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PERFORMANCE EVALUATION OF HISTONE DEACETYLASES IN LUNGS OF MICE EXPOSED TO OVALBUMIN AEROSOLS

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This study was to investigate expression levels and functional activities of histone deacetylases (HDACs) with potential therapeutic targets selected in animal model of allergic asthma. Mice were sensitized and then challenged with saline (control) or ovalbumin (OVA) for 8 weeks. Airway resistance was determined by increasing concentrations of acetyl- β -methacholine chloride (0 – 50 mg/ml). The number of cells and cytokine production in bronchoalveolar lavage fluid (BALF) were determined by ELISA. Pathological changes of lung specimens were examined by histochemical staining methods under the light microscope. Expression and quantification of HDACs in lungs were measured using immunohistochemistry and Western blotting analysis. HDAC activity was identified using colorimetric and fluorometric methods. The OVA-treated mice had a significant enhancement in airway resistance with a large number of cells and increased interleukin (IL)-4 and -5 levels in BALF. Morphologically, an infiltration of inflammatory cells into epithelial layer with mucus accumulation and subepithelial fibrosis were seen in the OVA-exposed lungs. The expression levels for HDAC1, HDAC5, HDAC6, and HDAC8 were significantly elevated with weak induction of HDAC 2-4, which was identical with their catalytic activities detected in the lungs. In contrast, HDAC1 and HDAC5 activities were higher than others in the lungs. Individual HDACs are differently regulated in expression levels and functional activities in animal model of allergic asthma. Selective targeting of HDAC1/5 offers an opportunity to improve therapeutic effects of the disease.

Key words: *histone deacetylases, allergic asthma, mucus hyperplasia, subepithelial fibrosis, airway resistance, ovalbumin*

INTRODUCTION

Allergic airway inflammation and airway hyperresponsiveness (AHR) are characteristics of bronchial asthma (1, 2). Inhalation antigen induces inflammatory cell recruitment, cytokines released and AHR in animal model of asthma (3, 4). Therefore, many studies have used this model to investigate the roles of enzymes in asthma pathophysiology and to explore efficacy of therapies before starting clinical trials (3, 5). Though animal models are limited for not being able to mimic all features and phenotypes of human asthma (6), they do provide the knowledge of many inflammatory, structural and pathological characteristics of asthma (7).

Histone deacetylases (HDACs) are classified in four classes depending on sequence homology to the yeast original enzymes and domain organization (8). HDACs are part of a vast family of enzymes that have crucial roles in numerous biological processes, largely through their repressive influence on transcription (9). It has been reported that performance of HDACs is influenced in human asthmatic airways (10, 11). Although expression patterns of individual HDACs in asthma are not fully elucidated, HDAC inhibitors have been described for inhibition of AHR and anti-inflammatory effects in asthma (12). Since HDACs act to regulate the expression of distinct subsets of inflammatory/immune genes in asthma and allergic diseases (13),

targeting specific enzymes involved in this process might lead to new therapeutic intervention, particularly in situations in which anti-inflammatory therapies are suboptimal (14).

The purpose of this study was to investigate features of individual HDAC isoforms in OVA-exposed lungs. Our data not only reveal performance of individual HDACs in their expression levels and functional activities in the lungs but also suggest the importance of selective HDAC isoforms as potential therapeutic targets in allergic asthma.

MATERIALS AND METHODS

Animals

Specific pathogen-free, female BALB/C mice (6 – 8 weeks, 18 – 22 g) were purchased from the Liaoning Changsheng Biotech Co., Ltd (Animal license number: SCXK (Liao 2010-0001). The mice were housed in an environmentally controlled animal facility of our hospital for the duration of the experiments.

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of The First Affiliated Hospital of China Medical University.

Sensitization and challenge

Twenty-four mice were divided randomly into two groups of twelve animals. The protocol for making animal model is modified according to previous studies (15, 16). Mice were sensitized with intraperitoneal (i.p.) injection of an equivalent volume of saline (NS) or OVA (20 µg in PBS) complexed with 2 mg Al (OH)₃ in 0.2 ml NS on days 0, 7 and 14. Commencing on day 21, mice received aerosol inhalation of NS or OVA at a flow rate of 3 ml/min by an ultrasonic nebulizer (Jiangsu Yuyue Medical Equipment & Supply Co. Ltd, China). This procedure was carried out three times per week for eight weeks. Measurements described in this study were performed 24 h after the final aerosol.

Six mice in each group were lavaged and the others were harvested with lung specimens. The left lungs were fixed with 4% paraformaldehyde for H&E and IHC staining. The right lungs were frozen and stored at -80°C for evaluating functional activities of HDACs.

Determination of cells and cytokines in bronchoalveolar lavage fluid

Lungs were lavaged by instillation and withdrawal of 0.5 ml of NS ($\times 3$ times) through a tracheal cannula, and an equal volume of BALF was collected from individual mice. The BALF sample was centrifuged (1200 rpm $\times 5$ min) at 4°C and the cells were counted using a hemocytometer under a microscope after supernatant was stored at -80°C for the enzyme-linked immunosorbent assays (ELISA). The cells (3.0×10^4 cells) from each sample were applied to a glass slide using a cytospin (1000 rpm $\times 10$ min) and then the slide was stained with Wright-Giemsa for the differential count of cells. Each cell type was expressed as a percentage by counting 200 cells/slide.

A total protein concentration was measured by BCA Assay (Pierce Biotechnology). The cytokine levels in supernatant were determined using antibodies against mouse IL-4 and IL-5 (CUSABIO, Hubei, PR China) according to the manufacturer's directions. Briefly, 100 µl of the substrate solution was added to 100 µl of sample in microtiter plates and incubated for 30 min at room temperature. The reaction was stopped by adding 50 µl of 4 M sulfuric acid, and the optical density (OD) values were detected at 490 nm using a fluorescence spectroscopy (TECAN infinite M200Pro, Switzerland).

