

LncRNA OIP5-AS1 aggravates house dust mite-induced inflammatory responses in human bronchial epithelial cells via the miR-143-3p/HMGB1 axis

XING-JUN CAI, LIN-HUI HUANG, YI-KE ZHU and YI-JIANG HUANG

Department of Respiratory and Critical Care Medicine, Hainan General Hospital, Haikou, Hainan 570311, P.R. China

Received November 27, 2019; Accepted June 23, 2020

DOI: 10.3892/mmr.2020.11536

Abstract. Bronchial asthma poses a serious threat to human health. Previous studies have documented the role of long non-coding RNAs (lncRNAs) in asthma. However, the molecular mechanism underlying bronchial asthma remains unclear. The aim of the present study was to evaluate the role of the lncRNA Opa-interacting protein 5 antisense RNA1 (OIP5-AS1) in the house dust mite-induced inflammatory response in human bronchial epithelial cells. BEAS-2B cells were treated with *Dermatophagoides pteronyssinus* peptidase 1 (Der p1) to establish an *in vitro* model of asthma. OIP5-AS1 expression levels increased in BEAS-2B cells following Der p1 treatment, while microRNA (miR)-143-3p was downregulated. Additionally, the levels of the pro-inflammatory factors tumor necrosis factor- α , interleukin (IL)-6 and IL-8 were measured, and apoptosis was evaluated following OIP5 silencing. OIP5-AS1 knockdown reduced the inflammatory response and apoptosis in BEAS-2B cells. Furthermore, using dual luciferase reporter assays and co-transfection experiments, it was demonstrated that the function of OIP5-AS1 was mediated by miR-143-3p. miR-143-3p overexpression attenuated the Der p1-induced inflammatory response and apoptosis of BEAS-2B cells by targeting high mobility group box 1 (HMGB1). In summary, OIP5-AS1 exacerbated Der p1-induced inflammation and apoptosis in BEAS-2B cells by targeting miR-143-3p via HMGB1.

Introduction

Bronchial asthma is a heterogeneous disease characterized by chronic airway inflammation and often associated with airway hyper-responsiveness (1,2). Bronchial asthma usually results in extensive and variable reversible airflow limitation resulting in recurrent attacks (3,4). Severe cases can endanger life (5,6). Common causes of asthma include air pollution, weather changes, upper respiratory tract infection, bronchitis and colds, allergic reactions and certain drugs, such as aspirin and penicillin (7,8). Common substances such as dust, pollen, carpets, clothing fibres and urine can trigger asthma. *Dermatophagoides pteronyssinus* peptidase 1 (Der p1) contained in air dust is the most frequent human allergen (9,10). Thus, even innocuous compounds can act as allergens and have a very serious impact on quality of life (11).

Airway epithelial cells constitute the first line of defence against inflammatory triggers, and injury to the airway epithelium is one of the characteristics of asthma (12,13). Increased epithelial cell apoptosis is detectable in patients with asthma and is associated with disease severity (14). Moreover, previous studies have suggested that apoptosis results in loss of epithelium integrity and exposes the airway and lungs to excess pathogens and allergens, which in turn exacerbates inflammation and compromises homeostasis of the airway epithelium (15,16). Thus, apoptosis of bronchial epithelial cells contributes to asthma (17,18). However, whether Der p1 can cause apoptosis of bronchial epithelial cells remains unknown and the underlying molecular mechanisms of Der p1-induced asthma are poorly characterized.

Long non-coding RNAs (lncRNAs) serve an important role in many bioactivities, such as the regulation of cell cycle progression, apoptosis, cell differentiation and autophagy (19). A previous study has demonstrated that the levels of lncRNAs in serum vary greatly between healthy people and patients with asthma, suggesting that lncRNAs may participate in the occurrence and development of asthma (20). In a recent study, 17 lncRNAs were detected in the serum of patients with asthma, and the expression levels of several lncRNAs were significantly lower, compared with serum from individuals without asthma (21). Other studies have also indicated that lncRNAs TCF7 and GAS5 promote the development of asthma (22,23). Among these lncRNAs, lncRNA Opa-interacting protein 5 antisense RNA1 (OIP5-AS1) was demonstrated to promote

Correspondence to: Dr Yi-Jiang Huang, Department of Respiratory and Critical Care Medicine, Hainan General Hospital, 19 Xiuhua Road, Xiuying, Haikou, Hainan 570311, P.R. China
E-mail: yijianghuang33@163.com

Abbreviations: HDM, house dust mite; lncRNA, long non-coding RNA; Der p1, *Dermatophagoides pteronyssinus* peptidase 1; miRNA/miR, microRNA; FITC, fluorescein isothiocyanate; HMGB1, high mobility group box 1

Key words: bronchial asthma, miR-143-3p, lncRNA, Opa-interacting protein 5 antisense RNA1, HMGB1, inflammatory factors

inflammation in several cell types, including macrophages and endothelial cells (24,25). Since inflammation is one of the pathological causes of asthma, it may be hypothesized that OIP5-AS1 could serve a functional role in bronchial asthma.

MicroRNAs (miRNAs) are endogenous small RNAs 20-24 nucleotides in length and have been implicated in asthma (26,27). For instance, it has been reported that miR-19a was upregulated in T cells and promoted the production of T helper 2 (Th2) cytokines in the asthmatic airway (28). A previous study also demonstrated that the levels of microRNA-18a, -27a, -128 and -155 were decreased in asthmatic bronchial epithelial cells (29). Moreover, miR-143-3p was downregulated in smooth muscle cells (SMCs) following stimulation with transforming growth factor- β 1 (TGF- β 1) (30). In addition, miR-143-3p was significantly downregulated in SMCs in patients with asthma compared with healthy controls (31). However, the role of miR-143-3p in asthma inflammation is still elusive and poorly understood.

The aim of the present study was to evaluate the role of OIP5-AS1 in asthma using an *in vitro* model. lncRNA OIP5-AS1 expression was evaluated in Der p1-exposed BEAS-2B cells. Moreover, the function of OIP5-AS1 in Der p1-induced inflammation and apoptosis was also evaluated. The findings of the present study could provide deeper insight to the role of OIP5-AS1 in asthma development and identify novel molecular mechanisms and treatment targets for asthma research.

Materials and methods

Cell culture and transfection. The human bronchial epithelial cell line BEAS-2B was obtained from American Type Culture Collection. Cells were cultured in Dulbecco's Modified Eagle Medium (Gibco; Thermo Fisher Scientific, Inc.) with 100 μ g/ml penicillin-streptomycin (Sigma-Aldrich; Merck KGaA) at 37°C with 5% CO₂. To establish an *in vitro* asthma model, BEAS-2B cells were incubated in the presence of 10 μ g/ml Der p1 (Sigma-Aldrich; Merck KGaA) for 24 h. Untreated BEAS-2B cells were used as a control.

Cells were transfected with miR-143-3p mimics (5 nM), inhibitor (5 nM) or negative control (NC, 5 nM), as well as OIP5-AS1 small interfering (si)-RNA (5 nM), si-NC (5 nM), pcDNA3.1-HMGB1 (p-HMGB1; 5 nM), or pcDNA3.1 NC (p-NC; 5 nM). All nucleic acids used for transfection were from Shanghai GeneChem Co., Ltd. Transfections were performed using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) in serum-free Opti-MEM medium (Gibco; Thermo Fisher Scientific, Inc.). The sequences of the nucleic acids used were as follows: i) miR-143-3p mimics, 5'-UGA GAUGAAGCACUGUAGCUC-3'; ii) mimics NC: 5'-UCA CAACUCCUAGAAAGAGUAGA-3'; iii) miR-143-3p inhibitor, 5'-GAGCUACAGUGCUUCAUCUCA-3'; iv) inhibitor NC, 5'-UUCUCCGAACGUGUCACGUTT-3'; v) si-OIP5-AS1 sense, 5'-GCAGCAGCUGUGGCAAA-3' and antisense, 5'-ACGUGACACGUUCGGAGAATT-3'; and vi) si-NC sense, 5'-UUCUCCGAACGUGUCACGUTT-3' and antisense, 5'-CUUGAGGCUGUUGUCAUACTT-3'. The transfection efficiency was evaluated 48 h after transfection.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from BEAS-2B cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.).

