

The Protective Effects of KAT5 Inhibition on Ocular Inflammation by Mediating the PI3K/AKT Pathway in a Murine Model of Allergic Conjunctivitis

Fei Luo,² Yu Tao,¹ Mengyu Wang,¹ Liuqing Yang,¹ Ruifeng Su,¹ Zhiqiang Pan,² and Xiaobo Tan¹

¹Department of Ophthalmology, the Affiliated Hospital of Chengde Medical University, China

²Beijing Tongren Eye Center, Beijing Institute of Ophthalmology, Beijing Tongren Hospital, Capital Medical University, Beijing Key Laboratory of Ophthalmology and Visual Sciences Key Lab, China

Correspondence: Xiaobo Tan, Department of Ophthalmology, the Affiliated Hospital of Chengde Medical University, Chengde 067000, China;

tanxb79@126.com.

Zhiqiang Pan, Beijing Tongren Eye Center, Beijing Institute of Ophthalmology, Beijing Tongren Hospital, 17 Hougou Street, Chong Wen Men, Beijing 100005, China; panyj0526@ccmu.edu.cn.

Received: November 9, 2021

Accepted: April 14, 2022

Published: May 3, 2022

Citation: Luo F, Tao Y, Wang M, et al. The protective effects of KAT5 inhibition on ocular inflammation by mediating the PI3K/AKT pathway in a murine model of allergic conjunctivitis. *Invest Ophthalmol Vis Sci.* 2022;63(5):4. <https://doi.org/10.1167/iovs.63.5.4>

PURPOSE. We aimed to explore the effect of lysine acetyltransferase KAT5 on allergic conjunctivitis (AC).

METHODS. The effect of KAT5 on inflammatory response during AC progression was analyzed in the experimental allergic conjunctivitis (EAC) mouse model.

RESULTS. The clinical score, permeability, total IgE, ovalbumin (OVA)-specific IgE, and IgG1/IgG2a were induced in the EAC mice, in which the overexpression of KAT5 could further enhance but KAT5 inhibitor NU9056 reduce the phenotypes. The eosinophilic infiltration was induced in EAC mice, in which the overexpression of KAT5 was able to further promote but NU9056 attenuate the phenotype. The expression of Eotaxin and RANTES and the inflammatory factors were upregulated in EAC mice and KAT5 overexpression increased, but NU9056 decreased the expression in the model. Significantly, the CD11c+ dendritic cells and CD4+ T cells infiltration in the conjunctiva was enhanced in EAC mice, whereas KAT5 overexpression induced but NU9056 suppressed the effect in the model. Mechanically, the phosphorylation of PI3K and Akt and the levels of histone H3 lysine 27 acetylation (H3K27ac) were enhanced in EAC mice, whereas the overexpression of KAT5 promoted and NU9056 repressed the phenotype in the mice. The enrichment of KAT5 and H3K27ac on PI3K promoter was increased in EAC mice, and the overexpression of KAT5 further enhanced the enrichment in the mice. Significantly, we observed similar results in the KAT5 knockout mice as well. Moreover, PI3K/AKT signaling inhibitor LY294002 reversed KAT5 overexpression-mediated phenotypes and inflammatory response after induction AC in vivo.

CONCLUSIONS. Therefore we concluded that KAT5 inhibition protected against ocular inflammation by mediating the PI3K/AKT pathway in EAC mouse model.

Keywords: allergic conjunctivitis, inflammation, KAT5, PI3K/AKT pathway, NU9056

Allergic conjunctivitis (AC) is a group of allergic diseases that affects a large population globally.^{1,2} AC is defined as an inflammatory response triggered by type I hypersensitivity reaction, which is mediated by the IgE and mast cell, or type IV hypersensitivity reaction, which is mediated by T cells, after exposure to allergens.^{3,4} Noteworthy, abnormal T-helper type 2 (Th2) orchestrates inflammatory responses during allergic reactions, such as asthma, atopic dermatitis, food allergy, as well as AC.^{5,6} The Th2 inflammatory responses cause interplay between various immune cells, including mast cells, dendritic cells (DCs), B cells, T cells, basophils, and eosinophils, simultaneously stimulate release of inflammatory cytokines such as IL-4, IL-5, and IL-13.⁷ For example, after reaction between allergen and IgE, activation of IgE receptors on the surface of mast cells promotes the degranulation of cells and secretion of inflammatory factors and subsequently causes elevated vascular permeability, vasodilation, and infiltration of inflammatory

cells into the conjunctiva, which promotes clinical symptoms including bilateral chemosis, hyperemia, pruritus, and lid edema.⁸

Clinically, the therapeutic methods for extraocular inflammatory symptoms such as AC mainly include antihistamines and mast cell membrane stabilizers, as well as corticosteroids for patients with severe reactions and high risk of side effects.⁹⁻¹² In this respect, exploration of novel, safe, and effective agents for control of inflammatory response becomes critical. KAT5, also known as TIP60, is a lysine acetyltransferase that modulates acetylation of genes and even proteins.^{13,14} KAT5 participates in multiple cellular process such as cell proliferation, differentiation, metabolism, and inflammatory responses.^{15,16} Studies have revealed the remarkable role of KAT5 during pathogenesis of various diseases and its potential as therapeutic targets.¹⁷ For instance, KAT5 is downregulated in rheumatoid arthritis (RA) T cells, which reduced acetylation of

Foxp3 and subsequently modulated function of regulatory T cells.^{18,19} A recent study suggested that KAT5 enhanced acetylation at the N terminal domain of DNA sensor cGMP-AMP synthase, which promoted its DNA binding ability and played an important role in initiating innate immune response.²⁰ Besides, mice with inactivated KAT5 function present decreased release of cytokines in serum on DNA virus infection and are more susceptible to DNA-virus-induced death.²⁰

In this work, we investigated the function of KAT5 during AC development by using an experimental AC (EAC) murine model and determined that overexpression of KAT5 accelerated the progression of AC through epigenetically modulated activation of the PI3K/AKT pathway. Our findings present KAT5 as a novel target of AC treatment.

MATERIALS AND METHODS

Murine EAC Model

Female BalB/c mice aged six to eight weeks old were obtained from the Charles River Laboratories (Beijing, China). All animal experiments were conducted following the guideline of the Medical Ethic Committee of Affiliated Hospital of Chengde Medical University. To establish the EAC model, mice were sensitized with 50 µg of short ragweed pollen (SRW) pollen (Greer Laboratories, Lenoir, NC USA) by footpad injection on day 0 and a secondary injection on day 5. Subsequently, 1.5 mg of SRW dissolved in 10 µL of PBS (pH 7.2) was given to each mouse from day 10 to 14 through eye drops. For KAT5 overexpression treatment, pcDNA-KAT5 plasmid was brought from Guangzhou Ribio-Bio (Guangzhou, China). Plasmid 2 µg was administered through tail vein injection 30 minutes before the SRW pollen treatment from day 10 to 14. Additionally, the plasmids were also administered by subconjunctival injection in the eyes at days 10 and 14. The PI3K/AKT inhibitor LY294002 (10 nmol/2 µL), PI3K/AKT activator SC79 (4 µg/mL), and KAT5 inhibitor NU9056 (0.3 mg/kg) were administered intraperitoneally. Each group contained six mice.

Meanwhile, the C57BL/6 mice and C57BL/6 KAT5 knock-out mice were purchased from Cyagen Biosciences Inc. (Hangzhou, China), followed by the establishment of the EAC model. All animal experiments were conducted following the guidelines of the Medical Ethics Committee of Affiliated Hospital of Chengde Medical University. To establish the EAC model, mice were sensitized with 50 µg of SRW pollen (Greer Laboratories) by footpad injection on day 0 and a second injection on day 5. Subsequently, 1.5 mg of SRW dissolved in 10 µL of PBS (pH 7.2) was given to each mouse from days 10 to 14, through eye drops.