Measurement of airway responsiveness

Enhanced pause (Penh) as a measure of airway hyperresponsiveness (AHR) was assessed by acetyl- β -methacholine chloride (MCh)-induced airflow limitation using single chamber whole body plethysmography (EMKA Technologies, Paris, France) (17, 18). Briefly, MCh dose responses were generated after baseline readings of NS inhalation were performed. Mice were aerosolized with increasing concentrations of MCh ranging from 0, 3.125, 6.25, 12.5, 25 and 50 mg/ml. The readings of Penh were taken with each dose of MCh over a 3-min period. The degree of bronchoconstriction was expressed as a percentage of the change in Penh relative to the baseline.

Histopathologic study

Left lungs were surgically removed and were inflated in 4% paraformaldehyde for 24 hours after mice were sacrificed. The lung tissues from groups were embedded in paraffin and cut into a 5-µm section. Histopathologic examination for lung specimens

was photographed under a light microscope at a magnification of 40 to compare morphological changes. Briefly, the lung sections of the grouped mice were stained with hematoxylin and eosin (H&E) solution for evaluating peribronchiolar inflammation, with Periodic Acid-Schiff (PAS) solution specifically for the presence of mucus production and with Masson's Trichrome for subepithelial fibrosis in parenchyma of lungs.

Immunohistochemical detection of histone deacetylases expression

For IHC staining, the slices of the lung tissues were mounted on slides, dehydrated using alcohol washes of increasing concentration (75%, 80%, 90%, 95%, and 100%) for different time periods, and cleared using a detergent like xylene before being imaged under a microscope. HDACs expression in lungs was carried out using concentrations of individual antibodies. Briefly, the sections were incubated with rabbit polyclonal antibodies of HDAC1-7 (1:500, Santa-Cruz Biotechnology, CA, US), HDAC8 (1:400, Novus Biologicals, CO, US), HDAC9 (1:100, Sigma-Aldrich, MO, US), and HDAC10 (1:200, abcam, MA, US), respectively. The primary antibodies required to be incubated for at least 18 hours at 4°C. The following day, the sections were incubated with a HRP-conjugated secondary antibody (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) for 1 hour at 37°C. Reaction with a 3,3'-diaminobenzidine (DAB) on the sections can produce a brown product at the site of the target antigen that is insoluble in alcohol and xylene. Slides were washed three times with PBS before being analyzed using a light microscope (Olympus BX51, Japan) at $\times 200$ magnification for visualizing expression of the targets in the investigated tissues.

Electrophoresis of histone deacetylases proteins

Lung tissues (right upper lobe) were placed in cell lysis buffer (1% Triton, 0.5% Deoxycholic Acid, 0.1% SDS in PBS, pH 7.3) containing a protease inhibitor cocktail (Pierce Biotechnology, Rockford, IL) and homogenized using a rapidly oscillating masher. Tissue supernatants were centrifuged ($12,000 \times g$) for 60 min at 4°C and the protein concentration of the cleared supernatant was determined by the BCA method (Pierce Biotechnology, Rockford, IL). Cleared supernatants were stored at -70°C until use. Briefly, aliquots of lung tissue lysate (100 µg/well) were loaded onto a 4 – 20% SDS polyacrylamide gel. The electrophoresed proteins were transferred to nitrocellulose membranes and blocked with 5% nonfat dried milk in Tris-buffered saline (TBS) at 4°C for 120 min. HDAC proteins were identified by using same antibodies described in the IHC method. The membranes were incubated with rabbit anti-mouse HDACs (1:1000) overnight and then washed with TBST (TBS, 0.1% Tween-20). The membrane was incubated with peroxidase-conjugated anti-rabbit IgG (1:3000) (Abcam, Cambridge, UK) for 60 min and washed with TBST again. A mixture of western blotting detection reagent I and II (GE Healthcare Life Sciences, Piscataway, NJ) was poured on the membrane at room temperature. The bands for HDACs on the membrane were visualized by autoradiography. Proteins were quantified using Image-J and relative band intensity was calculated as a percentage of the intensity of the GAPDH protein band.

Assay for histone deacetylases activity

Nuclear HDAC activity in lung lysates (right lower lobe) was determined using colorimetric and fluorometric assay kits (BioVision, Epigentek, US) according to the manufacturer's

protocol. Briefly, a sample (100 µg) was diluted to ddH₂O (85 µl) in each well in a 96 well plate. 10 µl of the 10X HDAC assay buffer and 5 µl of the HDAC colorimetric substrate were added to each well. The plate was incubated at 37°C for 1 hour after mixing thoroughly. The reaction was stopped by adding 10 µl of Lysine Developer and the plate was incubated at 37°C for 30 min. HDAC activity was determined at 405 nm in a microplate reader and expressed as the relative OD value per µg protein sample.

A sample (50 µg) was diluted to ddH₂O (85 µl) in each well in a 96 well plate. 10 µl of the 10X HDAC assay buffer and 5 µl of the HDAC fluorometric substrate were added to each well. The plate was incubated at 37°C for 30 min after mixing thoroughly. The reaction was stopped by adding 10 µl of Lysine Developer. HDAC activity was determined in a fluorescence plate reader and expressed as the relative fluorescence units per µg protein which was converted into ng/µg protein in the analysis of final results.