RNA was reverse transcribed using the Prime-Script One Step RT-PCR kit (Takara Biotechnology Co., Ltd.) at 65°C for 5 min, followed by 37°C for 50 min, then 70°C for 15 min. RT-qPCRs were performed in an Applied Biosystems 7500 Real Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) using SYBR GREEN Mastermix (Beijing Solarbio Science & Technology Co., Ltd.) according to manufacturer's instructions. The miR-143-3p, OIP5-AS1, high mobility group box 1 (HMGB1), tumor necrosis factor- α (TNF- α), interleukin (IL)-6, IL-8 genes were amplified, and GAPDH and U6 served as internal controls. The primer sequences were as follows: i) miR-143-3p-forward (F), 5'-CTTCTCAGACGTGCGCAA GG-3'; ii) miR-143-3p-reverse (R), 5'-GTTGATGTTGAA ACAGCTCTC-3'; iii) OIP5-AS1-F, 5'-TGCGAAGATGGC GGAGTAAG-3'; iv) OIP5-AS1-R, 5'-TAGTTCCTCTCCTCT GGCCG-3'; v) HMGB1-F, 5'-TGATGCGAACACGGCGTG CTCTA-3'; vi) HMGB1-R, 5'-GCACAAAGAATGCATATG AGGAC-3'; vii) TNF- α -F, 5'-AAGCCTGTAGCCCACGTC GTA-3'; viii) TNF- α -R, 5'-GGCACCCTAGTTGGTTGTCT TTG-3'; ix) IL-6-F, 5'-CCGGAGAGGAGACTTCACAG-3'; x) IL-6-R, 5'-TCCACGATTTCCCAGAGAAC-3'; xi) IL-8-F, 5'-GCTCTGTGTGAAGGTGCAGTT-3'; xii) IL-8-R, 5'-ACC CAGTTTTCTTGGGGTC-3'; xiii) U6-F, 5'-ATTGGAACG ATACAGAGAAGATT-3'; xiv) U6-R, 5'-GGAACGCTTCAC GAATTTG-3'; xv) GAPDH-F, 5'-CCACAGTCCATGCCA TCAC-3'; and xvi) GAPDH-R, 5'-GCTTCACCACCTTCT TGATG-3'. Relative RNA levels were calculated using the 2^{- $\Delta\Delta$ C_q} method (32).

Western blotting assay. Total protein was extracted from BEAS-2B cells using radio-immunoprecipitation assay (RIPA) buffer (Vazyme Biotech Co., Ltd.), then quantified using the Protein Concentration Determination kit (Bio-Rad Laboratories, Inc.). Proteins (20 μ g) were then resolved by SDS-PAGE on 10% gels, transferred to PVDF membranes, and blocked with 5% non-fat milk for 2 h at room temperature. The samples were then incubated with primary antibodies specific for HMGB1 (1:10,000; cat. no. ab79823, Abcam) or GAPDH (1:10,000; cat. no. ab181602; Abcam) at 4°C overnight and subsequently probed with the HRP-conjugated secondary antibody (goat anti-rabbit IgG H&L; 1:2,000; cat. no. ab205718; Abcam) at 37°C for 45 min. Protein bands were scanned using an ECL reagent (Bio-Rad laboratories, Inc.) and imaged. GAPDH served as an internal control. Relative protein expression was quantified using Image-Pro Plus software (version 6.0; Media Cybernetics, Inc.).

ELISA. ELISA was conducted to determine the levels of the inflammatory factors. Briefly, cells were centrifuged at 1,500 x g for 15 min at room temperature and the supernatant was collected. The levels of TNF- α , IL-6 and IL-8 were evaluated using commercial ELISA kits (cat. nos. ab181421, ab178013 and ab46032, respectively; all from Abcam) according to the manufacturer's instructions.

Annexin V-Fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining. For cell apoptosis measurements, cells were incubated with trypsin, then stained with Annexin V-FITC/PI double staining kit (cat. no. 556547, BD Biosciences) according to the manufacturer's instructions.

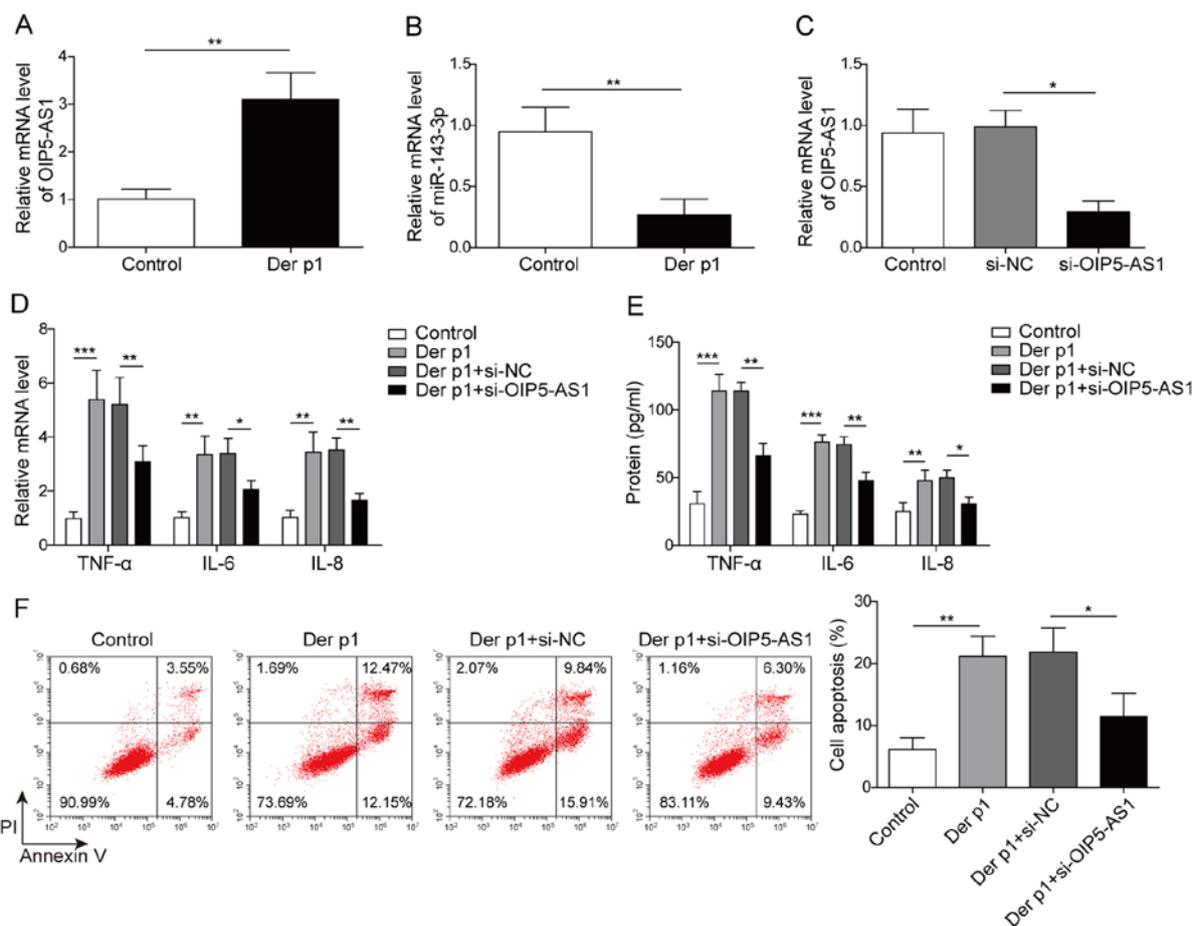


Figure 1. OIP5-AS1 knockdown reduces Der p1-induced inflammatory responses and apoptosis of BEAS-2B cells. (A) OIP5-AS1 mRNA expression was measured in BEAS-2B cells treated with 10 μ g/ml Der p1 for 24 h. (B) Expression of miR-143-3p in BEAS-2B cells treated with 10 μ g/ml Der p1 for 24 h. (C) OIP5-AS1 expression in BEAS-2B cells following transfection with si-OIP5-AS1 or si-NC. IL-6, IL-8 and TNF- α (D) mRNA and (E) protein levels following Der p1 treatment and OIP5-AS1 knockdown. (F) Apoptosis in BEAS-2B cells Der p1-treated cells transfected with si-OIP5-AS1. * P <0.05, ** P <0.01, *** P <0.001. OIP5-AS1, Opa-interacting protein 5 antisense RNA1; Der p1, *Dermatophagoides pteronyssinus* peptidase 1; RT-qPCR, reverse transcription-quantitative PCR; miR, microRNA; si, small interfering; NC, negative control; IL, interleukin; TNF- α , tumor necrosis factor- α ; PI, propidium iodide.

A FACScan flow cytometer (BD Biosciences,) was used for analysis. The data were analyzed using CellQuest software (version 3.3, BD Biosciences).