Clinical Evaluation

The clinical evaluation of mouse AC was performed under a SL500 Shin Nippon Slit Lamp (Ajinomoto Trading Inc., Tokyo, Japan) on the day before the first administration of SRW and daily from days 10 to 14 about 15 to 30 minutes after treatment. The level of AC symptoms was confirmed microscopically by two researchers not relevant to the study, basing on four clinical parameters (chemosis, lid edema, conjunctival hyperemia, and tearing). The severity of AC was assessed following previous studies²¹: score 0 represents no severity; score 1 represents mild; score 2 represents moderate; score 3 represents severe.

The vascular permeability was assessed via tail vein injection of 0.1% Evans blue dye solution (12 mL/kg in PBS) in vivo. One hour after injection, the mice were killed. Then 24 hours later, the Evans blue dye was extracted from the eyelid and conjunctival tissue and detected by a microplate reader at 620 nm.

Histologic Analysis

The eyes with eyelids and the cervical lymph nodes (CLNs) were collected 24 hours after the last treatment. Tissues were fixed with 4% paraformaldehyde and made into paraffin-embedded 4-µm-thick slides. To evaluate the amount of infiltrating cellular components and eosinophils in the ocular surface, the hematoxylin and eosin staining was conducted. To evaluate the number of CD4+ T cells and CD11c+ dendritic cells, the eyeballs and the CLNs were embedded by OCT, sliced into 5-µm-thick sections, and subjected to immunofluorescent staining. The samples were blocked with goat serum for 30 minutes at room temperature, incubated with anti-CD4 (5 µg/mL; Abcam, Cambridge, MA, USA), or anti-CD11c (5 µg/mL; Abcam) antibody overnight at 4°C. The next day, the sections were hatched with secondary antibodies conjugated with Alexa Fluor 488 or 633 (Thermo Fisher Scientific, Waltham, MA, USA) for one hour at room temperature. The nuclei were stained with DAPI (Solarbio Life Science, Beijing, China). Five random areas were captured under a fluorescence microscope (Nikon Inc., Melville, NY, USA).

ELISA

Blood samples were collected after the last treatment, coagulated, and spun in a centrifuge at 1500 rpm for 10 minutes to isolate the serum. The levels of the specific IgE antibody, IgG1/IgG2a ratio were evaluated by ELISA kits (Abcam) in accordance with the manufacturer's instructions.

The eyeballs and CLNs were lysed and spun in a centrifuge to obtain organ supernatants. The levels of inflammatory cytokines IL-4, IL-5, IL-10, and IL-13 were detected by ELISA kits (BioLegend, San Diego, CA, USA) following the manufacturer's instructions.

Western Blotting

Eyes were isolated from mice, and lysed by sonication in lysis buffer (50 mM TrisHCl, 150 mM NaCl, and 1% TritonX-100, pH 7.4) that contains protease inhibitor cocktail (Thermo Fisher Scientific). Protein concentrations were determined by BCA kit (Beyotime Institute of Biotechnology, Jiangsu, China). The protein samples were separated by SDS-PAGE and shifted to nitrocellulose membranes. The membranes were blocked in 5% non-fat milk at room temperature for 2 hours before incubating with primary antibodies against PI3K, AKT, H3K27ac, and GAPDH. Subsequently, the membranes were incubated at room temperature for 1 hour with HRP-conjugated secondary anti-mouse or anti-rabbit IgG. The membranes were detected by enhanced chemiluminescent substrate (Thermo Fisher Scientific).

Quantitative Real Time PCR (qRT-PCR)

The conjunctiva was lysed by RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols.

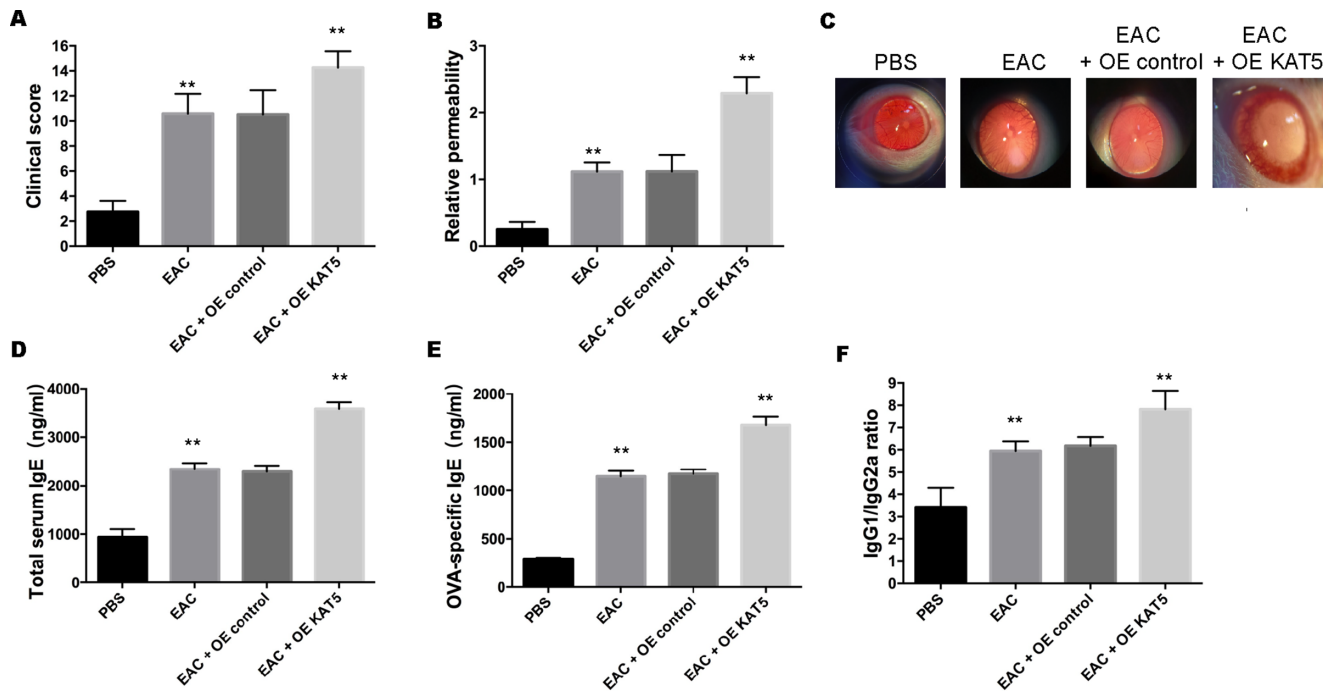


FIGURE 1. Overexpression of KAT5 leads to enhancement of phenotypes following induction AC in vivo. (A–F) The EAC mouse model was established followed by the indicated treatment. (A–C) The clinical score and permeability was analyzed. (D–F) The total IgE, OVA-specific IgE, and IgG1/IgG2a ratio were detected. OE control, overexpression control (empty vector of pcDNA); OE KAT5, overexpression KAT5 (pcDNA-KAT5). ** $P < 0.01$.