Statistical analysis

Values were expressed mean $\pm$ standard deviation (SD) on the experimental results. Statistical analysis was performed using Statistical Package for the Social Science (SPSS, version 19.0). Comparisons from groups were performed by one-way analysis of variance (ANOVA). Student's paired t-test was used to compare measurements of individual groups. P values less than 0.05 were considered significant.

RESULTS

Characterization of animal model

A model of allergic asthma was determined in mice exposed to NS or OVA and the results are shown in Fig. 1. The Penh values serving as an indirect measure of airway resistance gradually increased with increased concentrations of MCh in mice. In contrast, the value in OVA-challenged mice rose to threefold above the control value at the highest dose of MCh (Fig. 1A). In statistical analysis, there were significant differences in the Penn values measured at five doses of MCh inhalation between NS- versus OVA-treated mice ($P < 0.05$, $n = 6$).

To confirm an inflammatory process existed in OVA-exposed lungs, the numbers of total cells and individual cells in the BALF samples were determined (Fig. 1B-1D). Average values ($\times 10^4/\text{ml}$) for cell counts in the samples from NS- and OVA-treated mice were 13.42 ± 4.28 and 98.04 ± 9.27 in BALF cells, 0.88 ± 0.3 and 23.57 ± 6.08 in lymphocytes and 0.61 ± 0.28 and 37.64 ± 6.98 in eosinophils. In contrast, the counts of the BALF cells, lymphocytes and eosinophils were significantly increased in the challenged mice as compared to those in the control animals. ($P < 0.05$, $n = 6$).

IL-4 and IL-5 levels in BALF from experimental mice were examined (Fig. 1E and 1F). Average values for the concentrations (ng/ml) of the cytokines in BALF from NS- and OVA-treated mice were shown as 7.98 ± 1.73 and 16.88 ± 2.78

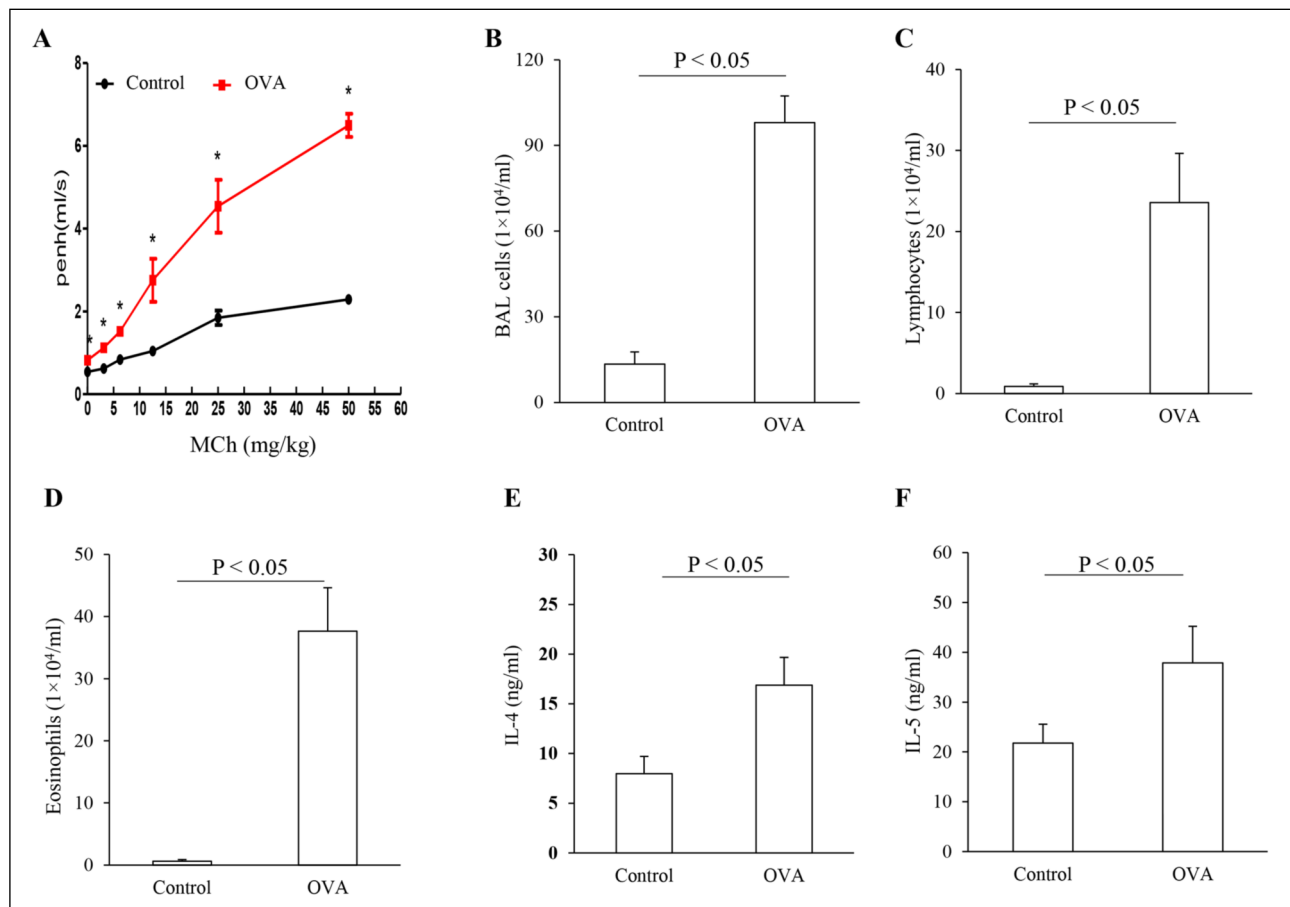


Fig. 1. Features of OVA model. Airway responsiveness (A) was determined with increasing MCh doses from 5 – 50 mg/kg. Counts of total cells (B), lymphocytes (C) and eosinophils (D) from the BAL were examined in these two groups of animals. The IL-4 (E) and IL-5 (F) levels in BAL of OVA-treated mice increased. The results were expressed as mean $\pm$ SD ($n = 6$).

in IL-4, 21.75 ± 3.82 and 37.86 ± 7.34 in IL-5. In contrast, both cytokine levels in the BALF samples from the OVA-challenged mice was higher than these in the samples from the control mice. In statistical analysis, there were significant differences in these cytokine contents between the two treatments (both $P < 0.05$, $n = 6$).

