



MIF signaling blocking alleviates airway inflammation and airway epithelial barrier disruption in a HDM-induced asthma model

Haibing Lan^{a,1}, Liang Luo^{b,1}, Yu Chen^a, Meng Wang^a, Zhihong Yu^a, Yuanqi Gong^{a,*}

^a Department of Intensive Care Unit, The Second Affiliated Hospital of NanChang University, Nanchang 330006, People's Republic of China

^b Department of Intensive Care Unit, The Seventh Affiliated Hospital of Sun Yat-Sen University, Shenzhen 518000, People's Republic of China

ARTICLE INFO

Keywords:

Asthma
Airway inflammation
Airway epithelial barrier disruption
MIF
Aerobic glycolysis

ABSTRACT

Recent studies have indicated that Macrophage migration inhibitory factor (MIF) plays an important role in the prevention and treatment of asthma. However the role of MIF in airway inflammation and airway epithelial barrier disruption in house dust mite (HDM)-induced asthma has not been addressed. We hypothesized that MIF contributed to HDM-induced the production of Th2-associated cytokines and E-cadherin dysfunction in asthmatic mice and 16HBE cells. In vivo, a HDM-induced asthma mouse model was set up and mice treated with MIF antagonist ISO-1 after HDM. The mice treated with the ISO-1 ameliorated airway hyper-reactivity, airway inflammation, increased serum IgE levels, the aberrant arrangement of E-cadherin as well as the release of Th2 cytokines induced by HDM. In vitro, the exposure of 16HBE cells to HDM and rhMIF resulted in airway epithelial barrier disruption, inflammatory cytokine production and enhanced glycolytic flux. While these changes were attenuated by MIF siRNA treatment. Sequentially, treatment of 16HBE cells with PFKFB3 antagonist PFK15 significantly lowered rhMIF-induced these changes in 16HBE cells. Therefore, these results indicate that MIF may be an important contributor in airway inflammation and airway epithelial barrier disruption of HDM-induced asthma. Moreover, HDM specifically induces airway inflammation and airway epithelial barrier disruption of 16HBE cells through MIF-mediated enhancement of aerobic glycolysis.

1. Introduction

Asthma is a common chronic nonspecific airway inflammatory diseases and the worldwide incidence, morbidity, and mortality of allergic asthma are increasing [1]. Allergic asthma is characterized by airway hyperresponsiveness (AHR) to a variety of specific and non-specific stimuli, airway inflammation, elevated serum immunoglobulin E (IgE), excessive airway mucus production and airway narrowing [2,3]. As a barrier to the external environment, the bronchial epithelium has typically been thought to function mainly as the first defensive barrier by impeding the access of a wide range of environmental materials present in inhaled air [4]. The physical barrier function of airway epithelial cells is dependent on the integrity of the cell, the coordinate expression and interaction of proteins in cell-cell junctional complexes especially adherens junctions, which consist of E-cadherin, β -catenin, and α -catenin [5]. Airway epithelium connected molecule E-cadherin is regarded as the 'gatekeeper' in the airway mucosa and can mediate immunological function of airway epithelium through its interaction with Tregs and DC as well as proliferation, differentiation, and

the release of growth factors and proinflammatory factor [6]. In asthma, epithelial barrier function is often compromised, with disrupted expression of the adhesion molecule E-cadherin and simultaneously the loss and redistribution of E-cadherin can facilitate the pro-inflammatory activities of the epithelium and contributes significantly to asthma pathogenesis [6,7].

Macrophage migration inhibitory factor (MIF) is originally described as a T lymphocyte-derived protein that could prevent the random migration of macrophages and a key modulator of both the inflammatory and immune responses [8]. Early studies showed that MIF is ubiquitously expressed by a variety of cells including primary macrophages, macrophage cell lines, eosinophils, neutrophils, endothelial cells, T lymphocyte cells and airway epithelial cells [9,10]. Overexpression of MIF was detected in the bronchoalveolar lavage fluid, serum, and sputum in asthma patients and MIF overexpression correlated with the release of a number of proinflammatory cytokines including TNF- α , interleukin (IL)-1 β and IL-6 [11,12]. Thus, MIF not only exerts proinflammatory effects, but also mediate resistance to the anti-inflammatory effects of steroids [13]. Furthermore, the local

* Corresponding author.

E-mail address: 760225787@qq.com (Y. Gong).

¹ These authors made equal contributions to this paper.

<https://doi.org/10.1016/j.cellimm.2019.103965>

Received 10 September 2018; Received in revised form 29 July 2019; Accepted 14 August 2019

0008-8749/ © 2019 Published by Elsevier Inc.

