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ORIGINAL RESEARCH

Identification of SIN3A as a Promising Epigenetic Target Against Allergic Rhinitis

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Purpose: The involvement of histone modifications in the development of other airway allergic diseases has been widely studied and followed, however, the role in allergic rhinitis(AR) has not been explored and discussed.

Methods: We downloaded GSE50223 from Gene Expression Omnibus database, obtained histone modification related genes in FACER database, and identified key functional genes related to histone modification in AR by WGCNA and single cell sequencing analysis. Finally, we collected nasal mucosa tissues from AR patients and healthy individuals to verify the expression of the target gene. At the functional level, a co-culture system of house dust mite (HDM)-sensitized human nasal epithelial cells and naive CD4+ T cells was employed. Using gene knockdown and overexpression techniques, the role of SIN3A in the differentiation of Th17 and Treg cells was analyzed.

Results: SIN3A was identified as a key functional gene related to histone modification with differential expression in AR, and single-cell data analysis showed that SIN3A might be involved in the immune infiltration difference by regulating Treg cells and thus. Upregulation of SIN3A was confirmed in nasal mucosal tissues of AR patients. Functional experiments demonstrated that SIN3A promotes the differentiation of naive T cells into Th17 cells and inhibits their differentiation into Treg cells, thereby disrupting the Th17/Treg balance.

Conclusion: Histone modification-related gene SIN3A is differentially expressed in AR and healthy populations. SIN3A may play a significant role in the pathogenesis of AR by regulating the Th17/Treg immune balance. This study first revealed a novel epigenetic mechanism by which SIN3A mediates immune imbalance in allergic rhinitis (AR), providing a theoretical basis for the development of intervention strategies targeting SIN3A.

Keywords: allergic rhinitis, histone modification, bioinformatics

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Introduction

Allergic rhinitis (AR) is an allergic condition of the nasal mucosa mediated by particular immunoglobulin E (IgE) and involves several immunoreactive cells and cytokines, among other elements.[1] Upon re-exposure of atopic persons to allergens, allergic rhinitis is clinically characterized by nasal congestion, rhinorrhea, sneezing, and pruritus of the nasal passages.[2] It frequently coexists with asthma or conjunctivitis. AR constitutes a worldwide health issue, affecting patients from many nations, ethnicities, socioeconomic statuses, and age demographics.[3,4] Statistical data reveals that between 20% to 30% of adults and as many as 40% of youngsters worldwide are impacted by this illness.[4] The prevalence is escalating, with a growing number of patients diagnosed each year.[5,6] This substantially affects patients' social interactions, sleep quality, academic achievement, and work efficiency. Approximately 90% of these individuals receive insufficient treatment, leading to a considerable economic burden.

Although the disease burden of allergic rhinitis (AR) is severe, its pathological mechanism remains incompletely understood. In recent years, the role of epigenetic regulation in allergic diseases has received extensive attention. Histone

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modifications (such as methylation, acetylation, etc.) as key epigenetic mechanisms can participate in the fine regulation of immune responses by altering chromatin structure and gene transcriptional activity. Existing studies have shown that abnormal histone modifications are closely related to allergic diseases such as asthma and atopic dermatitis.[7–9] Some studies have also found that specific probiotics can alleviate AR symptoms by affecting histone *H1.2* and *HMGB1*.[10] However, most epigenetic studies still focus on other allergic diseases, and there is a significant knowledge gap regarding the specific histone modification regulatory network in AR and its specific role in immune imbalance. In particular, which histone modification-related genes play a key role in AR and how they affect the differentiation and function of key immune cell subsets such as Th17/Treg have not been systematically explored.

To fill this gap, this study, based on bioinformatics methods, integrated the transcriptome data from the GEO database with the gene set related to histone modification, and screened the hub genes in AR through differential expression analysis and WGCNA. Further, the correlation with the immune microenvironment was revealed through immune infiltration analysis, and the expression was verified using clinical nasal mucosa samples. Finally, the regulatory effect of key genes on the differentiation direction of Th17/Treg was clarified through in vitro cell experiments, aiming to deepen the understanding of the immune mechanism of AR from the epigenetic level and provide a theoretical basis for finding potential therapeutic targets.