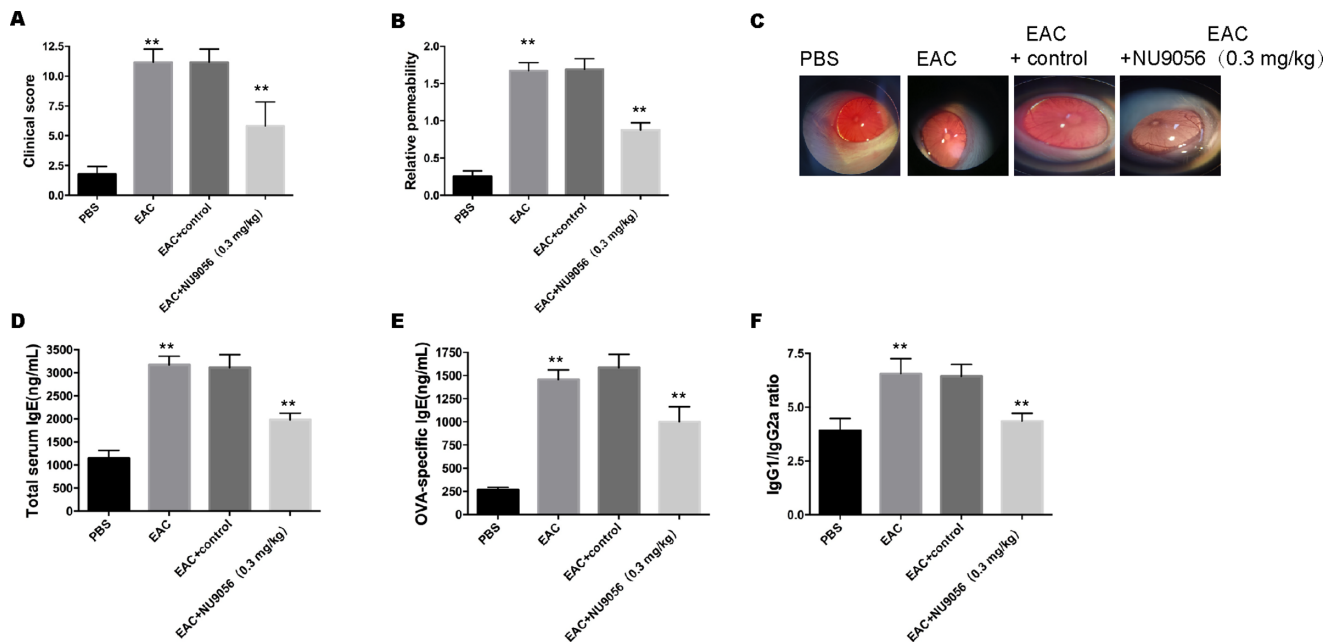


FIGURE 2. KAT5 inhibitor NU9056 leads to impairment of phenotypes following induction AC in vivo. (A–F) The EAC mouse model was established followed by the indicated treatment. (A–C) The clinical score and permeability was determined. (D–F) The total IgE, OVA-specific IgE, and IgG1/IgG2a ratio were analyzed. ** $P < 0.01$.

The cDNA was synthesized from the total RNA by SuperScript III (Thermo Fisher Scientific) and subjected to qRT-PCR for quantification of PI3K, Eotaxin, and RANTES by using SYBR qPCR Master Mix (Thermo Fisher Scientific). The relative expression of genes was normalized to GAPDH level and calculated by comparative threshold cycle method.

Chromatin Immunoprecipitation (ChIP)

ChIP assay was conducted by using ChIP assay kit (Millipore) following the manufacture's instruction. In brief, tissues were lysed and sonicated to extract chromatin fragments. The chromatin fragments were incubated with

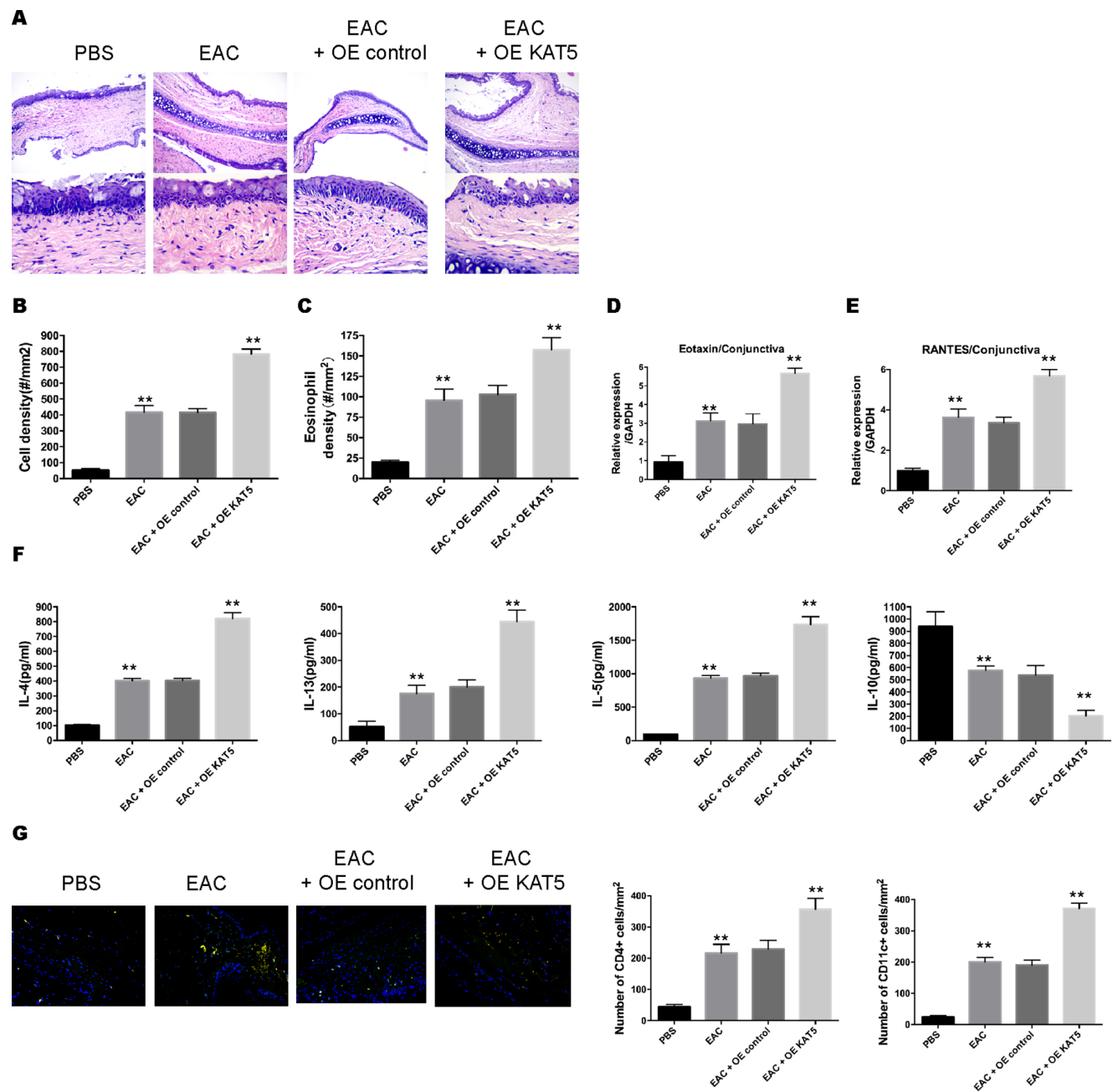


FIGURE 3. Overexpression of KAT5 leads to enhancement of inflammatory response in EAC mice. (A–G) The EAC mouse model was established followed by the indicated treatment. (A–C) The eosinophilic infiltration was analyzed by hematoxylin & eosin staining. (D and E) The expression of Eotaxin and RANTES was measured by qPCR. (F) The levels of IL-4, IL-13, IL-5, and IL-10 were detected by ELISA. (G) CD11c⁺ dendritic cells and CD4⁺ T cells infiltration in the conjunctiva were analyzed by fluorescence staining. ** $P < 0.01$.

anti-KAT5, anti-H3K27ac antibody, or rabbit IgG in rotation overnight at 4°C. The immunoprecipitated DNAs were purified by RNase A and proteinase K. The level of PI3K enrichment was detected by qRT-PCR assay.

