Received: 14 December 2023 | Accepted: 17 February 2024

DOI: 10.1002/wjo2.186

RESEARCH PAPER

World Journal of Otorhinolaryngology-Head and Neck Surgery

Sirt5 affects the metabolic remodeling of eosinophils by negatively regulating the level of succinylation modification of Pkm2 in eosinophilic chronic rhinosinusitis

Shun-Yu Wu[1] | Bo-Yu Cai[1] | Zhi-Wen Cao[2] | Tian-Yu Wang[1] | Cai-Quan Liang[1] | En-Hong Xu[3] | Hu Peng[1] | Jianchun-Liao[1] | Huan-Hai Liu[1]

[1]Department of Otolaryngology, The Second Affiliated Hospital of the Naval Military Medical University (Shanghai Changzheng Hospital), Shanghai, China

[2]Department of Otolaryngology, Jinshan Hospital of Fudan University, Shanghai, China

[3]Department of Otolaryngology, Naval Medical Center, Naval Medical University (Second Military Medical University), Shanghai, China

Correspondence

Jianchun-Liao and Huan-Hai Liu, Department of Otolaryngology, The Second Affiliated Hospital of the Naval Military Medical University (Shanghai Changzheng Hospital), Shanghai, China.
Email: abliaojc@163.com and liuhuanhaiok@126.com

Funding information

Naval Medical University; National Natural Science Foundation of China, Grant/Award Numbers: 81541038, 81670905, 81870702

Abstract

Objectives: This study aims to investigate the role of Sirt5 in regulating eosinophil maturation and activation, specifically focusing on primary eosinophils in mice at the genetic level. Additionally, the study aims to elucidate the underlying mechanism of Sirt5 in eosinophilic inflammation metabolism and identify potential drug targets for the treatment of chronic sinusitis. The findings of this study will provide new insights and a solid theoretical basis for the development of novel therapeutic strategies for eosinophilic chronic rhinosinusitis (eCRS).

Methods: Our study investigated the role of Sirt5 gene expression in both non-eCRS and eCRS. We examined the correlation between Sirt5 gene expression and disease severity as well as eosinophil infiltration. Additionally, we utilized a mouse model of eCRS to assess the impact of Sirt5 gene deletion on the disease. To further understand the underlying mechanisms, we conducted experiments at the single-cell level using bone marrow-derived eosinophils. We validated our findings through in vitro culture of eosinophils and intervention experiments. Through these experiments, we aimed to elucidate how Sirt5 regulates target proteins and reshapes their related metabolic pathways.

Results: There is a positive correlation between the severity of eCRS and the expression level of Sirt5 in nasal mucosa. Inhibiting Sirt5 expression can effectively alleviate the abnormal activation of eosinophils and the resulting inflammatory response in eCRS-affected nasal mucosa. Sirt5 exerts its influence on eosinophil metabolism by negatively regulating the succinylation level of pkm2, a critical gene in the amino acid biosynthesis pathway.

Conclusions: The severity of eCRS is closely associated with the expression level of Sirt5. Sirt5 plays a negative regulatory role in the succinylation level of Pkm2 in eosinophils, thereby influencing metabolic remodeling and functional activation in

Shun-Yu Wu, Bo-Yu Cai, and Zhi-Wen Cao contributed equally to the article.

eCRS. Investigating Sirt5 and its downstream metabolic pathways could offer valuable insights into the disease's pathogenesis and facilitate the development of targeted therapeutic strategies. This research holds significant implications for clinical practitioners involved in the diagnosis and treatment of patients with eCRS.

KEYWORDS
eosinophilic chronic rhinosinusitis, eosinophils, epigenetics, histone deacetylases, succinylation

Key points

- The severity of chronic rhinosinusitis in individuals with the eosinophilic phenotype is positively associated with the expression level of Sirt5 in nasal mucosa. Inhibition of Sirt5 expression attenuates abnormal activation of eosinophils and inflammatory response in the nasal mucosa of eosinophilic chronic rhinosinusitis.
- Sirt5 modulates transcriptional alterations associated with inflammatory response and cellular metabolism in eosinophils. Sirt5 exerts a negative regulatory effect on the expression of pkm2 at both the transcriptional and translational levels.
- Sirt5 plays a vital role in the metabolic reconfiguration of eosinophils by exerting a negative influence on the levels of succinylation of pkm2.

INTRODUCTION

According to the European Position Paper on Rhinosinusitis and Nasal Polyps 2020 (EPOS 2020), primary chronic rhinosinusitis (CRS) is classified into type 2 and nontype 2, which includes eosinophilic chronic rhinosinusitis eosinophilic chronic rhinosinusitis (eCRS).[1] eCRS is a subtype characterized by strong heterogeneity and high recurrence rate, with the main clinical features being the formation of nasal polyps and olfactory dysfunction.[2] In China, the histological definition of eCRS is an increase in the proportion of eosinophils in the tissue exceeding 10% under high magnification microscopy. In addition, diffuse polyp growth and thick eosinophilic mucin can also be observed under endoscopy.[3] However, these are only surface features of eCRS, and there are more complex pathological mechanisms behind them. Studies have shown that the pathogenesis of eCRS is related to multiple factors, including genetics, immune abnormalities, and environmental factors.[4] These factors interact with each other, leading to chronic inflammation and tissue remodeling of the nasal mucosa, ultimately resulting in the formation of nasal polyps. For patients with eCRS, in addition to the formation of nasal polyps, olfactory dysfunction is also a common problem.[5] Olfactory dysfunction not only affects the quality of life of patients but may also lead to the occurrence of other complications, such as taste abnormalities and emotional disorders.[6]

The interaction between immune cells and epithelial cells in the nasal sinus mucosa, along with exposure to environmental stimuli and the nasal bacterial microbiota, forms a dynamic immune barrier.[7] Sinusitis is often caused by immune imbalance and chronic activation of inflammatory cells.[8] Recent studies have highlighted the role of epigenetic modifications in immune dysregulation, leading to changes in the nasal microenvironment and function, which are key factors in eCRS.[9] However, the specific epigenetic mechanisms underlying immune dysregulation in eCRS, particularly the chronic activation of eosinophils, remain unclear. In the pathogenesis of eCRS, eosinophils undergo differentiation and maturation in the bone marrow, enter the peripheral blood, and are recruited to infiltrate the nasal mucosa and nasal polyps.[4] Elevated levels of IL-5 play a significant role in the increase and excessive activation of eosinophils.[10] Locally increased and activated eosinophils release various substances, such as granule proteins, Charcot-Leyden crystals protein, lipid mediators, reactive oxygen species, collagen and matrix metalloproteinases, cytokines, chemokines, tissue factors, and extracellular traps, which further promote type 2 inflammation, disrupt the epithelial barrier, and contribute to fibrosis, thereby exacerbating the disease.[11] Therefore, understanding the potential mechanisms and regulatory molecules involved in systemic and local eosinophil overactivation is crucial for the treatment of eCRS. Histone modification is a well-studied epigenetic change in current research, and histone deacetylases (HDACs) have been implicated in the development of various respiratory diseases.

The HDAC enzyme family contains a highly conserved deacetylase domain, which plays a crucial role in maintaining the balance of histone lysine acetylation and removing acetyl groups.[12] To date, 18 types of human HDACs have been identified and classified into four classes based on their function and genetic criteria.[13] Sirtuins, belonging to class III HDACs, rely on nicotinamide adenine dinucleotide (NAD) for their activity. They are further divided into seven types and are highly conserved throughout evolution.[14] Sirt5, in particular, possesses multiple modifying enzyme activities that contribute to the integrity and function of mitochondria, as well as the regulation of metabolic pathways such as the urea cycle and

glucose metabolism.[15] In mice, the expression of Sirt5 has been associated with age-related diseases including obesity, insulin resistance, fibrosis, neurodegenerative diseases, heart failure, and tumors.[16] However, the role of Sirt5 in eosinophil maturation and abnormal activation, particularly in the context of local inflammatory metabolism under physiological and eCRS pathological conditions, remains largely unexplored. Therefore, this study aims to investigate the impact of Sirt5 on the modification of mitochondrial-related proteins and its regulatory role in local inflammatory metabolism. By identifying relevant target proteins and metabolic regulatory pathways, this research aims to provide potential targets for the treatment and clinical classification diagnosis of CRS, as well as insights for the clinical development of Sirt5 small molecule modulators.

The desuccinylating modification activity of Sirt5 has emerged as a prominent research area in recent years.[17,18] Numerous scientists have dedicated their efforts to unraveling the crucial role of Sirt5 in cellular metabolism and disease progression. Through the application of gene editing technology, researchers have successfully deleted the Sirt5 gene in mice.[19] This experimental manipulation has yielded intriguing results, demonstrating that mice lacking Sirt5 exhibit a significantly accelerated weight gain rate when subjected to a high-fat diet. Subsequent investigations have revealed that the desuccinylating activity of Sirt5 plays a pivotal regulatory role in fatty acid metabolism. In the absence of Sirt5, the fatty acid synthesis pathway is impeded, leading to the accumulation of fatty acids within the body. Consequently, this metabolic disruption contributes to the development of obesity and metabolic disorders. This groundbreaking discovery has garnered considerable attention within the scientific community, prompting researchers to explore the involvement of Sirt5 in other diseases. Notably, the desuccinylating activity of Sirt5 has been found to exert a significant regulatory influence on tumor development.[20,21] However, a comprehensive understanding of the functional mechanisms of Sirt5 remains elusive, necessitating further investigations into its substrates and regulatory factors. These ongoing studies aim to elucidate additional details regarding the role of Sirt5 in cellular metabolism and disease progression.