Dual luciferase reporter assay. Binding between OIP5-AS1 and miR-143-3p was predicted using Starbase v2.0 (<http://starbase.sysu.edu.cn/>) and TargetScan 7.2 (<http://www.targetscan.org>), and the wild-type (WT) and mutant (MUT) fragments of OIP5-AS1 or HMGB1 were designed accordingly.

A dual luciferase reporter assay was performed to confirm the binding between OIP5-AS1 and miR-143-3p, as well as the binding between miR-143-3p and HMGB1. For the construction of the recombinant plasmid, the pre-miR-143-3p sequence was obtained from the National Center for Biotechnology Information database (accession no. MIMAT0000435; <https://www.ncbi.nlm.nih.gov>) and the fragment was extended to 80 bp both downstream and upstream for amplification. The sequences primer sequences were as follows: i) Pre-miR-143-3p-F, 5'-TGAGATGAAGCA CTGTAGCT-3'; ii) pre-miR-143-3p-R, 5'-TGAGATGAAGCA CTGTAGCTC-3'; iii) OIP5-AS1-3'UTR-F, 5'-CCGTCTGAA CTATCCTGCC-3'; iv) OIP5-AS1-3'UTR-R, 5'-TCAACG TCAAGGAGTCGCAG-3'; v) HMGB1-3'UTR-F, 5'-CCGAT

GCTTCTGTCAACTT-3'; and vi) HMGB1-3'UTR-R, 5'-GGG CGGTACTCAGAACAGAA-3'. The WT and MUT fragments of OIP5-AS1 or HMGB1 containing the putative miR-143-3p binding sequence, were amplified and subcloned into a pGL4.10 luciferase reporter vector (Promega Corporation). The PCR reaction condition was as described as above. The thermocycling conditions consisted of: i) 94°C for 2 min, 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec; ii) 30 cycles of denaturation at 94°C for 2 min, then annealing at 60°C for 25 sec; and iii) final extension at 72°C for 30 sec. Cells were then co-transfected with 5 nM miR-143-3p mimics or mimics NC, as well as luciferase reporter plasmids using Lipofectamine[®] 2000. (Invitrogen; Thermo Fisher Scientific, Inc.). After 48-h incubation, firefly and *Renilla* luciferase activities were measured using a Bright-Glo[™] Luciferase Assay system (Promega Corporation). Firefly luciferase activity was normalized to *Renilla* luciferase activity.

Statistical analysis. Data are presented as the mean \pm SD of three experiments. An unpaired Student's t-test was used for comparisons between two groups. Multi-group comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. P <0.05 was considered to indicate a statistically

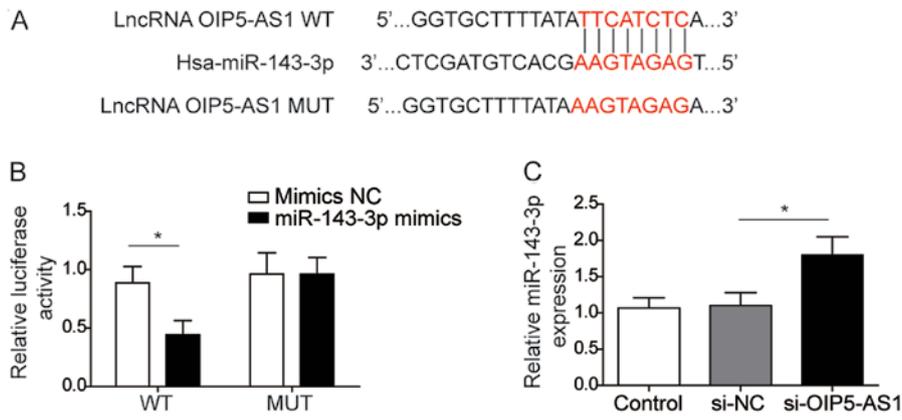


Figure 2. OIP5-AS1 directly targets and negatively regulates miR-143-3p in BEAS-2B cells. (A) Binding of OIP5-AS1 and miR-143-3p was predicted using the bioinformatics prediction software TargetScan 7.2. (B) Luciferase activity of WT-OIP5-AS1 or MUT-OIP5-AS1 cells co-transfected with miR-143-3p mimics or mimics NC. (C) miR-143-3p expression levels in BEAS-2B cells transfected with si-OIP5-AS1 or si-NC. *P<0.05. Opa-interacting protein 5 antisense RNA1; miR, microRNA, WT, wild-type; MUT, mutant; NC, negative control.

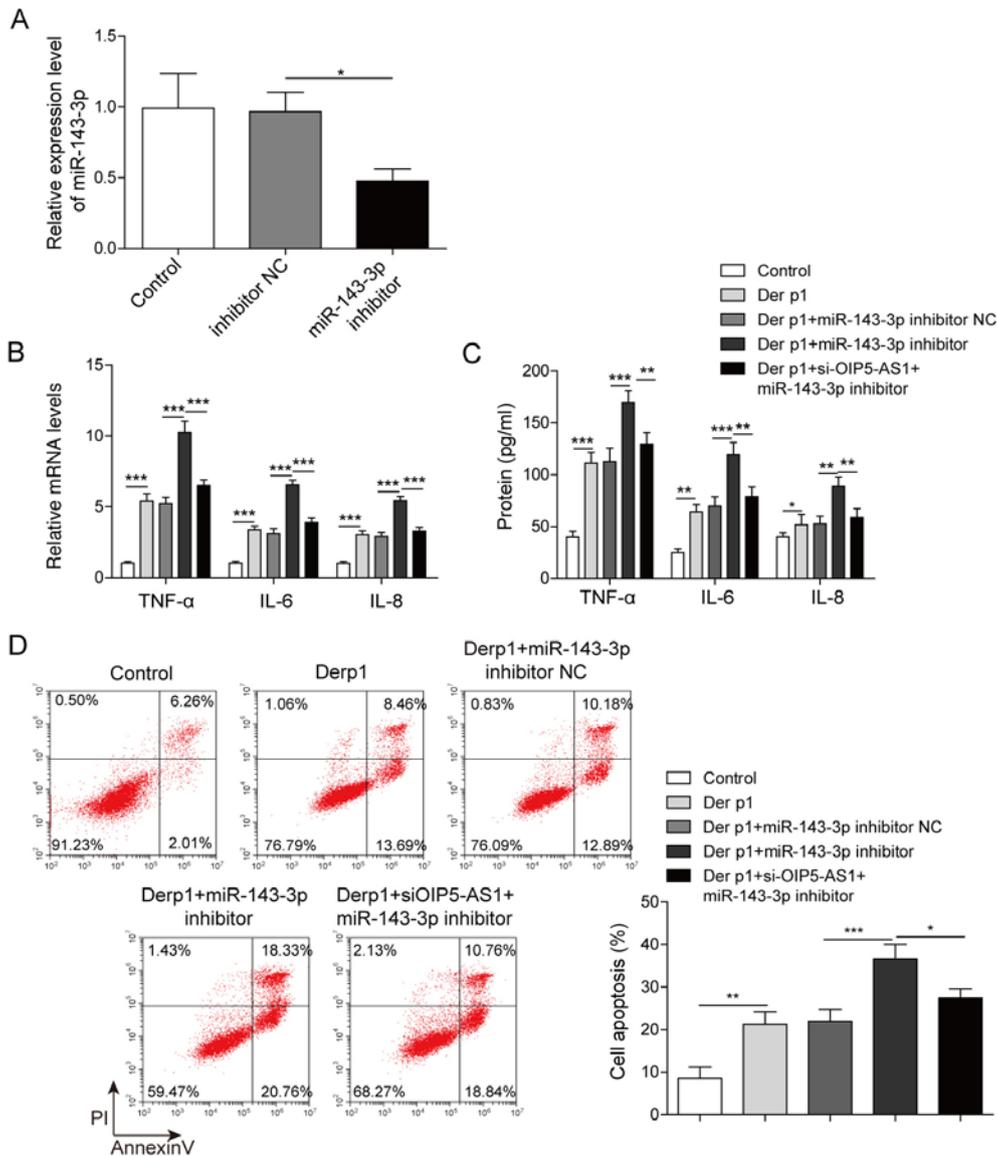


Figure 3. OIP5-AS1 knockdown exacerbates Derp1-induced inflammation and cell apoptosis through miR-143-3p. (A) miR-143-3p expression in BEAS-2B cells transfected with miR-143-3p inhibitor or inhibitor NC. IL-6, IL-8 and TNF-α (B) mRNA expression and (C) protein levels in Der p1-treated cells co-transfected with miR-143-3p inhibitor and si-OIP5-AS1. (D) Apoptosis in Der p1-treated cells co-transfected with miR-143-3p inhibitor and si-OIP5-AS1. *P<0.05, **P<0.01, ***P<0.001. OIP5-AS1, Opa-interacting protein 5 antisense RNA1; Der p1, *Dermatophagoides pteronyssinus* peptidase 1; miR, microRNA; si, small interfering; NC, negative control; IL, interleukin; TNF-α, tumor necrosis factor-α; PI, propidium iodide.

