Selective HDAC8 inhibition by PCI-34051 attenuates inflammation and airway remodeling in asthma *via* miR-381-3p-TGFβ3 axis

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ABSTRACT

Background and Objectives: Histone deacetylase (HDAC) families regulate various physical processes and the development of several diseases. The role of HDACs in asthma development and progression worths further investigation. This study aims to evaluate the effect of HDACs in a mouse model of asthma. **Methods:** HDAC8 selective inhibitor PCI-34051 was administered to a mouse model of ovalbumin-sensitized and challenged asthma. Airway responsiveness, serum cytokines, histological changes of the airway, and expression levels of α-SMA, β-actin, VEGFR, VEGF, GAPDH, HDAC8, TGF-β3, CD 105, p-ERK 1/2, ERK 1/2, PI3K, p-AKT, AKT, and PDK1 were evaluated. The miR-381-3p level was also measured. **Results:** All classic histologic and cellular changes of asthma in inflammation and airway remodeling were altered by HDAC8 inhibitor PCI-34051 via regulation of the miR-381-3p level and its downstream gene, TGF-β3. Inhibition of TGF-β3 further reduced the activation of ERK, PI3K, AKT, and PDK1. **Conclusion:** In a mouse model, HDAC8 inhibitor PCI-34051 exhibits comprehensive control of asthmatic changes, including inflammation and airway remodeling.

Key words: asthma, airway remodeling, histone deacetylase, inflammation, microRNA

INTRODUCTION

Asthma is typically characterized by chronic airway inflammation. This common respiratory disease affects an estimated 300 million people worldwide, and its prevalence is increasing.[1] Even with consistent clinical presentations, including wheezing, shortness of breath, and chest tightness, indicating airflow limitation, asthma is a heterogeneous disease that is difficult to define, diagnose, assess severity, and manage.[2] Current asthma control strategies rely mainly on glucocorticoids and long-acting β2 agonists.[3] However, using the available treatments, the disease is not well controlled in many patients, probably because of diverse pathogenic processes. Rather than being viewed as a single disease entity, asthma is now considered a clinical condition involving multiple pathological mechanisms. The underlying mechanisms can be classified into inflammation and airway remodeling. Developing new targeted therapies tailored to specific epigenotypes may be necessary for leveraging more detailed knowledge for better management through individualized treatment of these two key contributors to asthma.

The histone deacetylase (HDAC) family includes four classes of nuclear enzymes capable of modifying chromatin structure by removing acetyl functional groups from lysine residues of target proteins. HDACs regulate various physical processes, including the cell cycle, differentiation, and apoptosis.[4] In the pathogenesis of respiratory diseases, increased HDAC expression has been reported in chronic

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obstructive pulmonary disease,[5] allergic rhinitis[6-8] and asthma.[9,10] The role of HDACs in asthma development and progression in particular warrants further investigation. Because HDAC activity is reversible by pan- or specific inhibitors, the therapeutic effects of HDAC inhibitors have been evaluated in several diseases. Moreover, to better understand the disease mechanism, HDAC inhibitors can be used to clarify the function of each molecule involved in pathogenesis and the associations between the molecules.

In this study, the selective HDAC8 inhibitor PCI-34051 was administered to mice in an ovalbumin (OVA)-sensitized and challenged asthma model aiming to determine the role of HDAC8 in asthma development and explore the potential pathways involved. These results may help identify novel treatment targets and benefit the asthma population.

MATERIALS AND METHODS

Development of OVA-sensitized and challenged asthma model

The study protocol was reviewed and approved by our hospital's Institutional Animal Care and Use Committee (approval number AF-SOP-07-1.1-01). The OVA asthma model was used in the present study, as previously described.[11,12] Briefly, SPF-grade female BABL/c mice aged 6–8 weeks and weighing 20 ± 2 g were housed in an SPF-grade room for one week and provided water and food ad libitum. Mice were randomly divided into four groups (n = 6 for each group): healthy control (con), mice with asthma (ova), dexamethasone treatment (dex), and PCI-34051 treatment (pci). Mice of the ova, dex, and pci groups were sensitized by intraperitoneal injection of 0.2 mL OVA solution (20 μg OVA and 2 mg aluminum hydroxide [both Sigma-Aldrich, St. Louis, MO, USA] in 0.2 mL normal saline) at days 0, 7, and 14 to create the asthma model. The OVA challenge was conducted on day 21 using a 2% OVA saline solution with a nebulizer for 30 min daily, three times per week for eight weeks, while mice in the control group were administered saline. In the dex and pci groups, dexamethasone (2 mg/kg, Sigma-Aldrich) and PCI-34051 (0.5 mg/kg, Sigma-Aldrich), respectively, were administered intraperitoneally before each challenge, and the con and ova groups were administered saline as a control.

Airway responsiveness measurement

Airway resistance was estimated using noninvasive whole-body plethysmography (Emka Technologies, Paris, France). An enhanced pause (Penh) was recorded for 3 minutes following the administration of acetyl-β-methylcholine chloride (Sigma-Aldrich) at concentrations of 0, 3.125, 6.25, 12.5, 25, and 50 mg/mL, as previously described.[13]

Cytokine measurementSerum samples were prepared from blood collected by orbital puncture after anesthesia (2.5% isoflurane delivered in O_2, 2 L/minute) and centrifuged at 3500 rpm (with centrifugal radius 10 cm) at 4°C for 15 minutes. Cytokine levels, including IL-17/IL-17A, IL-17E/IL-25, IL-6, IL-13, and TNF-α, were measured by Luminex - based immunoassays.