Materials and Methods

Materials

This study selected the experimental group of AR patients who underwent postnasal neurectomy at the Department of Otorhinolaryngology Head and Neck Surgery of the Second Xiangya Hospital of Central South University. The control group consisted of patients with uncomplicated deviated nasal septum, devoid of allergic conditions such as allergic rhinitis and asthma, and was selected between February 2023 and September 2023. The Human Research Ethics Committee of the Second Xiangya Hospital of Central South University accepted the study (Ethics approval number: LYF2022222). All patients executed an informed consent document. The research complied with the principles of the Declaration of Helsinki.

RNA Isolation and Real-Time Quantitative PCR (RT-qPCR)

Total RNA was extracted from nasal mucosal tissue samples with TRIzol reagent (Takara, Japan). cDNA was generated using reverse transcription employing a PrimeScript™ RT kit (Takara, Japan) alongside random 6-polymers and oligo dT primers, by the manufacturer's protocol (Takara, Japan). The relative expression of the target genes was standardized to *GAPDH* mRNA. All responses were conducted in triplicate, and the data are presented as standard deviation ± mean. The following are the primer sequences:

*SIN3A*_forward: GCCTGTGGAGTTTAATCATGCC;
*SIN3A*_reverse: CCTCTTGCTCAGTCAAAGCTG;
*GAPDH*_forward: AGGTCGGTGTGAACGGATTTG;
*GAPDH*_reverse: GGCAGAGATGATGACCCTTTT。

Western Blot Analysis

Samples were lysed in RIPA buffer, and protein concentrations were measured with the BCA Protein Assay Kit (Beyotime, China). Proteins were separated via SDS-PAGE, electrotransferred to a PVDF membrane, treated with a primary antibody at 4°C overnight, washed three times, incubated with a secondary antibody for one hour at room temperature, and detected using chemiluminescent reagents. The Anti-*SIN3A* antibody was acquired from Proteintech (USA).

Immunofluorescence Staining

Following antigen repair, nasal mucosa tissue sections were incubated with 3% H2O2 for 15 minutes, and subsequently treated with 10% goat serum for 1 hour. Subsequently, sections were treated overnight at 4°C with a primary antibody, followed by a secondary antibody. Cell nuclei were labeled with DAPI (Beyotime, China). Angiogenesis was examined utilizing a confocal microscope (Olympus, Japan).

Cell Culture and Reagents

The human nasal mucosal epithelial cells (HNEpCs) line was procured from Ningbo Mingzhou Biotechnology Co., Ltd. The cell passage number was maintained at no more than 15. The cells were cultured in EpiCM (Procell, China), a complete epithelial cell culture medium. All cell cultures were carried out in a humidified incubator maintained at 37°C and 5% carbon dioxide (CO_2) concentration. The nasal mucosal epithelial cells were stimulated with 100 μg/mL of house dust mite extract (HDM, GREER, USA). Twenty-four hours post-stimulation, the expression levels of epithelial cell-derived cytokines, namely IL-25, TSLP, and IL-33, were detected.

Co-Culture of Transfected Cell Lines with Naive T Cells

HENpCs were seeded in 6-well plates and grown to approximately 70–80% confluence before transfection. *SIN3A* siRNA (AM16708; clone ID: 108731) was purchased from Invitrogen. The *SIN3A* overexpression plasmid (OHS6085-213584153; clone ID: ccsbBroad304-11788) was constructed for this study. Transfection reagents were diluted in serum-free medium using the RNAimax system (including NC, si-*SIN3A* and oe-*SIN3A*). The transfection efficiency of *SIN3A* was measured by RT-qPCR 48 hours after transfection to confirm successful transfection. Four groups were co-cultured with (1.5×10^6 cells/well) primary T cells (SAIOS, China) for 48 hours: untransfected HENpC without HDM intervention; empty vector-transfected HENpCs with HDM intervention; si-*SIN3A*-transfected HENpCs with HDM intervention; oe-*SIN3A*-transfected HENpCs with HDM stimulation.

Flow Cytometry for the Detection of Th17 and Treg Differentiation

After resuspending the cells, Cell Stimulation Cocktail was added and the cells were stimulated at 37°C for 4 hours, followed by centrifugation and washing. CD3 and CD4 antibodies were used for surface staining of the cells, which were incubated in the dark for 30 minutes and then washed with PBS. Subsequently, the cells were fixed with Intracellular Fixation Buffer for 30 minutes, permeabilized with 1×Permeabilization Buffer, and stained intracellularly with IL-17A antibody for 30 minutes in the dark. After washing, the cells were resuspended in PBS containing 0.5% BSA and analyzed on the flow cytometer to determine the proportion of CD3+CD4+IL-17A+ Th17 cells. The detection process for Treg cells was similar. CD4 and CD25 antibodies were used to label surface antigens of the cells, and Foxp3 antibody was used for intracellular staining. The cells were then analyzed on the flow cytometer to determine the proportion of CD4+CD25+Foxp3+ Treg cells.