Statistics

The data were presented as means \pm SD, and analyzed by using one-way ANOVA followed by the Bonferroni's post hoc test by using GraphPad Prism 7.0 software. P value < 0.05 was considered statistically significant.

RESULTS

Overexpression of KAT5 Leads to Enhancement of Phenotypes Following Induction AC In Vivo

To evaluate the effect of KAT5 on AC, we constructed EAC mouse model. We observed that the clinical score and permeability were induced in the EAC mice, in which the overexpression of KAT5 could further enhance the phenotypes (Figs. 1A–C). We observed the severe eyelid swelling, conjunctival edema, redness, and tearing in the EAC mice and KAT5 overexpression promoted these phenotypes in

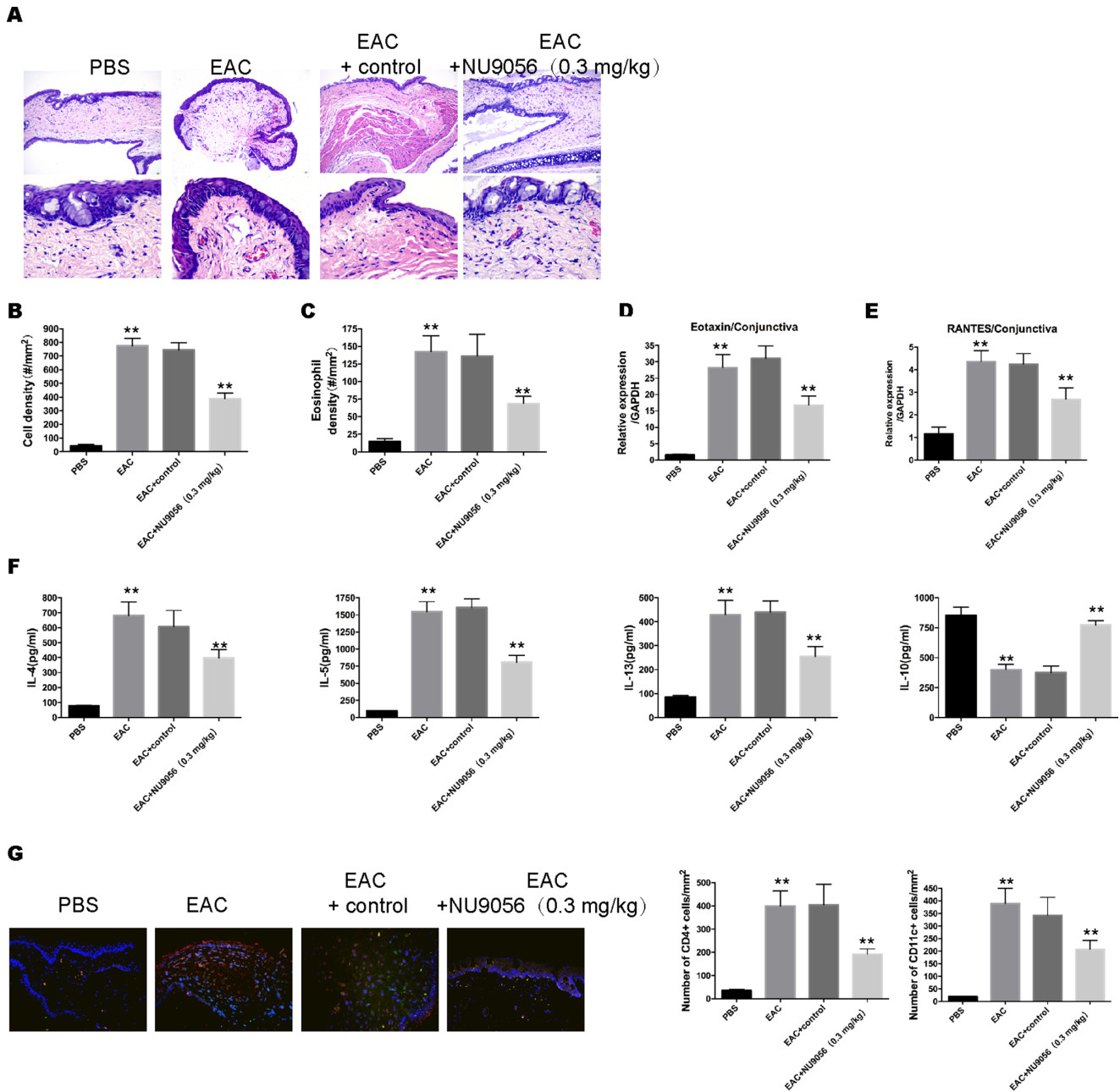


FIGURE 4. KAT5 inhibitor NU9056 leads to attenuation of inflammatory response in EAC mice. (A–G) The EAC mouse model was established followed by the indicated treatment. (A–C) The eosinophilic infiltration was analyzed by hematoxylin & eosin staining. (D and E) The expression of Eotaxin and RANTES was measured by qPCR. (F) The levels of IL-4, IL-13, IL-5, and IL-10 were detected by ELISA. (G) CD11c+ dendritic cells and CD4+ T cells infiltration in the conjunctiva were analyzed by fluorescence staining. ***P* < 0.01.

the model (Fig. 1C). Meanwhile, the levels of common clinical signs associated with conjunctivitis, such as total IgE, OVA-specific IgE, and IgG1/IgG2a ratio were enhanced in EAC mice, whereas the overexpression of KAT5 induced the levels in the model (Figs. 1D–F).

KAT5 Inhibitor NU9056 Leads to Impairment of Phenotypes After Induction of AC In Vivo

To further validate the function of KAT5 in AC, the EAC mice were treated with KAT5 inhibitor NU9056. We found that the clinical score and permeability were promoted in EAC mice, in which the treatment of NU9056 could attenuate the phenotypes (Figs. 2A–C). In

addition, the levels of total IgE, OVA-specific IgE, and IgG1/IgG2a ratio were increased in EAC mice, whereas the treatment of NU9056 reversed the levels in the model (Figs. 2D–F).

Overexpression of KAT5 Leads to Enhancement of Inflammatory Response in EAC Mice

Next, we assessed the function of KAT5 in the modulation of inflammatory response in EAC mouse model. We observed that eosinophilic infiltration was induced in EAC mice, in which the overexpression of KAT5 was able to further promote the phenotype (Figs. 3A–C). Meanwhile, the expression of Eotaxin and RANTES were upregulated in EAC