MATERIALS AND METHODS

Experimental samples

The nasal mucosal tissues used in this study were obtained from patients at the Department of Otolaryngology-Head and Neck Surgery, Shanghai Changzheng Hospital, between June 2020 and June 2021. Informed consent was obtained from all patients before surgery for the collection of clinical samples. Wild-type S129 background mice were purchased from Shanghai Bikai Experimental Animal Company, while Sirt5−/− mice with S129 genetic background were provided by Professor Han from the Naval Medical University. All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health in the United States. The experimental mice were housed, bred, administered, and subjected to behavioral tests in the SPF-level animal facility at the Naval Medical University of the People's Liberation Army of China. For in vitro studies on the function and regulation of human eosinophilic leukocytes, the Eol-1 (CB 15831161) cell line, a human eosinophilic leukemia cell line, was utilized. This cell line exhibits cytological characteristics of bone marrow progenitor cells and can differentiate into eosinophilic leukocytes upon stimulation. It is capable of synthesizing and releasing hematopoietic cell receptors and granule-degranulating proteins. The culture conditions for this cell line are well-established and provide a mature and stable environment for studying the function and regulation of human eosinophilic leukocytes.

Construction of eCRS animal model and cultivation of mouse bone marrow eosinophils

We optimized the experimental procedure by using *Aspergillus oryzae* proteinase (APO) extract combined with ovalbumin (Ova) for nasal stimulation in mice with a frequency of three times a week for a duration of 5 weeks. This allowed us to successfully establish a mouse model of eCRS. To further investigate the local eosinophil chemotaxis in the nasal mucosa of the mouse model, we performed immunofluorescence (IF) staining on two groups of wild-type mice. The control group received nasal drops of phosphate-buffered saline (PBS), while the APO + Ova nasal drop group showed an increased presence of eosinophil associated Mbp signals in the nasal mucosa of the mice. This indicates the successful construction of the eCRS mouse model.

Using 4- to 5-week-old male mice with S129 background, the heads were removed under deep anesthesia with 10% chloral hydrate. The heads were then disinfected with 75% ethanol and placed on a sterile technical platform. Under aseptic conditions, the tibiae of the mice were extracted, and the surface muscles were removed. The cells in the bone marrow cavity were washed repeatedly with DMEM medium using a syringe. After centrifugation, the cells were resuspended in red blood cell lysis buffer. After filtration and centrifugation, the cells were resuspended in Iscove's Modified Dulbecco's Medium containing recombinant mouse stem cell factor, and FMS-like tyrosine kinase 3 ligand. The cells were evenly seeded in cell culture dishes. After 4 days, the IL-5 medium was replaced every 2 days.

Real-time quantitative PCR (RT-qPCR) amplification

To obtain cDNA, reverse transcription was performed using the Takara Reverse Transcription Kit as per the manufacturer's instructions, with a reaction volume of 20 μL. Following the completion of RT-qPCR, the amplification and melting curves were confirmed, and the results were exported to Excel for subsequent processing and analysis. The $2^{-\Delta\Delta Ct}$ method was employed to calculate the relative expression level of the target mRNA, with GAPDH serving as the internal reference. The primers for the target genes used in the RT-qPCR were synthesized by Shanghai Biotechnology Company.

Western blot analysis (WB)

Prepare a precooled EDTA tissue lysis buffer containing 1 mM PMSF and 1 mM protease inhibitor. Add the lysis buffer to the target tissue and place it in a steel ball. Homogenize and centrifuge the mixture. Quantify the protein content of the preliminary protein sample and dilute the protein concentration to be within the range of the standard samples. Add five times the protein loading buffer to obtain the sample protein. Prepare SDS-PAGE gels with appropriate concentrations and thicknesses according to the size of the target protein. Place the gels in an electrophoresis tank filled with electrophoresis buffer. Add protein molecular weight markers and the loaded protein samples to the SDS-PAGE gels in a specific order. After electrophoresis and membrane transfer, cut the target bands according to the size of the target protein. Place the sealed target bands in a dilution solution of the primary antibody and incubate overnight. Then, transfer the washed target bands to a dilution solution of the secondary antibody. After drying the washed bands with absorbent paper, add a developing reagent to the protein-binding surface of the bands and perform visualization on a machine.

Immunofluorescence

The mouse nasal cavity or clinical samples were fixed in 4% formalin, dehydrated with ethanol, decalcified, and embedded in paraffin using an iron mold. Paraffin blocks were sectioned using a tissue slicer, baked, deparaffinized with xylene, and dehydrated with a gradient of ethanol. To remove endogenous peroxidase, the samples were treated with 3% H_2O_2. Antigen retrieval was performed by heating the white samples in a sodium citrate buffer. For target protein samples located within the cell membrane, a cell permeabilization treatment was applied. The pretreated white samples were incubated in PBS containing 0.2% Triton X-100, washed with PBS three times, and then incubated in a blocking solution containing 4% bovine serum albumin and 0.1% Tween at room temperature in a humid chamber for 1 h. The diluted primary antibody was incubated with the samples overnight in the blocking solution, followed by washing with prechilled PBS. The diluted secondary antibody was incubated with the samples in the blocking solution at room temperature in a dark environment. Subsequently, the samples were incubated with a concentration of 0.5 μg/mL DAPI at room temperature in a dark environment. An antifluorescence quenching agent was added to the center of the tissue samples, glass coverslips were sealed, and the samples were stored in a dark place at −20°C for fluorescence microscopy examination.

Immunohistochemistry (IHC)

The methods for preparing tissue sections and performing antigen retrieval are the same as those for IF. First, the sample is cooled to room temperature and washed twice with distilled water, followed by washing and soaking with PBS. Next, a 5% bovine serum albumin blocking solution is added at 37°C for blocking. The primary antibody dilution is incubated overnight in a refrigerator at 4°C. On the next day, the sample is soaked and washed with PBS, followed by incubation with the secondary antibody dilution in a 37°C incubator. After another round of soaking and washing with PBS, the sample is soaked in a premade DAB chromogenic reagent. After rinsing with tap water, the sample is soaked and stained with hematoxylin for 30 s. After rinsing with distilled water, the sample is dehydrated with a gradient of alcohol and finally soaked and stored in xylene. Lastly, the sample is sealed with neutral gum and observed under a microscope.

Statistical analysis

Data processing and statistical analysis were conducted using SPSS 23.0 and Graphpad Prism 8.0.2 software. The mean ± standard deviation (mean ± SD) was used to present the relevant data. Depending on the data type, clinical data were analyzed using *t*-tests or chi-square tests. Independent samples *t*-tests were employed to compare differences between two groups in basic experiments. Nonparametric tests were utilized for data involving more than two groups. A two-tailed *p*-value of less than 0.05 was considered statistically significant. In the notation, "ns" indicates $p > 0.05$; "*" indicates $p < 0.05$; "**" indicates $p < 0.01$; "***" indicates $p < 0.001$; "*****" indicates $p < 0.0001$.

RESULTS

The severity of chronic rhinosinusitis in individuals with the eosinophilic phenotype is positively associated with the expression level of Sirt5 in the nasal mucosa

Eosinophils play a crucial role in immune inflammation, as they can release granule contents that cause tissue damage and contribute to the progression of inflammation.[22] To better understand the clinical characteristics and histological manifestations of patients with eCRS, this study collected data from 100 hospitalized patients with sinusitis. The authors analyzed the patients' clinical baseline data and conducted univariate analysis (Figure 1A). Upon evaluating eosinophilic inflammation, it was observed that eCRS patients exhibited higher Lund-Mackay scores for sinus CT and Lund-Kennedy scores for nasal endoscopy compared to non-eCRS patients. Furthermore, these scores were found to be correlated with the proportion of eosinophils in peripheral blood (Figure 1B). Overall, these findings highlight the importance of eosinophils in the pathogenesis of eCRS and suggest that assessing eosinophilic inflammation can provide valuable insights into the severity and progression of the disease.

After obtaining informed consent from patients who underwent preoperative endoscopic sinus surgery (eCRS) and those who did not, nasal mucosal tissue samples were collected post-surgery for

(A)

Table 1. Baseline Data Sheets and Univariate Analyses

	Total	non-eCRS	eCRS	p-Value
Numbers	100	49	51	/
Female	43 [43]	19 [39]	24 [47]	0.403
Age	49 (36-57)	49 (36-57)	48 (35-56)	0.812
History of allergies	20 [20]	9 [18]	11 [22]	0.689
History of surgery for sinusitis	18 [18]	7 [14]	11 [22]	0.343
History of smoking	32 [32]	15 [31]	17 [33]	0.771
History of alcohol consumption	27 [27]	12 [24]	15 [29]	0.579
Height cm	168 (160-173)	168 (162.5-173.5)	167 (159-173)	0.358
Weight kg	65 (60-71.5)	67 (58.5-85)	64 (60-70)	0.612
BMI>25	25 [25]	14 [29]	11 [22]	0.419
Maxillary sinus	93 [93]	46 [94]	47 [92]	0.736
Anterior group ethmoid sinus	93 [93]	44 [90]	49 [96]	0.218
Posterior group ethmoid sinus	76 [76]	33 [67]	43 [84]	0.047
Sphenoid sinus	42 [42]	19 [39]	23 [45]	0.522
Frontal sinus	57 [57]	24 [49]	33 [65]	0.112
Ostiomeatalex, OMC	74 [74]	35 [71]	39 [76]	0.566
Bilateral polyps	79 [79]	38 [78]	41 [80]	0.727
LM score	9 (6-16)	7 (4-13.5)	12 (7-16)	0.023
LK score	6 (4-12)	5 (4-10.5)	8 (4-12)	0.133
Percentage of eosinophils	5.05 (1.375-7.150)	1.3 (0.75-1.7)	7 (5.8-9.2)	0.000
Eosinophils	0.175 (0.08-0.45)	0.08 (0.04-0.1)	0.45 (0.37-0.61)	0.000