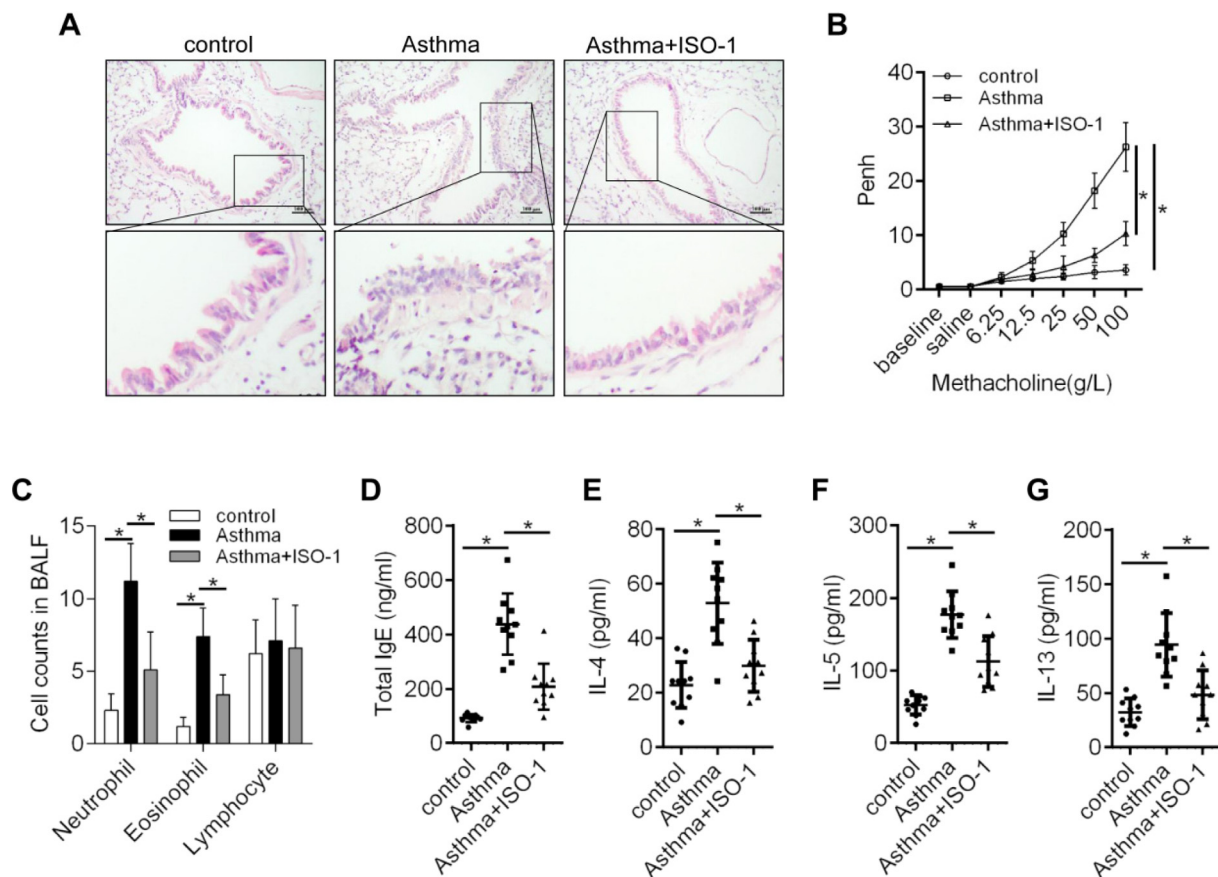


Fig. 1. MIF antagonist ISO-1 alleviates AHR and airway inflammation in the mouse model of HDM-induced asthma. (A) Representative H&E-stained lung sections from the different groups. Magnification, 200 $\times$ (upper panel) and 400 $\times$ (lower panel). (B) Airway hyper-responsiveness was measured by whole body plethysmography. (C) Neutrophils, eosinophils and lymphocytes number in the BALF of mice from different groups. (D) IgE was measured by ELISA in serum. (E) IL-4, (F) IL-5, and (G) IL-13 levels in BALF were measured by ELISA. Data are presented as the mean $\pm$ SD; n = 8–10/group; *P < 0.05.

application of the MIF antagonist ISO-1 prevented Th2-mediated airway inflammation and reduce airway remodeling in a murine model for chronic asthma [14]. Thus, MIF plays an important role in the context of allergic asthma. However, the precise molecular mechanisms leading to the dysfunction of airway epithelial adherens junctions E-cadherin in HDM-induced asthma remains incompletely understood. So we infer that MIF might also mediate E-cadherin signaling.

In the present study, we focused on the effects of MIF antagonist ISO-1 on lung inflammatory and the distribution of E-cadherin in a chronic HDM-induced mouse model of asthma and define the possible mechanism in normal human bronchial epithelial cells.

2. Materials and methods

2.1. Animals and experimental protocol

Specific-pathogen-free (SPF) BALB/c mice (male, 6–8 weeks old, 20–24 g) were purchased. The mice were housed in a SPF environment (room temperature 24 $^{\circ}$ C, humidity range 40–70%, and a 12-h light/dark cycle). Sterilized water and food were provided ad libitum. Standard guidelines for laboratory animal care followed the Guide for the Care and Use of Laboratory Animals. HDM was purchased from ALK-Abello A/S (Denmark) and ISO-1 was synthesized as described previously [15]. BALB/c mice were randomly assigned to one of 3 groups: (1) control group, in which the mice were received phosphate-buffered saline (PBS, Gibco, Life Technology); (2) HDM group; (3) ISO-1 + HDM group, in which mice were pretreated with ISO-1, followed by HDM. The HDM-induced asthma model were established as previously described with mildly modification [16]. Briefly, mice were

exposed to intranasal sevoflurane-anesthesia, then received treatment with 10 μ L PBS, HDM (400 U/mouse each day, dissolved in deionized water), ISO-1 (35 mg/kg). In the ISO-1 + HDM group, the anaesthetized mice were pretreated with ISO-1 60 min prior to the HDM administration. These treatment procedures were carried out daily for 5 consecutive days, followed by two days of rest, for 8 consecutive weeks. All treatments (except ISO-1) were administered via intranasal inhalation. ISO-1 was administered to the mice by intraperitoneal injection.

2.2. The measurement of airway hyper-reactivity (AHR)

As previously described [17], the measurement of AHR was carried out using methacholine (Sigma-Aldrich). Airway parameters were measured 24 h after the last challenge. Mice were placed in a barometric plethysmo-graphic chamber (Buxco Electronics, Inc., Troy, NY) and provoked with vehicle (normal saline), followed by increasing concentrations of methacholine (6.25, 12.5, 25, 50, and 100 mg/mL, respectively) via a nebulizer (Buxco Electronics, Inc.). The reading interval was set to 5 min following each nebulization. The broncho-pulmonary resistance was expressed as enhanced pause (Penh). After finishing the test, the mice were sacrificed with an overdose of sodium pentobarbitone (administered intraperitoneally at a concentration of 100 mg/kg body weight; Sigma, China).