Lung tissue preparation

Anesthetized mice were sacrificed by cervical dislocation 1 hour after the last OVA challenge. The left lung was fixed with 0.1 mL iced 4% polyoxymethylene, removed, and fixed in 4% polyoxymethylene for 24 hours. After rehydration by running tap water for 6 hours, dehydration and embedding in paraffin were performed, and 4 μm sections were cut for further analysis.

Histopathological analysis

Inflammation levels were evaluated by HE staining and a 5-point scoring system. Fibrosis levels were evaluated by collagen deposition or basement membrane thickness using Masson's trichrome staining. As determined by PAS staining, mucus metaplasia was evaluated based on the percentage of mucus-containing goblet cells.

Immunofluorescence staining

The sections were incubated with blocking solution (Beyotime Biotechnology, Shanghai, China) for 1 hour and then incubated overnight with the primary antibodies α-SMA and CD 31 (both Abcam, Cambridge, MA, USA) at 4°C and for 1 hour with Alexa Fluor-labeled secondary antibodies (Abcam) at 37°C. Nuclei were stained with DAPI (Abcam, Cambridge, UK). The stained sections were observed using a fluorescence microscope (Olympus, Tokyo, Japan).

Western blotting

Proteins were extracted with RIPA buffer, and concentrations were measured using a BCA protein concentration kit (Beyotime Biotechnology). Equal amounts of proteins were subjected to gel electrophoresis and transferred to polyvinylidene fluoride membranes. After blocking, membranes were incubated with the primary antibodies α-SMA, -actin, VEGFR, VEGF, GAPDH, HDAC8, TGF-3, CD 105, p-ERK 1/2, ERK 1/2, PI3K, p-AKT, AKT, and PDK1, followed by incubation with the corresponding HRP-conjugated secondary antibodies (all Abcam). The immunoblots were visualized using enhanced chemiluminescence (Bio-Rad, Hercules, CA, USA). Densitometry was performed using the ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

Immunohistochemistry

The sections were deparaffinized, hydrated, subjected

to antigen retrieval, and then incubated with the following primary antibodies: VEGF, VEGFR, TGF-3, CD 105, ERK 1/2, and AKT, and the corresponding biotinylated secondary antibodies (all Abcam). Color development was performed using a DAB solution (Vector Laboratories, Burlingame, CA, USA), and the sections were counterstained with Mayer's hematoxylin.

Real-time quantitative RT-PCRTotal RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was synthesized using a reverse transcription kit (Roche, Shanghai, China), and RT-qPCR was performed using SYBR Green-based reagent and amplified on a CFX real-time PCR detection system (Bio-Rad). U6 and GAPDH were internal references for miR-381-3p and VEGFR and VEGF mRNA, respectively.

Fluorescence in situ hybridization analysis

Sections were blocked with 3% BSA in 4× saline-sodium citrate, then treated with proteinase K (Roche), and hybridized with FITC-labeled miR-381-3p probe and stained with anti-digoxin rhodamine conjugate (Abcam) at 37°C for 1 hour in the dark. Nuclei were stained with DAPI (Abcam), and stained sections were observed under a fluorescence microscope (Olympus).

RESULTS

The Penh values increased with increasing concentrations of acetyl-β-methylcholine in all groups, and the value in the OVA group was significantly higher than in the other groups, indicating that the Penh value changed to near normal (con group) when mice received treatment (including the dex and pci groups). The airway resistance at the fixed dose of 50 mg/mL acetyl-β-methylcholine chloride showed the same effect (Figure 1A). HDAC8 inhibition reduced inflammation, fibrosis, and mucus secretion as evaluated by HE, Masson's trichrome, and PAS staining, respectively (Figure 1B). HDAC8 expression correlated with the pathogenesis of asthma, as the expression level increased in the ova group and decreased in the dex and pci groups, as evaluated by western blotting (Figure 1C).

Levels of the important Th 2 inflammatory cytokines IL-17A, IL-17E, IL-6, IL-13, and TNF-α were significantly increased in the ova group, compared to the con group ($P < 0.0001$) and significantly decreased in the dex and pci groups compared to the ova group ($P < 0.0001$ for all in the pci group, $P < 0.001$ for IL-17/IL-17A in the dex group and $P < 0.0001$ for the others in the dex group), indicating that HDAC8 inhibition reduces Th2-mediated airway inflammation (Figure 2).

Figure 3 shows the effects of HDAC8 inhibition on airway remodeling through the expression of α-SMA and CD 31, which represent the structural response of airway smooth muscle cells and the microvasculature in asthma. Immunofluorescence staining and western blotting indicated that HDAC8 inhibition reduced the number of smooth muscle and endothelial cells. These results indicate that HDAC8 inhibition may reduce the thickening of vessel walls and neovascularization in asthma. The percentages of vascular wall area, vascular endothelial cell numbers, and neogenetic microvessel density were all significantly increased in mice in the ova group compared to those in the con group ($P < 0.001$ for the percentage of vascular wall area, $P < 0.0001$ for the other two). After receiving treatments, mice of the dex and pci groups showed significant reductions in all three indicators, compared to those in the ova group ($P < 0.05$; Figure 3A). The protein expression of α-SAM significantly increased in mice of the ova group compared to those in the con group ($P < 0.0001$). Both decreased significantly in mice of the dex and pci groups, compared to those in the ova group ($P < 0.0001$). However, the expression of α-SMA in the dex and pci groups was still significantly higher than that of the con group ($P < 0.001$; Figure 3B).