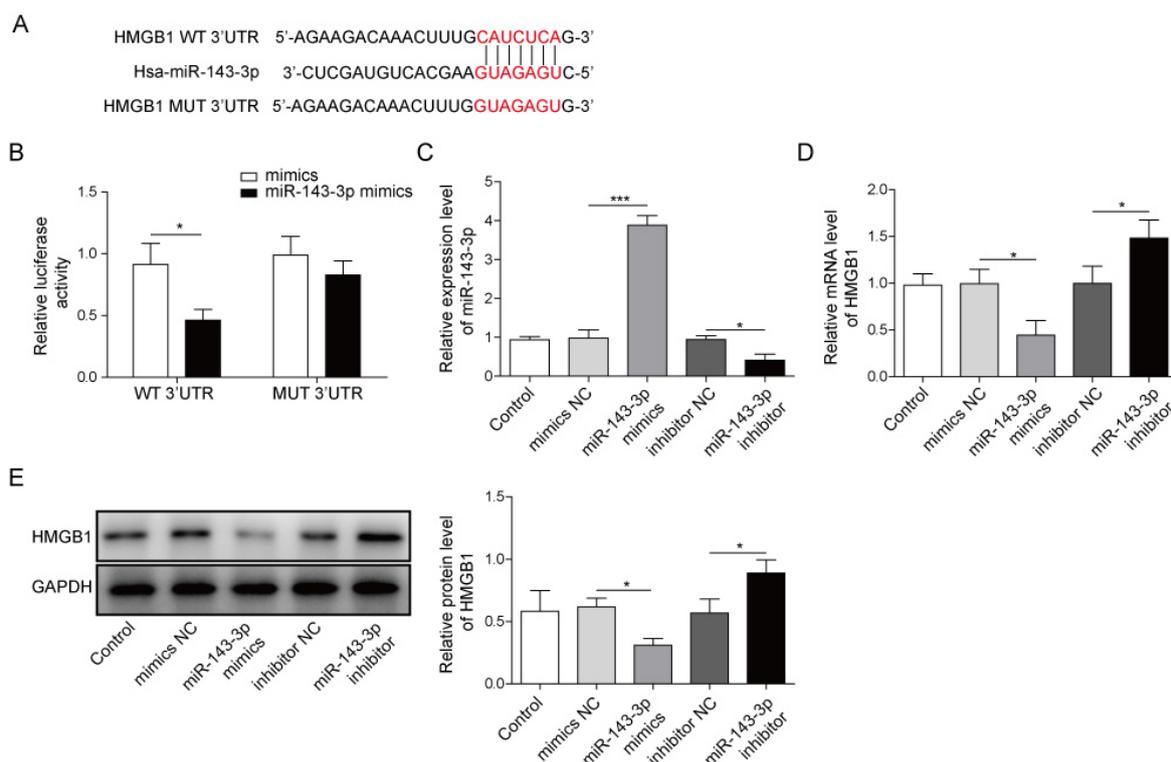


Figure 4. miR-143-3p directly targets and negatively regulates HMGB1. (A) Binding of miR-143-3p and HMGB1 was predicted using the bioinformatics prediction software TargetScan 7.2. (B) Luciferase activity in BEAS-2B cells co-transfected with miR-143-3p mimics or mimics NC, together with WT-HMGB1 or MUT-HMGB1. (C) miR-143-3p expression in cells transfected with miR-143-3p mimics or inhibitor. (D) HMGB1 mRNA expression and (E) protein levels in cells transfected with miR-143-3p mimics or inhibitor. * $P < 0.05$, *** $P < 0.01$. miR, microRNA; HMGB1, high mobility group box 1; WT, wild-type; MUT, mutant; UTR, untranslated region; NC, negative control.

significant difference. Data analysis was conducted using SPSS 20.0 statistical software (IBM Corp.).

Results

OIP5-AS1 knockdown reduces Der p1-induced inflammatory responses and apoptosis in BEAS-2B cells. OIP5-AS1 and miR-143-3p expression levels were evaluated in BEAS-2B cells following Der p1 treatment. OIP5-AS1 was significantly upregulated, while miR-143-3p was downregulated in Der p1-treated BEAS-2B cells, compared with the control (Fig. 1A and B). To further examine the potential function of OIP5-AS1, BEAS-2B cells were transfected with si-OIP5-AS1. OIP5-AS1 expression was significantly reduced si-OIP5-AS1-transfected cells, compared with si-NC, indicating successful transfection (Fig. 1C).

TNF- α , IL-6 and IL-8 levels were significantly upregulated in cells treated with Der p1, compared with untreated cells; however, OIP5-AS1 knockdown significantly reduced the expression of these inflammatory factors, relative to si-NC (Fig. 1D and E). Moreover, treatment with Der p1 significantly enhanced the apoptotic rate of BEAS-2B cells, compared with control. Transfection with si-OIP5-AS1 significantly reduced the frequency of apoptotic cells, compared with si-NC (Fig. 1F). Altogether, these results indicated that OIP5-AS1 silencing may attenuate Der p1-induced inflammatory responses and apoptosis *in vitro*.

LncRNA OIP5-AS1 directly targets and negatively regulates miR-143-3p in BEAS-2B cells. To further confirm the

association between OIP5-AS1 and miR-143-3p in BEAS-2B cells, potential binding partners for OIP5-AS1 were predicted using the Starbase software, which identified miR-143-3p as a target. Furthermore, luciferase activity was significantly reduced in BEAS-2B cells co-transfected with miR-143-3p and WT-OIP5-AS1 (Fig. 2A), but not with MUT-OIP5-AS1 (Fig. 2B), indicating that OIP5-AS1 directly targeted miR-143-3p. In addition, miR-143-3p was significantly upregulated following transfection with si-OIP5-AS1, compared with si-NC, which suggested that OIP5-AS1 may negatively regulate miR-143-3p expression (Fig. 2C). Collectively, these findings suggested that lncRNA OIP5-AS1 may target miR-143-3p and regulate its expression in BEAS-2B cells.

Suppression of OIP5-AS1 exacerbates Derp1-induced inflammation and apoptosis via miR-143-3p. The effect of miR-143-3p expression on Der p1-treated BEAS-2B cells was examined using miR-143-3p inhibitor transfection (Fig. 3A). Following transfection with miR-143-3p inhibitor, TNF- α , IL-6 and IL-8 were significantly upregulated at the mRNA and the protein levels, compared with inhibitor NC (Fig. 3B and C). However, in BEAS-2B cells co-transfected with the inhibitor and OIP5-AS1, the levels of these cytokines significantly decreased to levels comparable to inhibitor NC. Moreover, miR-143-3p inhibitor transfection also promoted cell apoptosis. This effect was also reversed following co-transfection with si-OIP5-AS1, reducing the frequency of apoptotic cells to the same levels as inhibitor NC (Fig. 3D). These results indicated that miR-143-3p attenuated Der p1-induced inflammatory