2.3. Analysis of serum and bronchoalveolar lavage fluid (BALF)

Blood samples were collected, stored for 2 h at room temperature, and then centrifuged (3000 rpm, 20 min). The supernatants were harvested and stored at -80° C. Total serum IgE was measured by ELISA

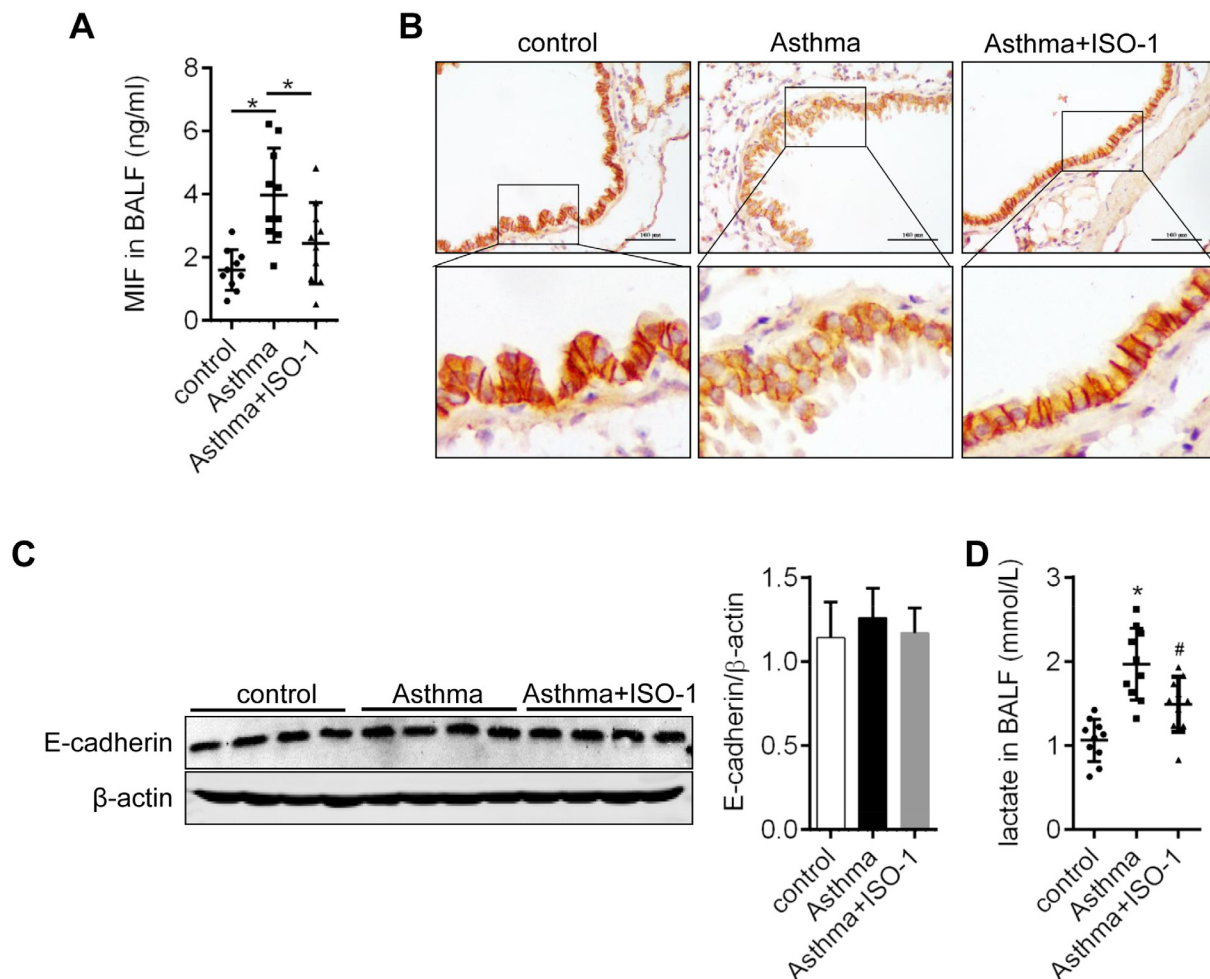


Fig. 2. MIF antagonist ISO-1 inhibits the disruption and delocalization of E-cadherin and the release of Th2 cytokines in the mouse model of HDM-induced asthma. (A) MIF was measured by ELISA in BALF. (B) Representative immunohistochemical staining images for E-cadherin in the bronchial regions. Magnification, 200× (upper panel) and 400× (lower panel). (C) Western blotting analysis of E-cadherin protein in whole lung tissue lysates and subsequent densitometric analysis of the blots. (D) Lactate in the BALF of mice from different groups. Data are presented as the mean ± SD; n = 8–10/group; *P < 0.05.

according to the manufacturer's instructions. Next, BALF samples were collected. Total cells in BALF were counted, and a cytospin sample was prepared and stained with hematoxylin and eosin (H&E) for blinded assessment of neutrophils cells, eosinophil cells and lymphocyte cells percentages in BALF. Then BALF samples were centrifuged (1000 rpm, 10 min). The supernatants were harvested and stored at -80°C . The levels of MIF, IL-4, IL-5 and IL-13 in the processed BALF samples were measured using ELISA according to the manufacturer's instructions. Lactate in BALF was measured with Lactate Colorimetric/Fluorometric Assay Kit according to the manufacturer's instructions.

2.4. Pathological staining and immunohistochemistry

The left lungs of mice were removed and embedded in paraffin. Lung sections (4 μm) were prepared and stained with hematoxylin and eosin (H&E) for blinded histopathologic assessment. Then immunohistochemistry for E-cadherin, lung sections submerged in citrate buffer (pH 6.0) for antigen retrieval. Samples were treated with H_2O_2 for 15 min to block endogenous peroxidase, and then incubated overnight at 4°C in recommended dilutions of anti-E-cadherin (Santa Cruz) antibodies. After washing with PBS, slices were incubated with a secondary antibody for 30 min at room temperature. Signals were visualized with a DAB peroxidase substrate kit.

2.5. Epithelial cell culture and treatment

The human bronchial epithelial cell line, 16HBEo- (16HBE) (Shanghai Fuxiang Biological Technology Co. Ltd., ATCC, USA) were grown in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco, Life Technology). The cells were then incubated in a humidified incubator at 37°C with an atmosphere of 5% CO_2 . When the cells reached 80–90% confluence, the cells were treated with trypsin and plated at a density of 10^4 – 10^5 cells/ cm^2 for use in the experiments. The medium was changed to serum-free RPMI 1640 when the cells reached 85% confluence, and 12 h after that, the cells were treated according to the experimental plan. In knockdown experiments, 16HBE cells were transfected with MIF-specific predesigned siRNA (Santa Cruz) or scrambled control siRNA (Santa Cruz) with Lipofectamine 2000 according to the manufacturer's instructions. Knockdown efficiency was assessed using western blot. Forty-eight h after transfection, the cells were detached from the test dish and used in experiments.