Data Sources

Gene expression datasets were queried utilizing allergic rhinitis as a keyword. The subsequent criteria were utilized during the screening: 1. It encompassed individuals with allergic rhinitis necessitating a clinically validated diagnosis; 2. The dataset had an extensive number of samples, incorporating both patients and healthy controls; and 3. The samples analyzed were human tissues. The dataset GSE50223 was acquired from the NCBI GEO public database with the annotation platform GPL6884. The dataset comprised 42 transcriptome samples, consisting of 21 control samples and 21 AR samples. From the FACER database, 510 histone modification-related genes were identified, of which 439 were expressed in the GSE50223 dataset.

Differential Expression Analysis

Differential expression analysis was conducted utilizing the most recent version of the “Limma” package in the R 4.0.3 program. Fold change (FC) was determined using the average gene expression from the AR and control groups. Genes with differential expression were identified using threshold values ($|\log FC|>1$ and $P < 0.05$).

Functional Enrichment Analysis of Differential Genes

To thoroughly investigate the functional significance of these differentially expressed genes (DEGs), we analyzed the DEGs in the regular and AR groups utilizing the “limma” package from Bioconductor in R. We examined the differentially expressed genes using the “clusterProfiler” package in R and conducted gene ontology (GO) and Kyoto Encyclopedia of Genes and

Genomes (KEGG) analyses. The "clusterProfiler" package in R was utilized to identify differentially expressed genes in the regular and AR groups, and to assess the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of these genes, applying a significance criterion of $P < 0.05$. Only upon meeting these criteria were the differentially expressed genes subjected to analysis. Significance criterion; only paths meeting the aforementioned conditions were deemed considerably enriched.

Immune Infiltration Analysis

To assess the impact of differentially expressed AR genes on immune infiltration, ssGSEA was employed to measure the degree of immune cell infiltration in each sample. A Pearson correlation analysis was conducted for gene expression and immune cell composition. A p-value of less than 0.05 was deemed statistically significant.

WGCNA Network Construction

To identify critical genes connected with pathophysiological pathways in allergic rhinitis patients, we employed the expression profiling data from GSE50223 to form a WGCNA network, thereby investigating the co-expression regulatory networks linked to allergic rhinitis. The modules exhibiting substantial association were identified as essential based on their relationship with sample categories.

ROC Analysis of Hub Genes in Histone Modification, Analysis of Associated Drugs, and Immune Cell Infiltration Assessment

The Receiver Operating Characteristic (ROC) function is graphically shown by plotting the true positive rate on the horizontal axis and the false positive rate on the vertical axis, used to evaluate the model's accuracy. The Area Under the Curve (AUC) represents the area beneath the ROC function, with values approaching 1 indicating superior performance. The efficacy of the effect was evaluated using the ROC function to compute the AUC value of the Hub genes for the sample categories, determining their suitability as indicators for these categories. Subsequently, the Drug-Gene Interaction databases (DGIdb) were employed to identify and present the medications interacting with the Hub gene. The relationship between histone-modified Hub genes and different immune cells was further analyzed.

Single-Cell Sequencing Analysis

The Seurat package was utilized for quality control and subsequent data analysis. The positional links among the clusters were determined by UMAP algorithm analysis, and the clusters were annotated utilizing MonacoImmuneData files. Ultimately, we refined each subgroup for marker genes utilizing the FindAllMarkers algorithm, with $|\text{avg\_log2FC}| > 1$ and a corrected $P\text{-value} < 0.05$ deemed as distinctive marker genes for each cell subtype.

Statistical Analysis

A two-tailed p-value of less than 0.05 was deemed significant. All analyses were conducted using R software (version 4.1.1) and Prism (version 8.1) for Windows 64.0.

