Monocyte chemotactic protein-inducing protein 1 negatively regulating asthmatic airway inflammation and mucus hypersecretion involving γ-aminobutyric acid type A receptor signaling pathway *in vivo* and *in vitro*

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Abstract

Background: Mounting evidence, consistent with our previous study, showed that γ-aminobutyric acid type A receptor (GABAAR) played an indispensable role in airway inflammation and mucus hypersecretion in asthma. Monocyte chemotactic protein-inducing protein 1 (MCPIP1) was a key negative regulator of inflammation. Recent studies showed that inflammation was largely suppressed by enhanced MCPIP1 expression in many inflammatory diseases. However, the role and potential mechanism of MCPIP1 in airway inflammation and mucus hypersecretion in asthma were still not well studied. This study was to explore the role of MCPIP1 in asthmatic airway inflammation and mucus hypersecretion in both mice and BEAS-2B cells, and its potential mechanism.

Methods: *In vivo*, mice were sensitized and challenged by ovalbumin (OVA) to induce asthma. Airway inflammation and mucus secretion were analyzed. *In vitro*, BEAS-2B cells were chosen. Interleukin (IL)-13 was used to stimulate inflammation and mucus hypersecretion in cells. MCPIP1 Lentiviral vector (LA-MCPIP1) and plasmid-MCPIP1 were used to up-regulate MCPIP1 in lung and cells, respectively. MCP-1, thymic stromal lymphopoietin (TSLP), mucin 5AC (MUC5AC), MCPIP1, and GABAARβ2 expressions were measured in both lung and BEAS-2B cells. Immunofluorescence staining was performed to observe the expression of GABAARβ2 in cells.

Results: MCPIP1 was up-regulated by LA-MCPIP1 ($P < 0.001$) and plasmid-MCPIP1 ($P < 0.001$) in lung and cells, respectively. OVA-induced airway inflammation and mucus hypersecretion, OVA-enhanced MCP-1, TSLP, MUC5AC, and GABAARβ2 expressions, and OVA-reduced MCPIP1 were significantly blunted by LA-MCPIP1 in mice (all $P < 0.001$). IL-13-enhanced MCP-1, TSLP, MUC5AC, and GABAARβ2 expressions, and IL-13-reduced MCPIP1 were markedly abrogated by plasmid-MCPIP1 in BEAS-2B cells (all $P < 0.001$).

Conclusion: The results of this study suggested that OVA and IL-13-induced airway inflammation and mucus hypersecretion were negatively regulated by MCPIP1 in both lung and BEAS-2B cells, involving GABAAR signaling pathway.

Keywords: Airway inflammation; Airway mucus hypersecretion; Gamma-aminobutyric acid type A receptor; GABAAR; IL-13; MCPIP1; Monocyte chemotactic protein-inducing protein 1; Ovalbumin

Introduction

Globally, asthma is one of the most common chronic airway disorders. It is estimated that the prevalence of asthma was about 1% to 18% in different regions, indicating more than 300 million people suffering from asthma in the world.[1,2] Airway mucus hypersecretion and persistent airway inflammation were the two most important features of asthma.[3-5] In the early stage of asthma, the response to the treatments, such as inhaled corticosteroids and long-acting β2-agonist (LABA), was well in most of the patients. Nevertheless, in the late stage of asthma, the control level was limited in a significant number of patients. Some investigations revealed that extensive and over-secretion of

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mucus and mucus plug accumulation inside of airway lumen were commonly observed in patients with refractory asthma and fatal asthma, leading to the poor response to bronchodilators and corticosteroids, even in higher dosage.[6,7] On the other hand, the underlying mechanisms on regulation of airway mucus secretion in asthma were still unclear. Then, new therapeutic targets are in great need.

It was well-known that airway inflammation contributed substantially to the pathogenesis of asthma.[8,9] Furthermore, Th2 cells over-activation and Th2 cytokines, such as interleukin (IL)-4, IL-5, and IL-13, over-releasing played the hub roles in airway inflammation in asthma.[10] Mounting evidence showed that Th2 inflammation also was essential for airway mucus hypersecretion in asthma.[8,10] However, the underlying mechanism was still not well explored. Recently, some investigations, consistent with our previous study, revealed that IL-13/γ-aminobutyric acid type A receptor (GABAAR) system played a critical role in airway mucus hypersecretion in asthma.[4,11,12] Airway mucus hypersecretion was largely attenuated by blocking or suppressing IL-13/GABAAR system.[4,11] Initially, Xiang *et al*[11] identified GABAAR expression in airway epithelium in both human and mice. Subsequently, they showed that the synthesis of mucin 5AC (MUC5AC), the main component of mucus, in airway was notably enhanced by IL-13 through up-regulation of GABAAR *in vivo* and *in vitro*. Furthermore, our previous study found that ovalbumin (OVA)-induced airway mucus hypersecretion was substantially alleviated by rosuvastatin through down-regulation of GABAAR in asthmatic mice.[4] However, the molecular mechanism of IL-13 on mediation of GABAAR in airway epithelium was still unknown. Additionally, monocyte chemotactic protein-inducing protein 1 (MCPIP1) was a critical negative inflammatory regulator, and was widely expressed in a variety of tissues. Some inflammatory diseases and conditions were remarkably obliterated or attenuated by MCPIP1 overexpression.[13-16] Li *et al*[16] demonstrated that lipopolysaccharide (LPS)-induced liver injury and inflammation were significantly suppressed by up-regulation of MCPIP1 in mice. Li *et al*[13] figured out that LPS-induced lung inflammation, lung injury, and mortality were substantially improved by suppression of MCPIP1 degradation in myeloid cells in mice. Furthermore, recently, it was reported that the development and function of IL-5-producing Th2 cells was mainly regulated by MCPIP1. And, MCPIP1 knockout led to more severe airway inflammation in asthmatic mice.[17]

Therefore, the purpose of this study was to explore the role of MCPIP1 in asthmatic airway inflammation and mucus hypersecretion in both mice and BEAS-2B cells, and its potential mechanism.

Methods

Animal

All procedures on animals were approved by the Animal Experimental Ethics Committee of Chongqing Medical University (No. 2018-199). This study was performed according to the recommendations in the Guide for the Care and Use of Laboratory Animals. All surgery was performed using sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. Specific pathogen-free (SPF) male BALB/c mice (18–22 g, age 6–10 weeks), were maintained under SPF conditions in the animal center facilities of our university. The mice were fed in a temperature-controlled room (12-h dark and light cycles) and offered *ad libitum* access to food and water. Animals underwent an acclimatization period of at least 1 week before the study.