Evaluation of VEGF and VEGFR, which are involved in Th2-mediated airway inflammation and angiogenesis in asthma, demonstrated that HDAC8 inhibition reduced the protein and mRNA levels of VEGF and VEGFR (Figure 4). Immunohistochemical staining showed that VEGF expression was significantly increased in mice of the ova group compared to those of the con group ($P < 0.0001$) and significantly reduced in those of the dex and pci groups compared to the ova group ($P < 0.0001$; Figure 4A). Western blotting indicated that both VEGFR and VEGF expression increased significantly in the ova group compared to in the con group ($P < 0.05$, $P < 0.0001$, respectively). VEGF expression decreased significantly in the dex and pci groups compared to the ova group ($P < 0.05$ and $P < 0.01$, respectively). However, the levels were still significantly higher than in the con group ($P < 0.0001$ and $P < 0.001$, respectively). VEGFR expression decreased significantly in the dex and pci groups compared to in the ova group ($P < 0.01$ and $P < 0.05$, respectively; Figure 4B). Changes in the mRNA expression of VEGF and VEGFR showed similar trends as the western blot results (Figure 4C).

The miR-381-3-p levels were significantly lower than those in the control group ($P < 0.0001$). When mice received the HDAC8 inhibitor, PCI-34051, miR-381-3-p expression in the asthmatic airway was significantly higher than in the con group ($P < 0.0001$) or ova group ($P < 0.0001$), indicating that HDAC8 may be associated with miR-381-

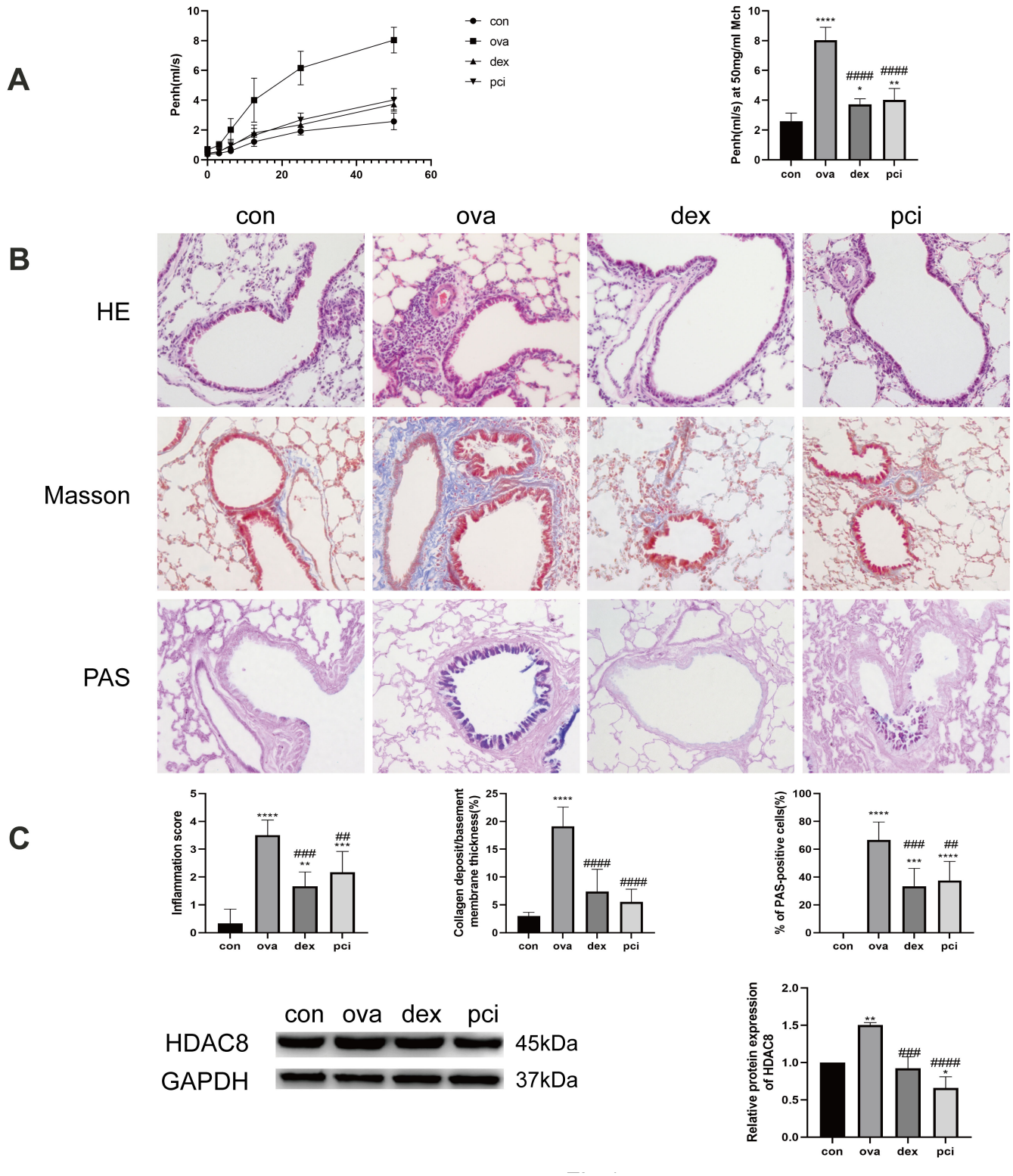

Figure 1: HDAC8 expression in airway related to asthma in ovalbumin-sensitized and challenged BALB/c mouse model. A. Enhanced Penh value was observed after asthma induction and significantly reduced to normal level by dexamethasone and HDAC 8 inhibitor. B. Results of HE, Masson's trichrome, and Periodic Acid-Schiff staining indicated that the inflammation level, collagen deposition and mucus secretion cell numbers significantly increased in the asthmatic airway, and both dexamethasone and HDAC 8 inhibitor reversed these changes. C. Increased HDAC8 was found in the asthmatic airway and significantly decreased by dexamethasone and HDAC 8 inhibitor, detected by Western blot. Con: control group; ova: ovalbumin induction group; dex: dexamethasone treatment group; pci: PCI-34051 treatment group; PAS: Periodic Acid-Schiff staining. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$ respectively compared to the con group. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ and $^{\#\#\#\#}P < 0.0001$ respectively compared to the ova group.