Histopathologic changes in lungs

Lung tissue sections from mice were stained and photographed at $\times 40$ magnification. The results are shown in Fig. 2. Morphologically, representative images of lung sections from the NS-treated mice showed an intact epithelium layer on the luminal side of a bronchiole and were free of cellular infiltration, mucus and fibrosis observed in the lung sections. Long-term exposure to OVA, however, induced a predominantly peribronchial inflammatory infiltrate with epithelial denudation

in the challenged lungs (Fig. 2 top). In contrast, a large amount of mucus secretion in the challenged lungs was shown by positive PAS staining (Fig. 2 middle) and subepithelial fibrosis with blue color by Masson's Trichrome can be clearly found in parenchyma of the lungs on day 56 (Fig. 2 down).

Expression of histone deacetylases in lungs

Image-based scale profiling of HDAC expression patterns in lung tissues was performed using an immunohistochemical staining method with visual features obtained from the lung specimens. The results are shown in Fig. 3. The HDAC-positive subsets were presented in a dark brown color under a light microscope. In analysis of imaging appearance, significant increases in the expression levels of HDAC 1, 5, 6 and 8 were visible in the OVA-exposed lungs as compared to these in the NS-exposed animals. However, the expression levels for HDAC

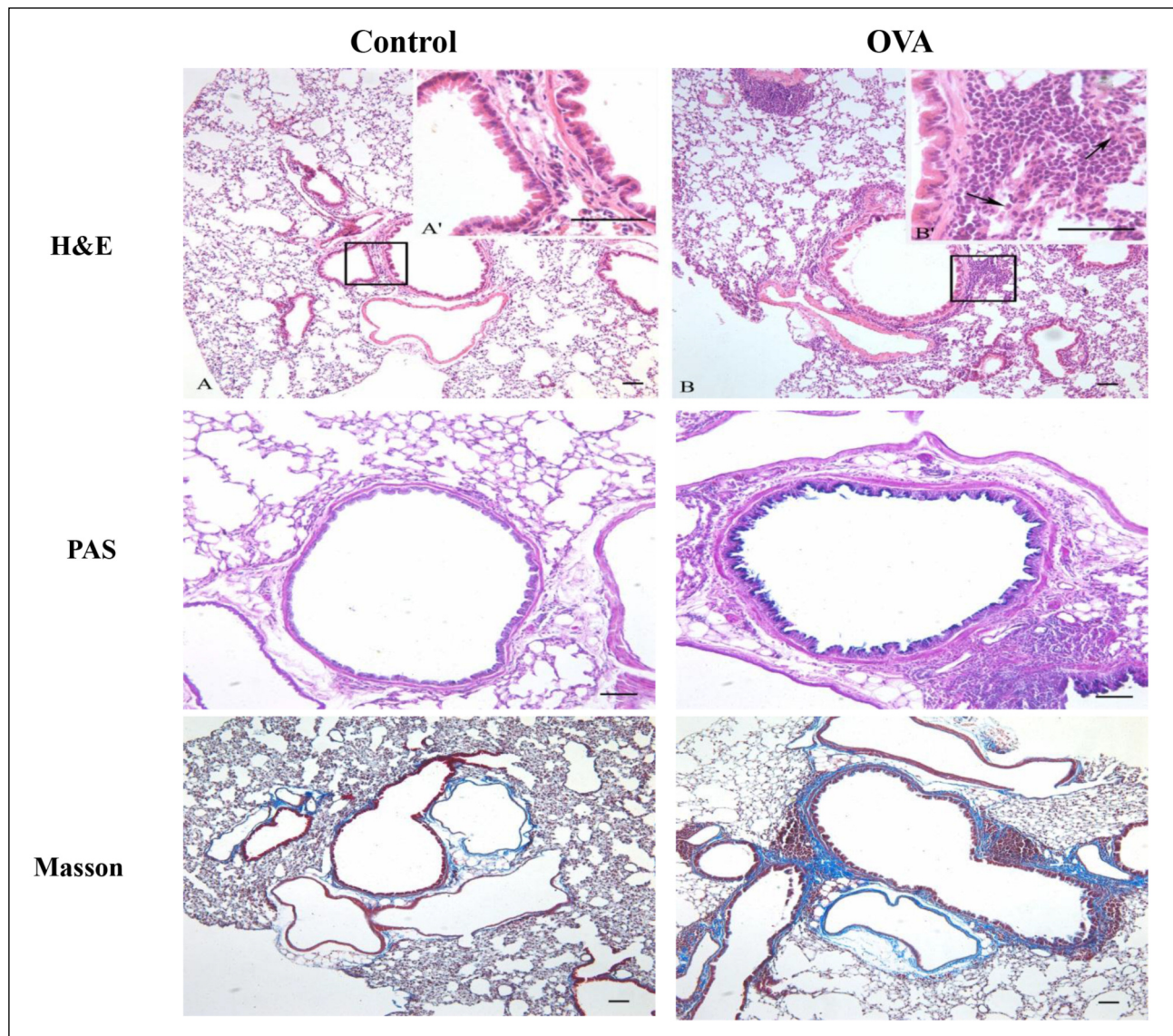


Fig. 2. Morphological analysis of lung tissues. Lungs specimens from PBS- and OVA-exposed mice were harvested and sections of the specimens were stained with H&E, PAS, and Masson's Trichrome, respectively. The morphological features with the status of airway epithelial layer, inflammatory cell infiltration, mucus accumulation and subepithelial fibrosis were observed on sections from the treated mice. Zoomed-up inset plots (top right corner) from the area in a rectangle with a black border were provided for H&E staining of the specimens. Histopathologic examination was performed under a microscope at $40 \times$ magnification. Scale bar (Red): $50 \mu\text{m}$.