Up-regulation of MCPIP1 by MCPIP1 lentiviral activation particles (LA-MCPIP1) in lung

According to our previous studies, the recombinant lentivirus vector for MCPIP1 (LA-MCPIP1, sc-432978-LAC; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used to up-regulate MCPIP1 expression in lung of mice.[18,19] And, negative control lentivirus expressing non-targeting sequence (sc-437282, Santa Cruz Biotechnology) was used as LA-scrambled. Briefly, 30 male BALB/c mice were divided into three groups ($n = 10$ each), Control group, LA-scrambled group, and LA-MCPIP1 group. After anesthesia, LA-MCPIP1 lentivirus vector (40 μL) for the LA-MCPIP1 group or negative control lentivirus (40 μL) for the LA-scrambled group was administrated by intratracheal injection. The mice in the control group were given sterile saline. And, 20 days after transfection, the left lower lung was resected. Histological changes were observed by Haemotoxylin and Eosin (H&E) staining. The efficiency of lentivirus vector transfection was detected by quantitative real-time polymerase chain reaction (qPCR) and western blot.

A murine model of OVA-induced chronic asthma

In this study, 30 male BALB/c mice were randomly divided into three groups: control group, OVA group, and OVA+ LA-MCPIP1 group, with ten mice in each. According to our previous studies, a murine model of asthma was induced by OVA sensitization and challenge.[3,18,20,21] In brief, mice were sensitized intraperitoneally with 10 μg of OVA (grade V; Sigma-Aldrich Chemical, St. Louis, MO, USA) and 100 μg of Al $(OH)_3$ in 100 μL saline on days 0, 7, and 14. From days 15 to 75, the mice were challenged with 5% OVA aerosol for 1.5 h once daily. LA-MCPIP1 (40 μL) was also given by intratracheal injection on days 15, 35, and 55.

H&E staining and Periodic Acid-Schiff (PAS) staining

The right lower lung of each mouse was fixed in 10% formalin, embedded in paraffin, cut into 5 μm sections, stained with H&E to observe the pathological changes of the lung. Meanwhile, PAS staining was performed to observe goblet cell hyperplasia and airway mucus secretion. According to our previous studies, the histological mucus index (the percentage of the mucus-positive area of the whole bronchial epithelium) was recorded.[3,4]

Cell transfection with plasmid

BEAS-2B cells, a cell line derived from human bronchial epithelial cells, were cultured in Dulbecco modified Eagle medium supplemented with penicillin (100 IU/mL),

streptomycin (100 μg/mL), and 10% heat-inactivated fetal bovine serum. BEAS-2B cells were cultured in an incubator maintained with 5% CO_2 at 37°C. According to our previous studies and the manufacturer's instruction, plasmid-MCPIP1 (sc-401790-ACT; Santa Cruz Biotechnology) or plasmid-negative (sc-437275; Santa Cruz Biotechnology) transfected BEAS-2B cells at 70% confluence.[18,19,22] Then, 24 h after transfection, BEAS-2B cells were used for further experiments. The expression of MCPIP1 was measured by qPCR and western blot.

Cell treatment

Plasmid-MCPIP1 transfected and non-transfected BEAS-2B cells were stimulated with IL-13 (10 ng/mL; R&D Systems, Minneapolis, MN, USA). And, 24 h after interventions, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to evaluate cell viability. Meanwhile, total and nuclear proteins and messenger RNA (mRNA) were extracted from cells and stored in −80°C.

Quantitative PCR

The mRNA expressions of MCP1, thymic stromal lymphopoietin (TSLP), MUC5AC, GABAARβ2, and MCPIP1 were measured by qPCR.[19,23,24] And, β-actin was used as an internal reference. Briefly, the right upper lung tissues were stored at −80°C. Total RNA was isolated from the lung tissue and BEAS-2B cells by Trizol reagent (Invitrogen, Carlsbad, CA, USA). PrimerScript® RT reagent kit with genomic DNA (gDNA) eraser (Takara Bio Inc., Otsu, Japan) was used for reverse transcription. PCR was then performed with iQ™5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and an SYBR Green PCR kit (Takara Bio Inc.) in a final volume of 20 μL, containing 1.6 μL complementary DNA template, forward and backward primers (0.8 μL each), 10 μL SYBR® Premix Ex Taq™ II and 6.8 μL dH_2O. The primers and TaqMan probes were designed using Primer Premier (PREMIER Biosoft International, Canada). The premier sequences were as follows: hMCP1: (forward) 5′-CCC CAGTCACCTGCT GTTAT-3′; (reverse) 5′-CCACA ATGGTCTTGAA GATCA C-3′; hMUC5AC: (forward) 5′-ATTTTT TCCCCACTCC TGATG-3′; (reverse) 5′-AAGACA ACCCAC TCCCAACC-3′; hTSLP (forward) 5′-CCAGAG CCTAACC TTCA ATCC-3′; (reverse) 5′-GTTGT GACTTTCCT TTTTCTC-CTC-3′; hGABAARβ2 (forward) 5′-GCAGAG TGTCAA-TGA CCCTAG-3′; (reverse) 5′-TGGCA ATGTCAA TGTTCATCC-3′; hMCPIP1 (forward) 5′- CTGGAG AA-GAAGAAG ATCCTGG-3′; (reverse) 5′-TGACGA AGGAG TACATG AGCAG-3′; hβ-actin: (forward) 5′-CTTAGTT GCGTTA CACC CTTTCTTG-3′; (reverse) 5′-CTGTCAC CTTCA CCGTTC CAGTTT-3′; mMCP1: (forward) 5′-TTAAAAA CCTGG ATCGGA ACCAA-3′ and (reverse) 5′-GCATTAGC TTCAG ATTTAC GGGT-3′; mMUC5AC (forward) 5′-GATGACT TCCAGACTAT CAGTG-3′ and (reverse) 5′-TGGCGT TAGTCA GCAGA-3′; mMCPIP1 (forward) 5′-CCACCTACCCATCCAGAGAG-3′ and (reverse) 5′-GGGAA GACACCA CACAGCTT-3′; mTSLP (forward) 5′-CCAGGCT ACCCTGA AACTGA-3′ and (reverse) 5′-TCTGGA GATTGCAT GAAGGA-3′; mGABA-ARβ2 (forward) 5′-CCCACC TCCGGG AAACTC-3′ and (reverse) 5′- GAAGAC AAAGCACCC CATTAGG-3′; mβ-actin (forward) 5′-GATTACT GCTCTGGCT CCTAGC-3′ and (reverse) 5′-ACTCATCGT ACTCCTG CTTGCT-3′. Changes in the expression of target genes were calculated using the $2^{-\Delta\Delta Ct}$ method, $\Delta\Delta Ct = (Ct_{target}\text{-}Ct_{\beta\text{-actin}})_{sample}\text{-}(Ct_{target}\text{-}Ct_{\beta\text{-actin}})_{control}$.[19,22]

Western blot

Western blot was used to analyze protein expression.[20,25,26] Briefly, protein lysates from the left upper lung tissues and BEAS-2B cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes. Antibodies against hMCP1, hMUC5AC, hTSLP, hGABAARβ2, hMCPIP1, hβ-actin, mMCP1, mMUC5AC, mTSLP, mGABAARβ2, mMCPIP1, and mβ-actin were all purchased from Santa Cruz Biotechnology. The relative protein levels of MCP1, MUC5AC, TSLP, GABAARβ2, and MCPIP1 were normalized to that of β-actin.