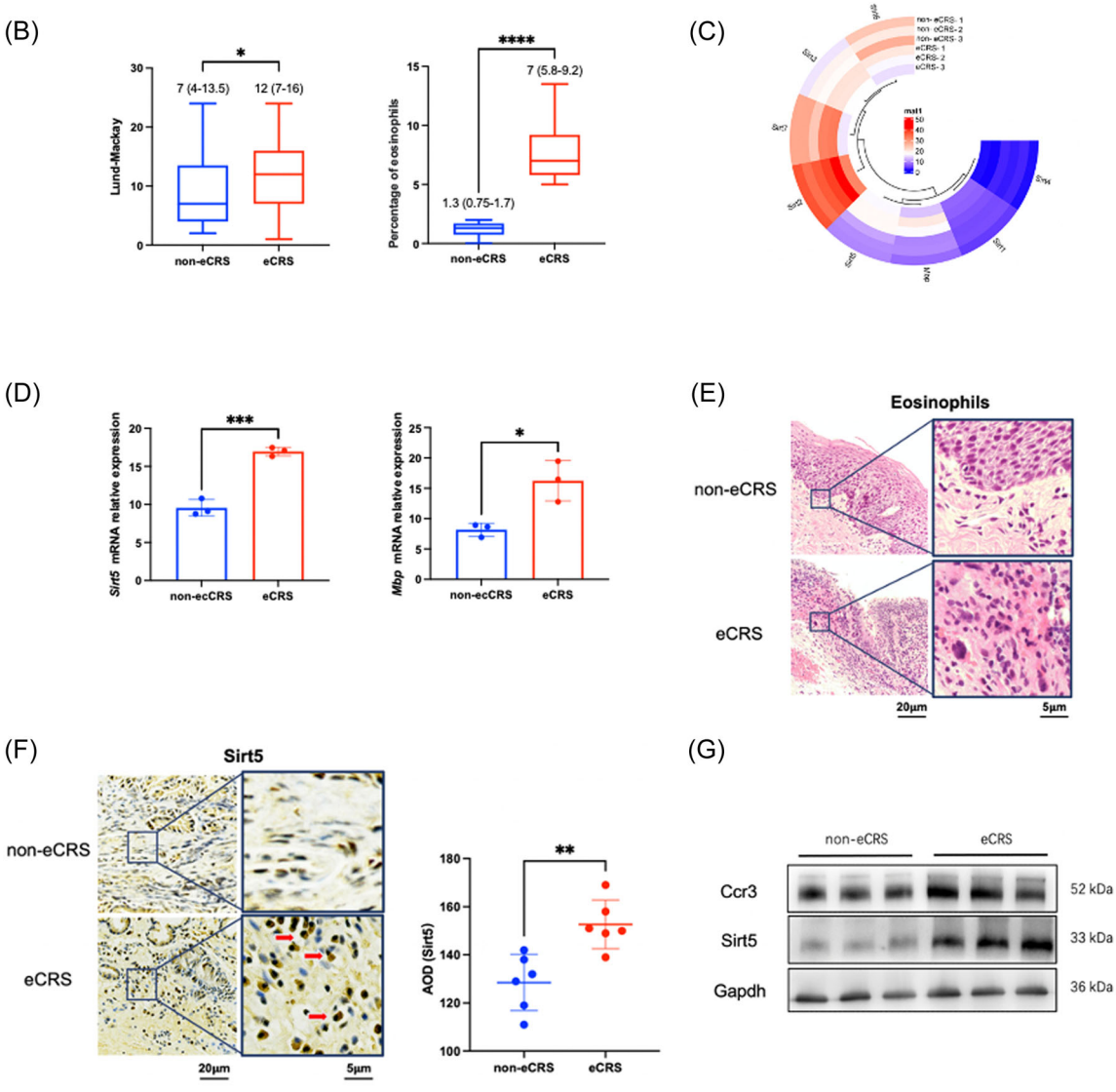

FIGURE 1 (See caption on next page).

transcriptome sequencing. The mRNA analysis revealed a positive correlation between the expression of Sirt5 and the eosinophil-related gene Mbp (Figure 1C). To further validate the association between Sirt5 expression and the development of eCRS, histological examination and IHC-staining were performed on nasal mucosa samples. The histological analysis showed disrupted ciliated columnar epithelial structure, extensive infiltration of inflammatory cells, and glandular hyperplasia in the nasal mucosa of eCRS patients, with a predominance of infiltrating eosinophils (Figure 1D). The IHC staining, followed by the calculation of average optical density (AOD), demonstrated that Sirt5 was highly expressed in the nasal mucosa of eCRS patients and correlated with the proportion of eosinophils (Figure 1E). Additionally, WB analysis confirmed the high expression of Sirt5 and the eosinophil-related protein Ccr3 in both nasal mucosa and nasal polyp tissues of eCRS patients, with higher expression observed in nasal polyps (Figure 1F). These findings suggest that Sirt5 may play a role in the development of eCRS.

By analyzing clinical tissue samples, we observed an elevation in the number of eosinophils and the expression of Sirt5 in the nasal mucosa tissue of patients with eCRS. To further investigate the association between Sirt5 and eosinophils, we conducted IF analysis on nasal mucosa tissue samples from both eCRS and non-eCRS patients. The findings revealed that eCRS patients exhibited higher levels of Sirt5 expression and eosinophil markers in the nasal mucosa tissue compared to non-eCRS patients, along with increased co-labeling signals. Quantitative analysis demonstrated a significantly higher positivity rate of Sirt5 + Mbp+ in the eCRS group compared to the non-eCRS group (Figure 1G). These results suggest that Sirt5 may potentially contribute to the development of eCRS by influencing eosinophils in the nasal mucosa.

Inhibition of Sirt5 expression attenuates abnormal activation of eosinophils and inflammatory response in the nasal mucosa of eCRS

The role of Sirt5 in the direct inhibition of abnormal activation and inflammatory response of eosinophils in the nasal mucosa of eCRS remains inconclusive. Further research is necessary to comprehensively understand the mechanism of Sirt5 in eCRS and its relationship with disease severity. Our preliminary findings in clinical tissue samples have shown an increase in Sirt5 expression in eosinophils of nasal mucosa in eCRS patients. To gain a deeper understanding of this phenomenon and explore the underlying mechanisms, we propose to utilize Sirt5 gene knockout mice with the S129 genetic background (Sirt5−/−) and establish an eCRS animal model for further validation (Figure 2A).

The research group conducted a comprehensive evaluation of the behavioral observation indicators in mice from each group at three different time points: the 1st, 3rd, and 5th weeks of intranasal administration. The primary focus of the study was to observe nasal symptoms, and the total score was recorded using a cumulative quantification method. The scoring system included parameters such as sneezing and nose scratching. In the control group of mice that were administered with PBS intranasally, no obvious abnormal behaviors were observed, and the mice remained calm throughout the study period. In contrast, the mice in the APO + Ova intranasal model group exhibited significant sneezing and nose scratching behaviors, which became more pronounced by the 5th week of modeling. Notably, among the APO + Ova intranasal model group mice, those with the Sirt5+/+ genotype displayed the most severe eCRS-related symptoms (Figure 2B). This finding suggests that the Sirt5 gene may be involved in the occurrence and development of eCRS and plays a crucial role in its pathogenesis. Overall, these results provide valuable insights into the behavioral manifestations of eCRS in mice and highlight the potential role of the Sirt5 gene in this disease. Further investigations are warranted to elucidate the underlying mechanisms and potential therapeutic targets associated with the Sirt5 gene in eCRS.

Before modeling, we extracted mouse nasal mucosa and fixed it with a 10% formaldehyde solution, followed by embedding it in paraffin. After consecutive sectioning, we performed HE-staining and observed the tissue structure of mouse nasal mucosa under an optical microscope. The results showed that the nasal mucosa of PBS-treated knockout mice and wild-type mice were intact, with orderly arranged ciliated epithelial cells, uniform basement membrane, normal cell size, and staining. In contrast, in Sirt5+/+ mice treated with APO + Ova, the nasal mucosa showed edematous connective tissue, infiltrating eosinophils, epithelial cell infiltration, and crypts. The cilia collapsed, showing typical polypoid changes, and there was obvious mucosal epithelial damage, infiltration of inflammatory cells, eosinophil infiltration, and lymphocyte infiltration. In comparison, the nasal mucosa of Sirt5−/− mice in the eCRS model group was intact,

FIGURE 1 Positive correlation between Sirt5 expression in nasal mucosa and disease severity in eosinophilic chronic rhinosinusitis (eCRS) patients (ns, $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). (A) Clinical baseline and univariate analysis revealed that eCRS patients had higher imaging scores, nasal endoscopy scores, and peripheral blood eosinophil proportions. (B) Sinus CT scores indicated more severe sinus obstruction in eCRS patients. However, eCRS patients had a higher proportion of peripheral blood eosinophils. Transcriptome sequencing of nasal mucosa samples showed higher expression of Sirt5 and Mbp in eCRS patients. (C) Gene clustering analysis based on sequencing data revealed higher expression of Sirt5 and eosinophil-associated gene Mbp in nasal mucosal tissues of eCRS patients. (D) RT-qPCR validation confirmed higher expression of Sirt5 and Mbp in nasal mucosa of eCRS patients. (E) HE-staining demonstrated increased eosinophil infiltration and more severe epithelial structure damage in nasal mucosal tissues of eCRS patients. (F) Immunohistochemical staining showed higher expression of Sirt5 in eCRS patients compared to non-eCRS patients (indicated by red arrows). Statistical analysis was performed based on the average optical density (AOD) values of immunohistochemical staining. (G) Western blotting (WB) experiment detected high expression of Sirt5 and eosinophil-associated protein Ccr3 in nasal mucosal tissues of eCRS patients.