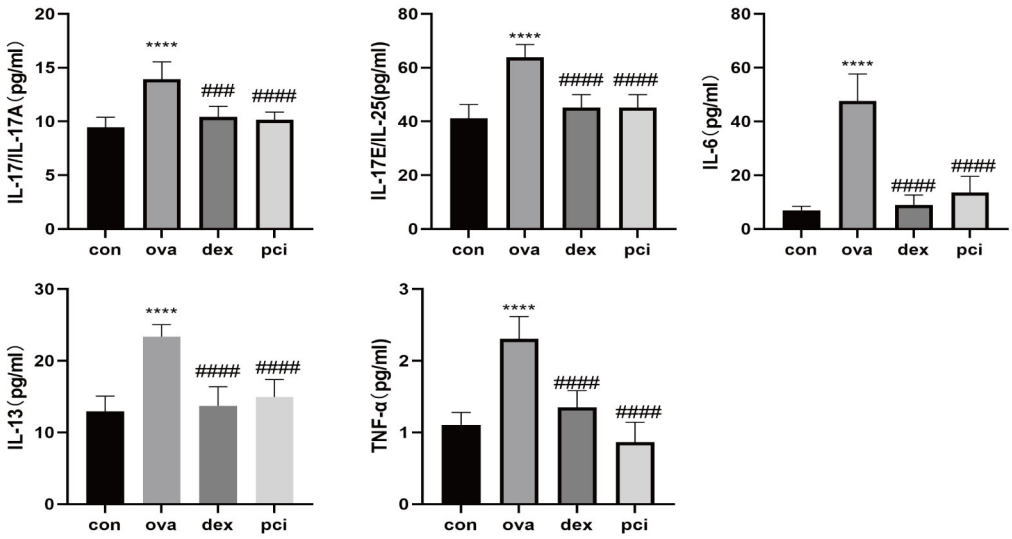

Figure 2: Changes of inflammatory cytokine levels in serum. IL-17/IL-17A, IL-17E/IL-25, IL-6, IL-13, and TNF-α significantly increased in asthma and decreased by dexamethasone and HDAC 8 inhibitor treatment. Con: control group; ova: ovalbumin induction group; dex: dexamethasone treatment group; pci: PCI-34051 treatment group. ****$P < 0.0001$ compared to the con group. ###$P < 0.001$ and ####$P < 0.0001$ respectively compared to the ova group.

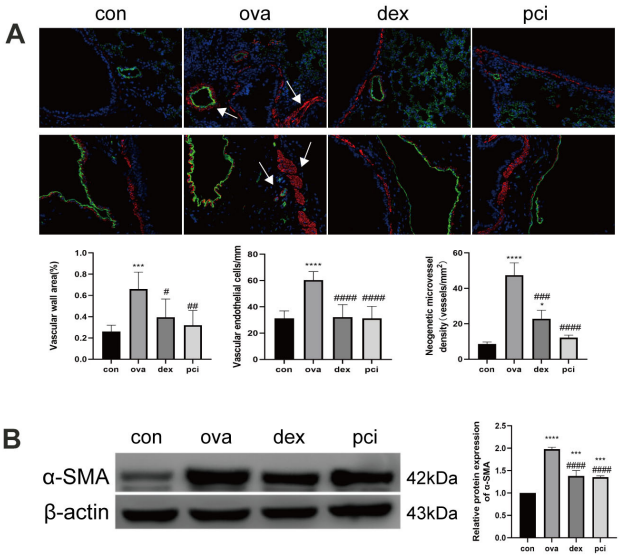

Figure 3: Airway remodeling in asthma was inhibited by dexamethasone and HDAC8 inhibitor. A. Immunofluorescence staining of α-SMA (red) and CD 31 (green) showed the smooth muscle hyperplasia in the arteries and neovascularization closed to the asthmatic airway decreased by dexamethasone and HDAC 8 inhibitor treatment. B. Results of western blot indicated that α-SMA expression increased in asthma significantly decreased by dexamethasone and HDAC 8 inhibitor treatment. Con: control group; ova: ovalbumin induction group; dex: dexamethasone treatment group; pci: PCI-34051 treatment group. ***$P < 0.001$ and ****$P < 0.0001$ respectively compared to the con group. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ and ####$P < 0.0001$ respectively compared to the ova group.

3-p expression in the lungs, as represented by the FISH and quantitative RT-PCR data (Figure 5). The levels of miR-381-3-p-regulated molecules TGF-β3 and CD 105 increased in the ova group and decreased in the dex and pci groups (Figure 6). Immunohistochemistry staining of TGF-β3 showed that the expression significantly increased in the mice of the ova group compared to those of the con group ($P < 0.0001$) and was significantly reduced in those of the dex and pci groups compared to the ova group ($P < 0.0001$). Western blotting revealed similar results (Figure 6A). Immunohistochemical staining of CD 105 showed that its expression significantly increased in mice of the ova group compared to those of the con group ($P < 0.0001$) and was significantly reduced in those of the dex and pci groups compared to the ova group ($P < 0.0001$). Western blotting revealed similar results (Figure 6B).