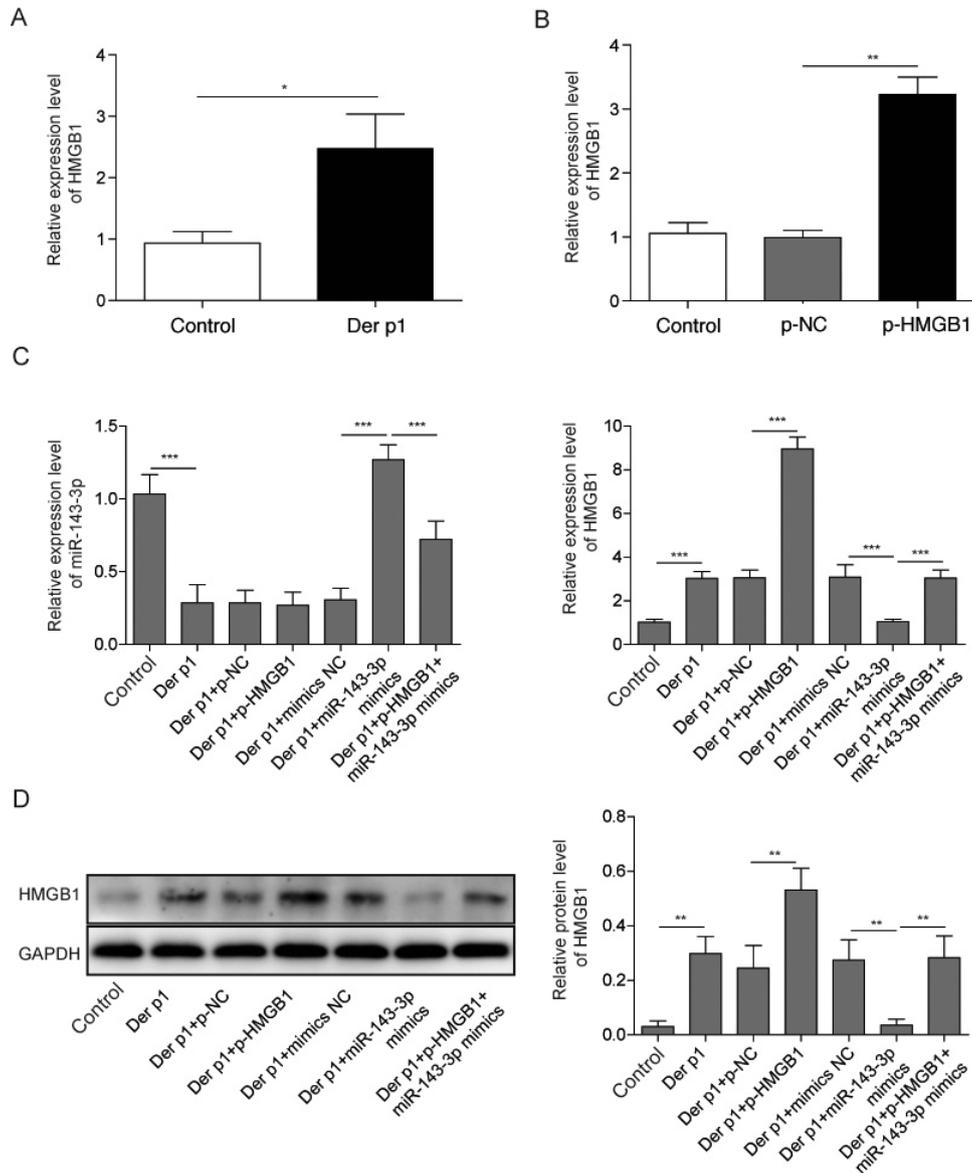


Figure 5. miR-143-3p overexpression reduces the Der p1-induced inflammatory response and apoptosis of BEAS-2B cells through HMGB1. (A) HMGB1 expression was determined in control and Der p1-treated cells using RT-qPCR. (B) Expression of HMGB1 in cells transfected with p-HMGB1 determined using RT-qPCR. (C) The expression of miR-143-3p and HMGB1 was determined in different groups of cells using RT-qPCR. (D) HMGB1 protein levels were determined in cells co-transfected with pcDNA3.1-HMGB1 or and miR-143-3p mimics using western blotting. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Der p1, *Dermatophagoides pteronyssinus* peptidase 1; miR, microRNA; HMGB1, high mobility group box 1; NC, negative control; RT-qPCR, reverse transcription-quantitative PCR.

responses and apoptosis in BEAS-2B cells and that OIP5-AS1 exacerbated these effects via miR-143-3p.

miR-143-3p directly targets and negatively regulates HMGB1.

To understand the mechanism by which miR-143-3p regulated the Der p1-induced inflammatory responses and apoptosis in BEAS-2B cells, potential binding targets for miR-143-3p were screened using TargetScan 7.2, which identified HMGB1 as a potential target gene (Fig. 4A). Additionally, luciferase activity was significantly decreased in BEAS-2B cells co-transfected with miR143-3p and WT-HMGB1, but not MUT-HMGB1 (Fig. 4B). Transfections with miR-143-3p mimics and miR-143-3p inhibitor were also carried out in order to assess the effects of miR-143-3p expression on BEAS-2B cells (Fig. 4C). Transfection with miR-143-3p mimics significantly

knocked down HMGB1 expression, compared with mimics NC. Conversely, BEAS-2B cells transfected with miR-143-3p inhibitor displayed significantly increased levels of HMGB1, compared with the inhibitor NC (Fig. 4D and E). Altogether, these observations suggested that miR-143-3p directly targeted HMGB1 and negatively regulated its expression *in vitro*.

Overexpression of miR-143-3p reduces Der p1-induced inflammation and apoptosis of BEAS-2B cells via HMGB1.

The role of HMGB1 in Der p1-induced inflammatory response and apoptosis of BEAS-2B cells was further examined. HMGB1 was significantly upregulated in cells treated with Der p1 (Fig. 5A). BEAS-2B cells were then transfected with miR-143-3p mimics, p-HMGB1 or both. Transfection with the p-HMGB1 vector significantly increased the expression of

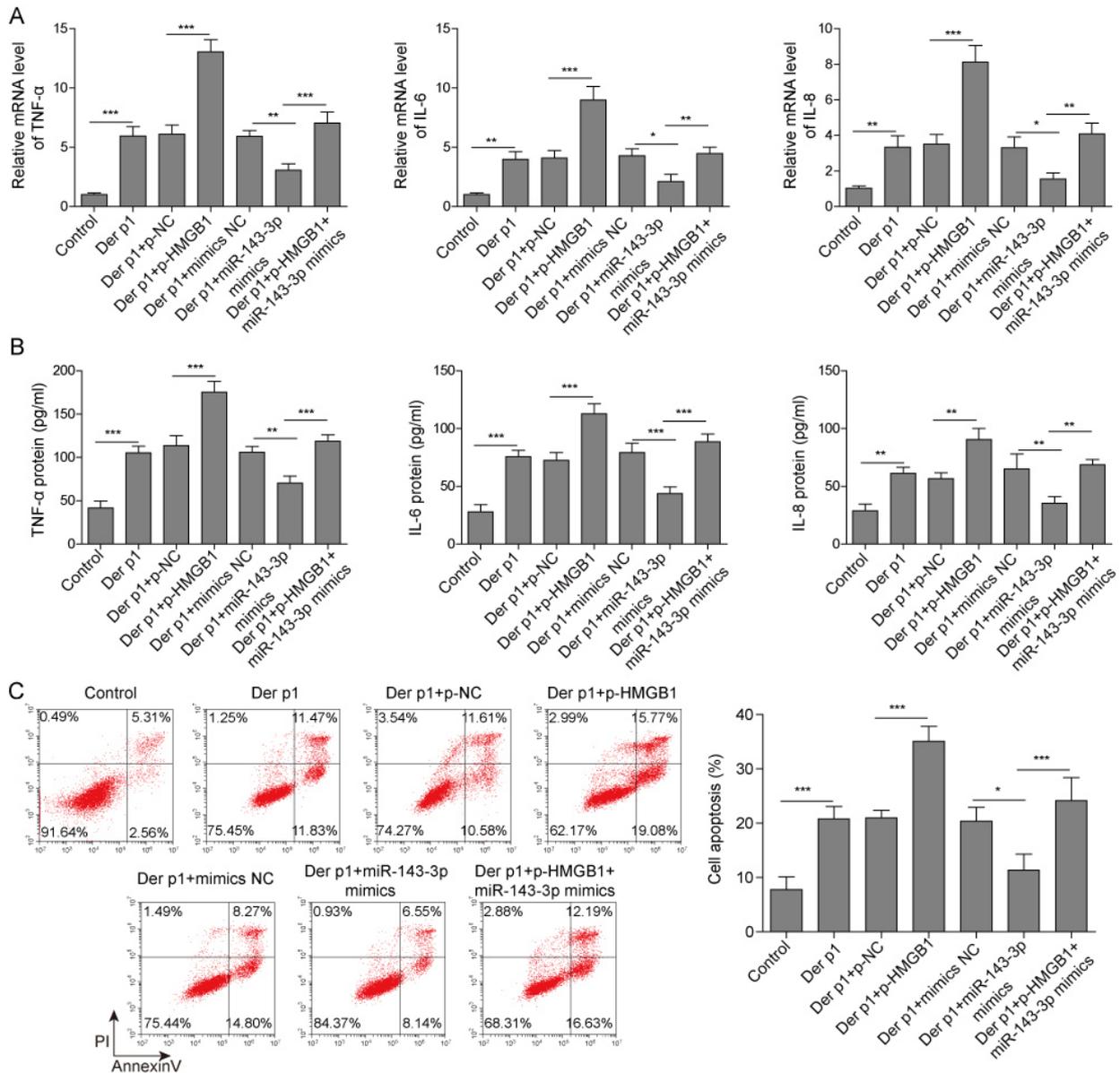


Figure 6. miR-143-3p overexpression reduces the expression of proinflammatory proteins and suppresses apoptosis of BEAS-2B cells by regulating HMGB1. IL-6, IL-8 and TNF- α (A) mRNA expression and (B) protein levels in Der p1-treated cells co-transfected with miR-143-3p mimics and p-HMGB1 over-expression vector. (C) Apoptosis in Der p1-treated cells co-transfected with miR-143-3p mimics and p-HMGB1. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Der p1, *Dermatophagoides pteronyssinus* peptidase 1; miR, microRNA; NC, negative control; IL, interleukin; TNF- α , tumor necrosis factor- α ; PI, propidium iodide.