2.6. The measurement of epithelial barrier function

Epithelial barrier function was assessed by measuring TER and fluorescein isothiocyanate (FITC)-dextran flux across the monolayers of cultured epithelial cells. TER and FITC was quantified as previously described [17]. Briefly, confluent monolayers of 16HBE cells, polarized

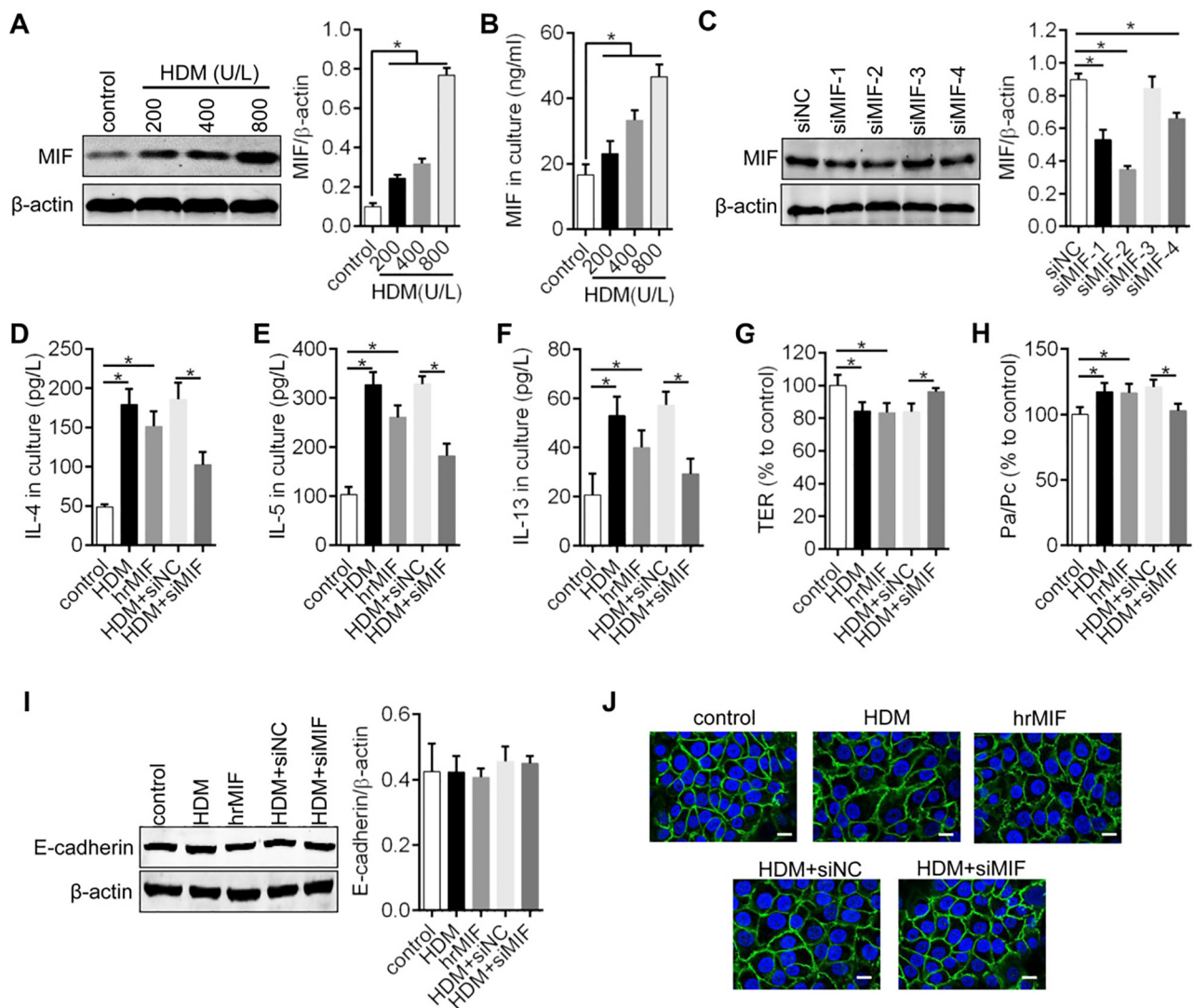


Fig. 3. Mediation of HDM-induced airway epithelial barrier disruption and the release of Th2 cytokines by elevating the MIF expression in 16HBE cells. (A) Western blot analysis of MIF protein levels in 16HBE stimulated with HDM and subsequent densitometric analysis of the blots. (B) MIF was measured by ELISA in the culture medium from different groups. (C) Western blot analysis of MIF protein levels in 16HBE cells and subsequent densitometric analysis of the blots. (D) IL-4, (E) IL-5, and (F) IL-13 levels in the culture medium were measured by ELISA. (G) TER and (H) FITC-Dx permeability was measured in the different groups of treated cells. (I) Western blotting analysis of E-cadherin protein in the different groups of treated cells and subsequent densitometric analysis of the blots. (J) The distribution of E-cadherin was assessed using immunofluorescence. Green represents E-cadherin and blue represents the nucleus (DAPI staining). Data are presented as the mean $\pm$ SD; $n = 3-5$ /group; * $P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

at an air-liquid interface, were cultured in 12-well Transwell inserts (Corning Costar). TER was measured using a Millicell ERS-2 Epithelial Volt-Ohm meter with an STX01 electrode (Millipore Corp, Billerica, MA, USA). Then the apical medium (luminal side) was replaced with 200 μ L phenol red-free RPMI-1640 containing 0.5 mg/mL fluorescein isothiocyanate-dextran (FITC-dextran), and the basal medium (non-luminal side) was replaced with 800 μ L phenol red-free RPMI-1640 without FITC-dextran, incubated at 37 $^{\circ}$ C for 90 min. Samples were respectively analyzed by fluorimetry (excitation 492 nm, emission 530 nm). Epithelial permeability was expressed as percent leakage of FITC-dextran from apical to basolateral compartments.

2.7. Immunofluorescence

The immunofluorescence of cultured cells was monitored as described in the previous study [16,17]. Primary antibodies against E-cadherin and the FITC (green)-linked anti-rabbit IgG were obtained from Santa Cruz Biotechnology, USA. 4',6-diamidino-2-phenylindole

dihydrochloride (DAPI) was obtained from Sigma-Aldrich.