Immunofluorescence staining of GABAARβ2 in BEAS-2B cells

According to our previous studies, immunofluorescence staining was performed to observe the expression of GABAARβ2 in BEAS-2B cells.[27,28] In brief, coverslips with BEAS-2B cells were fixed. Then, coverslips were blocked with PBS and incubated with anti-GABAARβ2 antibody at 4°C overnight, followed by incubation with Alexa Fluor 488-labeled secondary antibody (Bioworld Technology, Nanjing, China) for 1 h. And, 4′, 6-Diamidino-2-phenylindole (DAPI; KeyGen Biotech, Nanjing, Jiangsu, China) was used to stain the nuclei for 5 min. Finally, cells were observed by fluorescence microscopy (TE2000-U; Nikon, Tokyo, Japan).

Statistical analysis

Statistical analyses were performed with SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). All data were presented as mean ± standard error. One-way analysis of variance with Fisher's Least Significant Differences (LSD) and Student-Newman-Keuls (SNK)'s *post-hoc* test was used. A $P < 0.05$ was considered to be statistically significant.

Results

MCPIP1 was up-regulated after transfection of the lung with LA-MCPIP1

Twenty days after transfection, no pathological alterations were found in the three groups of mice [Figure 1A]. However, MCPIP1 expression (mRNA: $F = 282.611$, $P < 0.001$; protein: $F = 342.294$, $P < 0.001$) in the lung was noticeably increased by LA-MCPIP1 in mice [Figure 1B and 1C]. Additionally, no difference in MCPIP1 expression was found between Control group and LA-scrambled group [Figure 1B and 1C].

LA-MCPIP1 attenuated OVA-induced lung pathological alterations and airway mucus hypersecretion in mice

A murine model of asthma was established by 75 days of OVA sensitization and challenge. The severe and classical

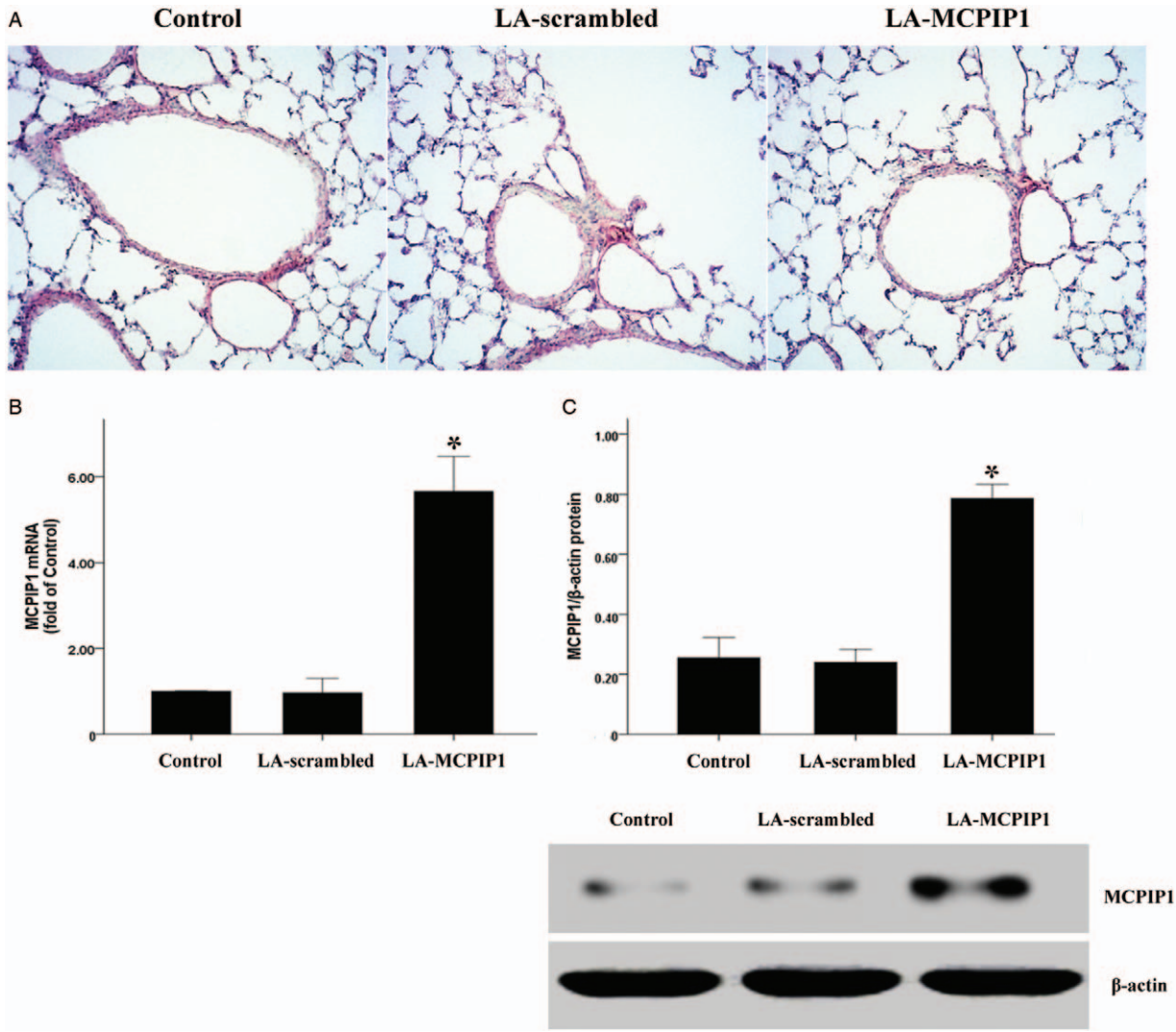

Figure 1: MCPIP1 expression was up-regulated after transfection of LA-MCPIP1 in lung. Mice were transfected with LA-MCPIP1 or LA-scrambled by intratracheal injection. Twenty days after transfection, the expression of MCPIP1 was measured. (A) Twenty days after transfection, mice were sacrificed and their left lower lungs were fixed. Then, the tissue sections were stained with hematoxylin-eosin. The figure demonstrates a representative view (original magnification, ×200) from each group; (B) Quantitative real-time polymerase chain reaction was performed to measure MCPIP1 mRNA expression; (C) Western blot was used to detect MCPIP1 protein expression. Each bar represents the mean ± standard error of ten mice. $^{*}P < 0.05$, compared with control. LA-MCPIP1: Lentivirus vector for MCPIP1; LA-scrambled: Lentivirus vector for negative; MCPIP1: Monocyte chemotactic protein-inducing protein 1.