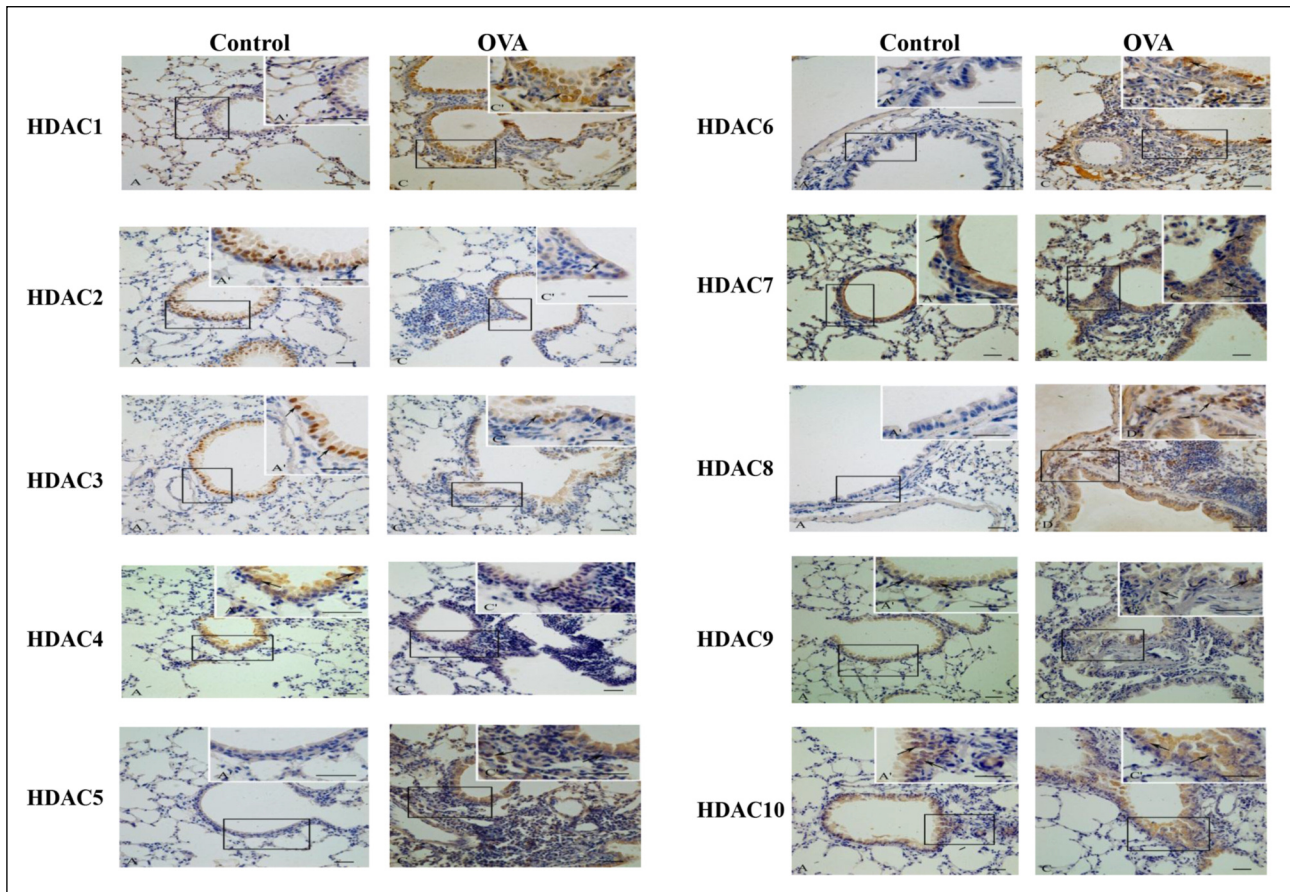


Fig. 3. Immunohistochemical staining of histone deacetylases in lungs. The immunohistochemical staining associated with HDAC1-10 were interpreted based on the signal intensity. HDAC1, 5, 6 and 8 in OVA-exposed lungs significantly increased in the visible light intensity, whereas HDAC2-4 obviously decreased as compared to the control. The HDAC7, 9 and 10 displayed the similar changes in intensity in the two grouped mice. Each figure contained a zoomed plot (top right corner) coming from the area in a rectangle with a black border.

isoforms such as 2 – 4 significantly decreased in the challenged lungs compared to the controls. Consequently, the expression levels of HDACs varied with performance of individual HDAC subtypes involved in the animal model.

Quantification of histone deacetylases in lungs

The expression levels of HDAC proteins in lung tissues were quantitated by Western blots and were calculated as relative density (%) of the GAPDH protein band. The results are shown in *Fig. 4*. A specific band visually recognized for each subtype of histone deacetylase was identified according to a specific antibody. The density for the enzyme varied with appearance of individual subsets in Western blot bands (*Fig. 4A*). In contrast, the expression levels of HDAC 7, 9 and 10 in the tissues from OVA-exposed mice were similar to these from the controls. However, HDAC 1, 5, 6 and 8 expression were significantly increased with obvious decreases in HDAC 2-4 expression in the challenged lungs compared to these in the controls (*Fig. 4B*). In statistical analysis of the relative density, there were significant differences in the expression levels of HDAC 1 – 6 and 8 between these two grouped animals (all $P < 0.05$, $n = 3$).