In the ova group, the expression of p-ERK1/2, ERK 1/2, PI3K, p-AKT, AKT, and PDK1 was higher than that in the con group. The levels of all proteins decreased in the dex and pci groups (Figure 7), and immunohistochemical staining of p-ERK 1/2 showed that the expression was significantly increased in mice of the ova group compared to those of the con group ($P < 0.0001$) and significantly reduced in those of the dex and pci groups, compared to the ova group ($P < 0.0001$). Western blotting revealed similar results (Figure 7A). Immunohistochemical staining of AKT showed that the expression was significantly increased in mice of the ova group compared to those of the con group ($P < 0.0001$) and significantly reduced in those of the dex and pci groups compared to the ova group ($P < 0.0001$; Figure 7B). Western blotting results showed significant increases in PI3K ($P < 0.05$), p-AKT ($P < 0.05$), and PDK1 ($P < 0.0001$) levels in mice in the ova group compared to those in the con group. After both dexamethasone and PCI-34051treatments, the expression

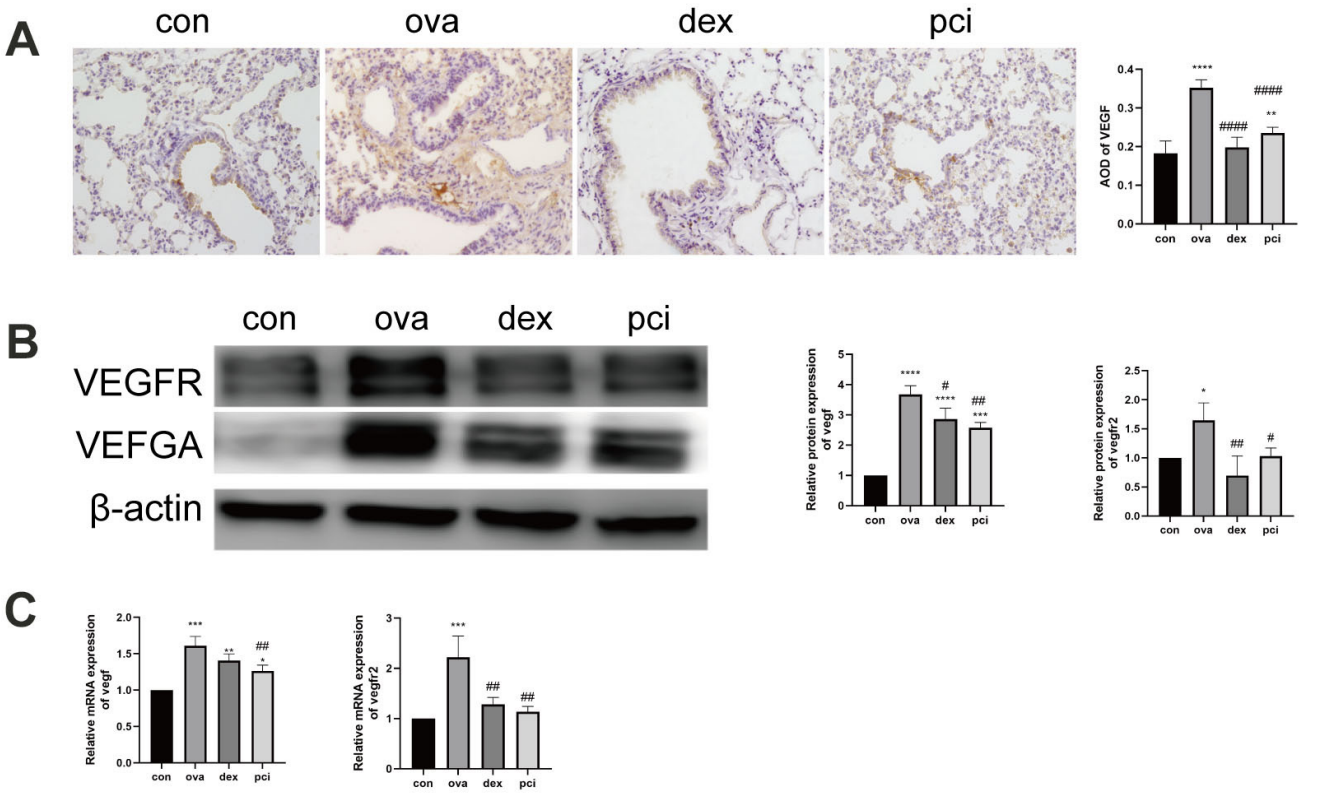

Figure 4: VEGF and VEGFR increased in asthma and decreased by dexamethasone and HDAC 8 inhibitor. A. Immunohistochemical staining. B. Western blot. C. Quantitative RT-PCR. Con: control group; ova, ovalbumin induction group; dex: dexamethasone treatment group; pci: PCI-34051 treatment group; AOD: average optical density. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and **** $P < 0.0001$ respectively compared to the con group. #$P < 0.05$, ##$P < 0.01$ and ####$P < 0.0001$ respectively compared to the ova group.

levels of PI3K ($P < 0.01$), p-AKT ($P < 0.05$), and PDK1 ($P < 0.0001$) were significantly reduced in the ova group (Figure 7B).