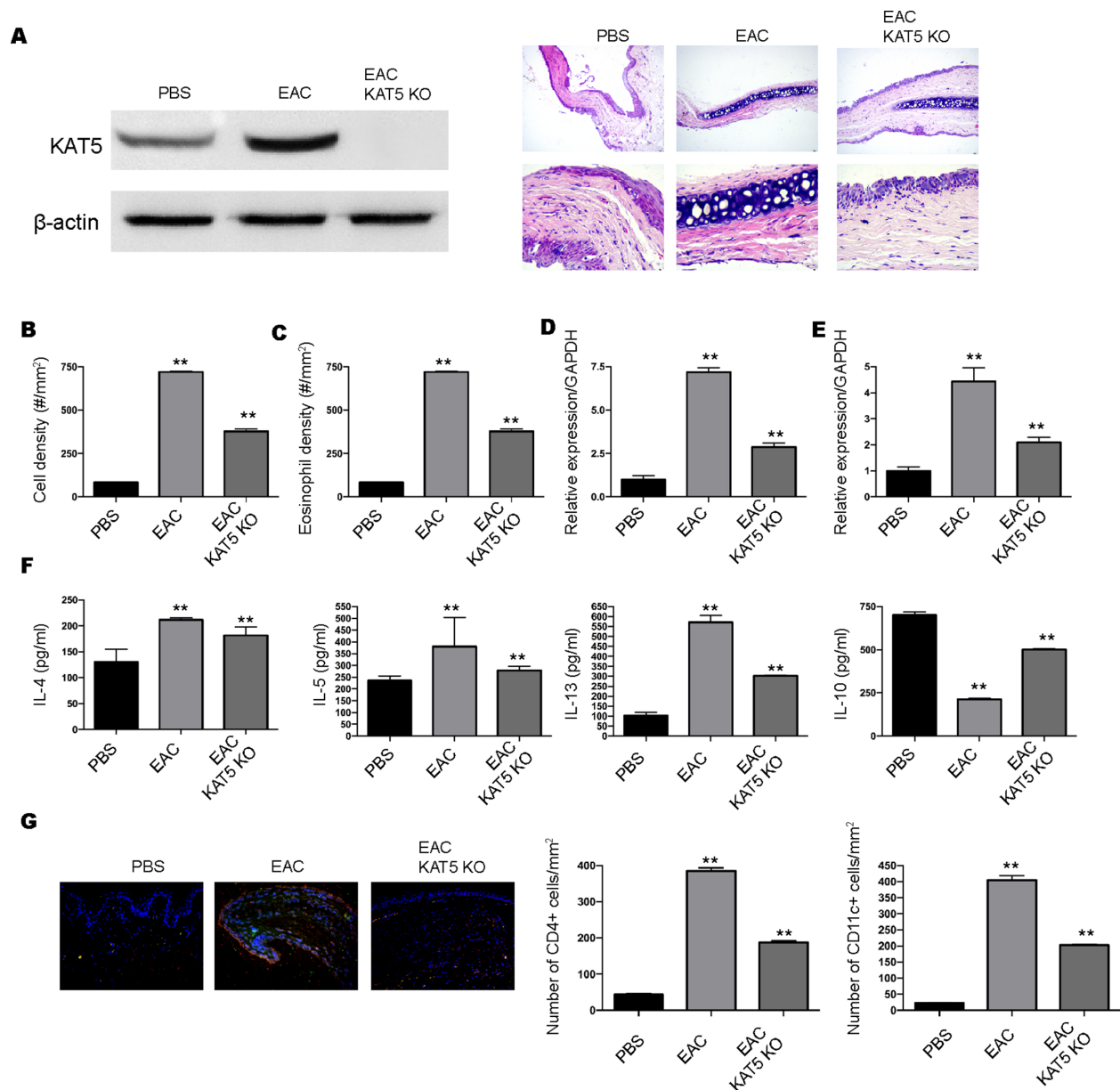


FIGURE 5. KAT5 knockout inhibits inflammatory response in EAC mice. (A–G) The EAC mouse model was established in wild type control mice (C57BL/6 mice) or C57BL/6 KAT5 knockout mice. (A–C) The eosinophilic infiltration was analyzed by hematoxylin & eosin staining. (D and E) The expression of Eotaxin and RANTES was measured by qPCR. (F) The levels of IL-4, IL-13, IL-5, and IL-10 were detected by ELISA. (G) CD11c+ dendritic cells and CD4+ T cells infiltration in the conjunctiva were analyzed by fluorescence staining. ** $P < 0.01$.

mice and KAT5 overexpression increased the expression in the model (Figs. 3D, 3E). The levels of IL-4, IL-13, IL-5 increased whereas IL-10 decreased in EAC mice, which were reinforced by the overexpression of KAT5 (Fig. 3F). Significantly, the CD11c+ dendritic cells and CD4+ T cells infiltration in the conjunctiva were enhanced in EAC mice, whereas KAT5 overexpression induced the effect in the model (Fig. 3G).

KAT5 Inhibitor NU9056 Leads to Attenuation of Inflammatory Response in EAC Mice

Then we confirmed the effect of KAT5 inhibitor NU9056 on inflammatory response in EAC mouse model. We found that eosinophilic infiltration was induced in EAC mice, in which the treatment of NU9056 was able to repress

the phenotype (Figs. 4A–C). Meanwhile, the expression of Eotaxin and RANTES were upregulated in EAC mice, and NU9056 decreased the expression in the model (Figs. 4D, 4E). The levels of IL-4, IL-13, and IL-5 increased whereas IL-10 decreased in EAC mice, which were reversed by NU9056 (Fig. 4F). Remarkably, the CD11c+ dendritic cell and CD4+ T cell infiltration in the conjunctiva was enhanced in EAC mice, whereas NU9056 reversed the effect in the model (Fig. 4G).

KAT5 Knockout Inhibits Inflammatory Response in EAC Mice

Then we validated the effect of KAT5 knockout on the inflammatory response in the EAC mouse model. We found that eosinophilic infiltration was induced in EAC mice, in

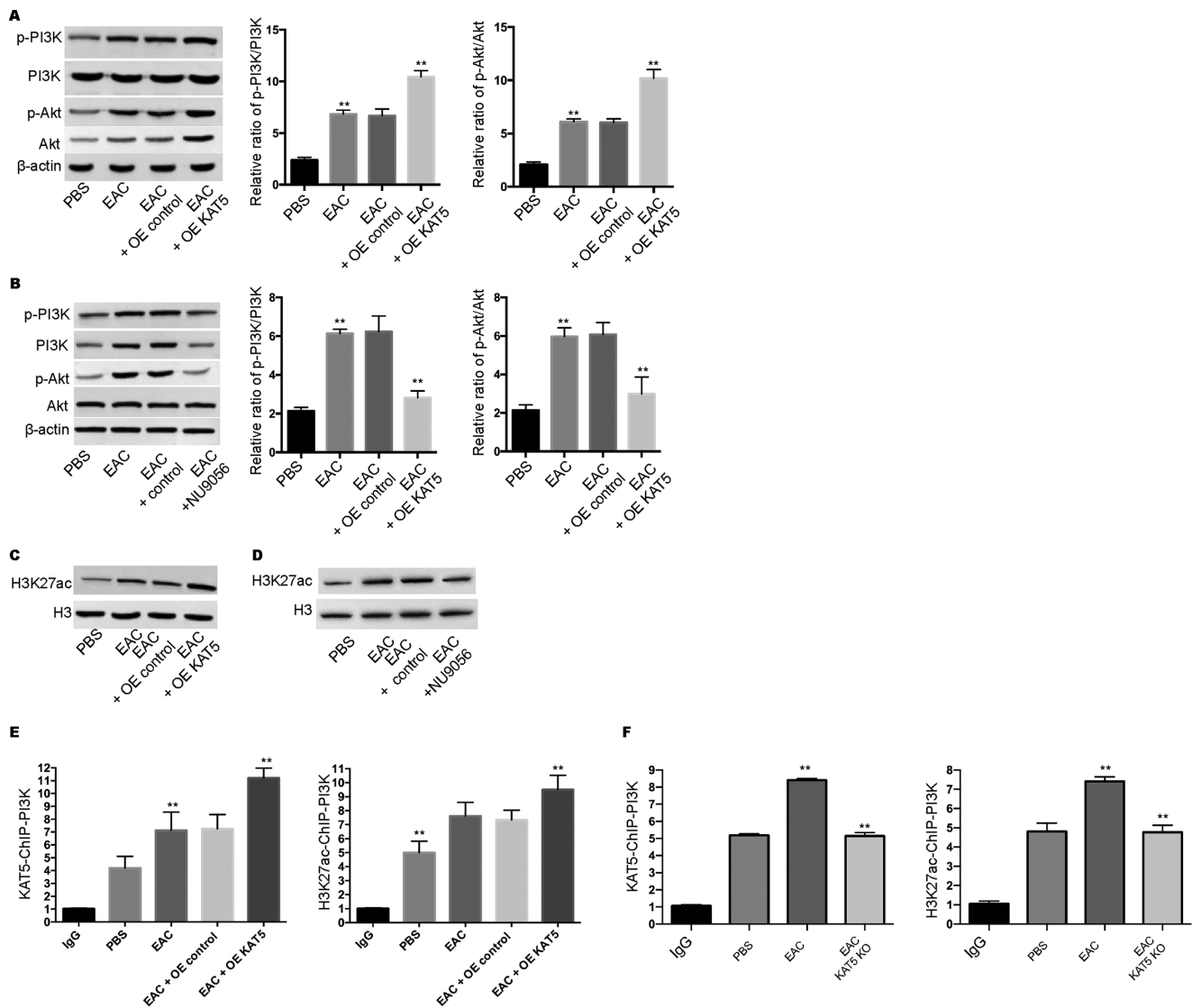


FIGURE 6. KAT5 promotes PI3K/Akt signaling by epigenetically inducing PI3K expression in EAC mice. (A–F) The EAC mouse model was established followed by the indicated treatment. (A and B) The expression of PI3K and Akt and the phosphorylation of PI3K and Akt were measured by Western blotting. (C and D) The levels of H3K27ac were detected by Western blotting. (E and F) The enrichment of KAT5 and H3K27ac on the promoter of PI3K was analyzed by ChIP. ** $P < 0.01$.