2.8. Biochemical assays and analysis of the culture medium

Glucose concentration of the culture medium was measured by using Glucose Colorimetric/Fluorometric Assay Kit according to the manufacturer's instructions. Lactate concentration of the culture medium was measured by using Lactate Colorimetric/Fluorometric Assay Kit according to the manufacturer's instructions. The levels of MIF, IL-4, IL-5 and IL-13 in the culture medium were measured using ELISA according to the manufacturer's instructions.

2.9. Western blotting analysis

The treated cells and the tissue lysates of right lungs were collected and boiled in standard SDS sample buffer and western blotting for detection of the following antigens: Antibodies against MIF (Abcam) and E-cadherin (Santa Cruz Biotechnology). After incubation with a

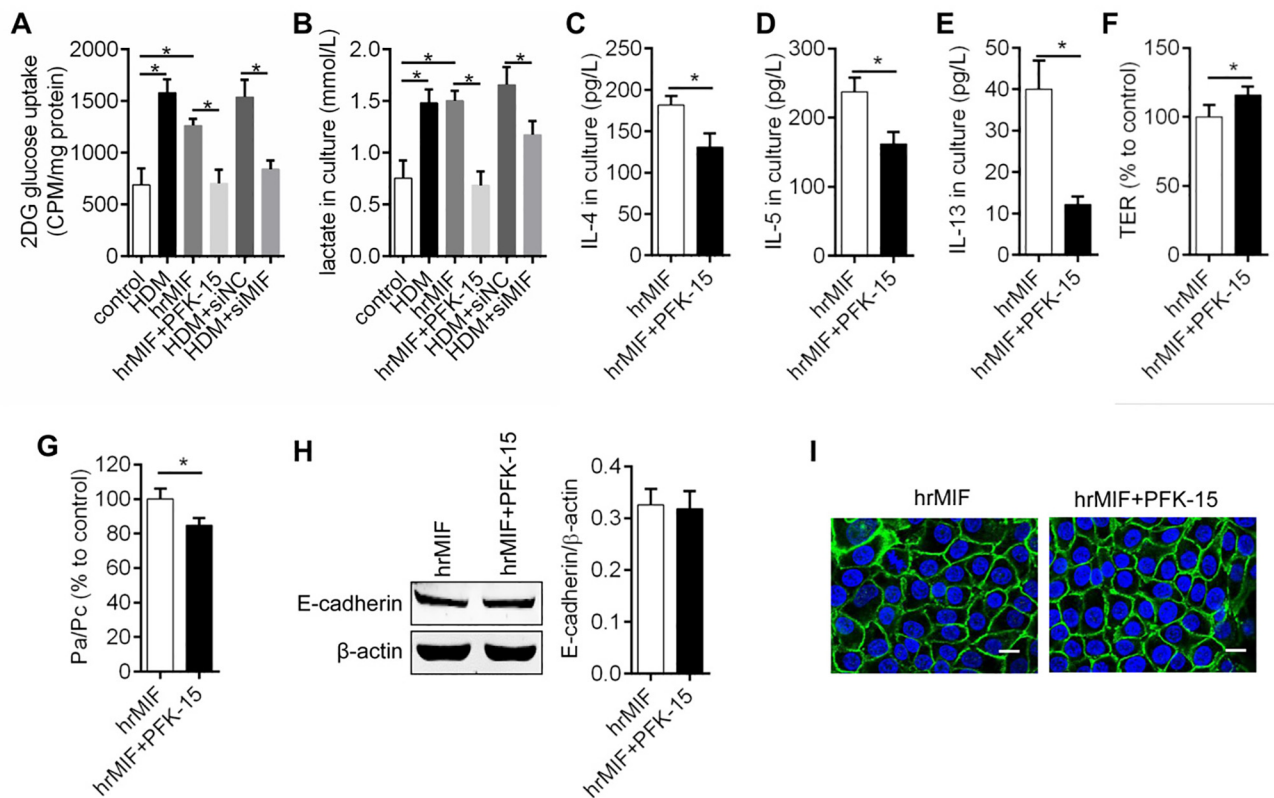


Fig. 4. HDM promotes MIF expression, which in turn induce airway epithelial barrier disruption and the release of Th2 cytokines by accelerating aerobic glycolysis in 16HBE cells. (A) Glucose consumption in 16HBE cells from different groups were determined. (B) The concentrations of lactate in the culture medium from different groups were determined. (C) IL-4, (D) IL-5, and (E) IL-13 levels in the culture medium were measured by ELISA. (F) TER and (G) FITC-Dx permeability was measured in the different groups of treated cells. (H) Western blotting analysis of E-cadherin protein in the different groups of treated cells and subsequent densitometric analysis of the blots. (I) The distribution of E-cadherin was assessed using immunofluorescence. Green represents E-cadherin and blue represents the nucleus (DAPI staining). Data are presented as the mean $\pm$ SD; $n = 3-5$ /group; $*P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

secondary antibody, immunoreactive bands were exposed to Odyssey® CLx Imager for image capture. The densitometry results were first normalized with that of β -actin and then compared with the control to obtain relative fold changes. Data analysis was done with Odyssey Software.

2.10. Statistical analysis

Statistical analysis was carried out using the SPSS (version 19.0) software package. All data are presented as the mean $\pm$ standard deviation (SD). One-way ANOVA and Bonferonni's post hoc test for multiple comparisons were utilized to compare differences among groups. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. MIF antagonist ISO-1 alleviates AHR and airway inflammation in the mouse model of HDM-induced asthma

To confirm that the HDM-induced asthmatic mice model was successfully established and to address the role of MIF and neutralization of MIF in the murine asthma model, we first evaluated AHR in the HDM-induced asthma model by assessing the enhanced pause (Penh). The Penh values were significantly increased in the HDM-treated mice, compared with the saline-treated control group following stimulation with 25, 50, and 100 mg/mL methacholine (Fig. 1B). Treatment with ISO-1 significantly inhibited the AHR to levels similar to that in mice that inhaled methacholine (Fig. 1B). Then we found MIF protein expression levels were upregulated in the HDM-exposed mice, whereas

pretreatment with MIF antagonist ISO-1 resulted in a significant inhibition of MIF expression, as expected (Fig. 2A). At the same time, compared to the control group, histological examination of lung sections from HDM-treated mice indicated markedly large numbers of infiltrating inflammatory cells in the peribronchial regions, epithelial hyperplasia and evident epithelial hyperplasia and a degree of epithelial shedding, while treatment with the ISO-1 markedly mitigated inflammatory infiltration in the peribronchial regions (Fig. 1A). Total cell counts, as well as numbers of neutrophils, eosinophils and lymphocytes were assessed in BALF. In agreement with total cell counts, higher amounts of neutrophils and eosinophils were found after HDM stimulation (Fig. 1C). Higher total serum IgE, a marker of sensitization, was also detected in HDM-asthmatic mice, but this effect was inhibited by ISO-1 (Fig. 1D). These data suggest that our mouse model of HDM-induced asthma is appropriate for addressing the role of airway inflammation and that MIF is upregulated and released upon induction of asthma.