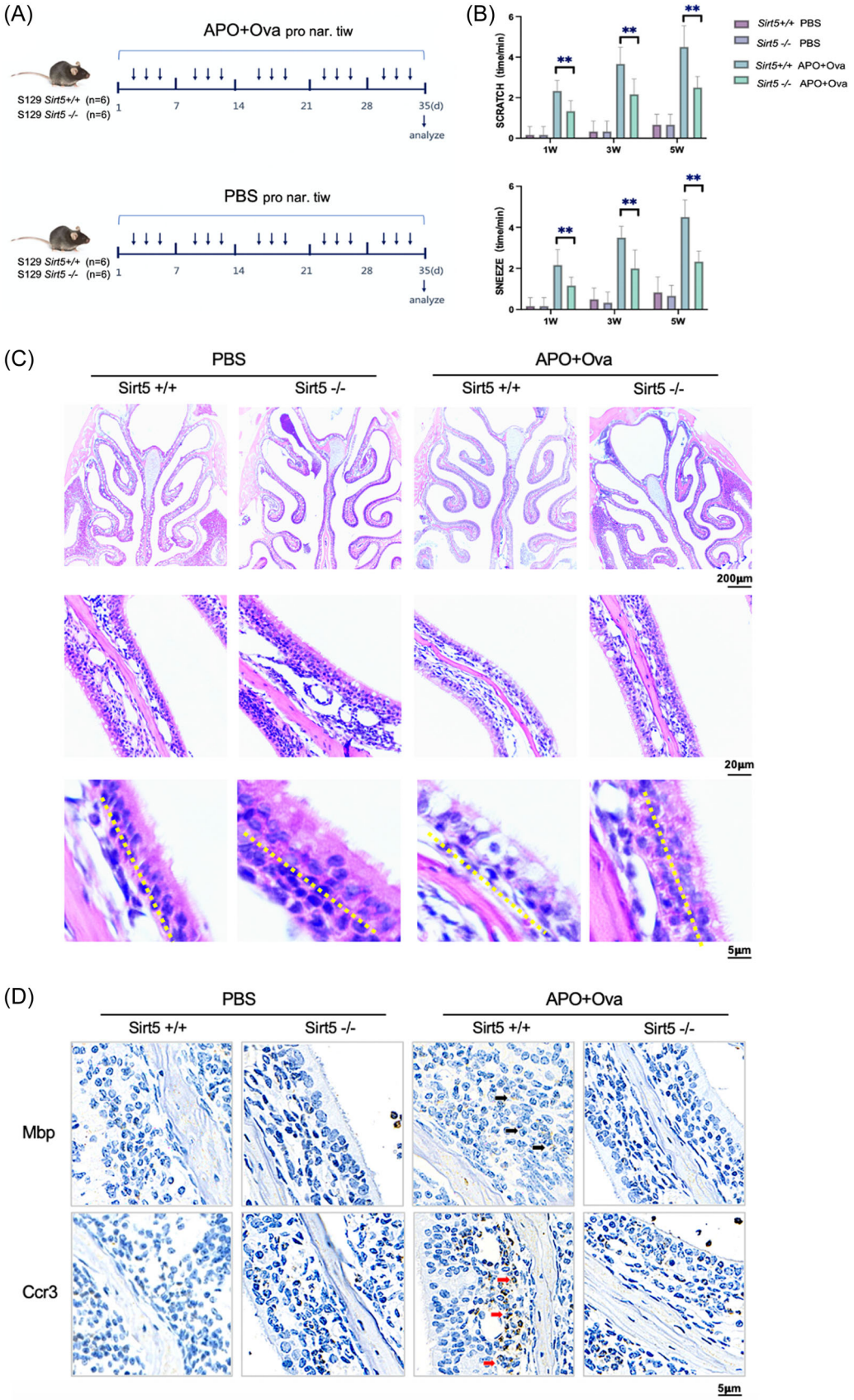

FIGURE 2 (See caption on next page).

with orderly arranged ciliated epithelial cells and a uniform basement membrane. Immunohistochemical staining results showed that compared with eCRS mice of the Sirt5+/+ genotype, the expression of eosinophil-related genes Mbp and Ccr3 was lower in eCRS mice of the Sirt5−/− genotype.

Sirt5 modulates transcriptional alterations associated with inflammatory response and cellular metabolism in eosinophils

Based on the aforementioned data mining and experimental validation, we speculate that eosinophils play an important role in the occurrence and development of eCRS disease. To further validate the relationship between Sirt5 and eosinophil function and identify potential regulatory factors, we extracted RNA from 14-day differentiated primary mouse bone marrow eosinophils and performed sequencing analysis to explore targets associated with eosinophilic diseases at the single-cell level (Figure 3A). Based on transcriptome sequencing technology, a total of 8418 differentially expressed genes (DEGs) were obtained. Compared with the control group, the experimental group upregulated 4387 genes, including Pkm, Rpl34ps1, Eno1b and Hal, and downregulated 4031 genes, including Apoe, Npy, Ccr3, Srp54b, and IL-13 (Figure 3B). RT-qPCR validation showed that genes associated with maturation and granule degranulation were generally downregulated in Sirt5−/− mouse bone marrow eosinophils, consistent with the transcriptome sequencing results (Figure 3C).

Inflammation and immune response dysfunction, as well as the extracellular microenvironment, are two key features of local pathological changes in the nasal mucosa of patients with eCRS. To better understand the functions of differentially expressed mRNAs and their relationships, we conducted GO term and KEGG pathway enrichment analysis. The GO enrichment analysis revealed that the differential genes between Sirt5 knockout mice and wild-type mice in primary bone marrow eosinophils are primarily involved in immune response, inflammatory response, and lipid metabolic processes, among other biological processes. This information is visually represented in the GO enrichment bubble plot (Figure 3D). Based on the results of the GO enrichment analysis, we focused on metabolic pathways and selected relevant pathways from the KEGG Pathway annotation category. Our findings indicate that the differential genes are mainly involved in biosynthesis of amino acids (01230), Pyruvate metabolism (00620), Oxidative phosphorylation (00190), Carbon metabolism (01200), and Glycolysis/Gluconeogenesis (00010). This information is presented in the form of a figure (Figure 3E). Understanding the enrichment of metabolic pathways in the transcriptome of eosinophils can provide valuable insights into the metabolic requirements and functional characteristics of these cells. Targeting specific metabolic pathways may offer potential therapeutic strategies for regulating eosinophil-related diseases and immune response dysregulation.

Pkm plays a crucial role as a central gene in the biosynthetic pathway of amino acids

Gene set enrichment analysis (GSEA) was utilized to assess the enrichment of gene sets and determine if there were significant differences in the expression of genes within specific pathway gene sets. A total of 199 pathways met the criteria of $p < 0.05$ and normalized enrichment score (NES) > 1. To further refine our analysis, we considered both the Q value and enrichment ratio, leading us to select five metabolic pathways for GSEA. Notably, the amino acid biosynthesis pathway (01230) exhibited significant enrichment, with 53 DEGs identified (Figure 4A).

In this study, we aimed to explore the role of protein-protein interactions in amino acid biosynthesis pathways. We utilized the STRING database to construct a comprehensive protein–protein interaction network, which consisted of 53 genes. Our analysis revealed that pyruvate kinase M (Pkm) interacts with multiple proteins, suggesting its involvement in various steps of amino acid biosynthesis in eosinophils. To further investigate genes or proteins associated with amino acid metabolism, we focused on those with regulatory relationships within the amino acid metabolism gene set and occupying major regulatory positions. Leveraging the protein-protein interaction network, we conducted key driver analysis (KDA) to identify key regulators. Our findings indicate that Pkm and its related genes, including Taldo1, Tkt, Pcx, and Eno3, may play crucial roles in the transcriptional regulation of eosinophils. These results provide valuable insights into the function and interaction relationships of proteins. Moreover, our study highlights the significant regulatory roles of Pkm and its related genes in eosinophils, thereby enhancing our understanding of amino acid metabolism. Overall, our study contributes to the growing body of knowledge of protein-

FIGURE 2 Animal models have provided valuable insights into the role of Sirt5 in the pathogenesis of eosinophilic chronic rhinosinusitis (eCRS). Inhibition of Sirt5 expression has been shown to significantly reduce the abnormal activation of eosinophils and mitigate inflammatory responses in the nasal mucosa associated with eCRS. (A) Schematic diagram illustrating the *Aspergillus oryzae* proteinase (APO) + ovalbumin (Ova) nasal instillation model for establishing the eCRS mouse model. Mice were intranasally stimulated with APO extract + Ova for 5 weeks, three times per week. The control group received phosphate-buffered saline (PBS) nasal instillation. (B) Quantitative evaluation of behavioral indicators in the modeled mice. The Sirt5−/− APO + Ova instillation group exhibited milder eCRS symptoms, such as nasal scratching and sneezing, as the modeling time increased. (C) HE-staining of the nasal mucosa in the four groups of modeled mice after successful construction of the eCRS animal model. The Sirt5−/− modeled mice showed less severe nasal epithelial damage and fewer infiltrating inflammatory cells compared to wild-type mice. (D) Immunohistochemical (IHC) staining demonstrated low expression of eosinophil-related proteins, Mbp (black arrow), and CCR3 (red arrow), in the nasal mucosa of Sirt5−/− modeled mice.