which the knockout of KAT5 inhibited the phenotype (Figs. 5A–C) and in which the expression of KAT5 was verified. Meanwhile, the expression of Eotaxin and RANTES were upregulated in EAC mice, and KAT5 knockout reduced the expression in the model (Figs. 5D, 5E). The levels of IL-4, IL-13, and IL-5 increased, whereas IL-10 decreased in EAC mice, which were reversed by KAT5 knockout (Fig. 5F). Remarkably, the CD11c⁺ dendritic cell and CD4⁺ T cell infiltration in the conjunctiva was promoted in EAC mice, whereas KAT5 knockout reversed the effect in the model (Fig. 5G).

KAT5 Promotes PI3K/Akt Signaling by Epigenetically Inducing PI3K Expression in EAC Mice

We then explored the potential mechanism underlying KAT5-mediated AC in the EAC mouse model. We observed that the phosphorylation of PI3K and Akt enhanced in EAC

mice, whereas the overexpression of KAT5 promoted and NU9056 repressed the phenotype in the mice (Figs. 6A, 6B). Meanwhile, the levels of histone H3 lysine 27 acetylation (H3K27ac) were induced in EAC mice, in which KAT5 overexpression promoted but NU9056 reversed the effect in the model (Figs. 6C, 6D). Significantly, the enrichment of KAT5 and H3K27ac on PI3K promoter was increased in EAC mice, and the overexpression of KAT5 further enhanced the enrichment in the mice (Figs. 6E, 6F).

PI3K/AKT Signaling Inhibitor LY294002 Reverses KAT5 Overexpression-Mediated Phenotypes After Induction AC In Vivo

Furthermore, we observed that the clinical score and permeability induced in the EAC mice was enhanced by the overexpression of KAT5, but the treatment of PI3K/Akt signaling inhibitor LY294002 inhibited the phenotypes (Figs. 7A,

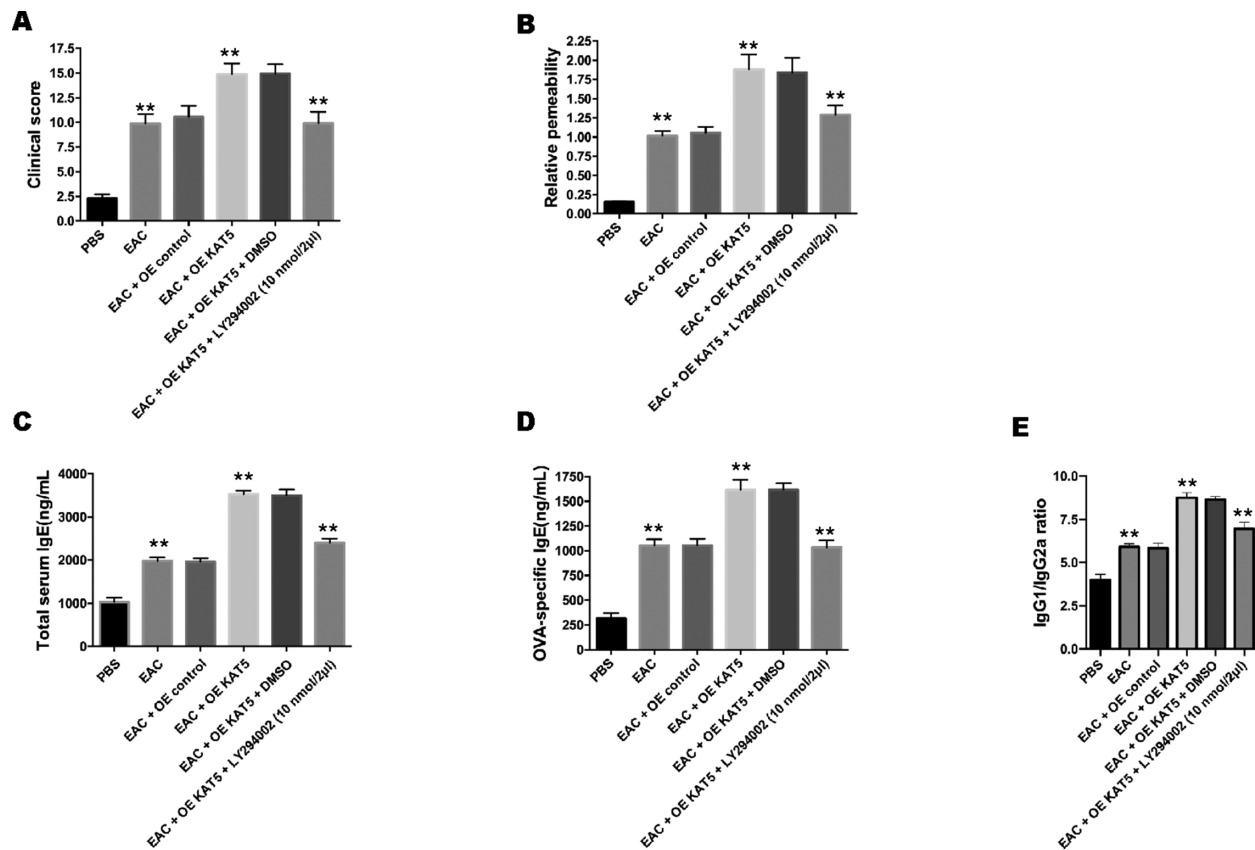


FIGURE 7. PI3K/AKT signaling inhibitor LY294002 reverses KAT5 overexpression-mediated phenotypes following induction AC in vivo. (A–E) The EAC mouse model was established followed by the indicated treatment. (A and B) The clinical score and permeability was analyzed. (C–E) The total IgE, OVA-specific IgE, and IgG1/IgG2a ratio were detected. ** $P < 0.01$.

7B). Meanwhile, the levels of total IgE, OVA-specific IgE, and IgG1/IgG2a ratio enhanced in EAC mice were induced by the overexpression of KAT5, whereas the treatment of LY294002 repressed the effect in the model (Figs. 7C–E).

PI3K/AKT Signaling Inhibitor LY294002 Reverses KAT5 Overexpression-Mediated Inflammatory Response in EAC Mice

Moreover, we found that eosinophilic infiltration induced in EAC mice was promoted by the overexpression of KAT5, whereas the treatment of LY294002 suppressed the phenotype (Figs. 8A, 8B). Meanwhile, the expression of Eotaxin and RANTES upregulated in EAC mice was increased by KAT5 overexpression, but LY294002 repressed the expression in the model (Figs. 8C, 8D). The upregulated levels of IL-4, IL-13, and IL-5 and the downregulated IL-10 levels by the overexpression of KAT5 were reversed by LY294002 (Fig. 8E). Significantly, the CD11c+ dendritic cells and CD4+ T cells infiltration in the conjunctiva enhanced in EAC mice were induced by KAT5 overexpression, in which the treatment of LY294002 blocked the effect in the mice (Fig. 8F).