3.2. MIF antagonist ISO-1 inhibits the disruption and delocalization of E-cadherin and the release of Th2 cytokines in the mouse model of HDM-induced asthma

Asthma was successfully induced by HDM. To further assess the effect of ISO-1 on the disruption and delocalization of E-cadherin and the release of Th2 cytokines in the HDM-induced asthma, the IL-4, IL-5 and IL-13 in BALF were measured by ELISA and the distribution of E-cadherin in lungs were detected by immunohistochemistry. There were notable increases in IL-4, IL-5 and IL-13 in BALF of HDM-induced asthma which were reduced by ISO-1 (Fig. 1E-G). E-cadherin mainly

localized at the lateral side and apicolateral border of the airway epithelial cells in unstimulated cells, while exposure to HDM resulted in a notable decrease in E-cadherin at the epithelial cell–cell contact regions and a diffused staining pattern in the cytoplasm (Fig. 2B). These effects were partially reversed by treatment with the ISO-1 (Fig. 2B). But western blotting analysis indicated a unchanged expression of E-cadherin proteins in lungs of HDM-treated mice (Fig. 2C). These findings clearly indicate that exposure to HDM results in a robust Th2-driven inflammation and the aberrant distribution of E-cadherin in mice, and the protective effect of the ISO-1.

3.3. Mediation of HDM-induced airway epithelial barrier disruption and the release of Th2 cytokines by elevating the MIF expression in 16HBE cells

Then in vitro we stimulated the 16HBE cells with 200, 400 and 800 U/ml HDM. We assessed the expression levels of MIF proteins by western blot analysis. These results revealed HDM caused a significant increase in the expression levels of MIF (Fig. 3A, B). Next, we examined the effects of HDM and hrMIF on barrier permeability in monolayers of 16HBE cells and the expression levels of inflammatory cytokines IL-4, IL-5 and IL-13 in the culture medium by ELISA. In the treated 16HBE cells, HDM exposure resulted in TER reduction (Fig. 3G), increase in fluorescent-dextran (FITC-Dx) permeability (Fig. 3H), the delocalization of E-cadherin (Fig. 3J) and a significant increase in the expression levels of Th2-associated cytokines IL-4, IL-5 and IL-13 (Fig. 3D–F). While the expression of E-cadherin proteins was not altered (Fig. 3I). We then infected 16HBE cells with MIF siRNAs and selected a lower silencing efficiency by conducting experiments 24 h after transfection (Fig. 3C). Interestingly, the downregulation of MIF prevented the HDM-induced these changes (Fig. 2E–G). Taken together, these results strongly suggest that the MIF pathway regulated HDM-induced the release of Th2 cytokines and airway epithelial barrier disruption in 16HBE cells, contributing to the pathogenesis of asthma.

3.4. HDM promotes MIF expression, which in turn induce airway epithelial barrier disruption and the release of Th2 cytokines by accelerating aerobic glycolysis in 16HBE cells

In mice, we have found the increase lactate in BALF of the HDM group. In 16HBE cells we also found HDM and hrMIF caused a significant increase in glucose uptake (Fig. 4A) and lactate level (Fig. 4B), indicative of an enhanced glycolytic flux. Then treatment of cells with MIF siRNA prevented the HDM-induced the increased glycolytic flux. Meanwhile, treatment of cells with PFKFB3 inhibitor PFK-15 prior to hrMIF stimulation inhibited the increased glycolytic flux, inflammatory cytokines release and airway epithelial barrier disruption induced by hrMIF in 16HBE cells. These results strongly suggest that the aerobic glycolysis mediates hrMIF-induced airway epithelial barrier disruption and the release of Th2 cytokines.

4. Discussion

In this study, ours is the first study to indicate that a crucial role of MIF in the mouse model of asthma and in HDM-induced airway epithelial barrier disruption and the release of Th2 cytokines in 16HBE cells. For the first time, we demonstrated that MIF antagonist ISO-1 reduced AHR, airway inflammation, serum IgE levels, the levels of Th2-associated cytokines, including IL-4, IL-5 and IL-13, and the redistribution of E-cadherin in the HDM-induced asthmatic mice through suppressing the MIF signaling pathways. Then we demonstrate that in 16HBE cells HDM resulted in MIF-mediated aerobic glycolysis, that in turn stimulate the release of Th2 cytokines and airway epithelial barrier disruption.

Asthma is one of the most common chronic inflammatory diseases which is characterized by airway hyperreactivity and inflammation, airway epithelial barrier dysfunction, Th2-mediated airway