Assessment of histone deacetylases activity in lung tissues

HDACs are a class of enzymes that include the class I HDACs (1, 2, 3, and 8) and class II HDACs (4, 5, 6, 7, 9, and 10). According to the positive changes in quantification of protein concentration, the activities of HDAC isoforms (1, 2, 3, 4, 5, 6, and 8) were examined using a colorimetric or fluorometric assay. HDAC-catalyzed hydrolysis of acetyllysine residues in quantitative colorimetric peptide assay and fluorescent protein-tagged HDACs were detected in the low to ng/ml. The results are shown in *Fig. 5*. The average values for the active levels of the HDAC isoforms in the NS- and OVA-exposed lungs were shown as 5.92 ± 2.68 and 22.07 ± 6.88 in HDAC1, 21.19 ± 3.40 and 8.66 ± 0.84 in HDAC2, 5.51 ± 0.79 and 2.43 ± 0.30 in HDAC3, 0.21 ± 0.02 and 0.10 ± 0.03 in HDAC4, 0.59 ± 0.06 and 1.60 ± 0.07 in HDAC5, 0.59 ± 0.06 and 1.27 ± 0.24 in HDAC6 and 0.39 ± 0.07 and 0.71 ± 0.11 in HDAC8, respectively. In contrast, the increased activities of HDACs (1, 5, 6 and 8) in OVA-exposed lungs reached to 3.73-, 2.71-, 2.15- and 1.83-fold over the controls. In further analysis, combination of HDAC1 and HDAC5 achieved 62% in the total activity from the four subsets overexpressed in the lungs. In contrast, activities of HDACs (2 – 4) in the lungs significantly decreased with 2.45-, 2.27- and 2.1-fold lower than the controls. There were statistical significances in functional activities of those subtypes between the two grouped mice (all $P < 0.05$, $n = 6$). Additionally, HDAC isoforms in lungs of the treated mice

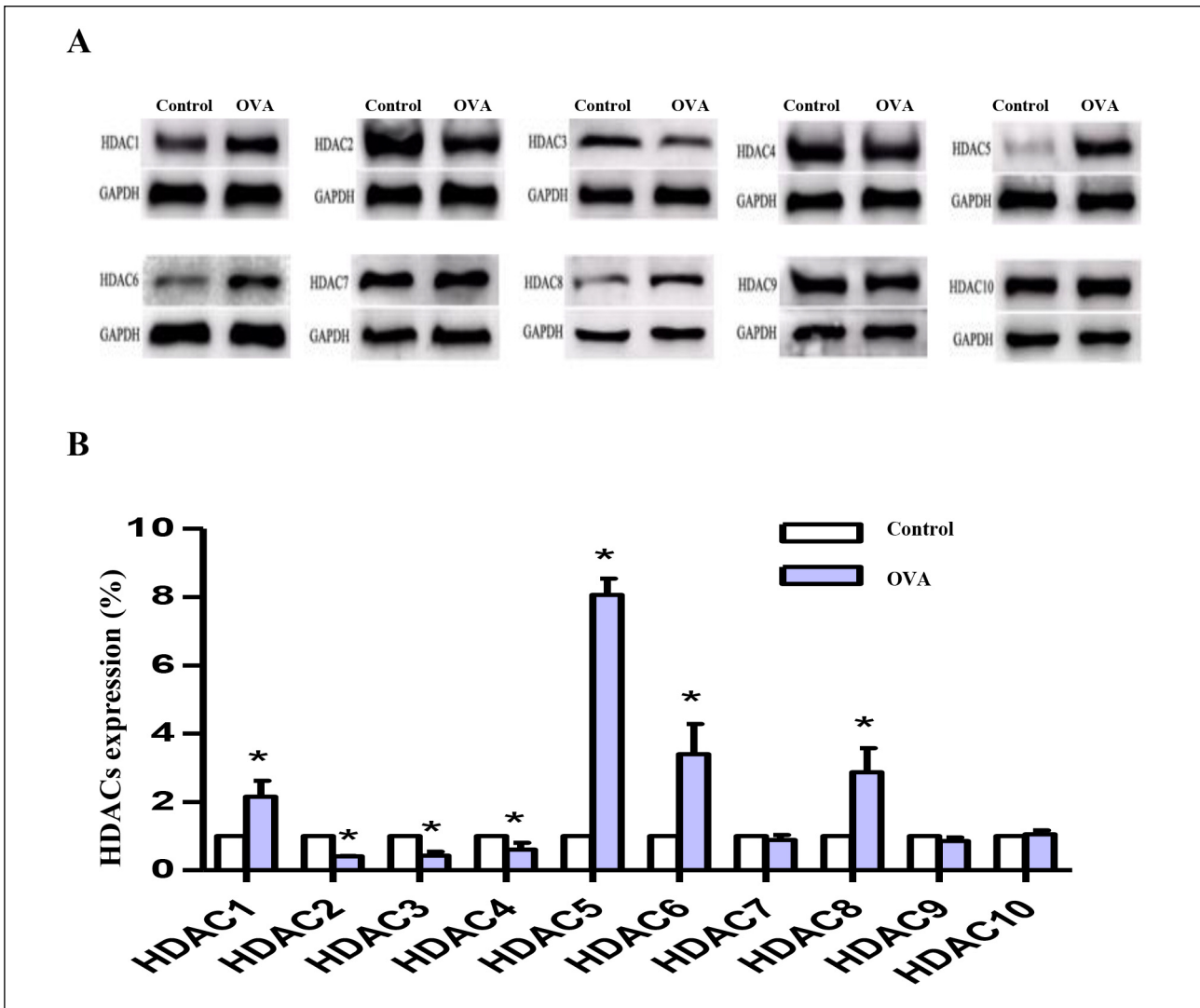


Fig. 4. Expression of HDACs in lungs. HDACs expression in the lung tissues lysates was measured by Western blot (A). Quantitation of the protein was performed using ImageJ and the relative band intensity was calculated as percentage in the reference intensity of the GAPDH protein band (B). The results were expressed as mean $\pm$ SD (n = 3). Scale bar: 50 μ m.

presented distinct and contrary activities in the same class of the enzyme.