DISCUSSION

AC serves as a group of allergic diseases and feature by the inflammatory response triggered by type I hypersensitivity reaction. KAT5 is a lysine acetyltransferase that modulate

acetylation of genes and even proteins and participates in multiple cellular processes such as cell proliferation, differentiation, metabolism, and inflammatory responses. Nevertheless, the effect of KAT5 on AC progression remains unclear. Here, we identified the crucial effect of KAT5 on AC in EAC mouse model.

The previous studies have found the critical roles of KAT5 in several disease models. It has been reported that KAT5 regulates the maintenance of hematopoietic stem cells.²² KAT5-modulated acetylation of SOX4 orchestrates chromatin remodeling in myoblast differentiation.²³ KAT5 regulates the metastasis and growth in a DPP4-dependent manner in papillary thyroid cancer.²⁴ The inhibition of KAT5 represses DNA repair and promotes DNA methylation in kidney podocytes.¹⁴ KAT5 contributes to metastasis and invasion by the stabilization of C-MYC.¹⁵ KAT5 acetylates cGMP-AMP synthase to enhance the response of innate immune to DNA virus.²⁰ In this study, we found that the clinical score and permeability were induced in the EAC mice, in which the overexpression of KAT5 could further enhance but KAT5 inhibitor NU9056 reduce the phenotypes. Meanwhile, the levels of total IgE, OVA-specific IgE, and IgG1/IgG2a ratio were enhanced in EAC mice, whereas the overexpression of KAT5 induced but NU9056 inhibited the levels in the model. The eosinophilic infiltration was induced in EAC mice, in which the overexpression of KAT5 was able to further promote but NU9056 attenuate the phenotype. The expression of Eotaxin and RANTES was upregulated in EAC mice and KAT5 overexpression increased but NU9056

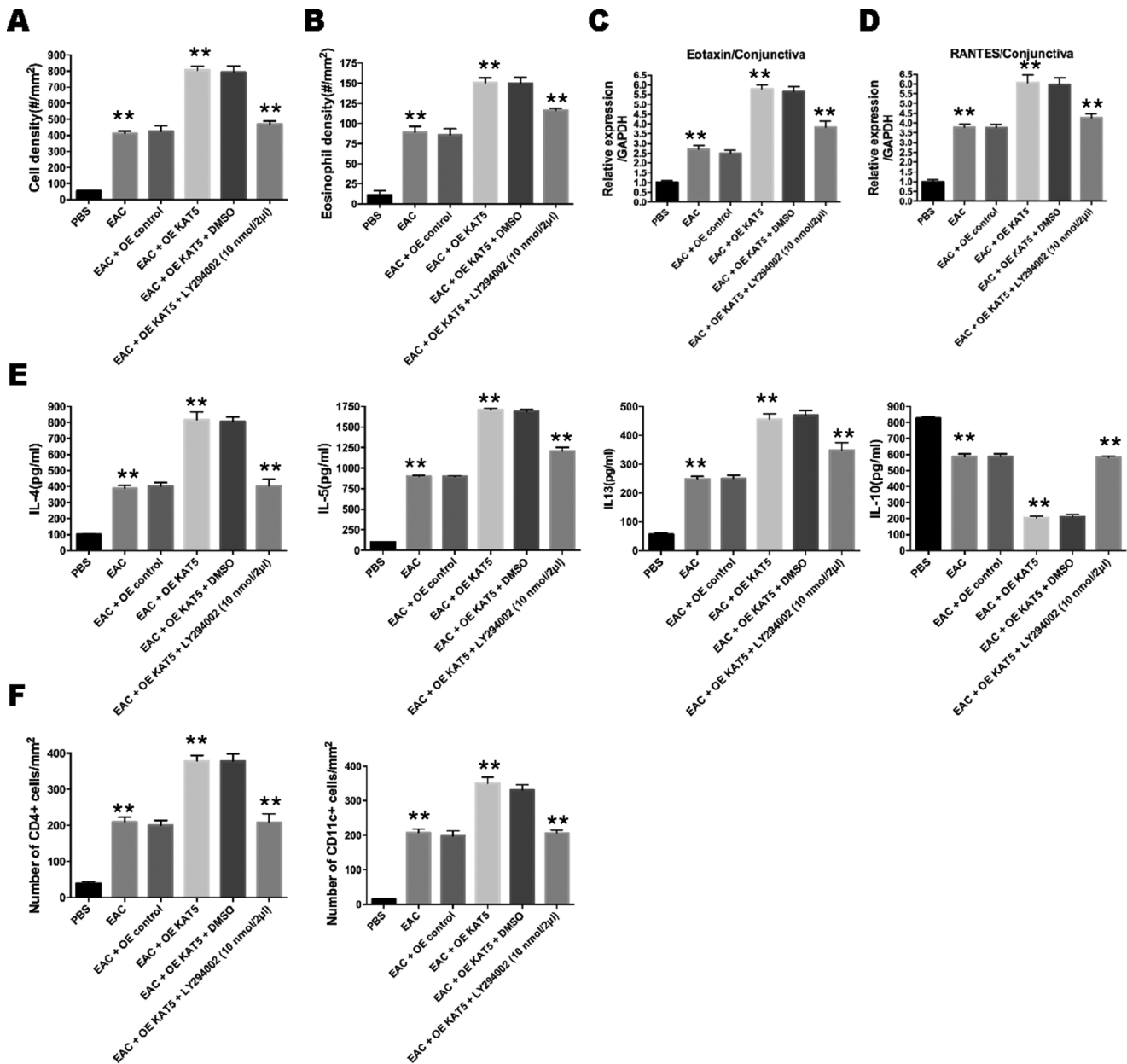


FIGURE 8. PI3K/AKT signaling inhibitor LY294002 reverses KAT5 overexpression-mediated inflammatory response in EAC mice. (A–F) The EAC mouse model was established followed by the indicated treatment. (A and B) The eosinophilic infiltration was analyzed by H&E staining. (C and D) The expression of Eotaxin and RANTES was measured by qPCR. (E) The levels of IL-4, IL-13, IL-5, and IL-10 were detected by ELISA. (F) CD11c+ dendritic cells and CD4+ T cells infiltration in the conjunctiva were analyzed by fluorescence staining. ** $P < 0.01$.

decreased the expression in the model. The levels of IL-4, IL-13, and IL-5 increased and IL-10 decreased in EAC mice were reinforced by the overexpression of KAT5 but blocked by NU9056. Significantly, the CD11c+ dendritic cells and CD4+ T cells infiltration in the conjunctiva was enhanced in EAC mice, whereas KAT5 overexpression induced but NU9056 suppressed the effect in the model. These data suggest that KAT5 contributes to ocular inflammation in AC, indicating the critical function of KAT5 in the modulation of AC. The clinical role of KAT5 in AC should be confirmed by more explorations. Moreover, it has been reported that targeting KAT5 attenuates the intestinal allergy.²⁵ In this study, we focused on the effect of KAT5 on AC in this study. Whether KAT5 participates in other allergic

diseases should be explored by more investigations. Similarly, whether KAT5 specifically enhances the allergic inflammation itself should be confirmed by more specific studies. Meanwhile, whether inhibitor of KAT5 NU9056 could control allergic reaction itself or not needed to be assessed in the future.