DISCUSSION

This study's results have shown that TGF-β3 plays a central role in regulating both the signaling of inflammation and airway remodeling in a mouse model of asthma. The HDAC8 inhibitor PCI-34051 altered all classic histologic and cellular changes of asthma *via* regulation of the miR-381-3p level and its downstream gene TGF-β3. Inhibition of TGF-β3 further reduced the activation of ERK, PI3K, AKT, and PDK1.

Inflammation and airway remodeling play vital roles in asthma's occurrence and progression, both of which are multicellular processes. Eosinophils, T cells, mast cells, neutrophils, and dendritic cells are recruited to the site of asthmatic inflammation. Structural changes in the airway, including epithelial fragility, goblet cell metaplasia, fibroblast hyperplasia, airway smooth muscle mass increase, and basement membrane thickening due to collagen deposition, are all significant typical features of asthma. The crosstalk between inflammation and airway remodeling is closed and frequent; inflammation leads to airway remodeling *via* factors secreted by inflammatory cells, including cytokines, chemokines, and growth factors, and drives changes in the structural cells of the airway,[14] which also produce factors that increase immune cell levels in the airway. For example, airway smooth muscle cells produce IL-6, which is required for IL-17A expression and promotes Th17 polarization.[15,16] Interactions between immune cells and airway structural cells increase the complexity of pathogenesis, asthma epigenotypes, and disease control because the treatment target is difficult to identify.[14,17]

MicroRNAs (miRs) are single-stranded RNA molecules that range from 19 to 24 nucleotides in length and are known to be involved in several important physical functions *via* post-transcriptional regulation of target gene expression. In addition, the role of miRNAs in the pathogenesis of many diseases has been investigated.[18-21] In a 4,4'-methylene diphenyl diisocyanate-induced occupational asthma (OA) mouse model, a 2- to 5-fold downregulation of circulating

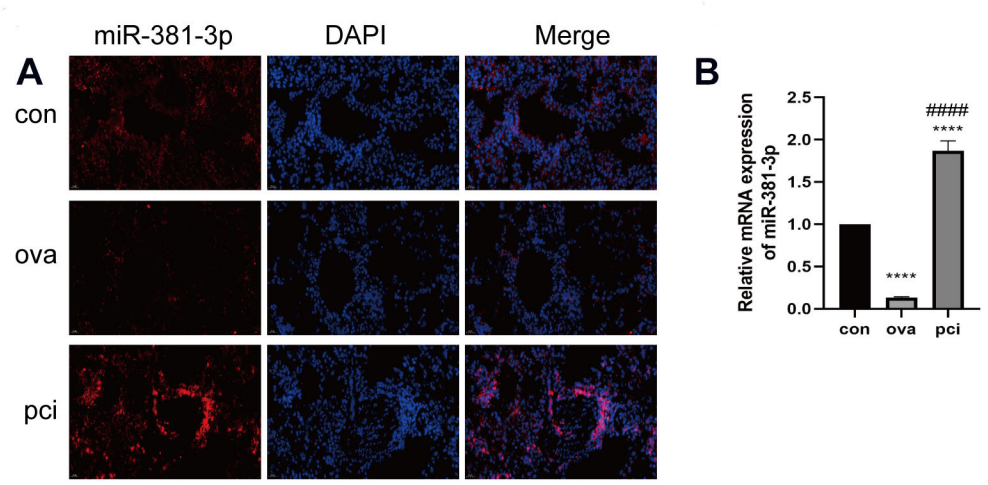

Figure 5. Decreased miR-381-3p in asthmatic airway was reversed and increased significantly after HDAC 8 inhibitor treatment. A. Fluorescence in situ hybridization. B. Quantitative RT-PCR. Con: control group; ova: ovalbumin induction group; dex: dexamethasone treatment group; pci: PCI-34051 treatment group. ****$P < 0.0001$ compared to the con group. ####$P < 0.0001$ compared to the ova group.

