupational asthma with higher odds ratios, indicating strong correlations [22]. Sabnis *et al.* [12] showed that aberrant function of the TRPM8 variant could account for the increases in diagnostic markers of disease states, such as cold-induced asthma. In the present study, IHC, western blot analysis, and qRT-PCR assays were performed and confirmed that TRPM8 expression was increased in the lung tissues of the OVA+cold group compared with OVA group, and was significantly decreased by downregulation of TRPM8. This result confirmed that cold air stimulus could induce TRPM8 overexpression and suggested that TRPM8 may play an important role in the pathogenesis of asthma.

Activation of the inflammatory response is a primary characteristic in the pathogenesis of asthma [23]. The development of chronic airway inflammation depends on the continuous recruitment of inflammatory cells (including eosinophils, lymphocytes, mastocytes, and neutrophils) and their subsequent activation [24,25]. Here, we found that inhibition of TRPM8 significantly attenuated OVA+cold air stimulus-induced inflammation and infiltration of lung tissues and reduced inflammatory cell accumulation (total cells, neutrophils, eosinophils, macrophages, and lymphocytes) in the BALF. Inflammatory cytokines released by inflammatory cells cause mucus hypersecretion, bronchial hyper-responsiveness, and initiation of airway remodeling [26].

Aberrant production of Th2 cytokines, such as IL-4, is involved in the pathogenesis of asthma and leads to the infiltration of inflammatory cells into the lung [27]. The hypersecretion of IL-4, IL-5, and IL-13 was required for IgE production, promotion of proliferation and activation of eosinophils. Activated eosinophils will

secrete a variety of pro-inflammatory components and lead to chronic inflammation of the airways [28]. Th1 cytokines, such as IFN-γ and IL-12, inhibit Th2 cytokine production and play critical roles in the pathogenesis of asthma [29]. Several studies have revealed that imbalance of Th1/Th2 cytokines is an important index in the evaluation of asthma [30]. Here, we found that downregulation of TRPM8 resulted in decreased levels of IgE and Th2 cytokines (IL-4 and IL-5) and increased levels of Th1 cytokines (IL-12 and INF-γ). IL-8 plays a key role in respiratory disease and acts as a regulator of pulmonary inflammation and immunity [31]. As an important upregulated cytokine for airway inflammation, IL-8 is mainly secreted during acute asthma [32]. The role of TRPM8 on IL-8 levels should be determined in asthma exposed to cold air in future research. Moreover, TRPM8 knockdown suppressed the expression and activity of MMP-2 and MMP-9, which have been identified as pathogenic factors in asthmatic airway remodeling. Thus, OVA with cold air stimulus increased inflammatory cytokines and stimulated cells involved in inflammation and remodeling, whereas suppression of TRPM8 expression inhibited these effects on the progression of inflammation and remodeling. However, further studies on the underlying molecular mechanisms are still required.

To elucidate the mechanisms through which shTRPM8 reduces the levels of Th2 cytokines, we explored the effects of shTRPM8 on NF-κB and MAPK pathways under cold stimulation. NF-κB is a master regulatory transcription factor that is crucial to the expression of inflammatory cytokines and related ligands in inflammation-related diseases, playing a major role in mediating mucus overproduction and pro-inflammatory cytokine expression [28]. MAPKs play critical roles in the expression and activation of inflammatory mediators in the airway, where both resident and infiltrating cells are essential [33]. Simultaneously, many studies have demonstrated that the production of inflammatory molecules is mainly regulated by NF-κB and MAPK pathway activation in asthma [34–36]. In our study, p-ERK, p-JNK, and p65 expression levels were found to be increased in the lung tissues of the OVA+cold group, whereas downregulation of TRPM8 significantly inhibited these effects in the OVA+cold+shTRPM8 group. These findings revealed that inhibition of TRPM8 alleviated airway inflammation and remodeling partially via MAPK- and NF-κB-mediated responses under cold stimulation.

In conclusion, downregulation of TRPM8 obviously attenuated cold air stimulus-induced inflammation and infiltration, alleviated the Th1/Th2 imbalance, and suppressed air remodeling. Thus, TRPM8 may represent a novel therapeutic target for combating obstructive airway diseases, such as asthma.

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