lung pathological alterations, including obvious inflammatory cells infiltration in the airway, goblet cell hyperplasia, smooth muscle hyperplasia and hypertrophy, collagen deposition and thickening of the airway basement membrane, were observed in mice [Figure 2A]. Simultaneously, 75 days after OVA sensitization and challenge, severe airway mucus hypersecretion and significantly increased mucus index (Control 0 *vs*. LA-scrambled 86.34 ± 3.63 *vs*. LA-MCPIP1 37.96 ± 2.70, $F = 2737.742$, $P < 0.001$) were observed in mice [Figure 2B and 2C]. And, MUC5AC expression (mRNA: $F = 726.869$, $P < 0.001$; protein: $F = 982.751$, $P < 0.001$) in lung was also markedly increased by OVA [Figure 2D and 2E]. However, OVA-induced lung pathological changes, airway mucus hypersecretion, increased mucus index, and MUC5AC expression were all significantly attenuated by LA-MCPIP1 [Figure 2].

LA-MCPIP1 abrogated OVA-reduced MCPIP1 expression in lung

Seventy-five days after OVA sensitization and challenge, the expression of MCPIP1 was markedly reduced in lung (mRNA: $F = 1159.404$, $P < 0.001$; protein: $F = 892.311$, $P < 0.001$) [Figure 3A and 3B]. However, OVA-reduced MCPIP1 was abrogated by LA-MCPIP1 in lung [Figure 3A and 3B].

LA-MCPIP1 blunted OVA-induced MCP1, TSLP, and GABAARβ2 in lung

Seventy-five days after OVA sensitization and challenge, the expressions of MCP1 (mRNA: $F = 687.245$, $P < 0.001$; protein: $F = 531.749$, $P < 0.001$), TSLP (mRNA: $F = 358.967$, $P < 0.001$; protein: $F = 811.616$,

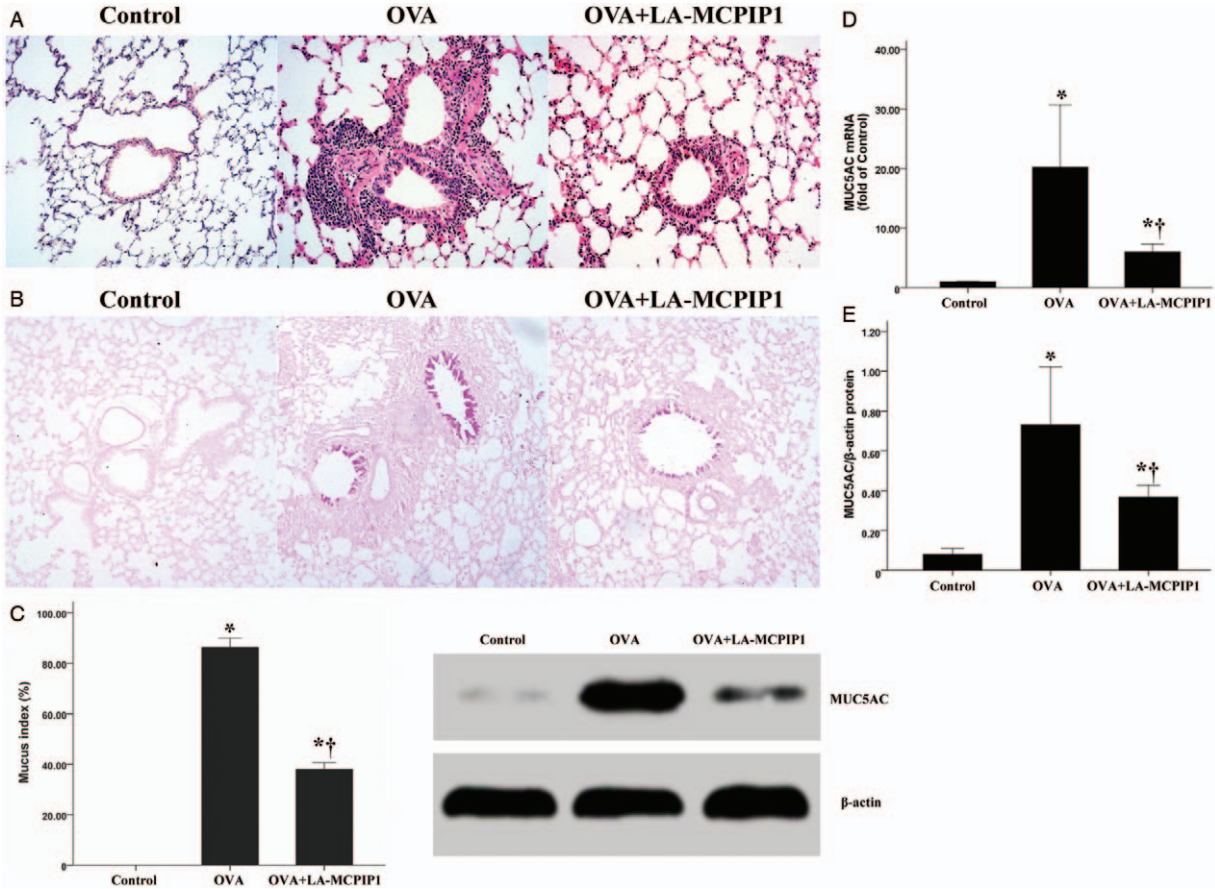

Figure 2: LA-MCPIP1 attenuated lung pathological alterations and airway mucus hypersecretion in OVA-induced asthma in mice. (A) Seventy-five days after OVA sensitization and challenge, mice were sacrificed and their right lower lungs were fixed. Then, tissue sections were stained with H&E. The figure demonstrates a representative view (original magnification, ×200) from each group; (B) tissue sections were stained with Periodic Acid-Schiff staining. The figure demonstrates a representative view original magnification, (×200) from each group; (C) mucus index was evaluated as the percentage of the mucus-positive area of the whole bronchial epithelium; (D) Quantitative real-time polymerase chain reaction was performed to measure the mRNA expression of MUC5AC; (E) western blot was used to detect the protein expression of MUC5AC. Each bar represents the mean ± standard error of ten mice. $^{*}P < 0.05$, compared with control, $^{\dagger}P < 0.05$, compared with OVA. LA-MCPIP1: Lentivirus vector for MCPIP1; MCPIP1: Monocyte chemotactic protein-inducing protein 1; MUC5AC: Mucin 5AC; OVA: Ovalbumin.