HMGB1 (Fig. 5B). miR-143-3p mimics transfection increased the levels of miR-143-3p in BEAS-2B cells. However, co-transfection with p-HMGB1 reduced miR-143-3p expression, compared with cells transfected with mimics alone (Fig. 5C). Moreover, BEAS-2B cells transfected with miR-143-3p mimics significantly decreased HMGB1 expression, both at the mRNA and protein levels, compared with controls. However, co-transfection with miR-143-3p mimics and p-HMGB1 increased HMGB1 levels, compared with mimics alone (Fig. 5D).

Furthermore, miR-143-3p mimics transfection decreased TNF- α , IL-6 and IL-8 secretion, compared with mimics NC. By contrast, HMGB1 overexpression significantly increased the levels of these cytokines, compared with mimics alone (Fig. 6A and B). Apoptosis was also reduced following miR-143-3p overexpression, but this effect was reversed by transfection with p-HMGB1

(Fig. 6C). Thus, miR-143-3p reduced Der p1-induced inflammation and apoptosis via HMGB1.

OIP5-AS1 regulates the expression of HMGB1 via miR-143-3p. Further validation experiments were conducted using si-OIP5-AS1, miR-143-3p inhibitor and p-HMGB1 co-transfection. OIP5-AS1 silencing resulted in a significant increase in miR-143-3p and a concomitant decrease in HMGB1 expression. However, co-transfection of si-OIP5-AS1 together with miR-143-3p inhibitor p-HMGB1 reversed these effects. Moreover, transfection miR-143-3p inhibitor alone or overexpression of HMGB1 both resulted in an increase in HMGB1. However, co-transfection with si-OIP5-AS1 downregulated HMGB1, compared with transfection with miR-143-3p inhibitor or p-HMGB1 alone. Altogether, these findings suggested that OIP5-AS1 may regulate HMGB1 via miR-143-3p in Der p1-treated BEAS-2B cells (Fig. 7).

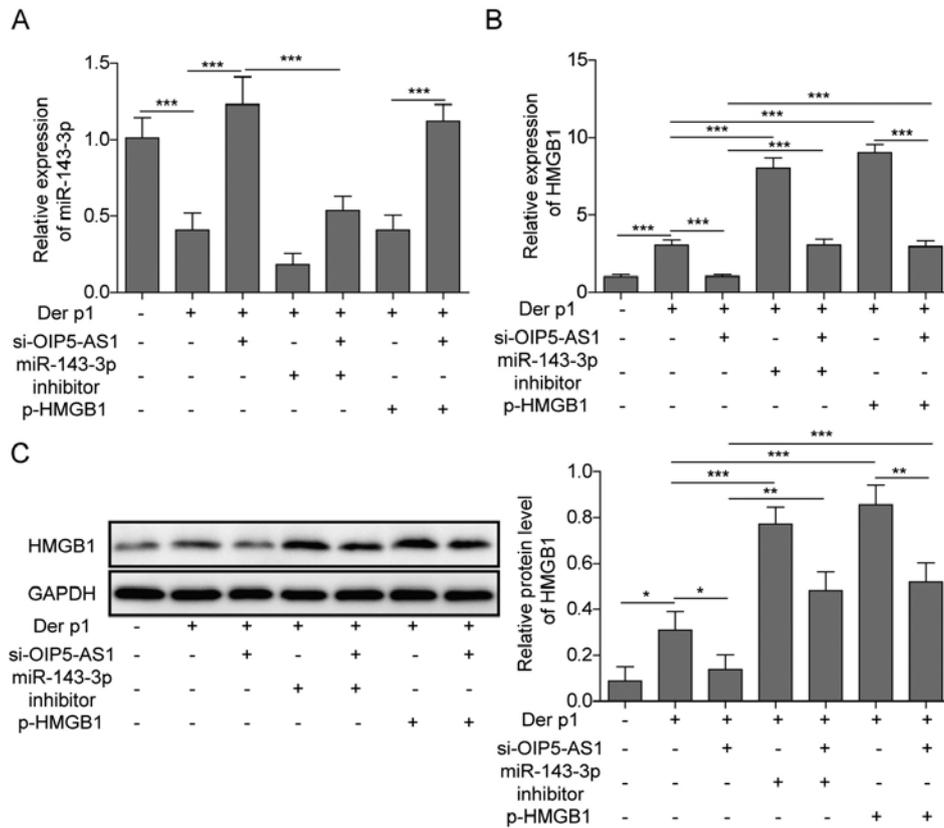


Figure 7. OIP5-AS1 regulates the expression of HMGB1 through miR-143-3p. (A) miR-143-3p and (B) HMGB1 expression in cells co-transfected with si-OIP5-AS1, miR-143 inhibitor or p-HMGB1. (C) Protein levels of HMGB1 in cells co-transfected with si-OIP5-AS1, miR-143 inhibitor or p-HMGB1. *P<0.05, **P<0.01, ***P<0.001. OIP5-AS1, Opa-interacting protein 5 antisense RNA1; Der p1, *Dermatophagoides pteronyssinus* peptidase 1; RT-qPCR, reverse transcription-quantitative PCR; miR, microRNA; si, small interfering; NC, negative control.

Discussion

Bronchial asthma is a chronic airway inflammatory disease characterized by allergic inflammation and airway hyper-responsiveness. Bronchial asthma can cause irreversible airway stenosis and remodelling, with serious adverse consequences on quality of life and mortality (7). During an asthma attack, inflammatory responses are initiated, releasing pro-inflammatory cytokines, such as IL-1β, IL-6, IL-8 and TNF-α (33,34). In addition, apoptosis is also involved in the development of asthma, and the inhibition of cell apoptosis may improve asthma (35,36). To the best of the authors' knowledge, few studies have reported the role of OIP5-AS1 in asthma. The present study demonstrated that OIP5-AS1 was upregulated in Der p1-treated BEAS-2B cells. OIP5-AS1 promoted Der p1-induced inflammation and apoptosis via miR-143-3p and HMGB1. Furthermore, OIP5-AS silencing resulted in a reduction in the expression levels of pro-inflammatory cytokines.

OIP5-AS1 can regulate several physiological functions, and several studies have documented its role in cancer development. For instance, Tao *et al* (37) demonstrated that inhibition of OIP5-AS1 inhibited the development of multiple myeloma. OIP5-AS1 also promoted cell apoptosis of myeloma cells (38). However, whether OIP5-AS1 also serves a role in asthma remains unknown. Since both activation of inflammation and induction of cell apoptosis, such as apoptosis of airway epithelial cells, are associated with incidence of asthma, we noticed

the promotion effects of OIP5-AS1 on cell apoptosis and inflammation and speculate it may also participate in asthma. Previous studies have demonstrated the effects of OIP5-AS1 on cell apoptosis. For instance, OIP5-AS1 promoted the apoptosis of oxidized low density lipoprotein (ox-LDL)-mediated vascular endothelial cells by targeting glycogen synthase kinase-3β, and silencing OIP5-AS1 reduced the apoptosis rate (25). Moreover, OIP5-AS1 silencing also inhibited cell apoptosis of myeloma cells (38). In a recent study, OIP5-AS1 could promote apoptosis of bladder cancer cells (39). Besides, OIP5-AS1 could also facilitate ox-LDL-induced inflammation and oxidative stress in macrophages, as well as induce ox-LDL mediated vascular endothelial cells apoptosis (24,25). Consistent with these previous findings, the present study demonstrated that OIP5-AS1 knockdown inhibited Der p1-induced apoptosis.