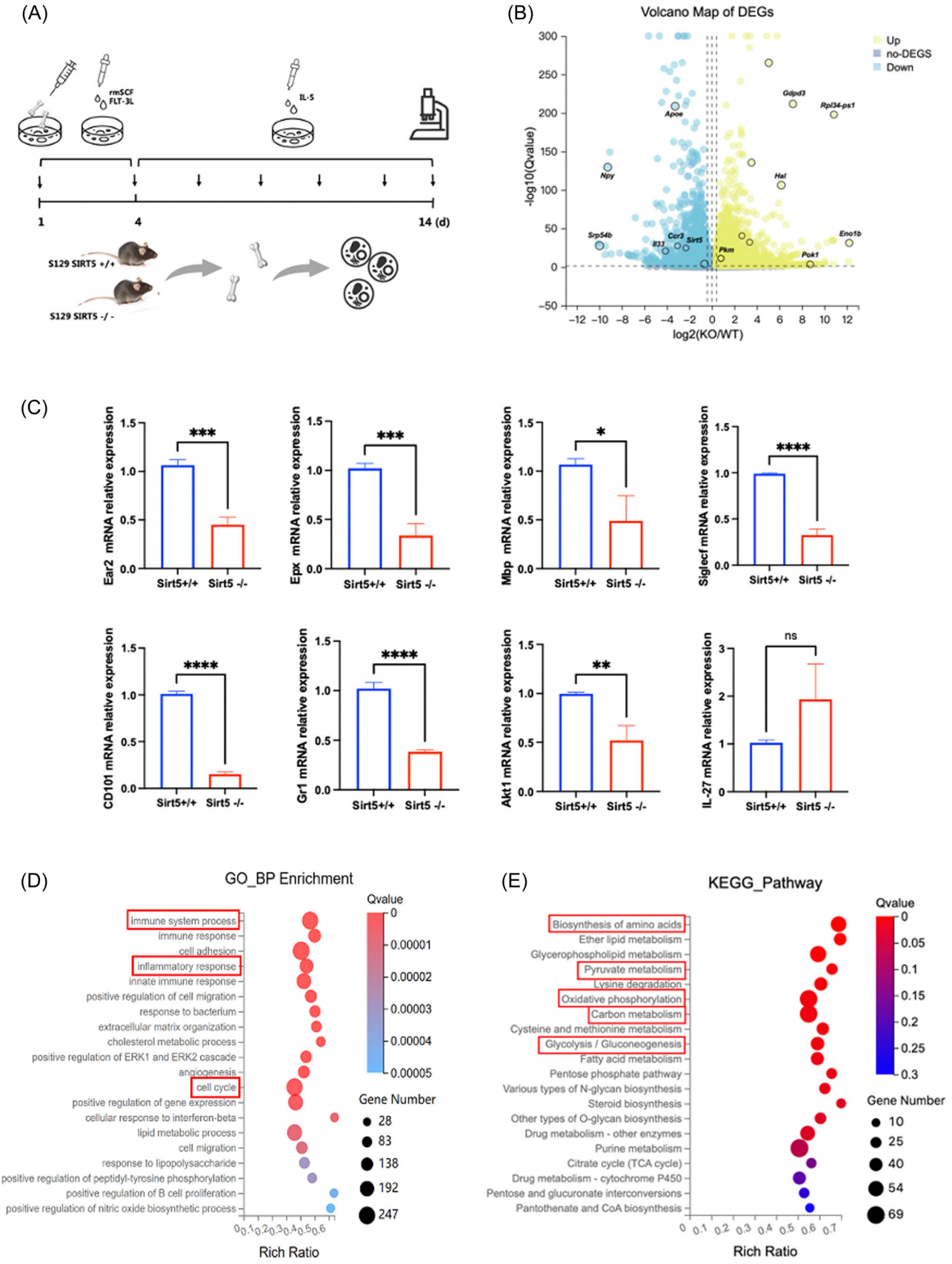

FIGURE 3 (See caption on next page).

protein interactions and their impact on amino acid biosynthesis pathways. Further investigations are warranted to elucidate the precise mechanisms underlying the regulatory role of Pkm and its associated genes in eosinophils.

Sirt5 exerts a negative regulatory effect on the expression of pkm2 at both the transcriptional and translational levels

This study aimed to investigate the role and mechanism of Sirt5 in primary mouse eosinophils at the genomic level. To achieve this, several analyses were conducted, including sample normalization, differential expression annotation analysis, and differential expression gene screening. Additionally, GO and KEGG enrichment analysis, GSEA, PPI network construction, and key driver analysis were performed. Compared to wild-type mice, the Sirt5−/− group of eosinophils exhibited differential expression of genes primarily enriched in the amino acid biosynthesis pathway. Among these genes, Pkm was identified as a key gene and its potential involvement in multiple steps of amino acid biosynthesis in eosinophils was explored. Notably, the Pkm gene undergoes alternative splicing to form Pkm2, which encodes Pkm2. Pkm2 regulates the final rate-limiting step of aerobic glycolysis and plays a crucial role in metabolic regulation. Consequently, Pkm2 has been extensively studied due to its significance. Overall, this study provides valuable insights into the role and mechanism of Sirt5 in primary mouse eosinophils at the genomic level. The findings highlight the importance of the amino acid biosynthesis pathway and the involvement of key genes, such as Pkm, in eosinophil metabolism. Further research on Pkm2 could contribute to a better understanding of eosinophil function and potentially lead to the development of novel therapeutic strategies for eosinophil-related disorders.

We extracted RNA from primary mouse bone marrow cells, eosinophilic cell lines, and mouse nasal mucosa, and performed RT-qPCR experiments to validate the negative correlation between Pkm2 and Sirt5 at the transcriptional level (Figure 5A). Subsequently, WB experiments showed that the expression of Pkm2 was upregulated in the nasal mucosal tissue of Sirt5−/− mice, while the eosinophilic cell line expressed the highest level of Pkm2 at the optimal concentration of Sirt5 inhibitor (Figure 5B). At the histological level, we found a higher expression of Pkm2 in the nasal mucosa of Sirt5−/− mice and the APO + Ova nasal drop model group in the nasal mucosa IF staining of the four groups of mice (Figure 5C).

Sirt5 plays a vital role in the metabolic reconfiguration of eosinophils by exerting a negative influence on the levels of succinylation of Pkm2

Previous studies conducted by our research group have focused on investigating the role of Sirt5 in the negative regulation of Pkm2 expression and its impact on amino acid biosynthesis pathways. Recently, there has been a surge of interest in studying the Sirt5-mediated desuccinylation modification activity, which plays a crucial role in various core energy metabolism pathways and its implications in mitochondrial-related diseases. Notably, Pkm2 has emerged as a potential substrate for succinylation by Sirt5. However, the precise mechanism by which Sirt5-mediated lysine desuccinylation regulates Pkm2 expression and its involvement in eosinophilic inflammatory responses remains to be elucidated.

We conducted further analysis on clinical nasal mucosa samples and mouse bone marrow eosinophil transcriptome sequencing data. Our findings revealed differential expression of succinylation-related genes, particularly Pkm (Figure 6A). To validate these results, we performed Western blot experiments, which confirmed that the levels of pan-succinylation and pan-acetylation in nasal mucosal tissues of Sirt5−/− mice were higher compared to the control group. Notably, the increase in pan-succinylation levels was more significant (Figure 2B). Similarly, in the eosinophil cell line, we observed an enhanced expression of pan-succinylation levels under the intervention of the optimal concentration of Sirt5 inhibitor, consistent with the results in mouse nasal mucosa (Figure 2C). To further investigate the mechanism behind these findings, we conducted immunoprecipitation experiments. These experiments revealed an increase in the co-precipitation of Pkm2

FIGURE 3 Data mining of eosinophil-related targets and validation of eosinophils in mouse bone marrow, revealing transcriptional changes related to inflammation and cellular metabolism induced by Sirt5. (A) Schematic diagram illustrating the isolation of mouse bone marrow cells and induction of eosinophils. After filtration and centrifugation of cells in the bone marrow cavity, stimulating factors were added, and the cells were evenly seeded in cell culture dishes. Differentiation was induced with IL-5 every 2 days starting from Day 4. (B) Volcano plot displaying differentially expressed genes (DEGs) obtained from transcriptome sequencing. Upregulated genes (yellow) in the experimental group include Pkm, Rpl34ps1, Eno1b, Hal, and so on, while downregulated genes (blue) include Apoe, Npy, Ccr3, Srp54b, IL-13, and so on. Gray represents non-DEGs. The *x*-axis represents the log2-transformed fold change, and the *y*-axis represents the −log10-transformed significance value. (C) RT-qPCR validation revealed that genes related to maturation and degranulation were generally downregulated in Sirt5−/− mouse bone marrow eosinophils, consistent with the results of clinical transcriptome sequencing (ns, $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). (D) GO enrichment analysis indicated that DEGs were mainly enriched in functions and processes such as protein binding and immune inflammatory response. Each dot in each row represents a significantly enriched pathway, with the color ranging from red to blue indicating the P-value after multiple corrections. The size of the circle represents the number of DEGs. The *Q*-value, obtained by FDR correction of the *p*-value, was used to determine significant enrichment (*Q*-value ≤:0.05). (E) Bubble plot of KEGG enrichment analysis showing that the DEGs are mainly involved in metabolic pathways such as amino acid biosynthesis, citric acid cycle, oxidative phosphorylation, carbon metabolism, and glycolysis/gluconeogenesis pathways.