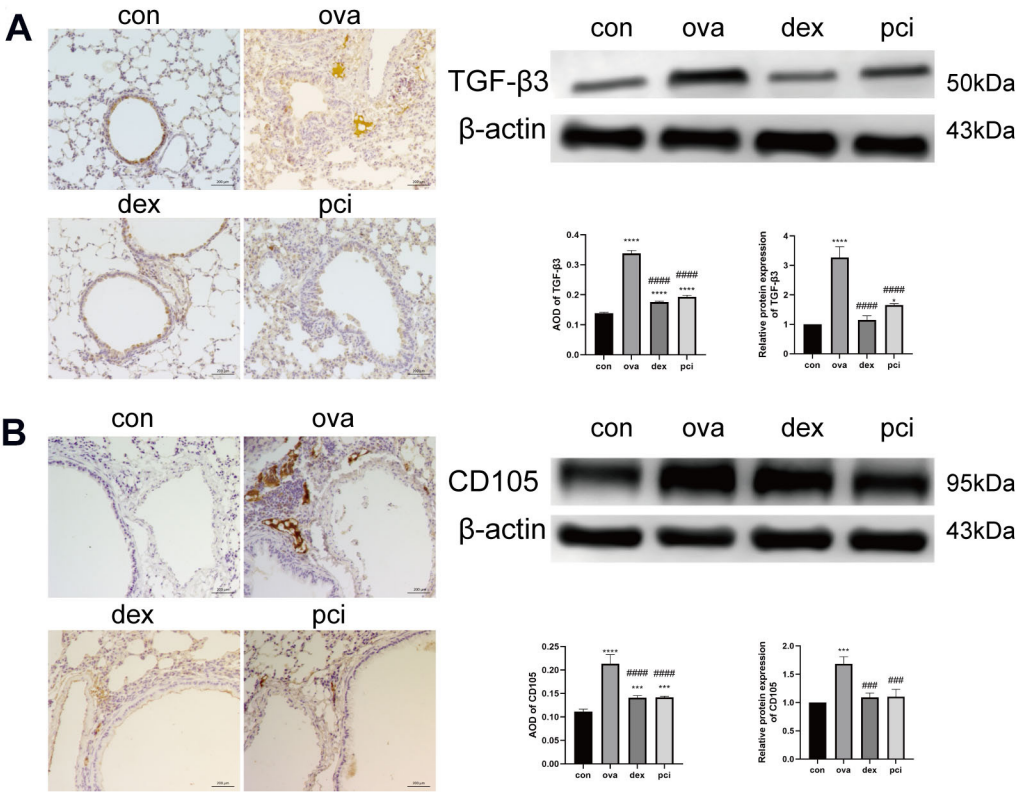

Figure 6: TGF-β3 and CD105 levels increased in asthma and decreased by dexamethasone and HDAC 8 inhibitor. Protein levels were evaluated by immunohistochemical staining and western blot. A. TGF-β3. B. CD105. Con: control group; ova: ovalbumin induction group; dex: dexamethasone treatment group; pci: PCI-34051 treatment group. ***$P < 0.001$ and ****$P < 0.0001$ respectively compared to the con group. ###$P < 0.001$ and ####$P < 0.0001$ compared to the ova group.

miR-381-3p has been observed in mice of the OA group compared to those of the control group. Pathway analysis results have indicated that miR-381-3p may be associated with immune system regulation.[22] However, a definitive mechanism has not yet been evaluated in this OA model. Our previous in vitro study found that miR-381-3p overexpression in human bronchial smooth muscle cells decreased the expression of α-SMA, fibronectin 1, and

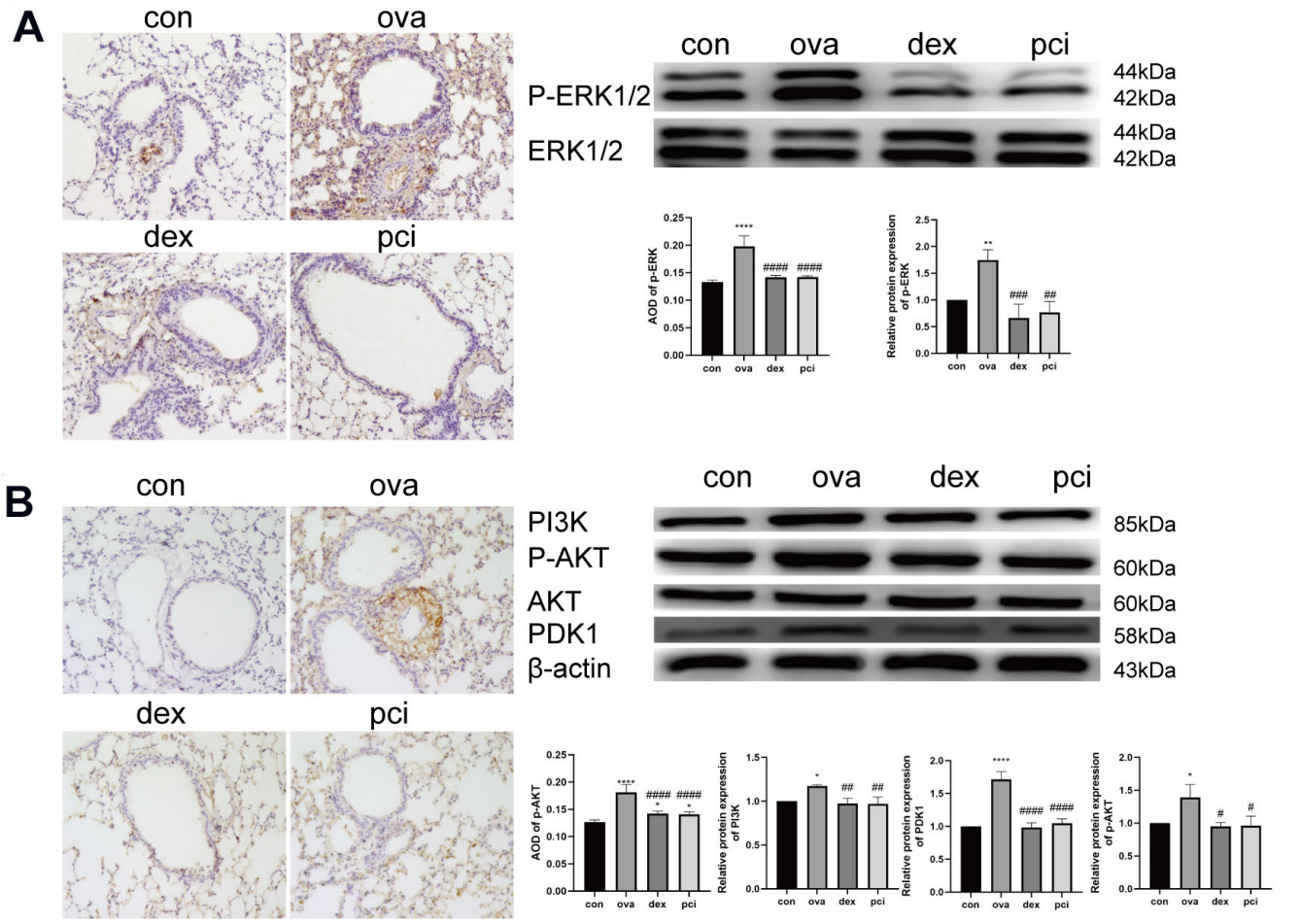

Figure 7: HDAC 8 involved in asthma via ERK and PI3K/AKT pathways. These proteins evaluated by immunohistochemical staining and western blot increased in the asthmatic airway and reduced significantly after dexamethasone and HDAC 8 inhibitor treatment. A. ERK1/2. B. PI3K, AKT, and PDK1. Con: control group; ova: ovalbumin induction group; dex: dexamethasone treatment group; pci: PCI-34051 treatment group. *$P < 0.05$, **$P < 0.01$ and ****$P < 0.0001$ respectively compared to the con group. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ and ####$P < 0.0001$ compared to the ova group.