LncRNAs regulate gene expression by serving as miRNA sponges by competitively binding to miRNA and acting as a competing endogenous RNA (ceRNA) of miRNA (40). Tao *et al* (37) suggested that OIP5-AS1 served as an endogenous sponge of miR-367-3p in gastric cancer cells. Moreover, OIP5-AS1 functioned as a sponge for miR-129-5p (38). In the present study, an association between miR-143-3p and OIP5-AS1 was predicted using bioinformatics analysis then validated in a dual-luciferase reporter assay. These results indicated that OIP5-AS1 acted as a sponge for miR-143-3p and negatively regulated its expression *in vitro*.

miR-143-3p is a short non-coding RNA that regulates the survival pathways of many cancer cell types (41-43). The role

of miR-143-3p in asthma has also been reported in several studies. Indeed, the expression of miR-143-3p was lower in asthmatic patients, compared with healthy controls (31). Cheng *et al* (30) also demonstrated that overexpression of miR-143-3p inhibited the abnormal proliferation of SMCs induced by TGF- β 1, suggesting a potential role for miR-143-3p in asthma. Consistent with these previous findings, miR-143-3p was downregulated in Der p1-induced BEAS-2B cells in the present study.

Mu *et al* (44) suggested that miR-143-3p inhibited hyperplastic scar formation by targeting connective tissue growth factor. However, it was also reported that miR-143-3p promoted inflammation in cardiac hypertrophy, suggesting that the roles of miR-143-3p might vary under different pathophysiological conditions (45). The present study demonstrated that miR-143-3p inhibited the expression of pro-inflammatory factors in Der p1-induced cells. Moreover, miR-143-3p was a direct target of OIP5-AS1, and its effects on Der p1-induced cells were mediated through HMGB1.

HMGB1 is associated with inflammation (46). HMGB1 activates NLR family pyrin domain containing 3 and initiates inflammatory responses (47-49). Several studies have demonstrated that HMGB1 is associated with the development of asthma (50-52). Zhang *et al* (50) demonstrated that recombinant HMGB1 inhibited Th17 responses in mice with neutrophilic asthma. Yanhua *et al* (51) also suggested that HMGB1 may induce asthmatic airway inflammation through 75-kDa glucose-regulated protein. HMGB1 also facilitates allergen-induced airway remodelling in chronic asthma and promotes lung fibrosis (52). In agreement with these previous findings, the presented study suggested that HMGB1 overexpression promoted inflammation and apoptosis in Der p1-treated cells. Besides, HMGB1 was reported to be a target of miR-143-3p in bladder cancer cells (47). In the present study, miR-143-3p directly targeted and negatively regulated the expression of HMGB1 in BEAS-2B cells. miR-143-3p attenuated apoptosis and inflammation *in vitro* by targeting HMGB1.

In conclusion, the present study demonstrated the effect of OIP5-AS1 on Der p1-induced inflammation and apoptosis *in vitro* and suggested a potential mechanism implicating OIP5-AS1, miR-143-3p and HMGB1 in bronchial asthma. Clinical evidence supporting the use of non-coding RNA as therapeutic targets may provide insight into novel treatment options for bronchial asthma.

Acknowledgements

Not applicable.

Funding

The present study was supported by The Key Research and Development Program of Hainan Province (grant no. ZDYF2018150) and The National Natural Science Foundation of China (grant no. 81860007).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

XJC conceived the study and methodology, acquired funding, wrote the original draft and validated the study. LHH conducted data curation, and contributed to data collection and analysis. YKZ performed data analysis and contributed to data collection. YJH supervised the study, reviewed and revised the manuscript, and contributed to data validation. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Meldrum K, Robertson SB, Römer I, Marczylo T, Dean LSN, Rogers A, Gant TW, Smith R, Tetley TD and Leonard MO: Cerium dioxide nanoparticles exacerbate house dust mite induced type II airway inflammation. *Part Fibre Toxicol* 15: 24, 2018.
- Izuhara K, Matsumoto H, Ohta S, Ono J, Arima K and Ogawa M: Recent developments regarding periostin in bronchial asthma. *Allergol Int* 64 (Suppl): S3-S10, 2015.
- Alam S, Li Z and Mahadeva R: S86 Formation of Oxidised Alpha-1 antitrypsin induces inflammatory response in human bronchial epithelial cells. *Thorax* 67: A41-A42, 2012.
- Ren YF, Li H, Xing XH, Guan HS, Zhang BA, Chen CL and Zhang JH: Preliminary study on pathogenesis of bronchial asthma in children. *Pediatr Res* 77: 506-510, 2015.
- Hellermann GR, Nagy SB, Kong X, Lockey RF and Mohapatra SS: Mechanism of cigarette smoke condensate-induced acute inflammatory response in human bronchial epithelial cells. *Respir Res* 3: 22, 2002.
- Tauber E, Herouy Y, Urbanek R, Urbanek R, Goetz M and Hagel E: Assessment of serum myeloperoxidase in children with bronchial asthma. *Allergy* 54: 177-182, 1999.
- Fattouh R, Algarawi A, Fattouh M, Arias K, Walker TD, Goncharova S, Coyle AJ, Humbles AA and Jordana M: Eosinophils are dispensable for allergic remodeling and immunity in a model of house dust mite-induced airway disease. *Am J Respir Crit Care Med* 183: 179-188, 2011.
- Larsen BB, Nielsen LP, Engelstätter R, Steinijans V and Dahl R: Effect of ciclesonide on allergen challenge in subjects with bronchial asthma. *Allergy* 58: 207-212, 2003.
- Cozens AL, Yezzi MJ, Kunzelmann K, Ohru T, Chin L, Eng K, Finkbeiner WE, Widdicombe JH and Gruenert DC: CFTR expression and chloride secretion in polarized immortal human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 10: 38-47, 1994.
- Hamasaki Y, Kohno Y, Ebisawa M, Kondo N, Nishima S, Nishimuta T, Morikawa A, Aihara Y, Akasawa A, Adachi Y, *et al*: Japanese pediatric guideline for the treatment and management of bronchial asthma 2012. *Pediatr Int* 56: 441-450, 2014.
- Viau E, Levischaffer F and Peccia J: Respiratory toxicity and inflammatory response in human bronchial epithelial cells exposed to biosolids, animal manure, and agricultural soil particulate matter. *Environ Sci Technol* 44: 3142-3148, 2010.
- Mertens TCJ, Karmouty-Quintana H, Taube C and Hiemstra PS: Use of airway epithelial cell culture to unravel the pathogenesis and study treatment in obstructive airway diseases. *Pulm Pharmacol Ther* 45: 101-113, 2017.
- Zhang H, Sun Y, Rong W, Fan L, Cai Y, Qu Q, Gao Y and Zhao H: miR-221 participates in the airway epithelial cells injury in asthma via targeting SIRT1. *Exp Lung Res* 44: 272-279, 2018.