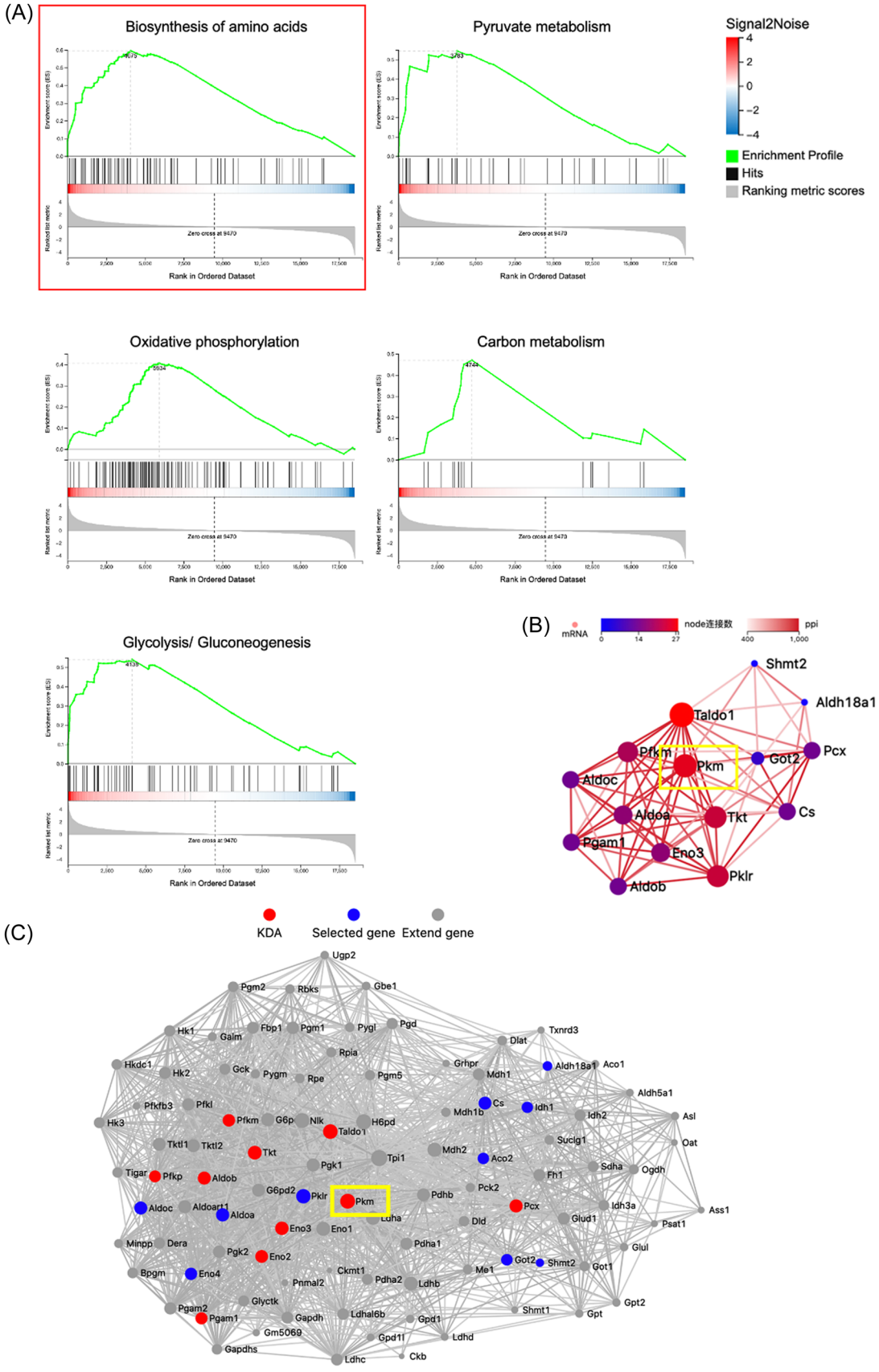

FIGURE 4 (See caption on next page).

and pan-succinylation antibody in eosinophils under Sirt5 inhibitor intervention. This suggests that Sirt5 affects the metabolic remodeling of eosinophils by inhibiting succinylation modification of Pkm2 (Figure 2D). Based on these results, we propose that Sirt5 may regulate the metabolic pathways and energy production of eosinophils by negatively regulating succinylation on Pkm2. This regulation may have implications for the function and behavior of eosinophils in immune responses and inflammatory processes.

DISCUSSION

The treatment of eCRS should be comprehensive. In the field of rhinology, researchers have been striving to find more effective treatment methods.[23] Some studies have shown that immunomodulators may have a certain effect on the treatment of eCRS.[24] For example, leukotriene receptor antagonists such as montelukast have been found to reduce the formation of nasal polyps and improve olfactory dysfunction.[25] Topical steroids have also been found to reduce inflammation and decrease the formation of nasal polyps.[26] However, long-term use of steroids may cause side effects for eCRS patients, so caution is needed. Surgical interventions, such as endoscopic sinus surgery, can help remove diseased tissue and nasal polyps in the sinuses, thereby improving symptoms.[27] However, it is important to note that surgery is not a permanent solution as eCRS has a high recurrence rate, and patients need regular follow-up and treatment.[28] Other treatment methods, such as photodynamic therapy, biofeedback therapy, and traditional Chinese medicine, have also been explored.[1] However, further research and validation are needed to determine their efficacy and safety, as they are associated with strong side effects, more complications, and higher recurrence rates. Therefore, there is a need for in-depth exploration of the pathogenesis of eCRS and the development of targeted blocking treatments, which has become a research hotspot in recent years.

Eosinophils, as integral components of the immune system, play a crucial role in defending the human body's health.[29] Their unique metabolic characteristics distinguish them from other cell types and enable them to fulfill their momentous functions in immune responses.[30] Research has demonstrated that the acidic substances generated during eosinophil metabolism are essential for combating pathogen invasion.[31] By releasing granules containing acidic substances directly into the surrounding environment, eosinophils effectively eliminate pathogens.[32] This exceptional metabolic feature positions eosinophils as a vital force within the immune system.

Our investigation has revealed a positive correlation between the severity of chronic sinusitis in eosinophilic patients and the expression level of Sirt5 in the nasal mucosa. Specifically, higher expression levels of Sirt5 are associated with more severe conditions. This finding suggests that elevated Sirt5 expression may induce alterations in specific metabolic pathways within the nasal mucosa, leading to abnormal activation of eosinophils and exacerbation of inflammatory reactions. Consequently, these changes may contribute to the worsening of symptoms and disease progression. In summary, eosinophils' metabolic characteristics, particularly their production of acidic substances, are crucial for their role in immune defense. Our research highlights the significance of Sirt5 expression in the nasal mucosa of eosinophilic patients, as it influences disease severity by modulating metabolic pathways and subsequent eosinophil activation. Understanding these mechanisms may provide valuable insights for developing targeted therapeutic strategies for chronic sinusitis.

Sirt5 is a mitochondrial NAD+-dependent deacetylase, which plays a crucial role in cellular metabolism and energy regulation.[14] In this study, we aimed to investigate the mechanisms underlying function of Sirt5 at the single-cell level using bone marrow-derived eosinophils. To validate our findings, we performed an in vitro culture of eosinophils and conducted intervention experiments. Our results revealed that a reduction in Sirt5 activity led to an increase in the succinylation modification level of pkm2, resulting in metabolic pathway remodeling in eosinophils. Through further experiments, we demonstrated that Sirt5 directly interacts with pkm2, thereby regulating its succinylation modification level. This interaction was found to influence the activity of pkm2, ultimately affecting the metabolic pathways of eosinophils. In summary, our study provides novel insights into the role of Sirt5 in regulating protein acetylation modifications and metabolic pathways in eosinophils. These findings contribute to a better understanding of cellular metabolism and energy regulation and may have implications for the development of therapeutic strategies targeting eosinophil-related diseases.

The outcomes of this study have profound implications in enhancing our understanding of the metabolic regulation mechanisms of eosinophils. Additionally, these findings present novel perspectives that can revolutionize the development of therapeutic strategies.

FIGURE 4 Transcriptome sequencing analysis of mouse bone marrow eosinophils reveals enrichment of differentially expressed genes (DEGs) in metabolic pathways, particularly the amino acid biosynthesis pathway. Pkm is identified as a key hub gene in this pathway. (A) Gene set enrichment analysis demonstrates significant enrichment of DEGs in the amino acid biosynthesis pathway (01230). The *x*-axis represents sorted genes, while the *y*-axis represents the corresponding running enrichment score (ES). The peak in the line graph indicates the ES of this gene set. The black line represents the position of the genes in the sorted expression data set within the analyzed functional gene set. The red-blue heatmap represents the expression abundance, with darker red indicating higher logFC of genes at that position, and darker blue indicating lower logFC. The gray area plot reflects the signal-to-noise ratio (Signal2noise) between groups. (B) The top 15 hub genes with the highest node degree in the amino acid biosynthesis pathway were selected, and a protein-protein interaction network was constructed using mRNA in the effective relationship. Nodes represent proteins, and lines represent protein interactions. (C) Key driver gene analysis reveals the potential involvement of key genes, such as Pkm, in regulating eosinophil transcriptional levels.