DISCUSSION

Increased airway resistance was achieved by an 8-week OVA challenge in the animal model of allergic asthma, indicating the existence of airway flow limitation characterized by one of the pathological changes in asthma (19, 20). In further observation, there was a large inflammatory cell infiltrate, comprised mainly of lymphocytes and eosinophils, recruited into BALF, demonstrating an airway inflammatory response as an important contributor involved in the event of asthma. Many studies have been reported that the molecular and cellular mechanisms mediating the allergic inflammatory cascade involve release of cytokines including IL-4 and IL-5 (21-23). Our results showed a significant increase in levels of IL-4 and IL-5 in BALF from OVA-exposed mice, confirming the importance of the cytokines in the ongoing inflammatory process in the challenged lungs. In morphological analysis, the long-term exposure to OVA not only led to inflammatory cell infiltrate with epithelial shedding and

mucus accumulation along with the airways but also induced subepithelial fibrosis in parenchyma of the lungs. These findings were similar to morphologic alterations of asthmatic airways seen in patients (24, 25). In association with airway flow obstruction and airway inflammation, it led us to conclude that this animal model can be used to explore the pathophysiologic changes of asthma and evaluate novel therapeutic targets for the disease.

Expression of HDACs in NS- and OVA-exposed lungs was examined by the IHC method. Our results manifested that staining intensity of HDAC1, 5, 6 and 8 was increased with decreased intensity of HDAC2-4 in the challenged lungs, indicating expression patterns of HDACs and suggesting the necessity of selective interference with HDAC isoforms in treatment of asthma. It has been reported that intervention of HDAC inhibitors attenuates airway inflammation and airway hyperresponsiveness in mouse model of allergic airways disease (26-28). However, many inhibitors are nonselective with a wide range of effects on the enzyme subtypes (29, 30), raising the possibility that some HDAC isoforms in low expression were also influenced due to the absence of on-target effects. This could lead to the undesired effects of these HDAC inhibitors on treatment. Therefore, the development of highly potent and isoform selective HDAC inhibitors is essential

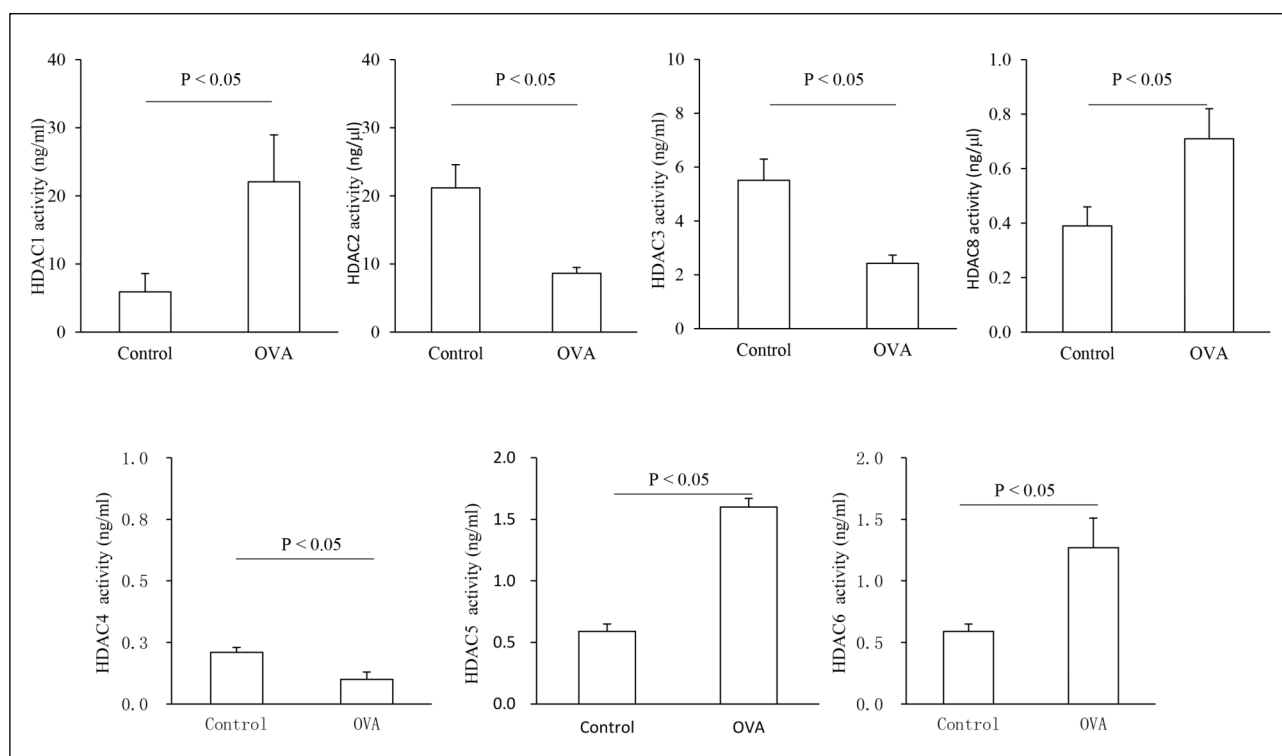


Fig. 5. HDAC activity in lungs. In association with the findings by Western blot analysis, HDAC activity in lung tissue was determined in the grouped mice. Functional activities of HDACs (1, 5, 6 and 8) in OVA-exposed lungs obviously increased with low activities of HDACs (2 - 4) in the lungs as compared to the controls. The data were expressed as mean $\pm$ SD ($n = 3$).