14. Zhou C, Yin G, Liu J, Liu X and Zhao S: Epithelial apoptosis and loss in airways of children with asthma. *J Asthma* 48: 358-365, 2011.
15. Yang LI, Na CL, Luo S, Wu D, Hogan S, Huang T and Weaver TE: The phosphatidylcholine transfer protein stard7 is required for mitochondrial and epithelial cell homeostasis. *Sci Rep* 7: 46416, 2017.
16. Pu Y, Liu YQ, Zhou Y, Qi YF, Liao SP, Miao SK, Zhou LM and Wan LH: Dual role of RACK1 in airway epithelial mesenchymal transition and apoptosis. *J Cell Mol Med* 24: 3656-3668, 2020.
17. Lee HS, Park DE, Song WJ, Park HW and Sohn SW: Effect of 1,8-cineole in *Dermatophagoides pteronyssinus*-stimulated bronchial epithelial cells and mouse model of asthma. *Biol Pharm Bull* 39: 946-952, 2016.
18. Bucchieri F, Gammazza AM, Pitruzzella A, Fucarino A, Farina F, Howarth P, Holgate ST, Zummo G and Davies DE: Cigarette smoke causes caspase-independent apoptosis of bronchial epithelial cells from asthmatic donors. *PLoS One* 10: e0120510, 2015.
19. Kim J, Abdelmohsen K, Yang X, De S, Grammatikakis I, Noh JH and Gorospe M: LncRNA OIP5-AS1/cyranosponges RNA-binding protein HuR. *Nucleic Acids Res* 44: 2378-2392, 2016.
20. Austin PJ, Tsitsiou E, Boardman C, Jones SW, Lindsay MA, Adcock IM, Chung KF and Perry MM: Transcriptional profiling identifies the long noncoding RNA plasmacytoma variant translocation (PVT1) as a novel regulator of the asthmatic phenotype in human airway smooth muscle. *J Allergy Clin Immunol* 139: 780-789, 2017.
21. Naemura M, Kuroki M, Tsunoda T, Arikawa N, Sawata Y, Shirasawa S and Kotake Y: The long noncoding RNA OIP5-AS1 is involved in the regulation of cell proliferation. *Anticancer Res* 38: 77-81, 2018.
22. Fan M, Xu J, Xiao Q, Chen F and Han X: Long non-coding RNA TCF7 contributes to the growth and migration of airway smooth muscle cells in asthma through targeting TIMMDC1/Akt axis. *Biochem Biophys Res Commun* 508: 749-755, 2019.
23. Zhang XY, Tang XY, Li N, Zhao LM, Guo YL, Li XS, Tian CJ, Cheng DJ, Chen ZC and Zhang LX: GAS5 promotes airway smooth muscle cell proliferation in asthma via controlling miR-10a/BDNF signaling pathway. *Life Sci* 212: 93-101, 2018.
24. Li X, Cao Q, Wang Y and Wang Y: LncRNA OIP5-AS1 contributes to ox-LDL-induced inflammation and oxidative stress through regulating the miR-128-3p/CDKN2A axis in macrophages. *RSC Advances* 9: 41709-41719, 2019.
25. Wang M, Liu Y, Li C, Zhang Y, Zhou X and Lu C: Long noncoding RNA OIP5-AS1 accelerates the ox-LDL mediated vascular endothelial cells apoptosis through targeting GSK-3 β via recruiting EZH2. *Am J Transl Res* 11: 1827-1834, 2019.
26. Williams AE, Larnersvenson H, Perry MM, Campbell GA, Herrick SE, Adcock IM, Erjefalt JS, Chung KF and Lindsay MA: MicroRNA expression profiling in mild asthmatic human airways and effect of corticosteroid therapy. *PLoS One* 4: e5889, 2009.
27. Feng MJ, Shi F, Qiu C and Peng WK: MicroRNA-181a, -146a and -146b in spleen CD4+ T lymphocytes play proinflammatory roles in a murine model of asthma. *Int Immunopharmacol* 13: 347-353, 2012.
28. Simpson LJ, Sana P, Bhakta NR, Choy DF, Brightbill HD, Ren X, Wang Y, Pua HH, Baumjohann D, Montoya MM, *et al.*: A microRNA upregulated in asthma airway T cells promotes TH2 cytokine production. *Nat Immunol* 15: 1162-1170, 2014.
29. Martinez-Nunez RT, Bondanese VP, Louafi F, Francisco-Garcia AS, Rupani H, Bedke N, Holgate S, Howarth PH, Davies DE and Sanchez-Elsner T: A microRNA network dysregulated in asthma controls IL-6 production in bronchial epithelial cells. *PLoS One* 9: e111659, 2014.
30. Cheng W, Yan K, Xie LY, Chen F, Yu HC, Huang YX and Dang CX: MiR-143-3p controls TGF- β 1-induced cell proliferation and extracellular matrix production in airway smooth muscle via negative regulation of the nuclear factor of activated T cells 1. *Mol Immunol* 78: 133-139, 2016.
31. Sorianoarroquia A, McCormick R, Molloy AP, Mcardle A and Goljanekwhysall K: Age-related changes in miR-143-3p: Igfbp5 interactions affect muscle regeneration. *Aging Cell* 15: 361-369, 2016.
32. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
33. Persson IM, Menzel M, Ramu S, Cerps S, Akbarshahi H and Uller L: IL-1 β mediates lung neutrophilia and IL-33 expression in a mouse model of viral-induced asthma exacerbation. *Respir Res* 19: 16, 2018.
34. Jiang XG, Yang XD, Lv Z and Zhuang PH: Elevated serum levels of TNF- α , IL-8, and ECP can be involved in the development and progression of bronchial asthma. *J Asthma* 55: 111-118, 2018.
35. Reece SW, Kilburg-Basnyat B, Madenspacher JH, Luo B, Capen A, Fessler MB and Gowdy KM: Scavenger receptor class B type I (SR-BI) modulates glucocorticoid mediated lymphocyte apoptosis in asthma. *Am Assoc Immunol* 198 (1 Suppl): S53, 2017.
36. Lv J, Su W, Yu Q, Zhang M, Di C, Lin X, Wu M and Xia Z: Heme oxygenase-1 protects airway epithelium against apoptosis by targeting the proinflammatory NLRP3-RXR axis in asthma. *J Biol Chem* 293: 18454-18465, 2018.
37. Tao Y, Wan X, Fan Q, Wang Y, Sun H, Ma L and Wu Y: Long non-coding RNA OIP5-AS1 promotes the growth of gastric cancer through the miR-367-3p/HMGA2 axis. *Dig Liver Dis* 52: 773-779, 2020.
38. Yang N, Chen J, Zhang H, Wang X, Yao H, Peng Y and Zhang W: LncRNA OIP5-AS1 loss-induced microRNA-410 accumulation regulates cell proliferation and apoptosis by targeting KLF10 via activating PTEN/PI3K/AKT pathway in multiple myeloma. *Cell Death Dis* 8: e2975, 2017.
39. Wang Y, Shi F, Xia Y and Zhao H: LncRNA OIP5-AS1 predicts poor prognosis and regulates cell proliferation and apoptosis in bladder cancer. *J Cell Biochem* 120: 7499-7505, 2019.
40. Huang YA, Chan KCC and You ZH: Constructing prediction models from expression profiles for large scale lncRNA-miRNA interaction profiling. *Bioinformatics* 34: 812-819, 2018.
41. Sun X, Dai G, Yu L, Hu Q, Chen J and Guo W: miR-143-3p inhibits the proliferation, migration and invasion in osteosarcoma by targeting FOSL2. *Sci Rep* 8: 606, 2018.
42. Chen L, Yao H, Wang K and Liu X: Long non-coding RNA MALAT1 regulates ZEB1 expression by sponging miR-143-3p and promotes hepatocellular carcinoma progression. *J Cell Biochem* 118: 4836-4843, 2017.
43. Du J, Zhang Y, Shen L, Luo J, Lei H, Zhang P, Pu Q, Liu Y, Shuai S, Li Q, *et al.*: Effect of miR-143-3p on C2C12 myoblast differentiation. *Biosci Biotechnol Biochem* 80: 706-711, 2016.
44. Mu S, Kang B, Zeng W, Sun Y and Yang F: MicroRNA-143-3p inhibits hyperplastic scar formation by targeting connective tissue growth factor CTGF/CCN2 via the Akt/mTOR pathway. *Mol Cell Biochem* 416: 99-108, 2016.
45. Yu B, Zhao Y, Zhang H, Xie D, Nie W and Shi K: Inhibition of microRNA-143-3p attenuates myocardial hypertrophy by inhibiting inflammatory response. *Cell Biol Int* 42: 1584-1593, 2018.
46. Yang H and Tracey KJ: Targeting HMGB1 in inflammation. *Biochim Biophys Acta* 1799: 149-156, 2020.
47. Luo J, Chen J, Li H, Yang Y, Yun H, Yang S and Mao X: LncRNA UCA1 promotes the invasion and EMT of bladder cancer cells by regulating the miR-143/HMGB1 pathway. *Oncol Lett* 14: 5556-5562, 2017.
48. Thankam FG, Roesch ZK, Dilisio MF, Radwan MM, Kovilam A, Gross RM and Agrawal DK: Association of inflammatory responses and ECM disorganization with HMGB1 upregulation and NLRP3 inflammasome activation in the injured rotator cuff tendon. *Sci Rep* 8: 8918, 2018.
49. Falcão AS, Carvalho LA, Lidônio G, Vaz AR, Lucas SD, Moreira R and Brites D: Dipeptidyl vinyl sulfone as a novel chemical tool to inhibit HMGB1/NLRP3-inflammasome and inflamma-miRs in A β -mediated microglial inflammation. *ACS Chem Neurosci* 8: 89-99, 2017.
50. Zhang F, Huang G, Hu B, Qian GS and Song Y: Recombinant HMGB1 A box protein inhibits Th17 responses in mice with neutrophilic asthma by suppressing dendritic cell-mediated Th17 polarization. *Int Immunopharmacol* 24: 110-118, 2015.
51. Yanhua L, Yanli L, Dandan Z, Anbing Z, Weihong G and Shunfang Z: HMGB1-induced asthmatic airway inflammation through GRP75-mediated enhancement of ER-Mitochondrial Ca²⁺ transfer and ROS increased. *J Cell Biochem* 119: 4205-4215, 2018.
52. Hou C, Kong J, Liang Y, Huang H, Wen H, Zheng X, Wu L and Chen Y: HMGB1 contributes to allergen-induced airway remodeling in a murine model of chronic asthma by modulating airway inflammation and activating lung fibroblasts. *Cell Mol Immunol* 12: 409-423, 2015.