inflammation, mucus overproduction and airway wall remodeling [2,18]. Consistent with the findings of previous experimental models [16,17], we successfully established a chronic asthmatic experimental mouse model and the HDM-exposed mice developed typical asthmatic features, including airway hyper-reactivity, airway inflammation, increased serum IgE levels, as well as the release of Th2 cytokines. During the progression of asthma, excessive Th2-driven inflammatory response in particular Th2 cytokines IL-4, IL-6 and IL-13 production and the loss integrity of the airway epithelial barrier contributes to IgE synthesis, AHR, cell injury and shedding [19,20]. During this process, airway inflammation and airway epithelial barrier dysfunction influence each other, which then contributes to the pathogenesis and exacerbation of asthma [16]. The integrity of the airway epithelial barrier is dependent on cellular integrity, the cell–cell contact integrity and strong cell–cell adhesion mediated by particular junctions especially adherens junctions [21]. Adherens junctions, comprised of E-cadherin, β -catenin, and α -catenin, mechanically connect adjacent cells and E-cadherin plays a crucial role in AJs and can provide the architectural support required for forming these junctional complexes [21,22]. In the mouse model we showed a HDM-induced aberrant arrangement of E-cadherin at epithelial cell–cell contact sites. Then we selected the airway epithelial cell line 16HBE to assess the effects of HDM on barrier properties and airway inflammation in vitro. We found that HDM exposure causes an increase in both ionic and macromolecular permeability (TER reduction and permeability increase), the distribution anomalies of E-cadherin in association with the release of Th2 cytokines in 16HBE cells. However, the expression of E-cadherin proteins in HDM-treated 16HBE cells and in lungs of HDM-treated mice remained unchanged. The above results suggested that airway inflammation and the damage to epithelial barrier function play an important role in HDM-induced asthma in mice and 16HBE cells.

MIF was originally described as a product derived from activated T lymphocytes and is considered to be a critical regulator of various inflammatory conditions [8,23]. Extensive studies have focused on the role of MIF in allergic inflammatory responses especially bronchial asthma and MIF plays a crucial role in airway inflammation as well as airway hyperresponsiveness in asthma [7,24]. MIF expression in bronchoalveolar lavage fluid were significantly elevated in Ovalbumin (OVA)-induced rat models of asthma and the elevated expression of MIF in the serum and induced sputum of patients with asthma was found [8,24]. Consistent with data from these previous studies, we showed that MIF expression was significantly increased in the exposure of 16HBE cells to HDM and in the mouse model of HDM-induced asthma, suggesting the possible involvement of MIF in the development of airway inflammation and airway epithelial barrier disruption. To further address the roles of MIF in the HDM-induced asthmatic mouse model, we investigated the effect of MIF neutralization with MIF antagonist ISO-1. In a previous study, the anti-MIF antibody suppresses the antigen-induced increase in the number of inflammatory cells in the airways and reduces airway hyperresponsiveness in OVA-immunized rats and the MIF antagonist ISO-1 has been shown to reduce the infiltration of inflammatory cells and airway remodeling in a murine model for chronic asthma [9,14]. Then we observe the influences of MIF antagonist ISO-1 on HDM-induced asthma in mice and the results showed that the blockade of MIF with ISO-1 ameliorated HDM-induced these changes especially the production of Th2-associated cytokines IL-4, IL-6, IL-13 and aberrant arrangement of E-cadherin in our mouse asthma model as well. Simultaneously, in vitro we found that HDM significantly induce the expression of MIF and pretreatment with MIF siRNA could inhibit the HDM-induced upregulation of MIF, airway epithelial barrier disruption and the release of inflammatory cytokines. Interestingly, we also found hrMIF exposure promote the release of Th2-associated cytokines and epithelial barrier disruption. These observations provide direct support for the conclusion that MIF participates in promoting the release of inflammatory cytokines and airway epithelial barrier disruption in HDM-induced asthma cell model.

However, further studies are warranted to delineate the specific mechanisms by which MIF regulate inflammatory cytokines release and airway epithelial barrier disruption in HDM-induced asthma cell model.

The metabolic syndrome components including increased glucose uptake, aerobic glycolysis and hyperlactatemia have been identified as an independent risk factor for worsening respiratory symptoms, greater pulmonary function impairment, pulmonary hypertension, and asthma [25,26]. Aerobic glycolysis is increased in asthma, which promotes T cell activation and the inhibition of lactate generation by a PDK inhibitor blocks T cell activation and development of asthma [27]. In our study, we showed that lactate expression in BALF was significantly increased in the mouse model of HDM-induced asthma mice, suggesting the possible involvement of aerobic glycolysis in the development of HDM-induced asthma. The present data place MIF play an important role in the control of peripheral glucose metabolism and in mediating certain of the catabolic effects induced by severe inflammatory responses [28]. Meanwhile an increasing number of studies have found that MIF has emerged as an important player in the physiological and pathological regulation of glucose metabolism and MIF inhibition could decrease glucose uptake and glycolysis in muscle and heart [29]. Then in our vitro experiment, the HDM and hrMIF could induce a increase in glucose uptake and lactate level in 16HBE cells and pre-treatment with MIF siRNA could inhibit the HDM-induced upregulation of glucose uptake and lactate level. These data show that there are interaction relationship between the upregulation of MIF and activation of the aerobic glycolysis pathway in regulation of HDM-induced inflammatory cytokines release and airway epithelial barrier disruption. Then in order to further explore the roles of aerobic glycolysis in hrMIF-induced inflammatory cytokines release and airway epithelial barrier disruption in 16HBE cells, the PFKFB3 antagonist PFK15 were used to block aerobic glycolysis as described previous reports [30,31]. In our study, inhibition of aerobic glycolysis with PFK15 can ameliorate the production of Th2-associated cytokines and airway epithelial barrier disruption induced by hrMIF in 16HBE cells. Our results further indicate that MIF induces the production of Th2-associated cytokines and airway epithelial barrier disruption by aerobic glycolysis in 16HBE cells.

In conclusion, our results demonstrate that the local application of a MIF antagonist ISO-1 prevents HDM-induced airway epithelial barrier disruption and airway inflammation in mice, and the aerobic glycolysis pathways may be involved in this process. Moreover, in 16HBE cells HDM can promote MIF expression to induce an enhanced aerobic glycolysis which then cause the production of Th2-associated cytokines and airway epithelial barrier disruption. These findings provide additional insights into the importance of MIF in asthma and MIF signaling blocking can be as a potential therapeutic target.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] B.N. Lambrecht, H. Hammad, Allergens and the airway epithelium response: gateway to allergic sensitization, *J. Allergy Clin. Immunol.* 134 (3) (2014) 499–507.
- [2] B.N. Lambrecht, H. Hammad, The immunology of asthma, *Nat. Immunol.* 16 (1) (2014) 45.