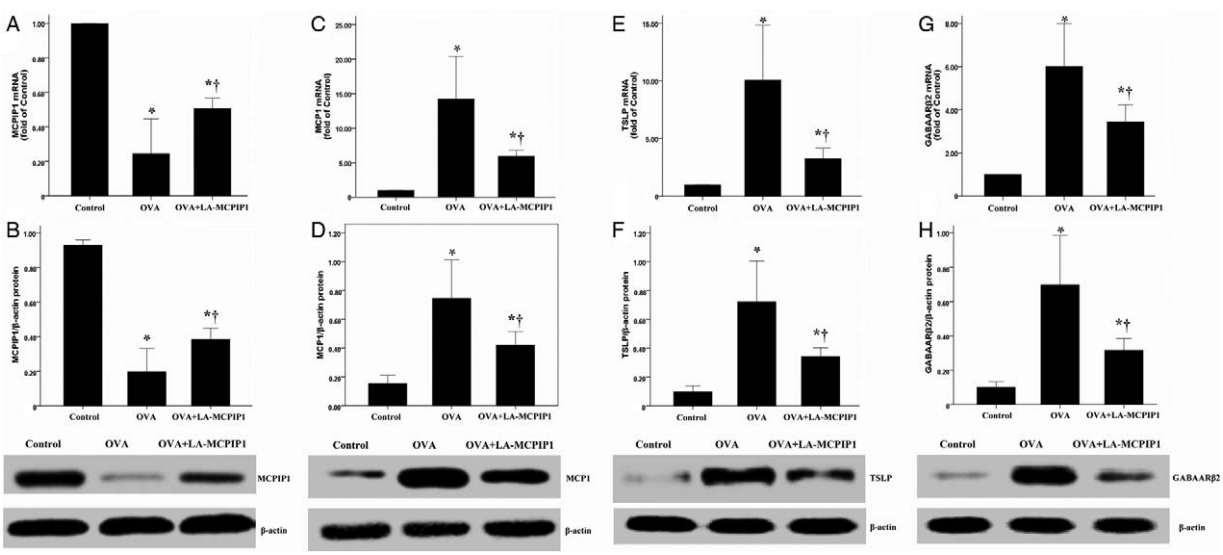

Figure 3: LA-MCPIP1 blunted OVA-induced MCPIP1, MCP1, TSLP, and GABAARβ2 in lung. (A, C, E, and G) Quantitative real-time polymerase chain reaction was performed to analyze the mRNA expression of MCPIP1, MCP1, TSLP, and GABAARβ2 in lung; (B, D, F, and H) western blot was used to detect the protein expression of MCPIP1, MCP1, TSLP, and GABAARβ2 in lung. Each bar represents the mean ± standard error of ten mice. $^{*}P < 0.05$, compared with control, $^{\dagger}P < 0.05$, compared with OVA. GABAAR: γ-Aminobutyric acid type A receptor; LA-MCPIP1: Lentivirus vector for MCPIP1; MCPIP1: Monocyte chemotactic protein-inducing protein 1; MCP-1: Monocyte chemotactic protein-1; OVA: Ovalbumin; TSLP: Thymic stromal lymphopoietin.

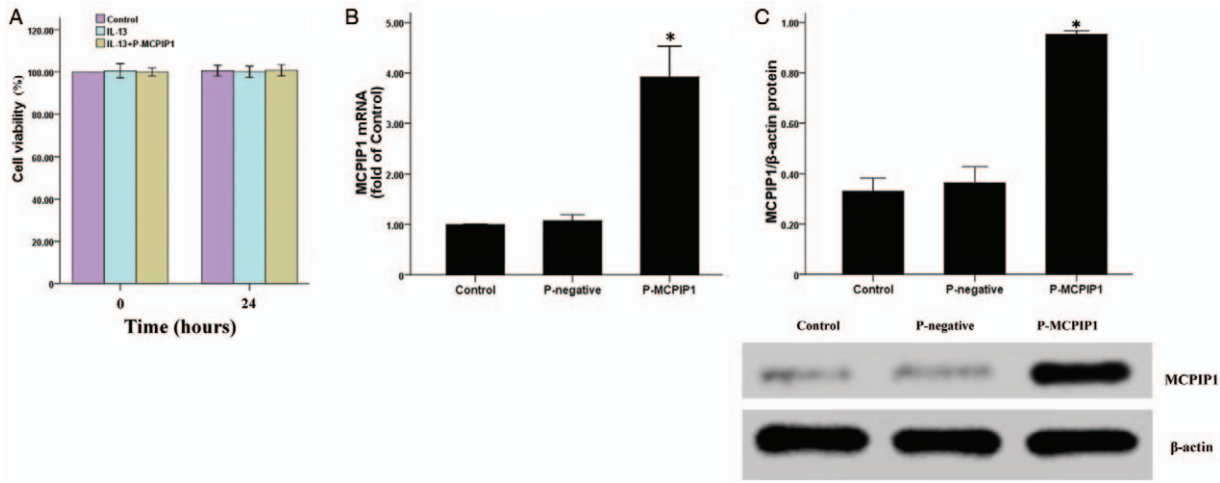

Figure 4: MCPIP1 was up-regulated after plasmid-MCPIP1 transfection in BEAS-2B cells. (A) Twenty-four hours after transfection, MTT assay was performed to evaluate the cell viabilities of BEAS-2B cells; (B) Quantitative real-time polymerase chain reaction was performed to measure MCPIP1 mRNA expression; (C) western blot was obtained to evaluate MCPIP1 protein expression. Quantitative data were presented as mean ± standard error ($n = 5$). $^{*}P < 0.05$, compared with control. IL: Interleukin; MCPIP1: Monocyte chemotactic protein-inducing protein 1; P-negative: Plasmid-negative; P-MCPIP1: Plasmid-MCPIP1.