Regarding the mechanism, we identified that the phosphorylation of PI3K and Akt enhanced in EAC mice, while the overexpression of KAT5 promoted and NU9056 repressed the phenotype in the mice. Meanwhile, the levels of histone H3 lysine 27 acetylation (H3K27ac) were induced in EAC mice, in which KAT5 overexpression promoted but NU9056 reversed the effect in the model. The enrichment of KAT5 and H3K27ac on PI3K promoter was increased in

EAC mice and the overexpression of KAT5 further enhanced the enrichment in the mice. Our data report the innovative mechanism by which KAT5 promotes AC by targeting PI3K/AKT signaling. In this study, we identified that the inhibition of KAT5 protects against ocular inflammation by mediating the PI3K/AKT pathway in a murine model of AC. We validated the mechanism in Figures 6 and 7. We observed that the clinical score and permeability, and the levels of total IgE, OVA-specific IgE, and IgG1/IgG2a ratio, the eosinophilic infiltration, the expression of Eotaxin and RANTEX, the levels of IL-4, IL-13, IL-5, and IL-10, and the CD11c+ dendritic cells and CD4+ T cells infiltration induced in the EAC mice was enhanced by the overexpression of KAT5, but the treatment of PI3K/Akt signaling inhibitor LY294002 inhibited the phenotypes. Our finding provides new insights in to the mechanism by which KAT5 contributes to EAC by PI3K/Akt signaling. Given that KAT5 may play an important role during pathogenesis of allergic diseases,²⁵ whether KAT5 regulates other allergic diseases, such as intestinal allergy, by PI3K/Akt signaling should be investigated in the future.

There are several limitations in the current study. For examples, It is hard to confirm the cell type of KAT5 overexpression and we added the related limitation of this study in the discussion section of the revised manuscript. The overexpression of KAT5 may in the conjunctival epithelial cell, but not fibroblasts or resident immune cells, because we observed the effect and mechanism of KAT5 in conjunctiva of the mice. We did not evaluate the interaction or correlation of conjunctiva with fibroblasts or resident immune cells. The further investigations should be performed in future to validated it. In this study, we only investigated the effect of KAT5 in conjunctiva during EAC, and we did not evaluate the interaction or correlation of conjunctiva with immune cells. The effect of KAT5 on innate immune responses during EAC is an interesting scientific question and should be investigated in the future.

Therefore we concluded that KAT5 inhibition protected against ocular inflammation by mediating the PI3K/AKT pathway in EAC mouse model. Targeting KAT5 may be applied as a promising therapeutic strategy for AC.

Acknowledgments

Supported by Hebei Provincial Natural Science Foundation of China (H2020406019); Technology Innovation Guidance Project-Science and Technology Work Conference of Hebei Provincial Department of Science and Technology; Post-graduate's Innovation Fund Project of Hebei Province (CXZZSS2021141).

Disclosure: **F. Luo**, None; **Y. Tao**, None; **M. Wang**, None; **L. Yang**, None; **R. Su**, None; **Z. Pan**, None; **X. Tan**, None

References

- Blais MS, Hammerby E, Robinson S, Kennedy-Martin T, Buchs S. The burden of allergic rhinitis and allergic rhinoconjunctivitis on adolescents: a literature review. *Ann Allergy Asthma Immunol*. 2018;121:43–52.e43.
- Miyazaki D, Fukagawa K, Okamoto S, et al. Epidemiological aspects of allergic conjunctivitis. *Allergol Int*. 2020;69:487–495.
- Fukushima A. Current research progress in allergic conjunctival diseases. *Allergol Int*. 2020;69:485–486.
- Leonardi A, Castegnaro A, Valerio AL, Lazzarini D. Epidemiology of allergic conjunctivitis: clinical appearance and treatment patterns in a population-based study. *Curr Opin Allergy Clin Immunol*. 2015;15:482–488.
- Asada Y. Roles of Type 2 Immune Response-Initiating Cytokines and Detection of Type 2 Innate Lymphoid Cells in Mouse Models of Allergic Conjunctivitis. *Cornea*. 2020;39(Suppl 1):S47–S50.
- Chen X, Deng R, Chi W, et al. IL-27 signaling deficiency develops Th17-enhanced Th2-dominant inflammation in murine allergic conjunctivitis model. *Allergy*. 2019;74:910–921.
- Patel DS, Arunakirinathan M, Stuart A, Angunawela R. Allergic eye disease. *BMJ*. 2017;359:j4706.
- Elieh Ali Komi D, Rambasek T, Bielory L. Clinical implications of mast cell involvement in allergic conjunctivitis. *Allergy*. 2018;73:528–539.
- Abelson MB, Shetty S, Korchak M, Butrus SI, Smith LM. Advances in pharmacotherapy for allergic conjunctivitis. *Expert Opin Pharmacother*. 2015;16:1219–1231.
- Castillo M, Scott NW, Mustafa MZ, Mustafa MS, Azuara-Blanco A. Topical antihistamines and mast cell stabilisers for treating seasonal and perennial allergic conjunctivitis. *Cochrane Database Syst Rev*. 2015;(6):Cd009566.
- Erdinest N, Ben-Eli H, Solomon A. Topical tacrolimus for allergic eye diseases. *Curr Opin Allergy Clin Immunol*. 2019;19:535–543.
- Ridolo E, Montagni M, Caminati M, Senna G, Incorvaia C, Canonica GW. Emerging drugs for allergic conjunctivitis. *Expert Opin Emerg Drugs*. 2014;19:291–302.
- Cregan S, McDonagh L, Gao Y, et al. KAT5 (Tip60) is a potential therapeutic target in malignant pleural mesothelioma. *Int J Oncol*. 2016;48:1290–1296.
- Hishikawa A, Hayashi K, Abe T, et al. Decreased KAT5 expression impairs DNA repair and induces altered DNA methylation in kidney podocytes. *Cell Rep*. 2019;26:1318–1332.e1314.
- Wei X, Cai S, Boohaker RJ, et al. KAT5 promotes invasion and metastasis through C-MYC stabilization in ATC. *Endocr Relat Cancer*. 2019;26:141–151.
- Li Z, Mbonye U, Feng Z, et al. The KAT5-Acetyl-Histone4-Brd4 axis silences HIV-1 transcription and promotes viral latency. *PLoS Pathog*. 2018;14(4):e1007012.
- Urban I, Kerimoglu C, Sakib MS, et al. TIP60/KAT5 is required for neuronal viability in hippocampal CA1. *Sci Rep*. 2019;9(1):16173.
- Zhang X, Wu J, Luan Y. Tip60: main functions and its inhibitors. *Mini Rev Med Chem*. 2017;17:675–682.
- Su Q, Jing J, Li W, et al. Impaired Tip60-mediated Foxp3 acetylation attenuates regulatory T cell development in rheumatoid arthritis. *J Autoimmun*. 2019;100:27–39.
- Song ZM, Lin H, Yi XM, Guo W, Hu MM, Shu HB. KAT5 acetylates cGAS to promote innate immune response to DNA virus. *Proc Natl Acad Sci USA*. 2020;117:21568–21575.
- Groneberg DA, Bielory L, Fischer A, Bonini S, Wahn U. Animal models of allergic and inflammatory conjunctivitis. *Allergy*. 2003;58:1101–1113.
- Numata A, Kwok HS, Zhou QL, et al. Lysine acetyltransferase Tip60 is required for hematopoietic stem cell maintenance. *Blood*. 2020;136:1735–1747.
- Jang SM, Kim JW, Kim CH, et al. KAT5-mediated SOX4 acetylation orchestrates chromatin remodeling during myoblast differentiation. *Cell Death Dis*. 2015;6:e1857.
- Du J, Fu L, Ji F, Wang C, Liu S, Qiu X. FosB recruits KAT5 to potentiate the growth and metastasis of papillary thyroid cancer in a DPP4-dependent manner. *Life Sci*. 2020;259:118374.
- Yang G, Cheng BH, Yang SB, et al. Targeting histone-acetyltransferase Tat-interactive protein 60 inhibits intestinal allergy. *Allergy*. 2018;73:387–394.