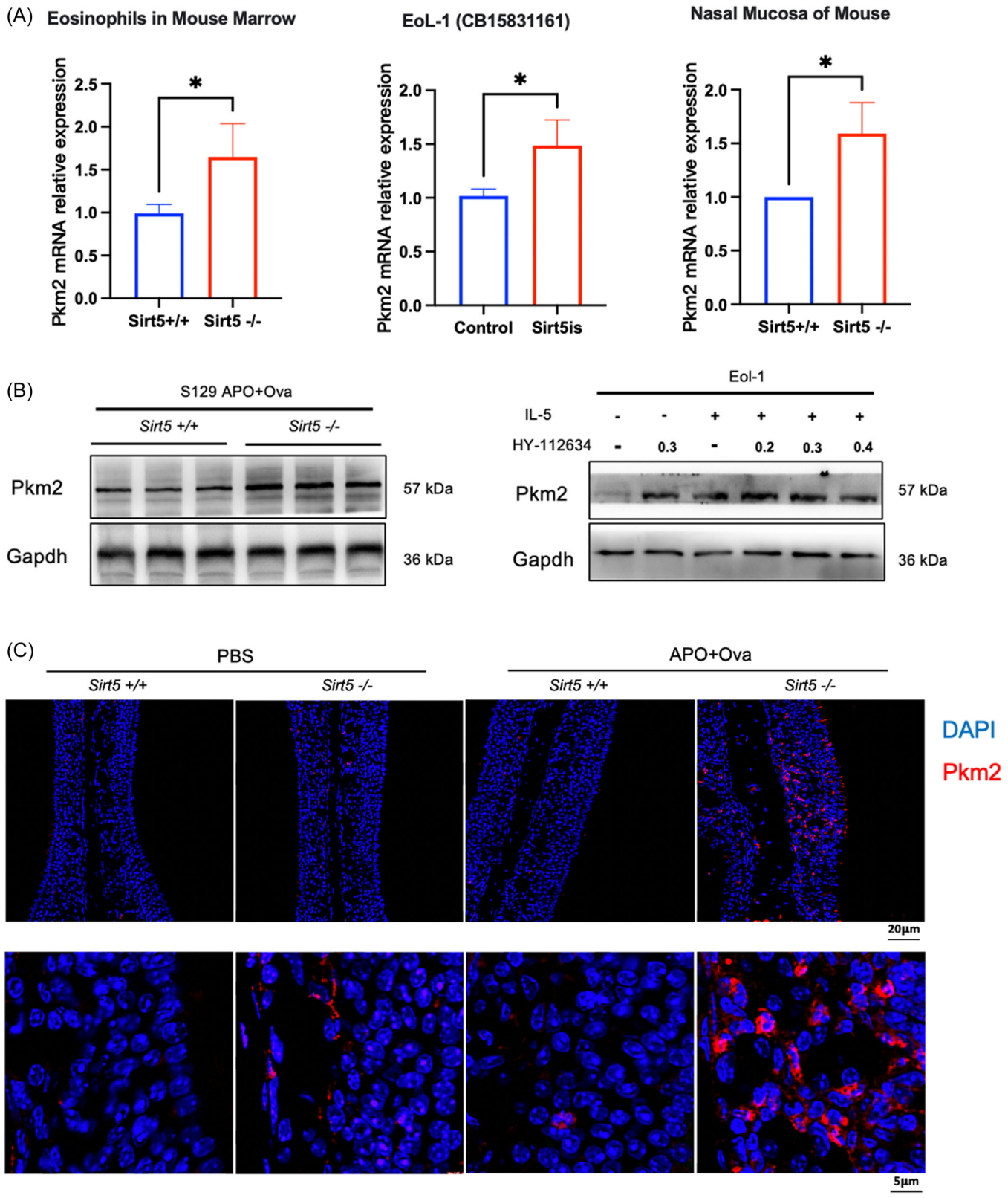

FIGURE 5 Sirt5 negatively regulates Pkm2 expression at the transcription and translation levels. (A) RT-qPCR analysis confirmed the negative correlation between Pkm2 expression and Sirt5 in mouse bone marrow primary cells, eosinophilic cell lines, and mouse nasal mucosa (ns, $p > 0.05$). (B) Western blot experiments showed increased Pkm2 expression in Sirt5−/− mice at the translation level. The eosinophilic cell line exhibited the highest Pkm2 expression when treated with the optimal concentration of Sirt5 inhibitor (representative images from three independent experiments). (C) Immunofluorescence staining of nasal mucosa from four groups of mouse models revealed high Pkm2 expression (red) in Sirt5−/− mice in the APO + Ova nasal drop model group.

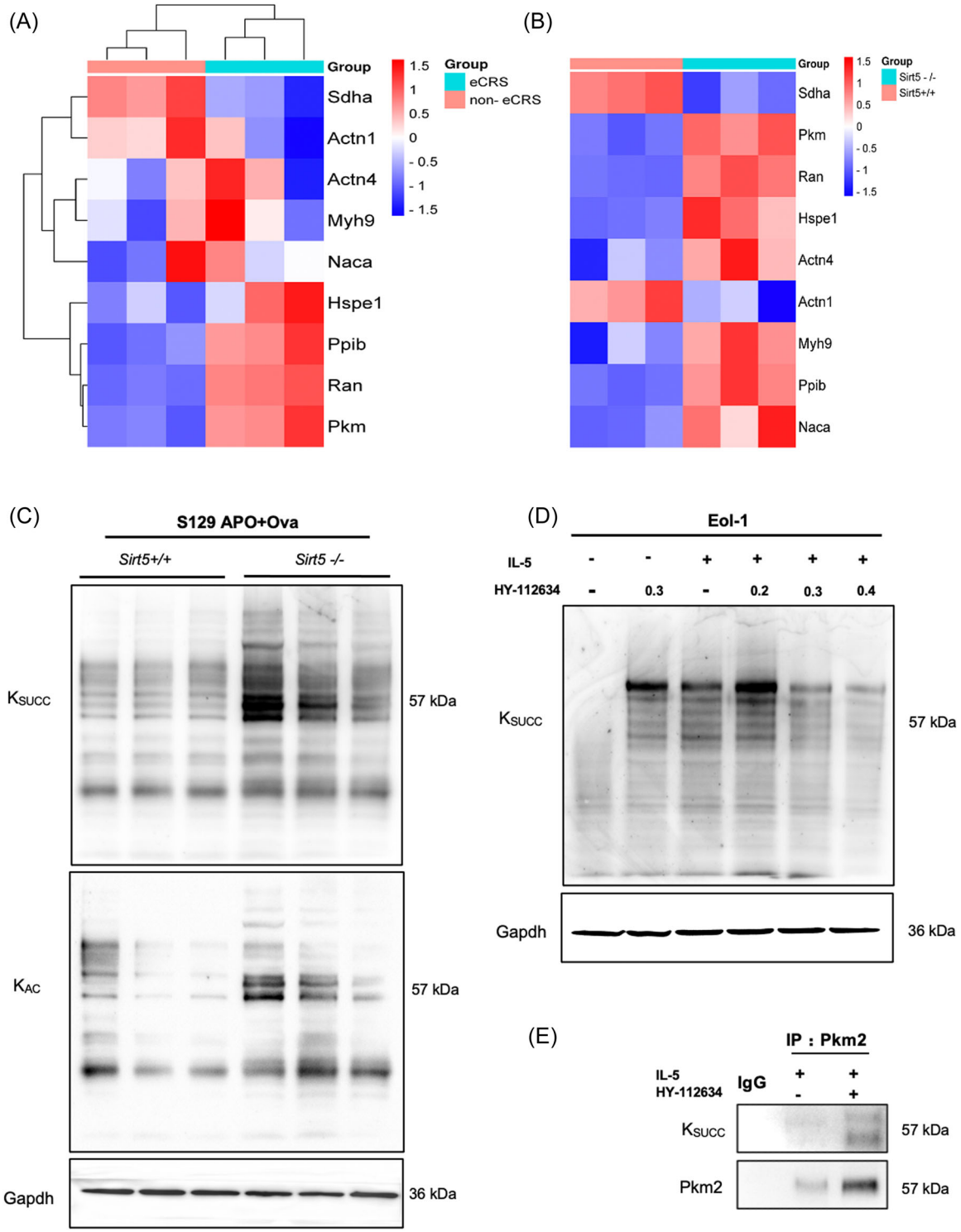

FIGURE 6 Sirt5 regulates metabolic remodeling of eosinophils by modulating the succinylation level of Pkm2. (A) Analysis of transcriptome sequencing data from clinical nasal mucosa samples revealed differential expression of succinylation-related genes, primarily Pkm. (B) Transcriptome sequencing data from mouse bone marrow eosinophils also showed differential expression of succinylation-related genes, primarily Pkm. (C) Western blot experiment confirmed that Sirt5−/− mouse nasal mucosal tissues exhibited higher levels of global succinylation and acetylation compared to the control group, with a more significant increase in global succinylation level. (D) Treatment of eosinophil cell line with the optimal concentration of Sirt5 inhibitor resulted in a significant enhancement in global succinylation level. (E) Immunoprecipitation experiment revealed increased co-immunoprecipitation of Pkm2 and global succinylation antibody in eosinophils treated with Sirt5 inhibitor (representative images from three independent experiments).