not only to refine our understanding regarding the relevant isoform(s) for on-target efficacy but also to mitigate potential mechanism-based, dose-limiting side effects caused by the inhibition of multiple HDACs (31). Given these findings by IHC staining, it leads us to conclude that HDACs as potential therapeutic targets contribute to asthma treatment. Furthermore, targeting multi-HDACs with imparting sufficient selectivity and specificity is superior to targeting one of the individual subsets.

Since most antigens under investigation are influenced significantly and adversely by formalin fixation (32, 33), widespread application of IHC has raised a critical issue concerning traditional formalin fixation. Western blotting analysis was used to verify the HDACs expression levels in lung tissues of mice. In contrast to the control, aberrant over-expression of HDACs (1, 5, 6 and 8) with low-abundance HDACs (2 - 4) in OVA-exposed lungs was observed by quantification of the enzymatic proteins. These findings not only displayed the expression patterns of HDACs in the lungs but also confirmed the features of the enzyme subsets found in the immunostained specimens. This may provide important clues to the enzyme's role in asthma and thus may develop novel therapeutic opportunities of the disease. Western blot in cell and molecular biology is an important technique used to separate, identify and quantify proteins (34). In most basic terms, it is used to detect the presence of a specific protein in a complex mixture extracted from cells or tissue. It has been found that the abnormal HDAC expression levels are associated with a number of autoimmune and inflammatory diseases (35, 36), therefore HDACs have been acknowledged as promising targets for treatment of respiratory diseases (34, 37). Although the mechanism of action through which HDACs function remains to be fully elucidated, it would appear that classical broad-spectrum HDAC inhibitors would be inappropriate as candidate potential therapeutics for asthma. Further, it's conceivable that a strategy for development of target-specific or isoform-selective drugs would

be feasible and efficacious in treating asthma as well as in decreasing the toxicity of HDAC therapy.

HDACs catalyze the removal of acetyl functional groups from the lysine residues of both histone and nonhistone proteins, allowing the histones to wrap the DNA more tightly (8, 38). There is increasing evidence that histone acetylation reversed by HDACs plays a critical role in the process of regulation of inflammatory genes and in mediating the anti-inflammatory effects of corticosteroids in asthma patients (39, 40). Accordingly, research is now progressing to targeting specific HDAC enzymes to improve efficacy of treatment as well as to reduce the risk of any unwanted side effects (41, 42). In assessment of enzyme activity, the catalytic activities of HDAC isoforms (1, 5, 6 and 8) in the OVA-exposed lungs were obviously higher than the NS-treated ones, whereas the activities of the isoforms (2 - 4) significantly decreased in the lungs. These results were consistent with quantification of the enzymatic proteins from Western blotting analysis, revealing that these individual HDAC isoforms had dissimilar functions in the diseased lungs. This renewed interest in the possible exploitation of such high affinity interactions to target drugs to specific subsets as a potential therapeutic intervention for asthma. Given airway inflammation in existence, it is most likely that the changes in the enzyme activity were accounted for the inflammatory process in the lungs (28, 43, 44). Conceivably, small-molecule drugs aimed to interfere with the selective HDAC subsets exhibit a favorable effectiveness in blocking the effects of HDACs on inflamed lungs. In analysis of HDAC activity, combination of HDAC1/5 reached over 62% of the total activity among the four subsets shown to have the increased activity levels in the lungs, suggesting that selective inhibition of HDAC1/5 by drugs was more conclusive than other subsets in treatment strategy for asthma. Since highly specific functions of individual HDACs are related to development of diseases in analysis of HDAC

knockout mice (45-47), it's reasonable to propose that inhibiting or restoring isoform-selective HDAC activity for recovery of the unique function of specific HDACs is an effective approach to enhancing efficacy and reducing the risk of adverse effect of a therapy in the treatment of asthma. Asthma treatment has widely been explored in animals, which relies on targeted therapies (48, 49). It has been reported that selective phosphodiesterase inhibitors and some drugs used for decreasing oxidative damage of lungs and a Ca^{2+} level in airway smooth muscle cells show anti-asthmatic effects in management of the disease (50-52). Based on observation of clinical outcomes, it seems that intervention of HDACs inhibitors would be more common than the enzyme activators in development of new therapeutic agents.

Taken together, this study indicates expression patterns of the individual HDAC isoforms with the changes in enzymatic activities in animal model of allergic asthma. Targeting specific isoforms such as HDAC1/5 should be a good strategy to improve therapeutic efficacy of existing drugs and to develop novel agents in the treatment of asthma.

Acknowledgements: This study was supported by the National Natural Science Foundation of China (No.81770021, The HSP90 acetylation modulation of HDAC6 inhibitor in the molecular mechanism of asthma), and the Liaoning Natural Science Foundation (No.20170540994, Regulatory effect and mechanism of HDAC8 on airway regeneration and remodeling in bronchial asthma).

Conflicts of interests: None declared.

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Received: January 9, 2018

Accepted: April 30, 2018

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