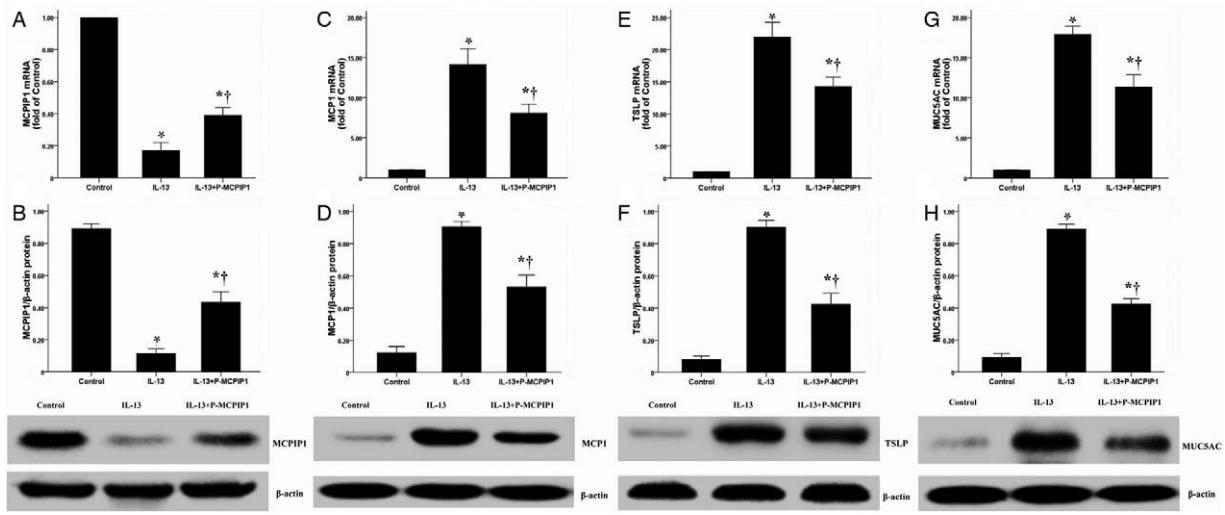

Figure 5: Plasmid-MCPIP1 abrogated IL-13-induced MCPIP1, MCP1, TSLP, and MUC5AC in BEAS-2B cells. Plasmid-MCPIP1-transfected and non-transfected BEAS-2B cells were stimulated with IL-13 (10 ng/mL) for 24 h. (A, C, E, and G) Quantitative real-time polymerase chain reaction was performed to analyze the mRNA expression of MCPIP1, MCP1, TSLP, and MUC5AC; (B, D, F, and H) western blot was used to measure the protein expression of MCPIP1, MCP1, TSLP, and MUC5AC. Quantitative data were presented as mean ± standard error ($n = 5$). $^{*}P < 0.05$, compared with control. $^{\dagger}P < 0.05$, compared with IL-13. GABAAR: γ-Aminobutyric acid type A receptor; IL: Interleukin; MCP-1: Monocyte chemoattractant protein-1; MCPIP1: Monocyte chemotactic protein-inducing protein 1; MUC5AC: Mucin 5AC; P-MCPIP1: Plasmid-MCPIP1; TSLP: Thymic stromal lymphopoietin.

$P < 0.001$), and GABAARβ2 (mRNA: $F = 163.974$, $P < 0.001$; protein: $F = 1079.529$, $P < 0.001$) were dramatically increased in lung [Figure 3C–H]. However, OVA-induced MCP1, TSLP, and GABAARβ2 were all noticeably blunted by LA-MCPIP1 in lung [Figure 3C–H].

The effect of plasmid-MCPIP1 in BEAS-2B cells

As shown in Figure 4A, no significant difference in cell viabilities (0 h: Control 100.00 ± 0.00 *vs.* IL-13 100.57 ± 3.34 *vs.* IL-13-P-MCPIP1 99.99 ± 1.89, $F = 0.1103$, $P = 0.8964$; 24 h: Control 100.68 ± 2.56 *vs.* IL-13 100.06 ± 2.68 *vs.* IL-13-P-MCPIP1 100.82 ± 2.69, $F = 0.1152$, $P = 0.8922$) was found in different groups at 24 h after administrations, indicating that plasmid-MCPIP1 was non-toxic to BEAS-2B cells. Meanwhile, MCPIP1 expression (mRNA: $F = 107.568$, $P < 0.001$; protein: $F = 262.066$, $P < 0.001$) was markedly up-regulated by plasmid-MCPIP1 in BEAS-2B cells [Figure 4B and 4C].

Plasmid-MCPIP1 abrogated IL-13-reduced MCPIP1 expression in BEAS-2B cells

After IL-13 stimulation, the expression of MCPIP1 (mRNA: $F = 590.106$, $P < 0.001$; protein: $F = 383.548$, $P < 0.001$) was significantly reduced in BEAS-2B cells [Figure 5A and 5B]. However, IL-13-reduced MCPIP1 expression was substantially abrogated by plasmid-MCPIP1 in BEAS-2B cells [Figure 5A and 5B].

Plasmid-MCPIP1 inhibited IL-13-induced MCP1, TSLP, MUC5AC, and GABAARβ2 expressions in BEAS-2B cells

After IL-13 stimulation, the expressions of MCP1 (mRNA: $F = 124.791$, $P < 0.001$; protein: $F = 291.235$, $P < 0.001$), TSLP (mRNA: $F = 226.524$, $P < 0.001$; protein: $F = 366.516$, $P < 0.001$), MUC5AC (mRNA: $F = 329.960$, $P < 0.001$; protein: $F = 924.855$, $P < 0.001$), and GABAARβ2 (mRNA: $F = 388.866$, $P < 0.001$; protein: $F = 1189.885$, $P < 0.001$) were significantly up-regulated in BEAS-2B cells [Figure 5C–H and Figure 6]. However, IL-13-induced MCP1, TSLP, MUC5AC, and GABAARβ2 expressions were noticeably inhibited by plasmid-MCPIP1 in BEAS-2B cells [Figure 5C–H and Figure 6].

Discussion

In this study, our results found that OVA-induced airway inflammation and mucus hypersecretion were substantially blunted by LA-MCPIP1 in mice. Meanwhile, OVA and IL-13-enhanced MCP1, TSLP, MUC5AC, and GABAARβ2 expressions were noticeably abrogated by LA-MCPIP1 and plasmid-MCPIP1 both *in vivo* and *in vitro*, respectively. Taken together, our data indicated that OVA and IL-13-induced airway inflammation and mucus hypersecretion were negatively regulated by MCPIP1 both *in vivo* and *in vitro*, involving GABAAR signaling pathway.