Specifically, manipulating the activity of Sirt5 or intervening in the succinylation modification level of pkm2 may hold immense potential in the treatment of diseases associated with eosinophils.

Nevertheless, despite the remarkable advancements made in this study, there remain several unresolved questions that warrant further exploration. For instance, it is imperative to investigate the potential involvement of other regulatory factors in the interaction mechanism between Sirt5 and pkm2. Furthermore, it is crucial to ascertain whether the influence of Sirt5 on eosinophil metabolism is also mediated by other proteins. Answering these inquiries will contribute to a more comprehensive understanding of the precise role of Sirt5 in the regulation of eosinophil metabolism.

In summary, this study focuses on the role of epigenetic regulation and its potential mechanisms in the context of eCRS. The aim is to provide theoretical references for identifying potential therapeutic drug targets for eCRS. Specifically, our findings suggest that Sirt5 plays a crucial role in the metabolic remodeling of eosinophils by negatively regulating the succinylation modification level of pkm2. This discovery provides valuable insights into the metabolic regulation mechanisms of eosinophils. Further research in this area is expected to unveil more exciting discoveries regarding the relationship between Sirt5 and eosinophil metabolism.

CONCLUSION

The study demonstrated a positive correlation between the severity of chronic eosinophilic sinusitis in patients and the expression level of Sirt5 in the nasal mucosa. Sirt5 was observed to directly interact with the pkm2 protein, regulating its succinylation modification level and impacting the metabolic pathways of eosinophils. These findings hold great significance in understanding the metabolic regulation mechanism of eosinophils and provide valuable insights for the development of new treatment strategies.

AUTHOR CONTRIBUTIONS

Shun-Yu Wu is responsible for experimental design, experimental operation, statistical analysis, and manuscript writing. Tian-Yu Wang is responsible for data analysis, result interpretation, and article writing. Bo-Yu Cai, Zhi-Wen Cao, Cai-Quan Liang, and En-Hong Xu are responsible for data organization, formatting, and article writing. Hu Peng and Huan-Hai Liu are responsible for article proofreading. Professor Liao has provided valuable feedback on the revision of this article and is acknowledged as a co-corresponding author.

ACKNOWLEDGMENTS

Thanks to the Naval Medical University for its training and funding. Also funded by the National Natural Science Foundation of China: 81541038; 81670905; 81870702.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data are available from the corresponding author on reasonable request.

ETHICS STATEMENT

All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health in the United States.

ORCID

Shun-Yu Wu http://orcid.org/0000-0003-2793-7015
En-Hong Xu https://orcid.org/0000-0002-9479-4053

REFERENCES

1. Fokkens WJ. EPOS2020: a major step forward. *Rhinol J*. 2020;58:1.
2. Steinke JW, Borish L. Chronic rhinosinusitis phenotypes. *Ann Allergy Asthma Immunol*. 2016;117:234-240.
3. Thaitrakool W, Sukswai N, Keelawat S, et al. Histopathology of ethmoid mucosa versus polyp tissue in diagnosing eosinophilic mucin rhinosinusitis. *Rhinol J*. 2019;57:67-72.
4. Bachert C, Zhang N, Holtappels G, et al. Presence of IL-5 protein and IgE antibodies to staphylococcal enterotoxins in nasal polyps is associated with comorbid asthma. *J Allergy Clin Immunol*. 2010;126:962-968.
5. Stevens WW, Peters AT, Hirsch AG, et al. Clinical characteristics of patients with chronic rhinosinusitis with nasal polyps, asthma, and aspirin-exacerbated respiratory disease. *J Allergy Clin Immunol Pract*. 2017;5:1061-1070.
6. Lee SE, Amin N, Mannent LP, et al. The relationship of sinus opacification, olfaction and dupilumab efficacy in patients with CRSwNP. *Rhinology*. 2023;61:531-540.
7. Gevaert P, Holtappels G, Johansson SGO, Cuvelier C, van Cauwenberge P, Bachert C. Organization of secondary lymphoid tissue and local IgE formation to *Staphylococcus aureus* enterotoxins in nasal polyp tissue. *Allergy*. 2005;60:71-79.
8. Alves-Fernandes DK, Jasiulionis MG. The role of SIRT1 on DNA damage response and epigenetic alterations in cancer. *Int J Mol Sci*. 2019;20:3153.
9. Loscalzo J, Handy DE. Epigenetic modifications: basic mechanisms and role in cardiovascular disease (2013 Grover Conference series). *Pulm Circ*. 2014;4:169-174.
10. Fulkerson PC, Schollaert KL, Bouffi C, Rothenberg ME. IL-5 triggers a cooperative cytokine network that promotes eosinophil precursor maturation. *J Immunol*. 2014;193:4043-4052.
11. Bochner BS, Stevens WW. Biology and function of eosinophils in chronic rhinosinusitis with or without nasal polyps. *Allergy Asth Immunol Res*. 2021;13:8-22.
12. Peserico A, Simone C. Physical and functional HAT/HDAC interplay regulates protein acetylation balance. *J Biomed Biotechnol*. 2011;2011:1-10.
13. Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. *Nat Rev Genet*. 2009;10:32-42.
14. Wu S, Liu H. Sirtuins-Novel regulators of epigenetic alterations in airway inflammation. *Front Genet*. 2022;13:862577.
15. Heinonen T, Ciarlo E, Le Roy D, Roger T. Impact of the dual deletion of the mitochondrial sirtuins SIRT3 and SIRT5 on anti-microbial host defenses. *Front Immunol*. 2019;10:2341.
16. Carrico C, Meyer JG, He W, Gibson BW, Verdin E. The mitochondrial acylome emerges: proteomics, regulation by sirtuins, and metabolic and disease implications. *Cell Metab*. 2018;27:497-512.

17. Du J, Zhou Y, Su X, et al. Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. *Science*. 2011;334:806-809.
18. Park J, Chen Y, Tishkoff DX, et al. SIRT5-mediated lysine desuccinylation impacts diverse metabolic pathways. *Mol Cell*. 2013; 50:919-930.
19. Bhatt DP, Mills CA, Anderson KA, et al. Deglutarylation of glutaryl-CoA dehydrogenase by deacylating enzyme SIRT5 promotes lysine oxidation in mice. *J Biol Chem*. 2022;298:101723.
20. Yang X, Wang Z, Li X, et al. SHMT2 desuccinylation by SIRT5 drives cancer cell proliferation. *Cancer Res*. 2018;78:372-386.
21. Hu T, Shukla SK, Vernucci E, et al. Metabolic rewiring by loss of Sirt5 promotes Kras-Induced pancreatic cancer progression. *Gastroenterology*. 2021;161:1584-1600.
22. Wechsler ME, Munitz A, Ackerman SJ, et al. Eosinophils in health and disease: a state-of-the-art review. *Mayo Clin Proc*. 2021;96:2694-2707.
23. Ho J, Earls P, Harvey RJ. Systemic biomarkers of eosinophilic chronic rhinosinusitis. *Curr Opin Allergy Clin Immunol*. 2020;20:23-29.
24. Zuo K, Guo J, Chen F, et al. Clinical characteristics and surrogate markers of eosinophilic chronic rhinosinusitis in Southern China. *Eur Arch Otrhinolaryngol*. 2014;271:2461-2468.
25. Bernstein JM, Lehman H, Lis M, et al. Humanized mouse model used to monitor MUC gene expression in nasal polyps and to preclinically evaluate the efficacy of montelukast in reducing mucus production. *Ann Otol Rhinol Laryngol*. 2012;121:307-316.
26. Becker SS, Duncavage JA. The role of steroid injection in the management of sinonasal polyps. *Curr Opin Otolaryngol Head Neck Surg*. 2008;16:38-43.
27. Homsi MT, Gaffey MM. *Sinus Endoscopic Surgery*. StatPearls Publishing LLC.; 2023.
28. Miglani A, Soler ZM, Smith TL, Mace JC, Schlosser RJ. A comparative analysis of endoscopic sinus surgery versus biologics for treatment of chronic rhinosinusitis with nasal polyposis. *Int Forum Allergy Rhinol*. 2023;13:116-128.
29. Rosenberg HF, Dyer KD, Foster PS. Eosinophils: changing perspectives in health and disease. *Nat Rev Immunol*. 2013;13:9-22.
30. Wen M, Li Y, Qin X, Qin B, Wang Q. Insight into cancer immunity: MHCs, immune cells and commensal microbiota. *Cells*. 2023;12: 1882.
31. Chu VT, Beller A, Rausch S, et al. Eosinophils promote generation and maintenance of immunoglobulin-a-expressing plasma cells and contribute to gut immune homeostasis. *Immunity*. 2014;40:582-593.
32. Chu DK, Jimenez-Saiz R, Verschoor CP, et al. Indigenous enteric eosinophils control DCs to initiate a primary Th2 immune response in vivo. *J Exp Med*. 2014;211:1657-1672.

How to cite this article: Wu S-Y, Cai B-Y, Cao Z-W, et al. Sirt5 affects the metabolic remodeling of eosinophils by negatively regulating the level of succinylation modification of Pkm2 in eosinophilic chronic rhinosinusitis. *World J Otorhinolaryngol Head Neck Surg*. 2025;11:86-101. doi:10.1002/wjo2.186