collagen I, which are all markers of airway remodeling, suggesting that miR-381-3p may be a moderator of airway remodeling in asthma.[23]HDAC8 belongs to class I HDACs. Since HDAC8 overexpression has been observed in several malignancies[24-26] and respiratory diseases, including asthma,[5-10] it has become a novel therapeutic target for these diseases. HDAC8 selective inhibitor PCI-34051, with > 200-fold selectivity over other class I HDACs, has been tested for its therapeutic effects in mouse models of asthma, demonstrating that PCI-34051 reduces inflammation and airway remodeling in asthma. Treatment with PCI-34051 ameliorates airway hyperresponsiveness by reducing immune cell counts in bronchoalveolar lavage fluid, significantly decreasing inflammatory cell infiltration and cytokine levels, and reducing M2 macrophage polarization in lung tissue in a mouse model of asthma.[5,10,27-29] The anti-airway remodeling function of PCI-34051 in asthma has been reported to regulate smooth muscle differentiation and contraction, decrease α-SMA expression, and reduce fibrosis in EMT, bronchial smooth muscle cells hyperplasia, and collagen deposition.[23,28,29]

An investigation of the effects of PCI-34051-induced bronchial epithelial cell-derived exosomes revealed that miR-381-3p expression was upregulated in human bronchial smooth muscle cells.[23] Further genetic analysis and dual-luciferase reporter assay results indicated that TGF-β3 is the target of miR-381-3p after HDAC8 inhibitor PCI-34051 treatment. This is consistent with the results of the present study, which demonstrated that the miR-381-3p level increased and TGF-β3 expression decreased in response to treatment with PCI-34051.

The TGF-β superfamily are key molecules in the asthma signaling pathway, as they are primary mediators of pro-inflammatory responses and fibrotic tissue remodeling

in the lungs. TGF-β, produced by epithelial cells, eosinophils, macrophages, and fibroblasts, is involved in epithelial transformation, fibrosis, airway smooth muscle proliferation, and microvascular changes associated with airway wall thickness, mucus production, collagen deposition, and cytokine dysregulation. These effects are observed in allergenic asthma models, including OVA sensitization and challenge and house dust mite induction models.[30,31] IL-13 induces TGF-β expression and then increases α-SMA expression, collagen deposition, and IL-6 production.[32,33] IL-6 induces VEGF expression and promotes neovascularization in asthma.[15,34,35] These changes were observed in the present study, and it may be concluded that TGF-β3 plays a central role in regulating inflammation signaling and airway remodeling in asthma. Furthermore, this signal may be amplified because not only TGF-β3 but also the receptor increased since the level of one of the components of the TGF-β3 receptor (CD105) in the ova group was elevated.

The TGF-β-centered signaling pathway has been investigated in several studies. It has been reported that TGF-β1 regulates airway smooth muscle hyperplasia in asthma *via* phosphorylation of PI3K and ERK, which both induce airway smooth muscle cell proliferation.[36,37] In this study, PCI-34051 treatment recovered the increases of TGF-β3, p-ERK, ERK, PI3K, p-AKT, AKT, and PDK1 in the asthmatic airway to normal levels, indicating the important role of HDAC8 in asthma, and the potential therapeutic effect of HDAC8 inhibitor PCI-34051 by controlling the key modulator TGF-β3.

CONCLUSION

In a mouse model, the HDAC8 inhibitor PCI-34051 exhibited comprehensive control of asthmatic changes, including inflammation and airway remodeling. PCI-34051 altered all classic histologic and cellular changes in inflammation and airway remodeling of asthma by regulating the miR-381-3p level and its downstream gene, TGF-β3. Inhibiting TGF-β3, as a key moderator of asthma occurrence and progression, reduces the activation of ERK, PI3K, AKT, and PDK1. This may improve asthma conditions through reduced airway responsiveness, inflammation infiltration, fibrosis, mucus secretion, microvasculature changes, and Th2 inflammatory cytokines, including IL-17A, IL-17E, IL-6, IL-13, and TNF-α.

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None.

Author Contributions

Shiyao Bai: study design, literature research, experimental studies, data analysis, statistical analysis and manuscript preparation; Xinming Su: research integrity, study concepts, study design, definition of intellectual content, manuscript editing and manuscript review; Delei Kong: experimental studies and statistical analysis; Chenye Feng: experimental studies and statistical analysis; Xiaochun Zhang: literature research; Jieyu Zhao: literature research and experimental studies; Jiamin Sun: experimental studies; Ying Pan: experimental studies; Wenyang Li: study design and manuscript editing.

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Ethical Approval

This study protocol was reviewed and approved by the institutional animal care use committee (IACUC) of our hospital, approval number AF-SOP-07-1.1-01.

Informed Consent

Not applicable.

Conflict of Interest

All authors declare that there is no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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