MCPIP1, also named ZC3H12A, belongs to CCCH Zn finger-containing proteins family. Mounting evidence revealed that MCPIP1 was a critical negative inflammatory regulator in a variety of pathological conditions, such as acute lung injury, hepatic ischemia/reperfusion injury, pulmonary fibrosis, and type 1 diabetes.[13,14,16,29-31] It was found that the expression of MCPIP1 negatively correlated with the severity of inflammation in different diseases.[13,15,16,29,31] Several studies showed that promoting MCPIP1 expression could suppress inflammation in many conditions.[13,15] Li *et al*[13] figured out that LPS-induced lung inflammation, lung injury, and mortality were substantially improved by suppression of MCPIP1 degradation in myeloid cells in mice. Yao *et al*[15] showed that miR-9-induced microglial activation and inflammatory responses resulted from down-regulation of MCPIP1. Recently, a study showed that OVA and house dust mite-induced airway inflammation and inflammatory mediators secretion, such as IL-5 and IL-13, were more severe in MCPIP1$^{-/-}$ mice than in wild type mice.[30] In this study, LA-MCPIP1 and plasmid-MCPIP1 were used to up-regulate MCPIP1 expression in lung and BEAS-2B cells, respectively. According to our previous studies, Control Lentiviral Activation Particles were also used as the negative control (LA-scrambled group) to exclude the potential impact of vector on mice lungs.[18,19] Twenty days after transfection, no pathological changes in lungs were observed in all groups, Control group, LA-scrambled group, and LA-MCPIP1 group [Figure 1]. Meanwhile, compared with the Control group, both mRNA and protein expression of MCPIP1 was significantly increased in the LA-MCPIP1 group. However, no difference in

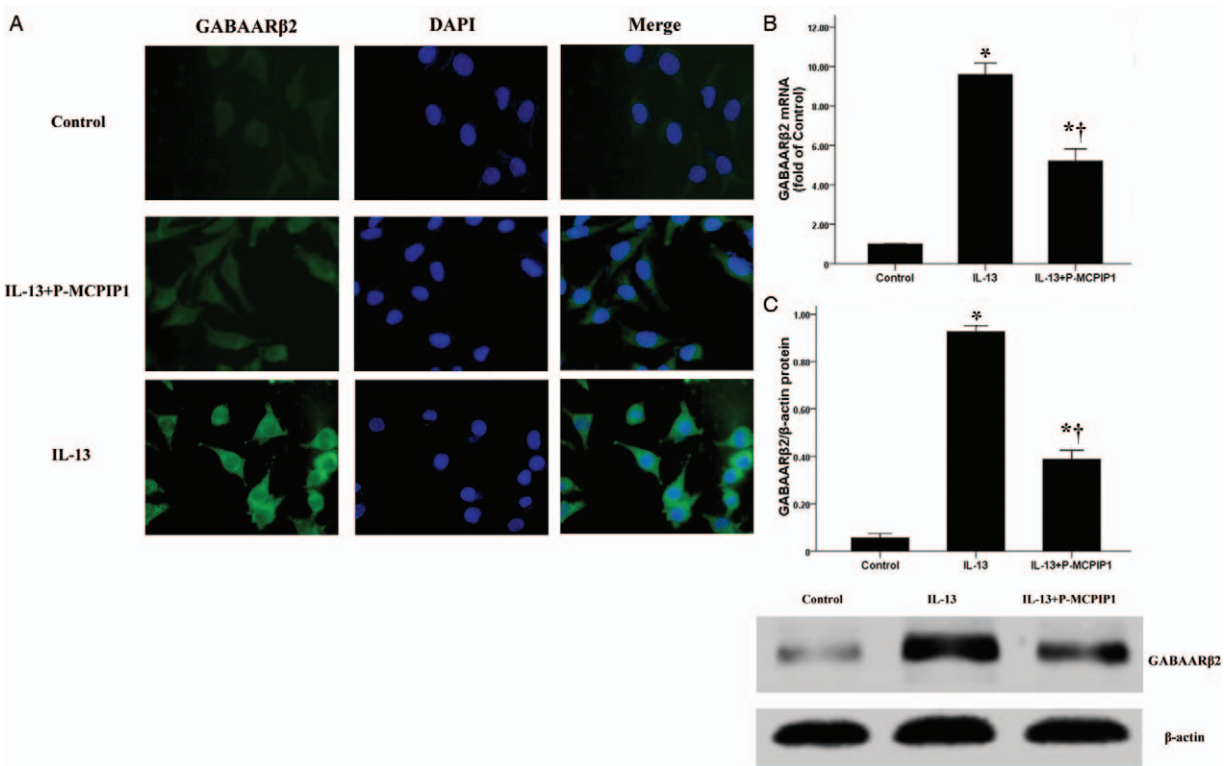

Figure 6: Plasmid-MCPIP1 attenuated IL-13-induced GABAARβ2 expression in BEAS-2B cells. Plasmid-MCPIP1-transfected and non-transfected BEAS-2B cells were stimulated with IL-13 (10 ng/mL) for 24 h. (A) Immunofluorescence was used to observe the expression of GABAARβ2 in BEAS-2B cells. The figure demonstrates a representative view (original magnification, ×400) from each group; (B) Quantitative real-time polymerase chain reaction was performed to measure the mRNA expression of GABAARβ2. (C) Western blot was used to measure the protein expression of GABAARβ2. Quantitative data were presented as mean ± standard error ($n = 5$). $^{*}P < 0.05$, compared with control, $^{\dagger}P < 0.05$, compared with IL-13. DAPI: 4′, 6-Diamidino-2-phenylindole; GABAAR: γ-Aminobutyric acid type A receptor; IL: Interleukin; MCPIP1: Monocyte chemotactic protein-inducing protein 1; P-MCPIP1: Plasmid-MCPIP1.

MCPIP1 expression was found between the Control group and the LA-scrambled group. These results indicated that LA-MCPIP1 effectively increased MCPIP1 in lung. And, lentiviral vectors showed no effect on mice lungs. Then, consistent with the designs in our previous studies, LA-MCPIP1 was used, and, LA-scrambled group was not included in the next step study.[18,19] Additionally, IL-13, belonging to Th2 cytokines, played a hub role in airway mucus hypersecretion and airway inflammation in asthma.[32,33] IL-13 was essential for the metaplasia and proliferation of goblet cells in lung, leading to mucus synthesis and secretion into the airway.[4,11] In our study, IL-13 was used to stimulate the inflammation and mucus hypersecretion in BEAS-2B cells.