- [3] M. Willkarp, J. Luyimbazi, X. Xu, et al., Interleukin-13: central mediator of allergic asthma, *Science* 282 (5397) (1998) 2258–2261.
- [4] C. Xiao, S.M. Puddicombe, S. Field, et al., Defective epithelial barrier function in asthma, *J. Allergy Clin. Immunol.* 128 (3) (2011) 549–556.e12.
- [5] D.E. Davies, Epithelial barrier function and immunity in asthma, *Annals ATS* 11 (Suppl. 5) (2014) S244–S251, <https://doi.org/10.1513/AnnalsATS.201407-304AW>.
- [6] M.C. Nawijn, T.L. Hackett, D.S. Postma, et al., E-cadherin: gatekeeper of airway mucosa and allergic sensitization, *Trends Immunol.* 32 (6) (2011) 248–255.
- [7] I.H. Heijink, M.C. Nawijn, T.L. Hackett, Airway epithelial barrier function regulates the pathogenesis of allergic asthma, *Clin. Exp. Allergy* 44 (5) (2014) 620–630.
- [8] E. Yamaguchi, J. Nishihira, T. Shimizu, et al., Macrophage migration inhibitory factor (MIF) in bronchial asthma, *Clin. Exp. Allergy* 30 (9) (2010) 1244–1249.
- [9] T. Amano, J. Nishihira, I. Miki, Blockade of macrophage migration inhibitory factor (MIF) prevents the antigen-induced response in a murine model of allergic airway inflammation, *Inflamm. Res.* 56 (1) (2007) 24–31.
- [10] T. Shimizu, A. Ohkawara, J. Nishihira, et al., Identification of macrophage migration inhibitory factor (MIF) in human skin and its immunohistochemical localization, *FEBS Lett.* 381 (3) (1996) 199–202.
- [11] H. Lan, N. Wang, Y. Chen, et al., Macrophage migration inhibitory factor (MIF) promotes rat airway muscle cell proliferation and migration mediated by ERK1/2 and FAK signaling, *Cell Biol. Int.* (2017) 42(1).
- [12] T. Calandra, J. Bernhagen, C.N. Metz, et al., MIF as a glucocorticoid-induced modulator of cytokine production, *Nature* 377 (6544) (1995) 68–71.
- [13] Sabroe, Pease, Williams, Asthma and MIF: innately Th1 and Th2, *Clin. Exp. Allergy* 30 (9) (2010) 1194–1196.
- [14] P.F. Chen, Y.W. Luo, J.X. Wang, et al., ISO-1, a macrophage migration inhibitory factor antagonist, inhibits airway remodeling in a murine model of chronic asthma, *Mol. Med.* 16 (9–10) (2010) 400–408.
- [15] Y. Alabed, D. Dabideen, B. Aljabari, et al., ISO-1 binding to the tautomerase active site of MIF inhibits its pro-inflammatory activity and increases survival in severe sepsis, *J. Biol. Chem.* 280 (44) (2005) 36541–36544.
- [16] Y. Hu, H. Dong, M. Zou, et al., TSLP signaling blocking alleviates E-cadherin dysfunction of airway epithelium in a HDM-induced asthma model, *Cell. Immunol.* 315 (2017) 56–63.
- [17] H. Dong, Y. Hu, L. Liu, et al., Distinct roles of short and long thymic stromal lymphopoietin isoforms in house dust mite-induced asthmatic airway epithelial barrier disruption, *Sci. Rep.* 6 (2016) 39559.
- [18] A. Kleinjan, Airway inflammation in asthma: key players beyond the Th2 pathway, *Curr. Opin. Pulmonary Med.* 22 (1) (2015) 46.
- [19] Q. Wu, Y. Tang, X. Hu, et al., Regulation of Th1/Th2 balance through OX40/OX40L signalling by glycyrrhizic acid in a murine model of asthma, *Respirology* 21 (1) (2016) 102–111.
- [20] S.T. Holgate, Epithelium dysfunction in asthma, *J. Allergy Clin. Immunol.* 120 (6) (2007) 1233–1244.
- [21] M. Varelle, E. Kieninger, M.R. Edwards, et al., The airway epithelium: soldier in the fight against respiratory viruses, *Clin. Microbiol. Rev.* 24 (1) (2011) 210–229.
- [22] G. Shyamala, A.T. Comstock, U.S. Sajjan, Barrier function of airway tract epithelium, *Tissue Barriers* 1 (4) (2013) –.
- [23] Korsgren M, Kallstrom L, Uller L, et al. Role of macrophage migration inhibitory factor (MIF) in allergic and endotoxin-induced airway inflammation in mice [In Process Citation], 2000.
- [24] M. Kobayashi, Y. Nasuhara, A. Kamachi, Y. Tanino, T. Betsuyaku, E. Yamaguchi, J. Nishihira, M. Nishimura, Role of macrophage migration inhibitory factor in ovalbumin-induced airway inflammation in rats, *Eur. Respir. J.* 27 (4) (2006) 726–734.
- [25] M.S. Bae, J.H. Han, J.H. Kim, et al., The relationship between metabolic syndrome and pulmonary function, *Korean J. Family Med.* 33 (2) (2012) 70–78.
- [26] C.W. Baffi, L. Wood, D. Winnica, et al., Metabolic syndrome and the lung, *Chest* 149 (6) (2016) 1525–1534.
- [27] M. Ostrokhova, N. Goplen, M.Z. Karim, et al., The role of low-level lactate production in airway inflammation in asthma, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 302 (3) (2012) L300.
- [28] F. Benigni, T. Atsumi, T. Calandra, et al., The proinflammatory mediator macrophage migration inhibitory factor induces glucose catabolism in muscle, *J. Clin. Invest* 106 (10) (2000) 1291.
- [29] C. Toso, J.A. Emamaullee, S. Merani, et al., The role of macrophage migration inhibitory factor on glucose metabolism and diabetes, *Diabetologia* 51 (11) (2008) 1937–1946.
- [30] W. Zhu, L. Ye, J. Zhang, et al., PFK15, a small molecule inhibitor of PFKFB3, induces cell cycle arrest, apoptosis and inhibits invasion in gastric cancer, *PLoS One* 11 (9) (2016) e0163768.
- [31] C. Wang, J. Qu, S. Yan, et al., PFK15, a PFKFB3 antagonist, inhibits autophagy and proliferation in rhabdomyosarcoma cells, *Int. J. Mol. Med.* 42 (1) (2018).