MCP1 (chemokine (C-C motif) ligand 2 [CCL2]) was considered to be a critical inflammatory mediator in the allergic inflammation through inducing mast cell activation and leukotriene C4 releasing, leading to airway hyperresponsiveness.[34-36] It was found that MCP1 expression was markedly up-regulated in allergic inflammation in BEAS-2B cells.[34,36] Additionally, Cevit *et al*[37] found that MCP-1 expression positively correlated with the severity of airway inflammation in asthma patients. MCPIP1 was essential for regulation of MCP1 in inflammation. Yi *et al*[38] showed that oxygen glucose deprivation reoxygenation-induced MCP1 expression was significantly inhibited by minocycline through up-regulation of MCPIP1 in H9c2 cells. TSLP, mainly synthesized and secreted by airway epithelial cells, was essential for mast cells, dendritic cell, and Th2 cells activation in asthma.[39-41] Some studies revealed that TSLP level correlated well with the severity of airway inflammation in asthma.[42,43] Berraïes *et al*[42] showed that the content of TSLP in induced sputum positively correlated with the severity of asthma in children. Furthermore, TSLP was also considered to be a potential therapeutic target of asthma. In a phase 2, randomized, double-blind, placebo-controlled trial, Corren *et al*[44] found that tezepelumab, an anti-TSLP drug, significantly reduced the exacerbation rates in asthma patients combined with LABA and medium-to-high doses of inhaled glucocorticoids treatments. In this study, we found that OVA-induced airway inflammation was substantially attenuated by LA-MCPIP1 in lung. Meanwhile, our data showed that OVA and IL-13-enhanced MCP1 and TSLP were largely inhibited by LA-MCPIP1 and plasmid-MCPIP1 in both lung and BEAS-2B cells, respectively. These findings suggested that asthmatic airway inflammation was dramatically blocked through up-regulation of MCPIP1 both *in vivo* and *in vitro*.

Airway mucus hypersecretion was one of critical features of asthma, accounting for extensive mucus plugs in small airways and poor bronchodilators response.[3-5] Many investigations revealed that the over-produced mucus in airway was mainly caused by goblet cell metaplasia and hyperplasia.[3,4,11,45,46] Many inflammatory mediators and molecules, particularly epidermal growth factor, leukotrienes, and Th2 cytokines, contributed substantially to this pathological process.[46] Autopsy studies showed that extensive and widely mucus plugs or even mucus casts occlusion of the airway was a critical feature of fatal asthma.[6,47] These mucus plugs caused mechanical airway irreversible obstruction, leading to increased airway resistance, expiratory dyspnea, and dynamic lung hyperinflation. Meanwhile, MUC5AC was the most abundant component of airway mucus in asthma.[5,7,48] MUC5AC was also a marker of airway hypersecretion in asthma.[4,20] It was estimated that the secretion of MUC5AC could increase 40 to 200 folds in the patient with chronic asthma and fatal asthma, compared with the health subjects.[5,7,48,49] In this study, our data revealed that OVA and IL-13-enhanced MUC5AC expression was markedly abrogated by LA-MCPIP1 and plasmid-MCPIP1 in both lung and BEAS-2B cells. Furthermore, our results also found that OVA-induced airway mucus hypersecretion was noticeably suppressed by LA-MCPIP1 in lung. These results indicated that OVA and IL-13-induced airway hypersecretion was negatively regulated by MCPIP1 both *in vivo* and *in vitro*.

However, the potential mechanism of airway mucus hypersecretion was still not very clear. Several studies, consistent with our previous study, found that GABAAR, a chloride ion channel, was a critical molecule in the regulation of mucus secretion in airway epithelium.[4,11] GABAAR was composed by five sub-units ($2\alpha2\beta\gamma$), $\alpha2$, $\alpha3$, $\beta2$, $\beta3$, and γ, in airway epithelium. Among them, the $\beta2$ sub-unit was the ligand binding site and active center.[4,11] GABAAR activation induced depolarization of airway epithelial cells by promoting chloride anion efflux, leading to mucus persistent production.[4,11] Several studies revealed that airway mucus hypersecretion was notably suppressed by down-regulation and/or blocking of GABAAR in asthma.[11,45,46] IL-13 was essential for regulation of GABAAR expression in airway epithelium in asthma.[4,11] Xiang *et al*[11] identified GABAAR expression in the apical membranes of airway epithelial cells. Subsequently, they also found that OVA and IL-13-induced goblet cells hyperplasia and airway mucus hypersecretion were markedly suppressed by selective GABAAR inhibitors. Furthermore, in our previous study, we found that OVA-induced airway mucus hypersecretion and airway inflammation were largely attenuated by rosuvastatin through inhibition of IL-13/GABAAR signaling pathway.[4] Nevertheless, the potential mechanism of IL-13 on the regulation of GABAAR was still unknown. In this study, our data showed that OVA and IL-13-induced GABAAR$\beta2$ expression was substantially compromised by LA-MCPIP1 and plasmid-MCPIP1 in both lung and BEAS-2B cells. Therefore, these findings indicated that OVA and IL-13-induced GABAAR$\beta2$ was dependent on MCPIP1 both *in vivo* and *in vitro*.

Although our results indicated that GABAAR was up-regulated by IL-13 through down-regulation of MCPIP1 in airway epithelium, the molecular mechanisms of MCPIP1 in the regulation of GABAAR was still unclear. However, Ligeza *et al*[50] found that Akt activity was significantly suppressed by up-regulation of MCPIP1 in Caki-1 cells. Meanwhile, It was reported that Akt activation was essential for regulation of GABAAR expression.[51-53] Chang *et al*[51] revealed that GABAAR expression in brain, particularly hippocampus, was markedly reduced in $Akt^{-/-}$ mice. Serantes *et al*[52] showed that GABAAR expression on the cellular membrane were increased by IL-1β though

activation of PI3K/Akt pathway in Xenopus oocytes. Wang *et al*[53] also identified the hub role of Akt in the regulation of GABAAR expression in neurons. Therefore, we presumed that GABAAR was down-regulated by MCPIP1 probably through inactivation Akt in airway epithelium in asthma. However, further study should be performed to explore this hypothesis.

In conclusion, our data suggested that asthmatic airway inflammation and mucus hypersecretion were negatively regulated by MCPIP1. Furthermore, OVA and IL-13-induced airway mucus hypersecretion was dependent on MCPIP1/GABAAR system both *in vivo* and *in vitro*. Meanwhile, further study should be performed to explore the underlying mechanism on the interactions between MCPIP1 and GABAAR in airway epithelium in asthma.

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Conflicts of interest

None.

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