Results

Identification and Functional Study of Histone-Associated Differentially Expressed Genes (DEGs)

Acquire the gene chip dataset of GSE50223 from the NCBI GEO public database, then employ the limma package to identify differentially expressed genes between regular and AR groups, with the screening criteria of $|\log FC|>1$ and $P<0.05$, resulting in the identification of 316 differentially expressed genes (Figure 1a and b). Subsequently, we intersected with 439 histone modification-related genes, resulting in the identification of 7 differentially expressed genes associated with histone modifications: *SIN3A*, *SIRT2*, *UBE2E1*, *PRDM10*, *BANP*, *YWHAZ*, and *SET*. To enhance our comprehension of the potential roles of histone modification-associated differentially expressed genes, we employed the clusterProfiler package in R to conduct Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes

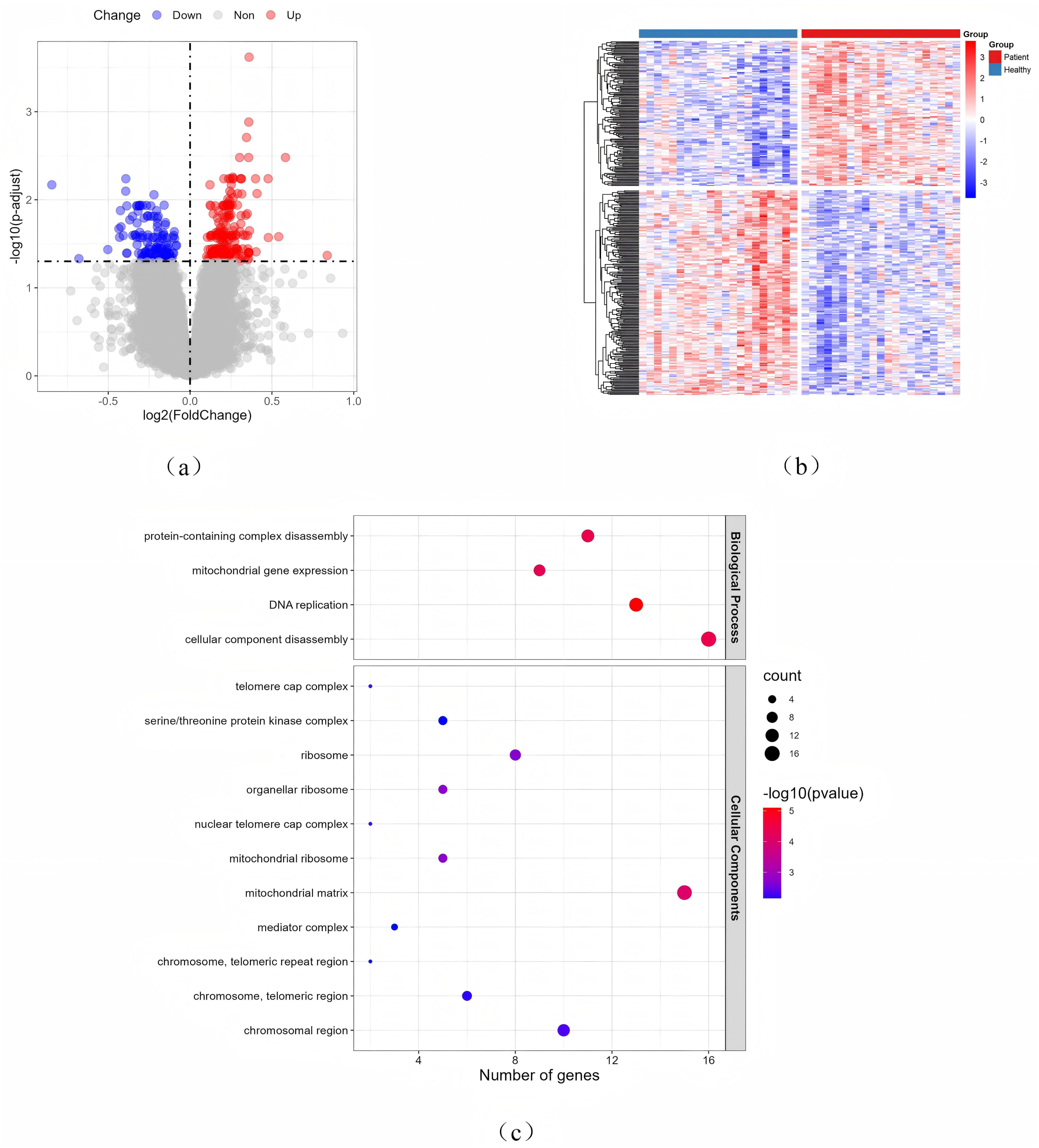

Figure 1 Identification and functional analysis of histone-related DEGs. (**a**) Volcano map of differentially expressed genes (DEGs) in GSE50223; (**b**) Heat map of DEGs expression; (**c**) GO enrichment pathway analysis of histone modification-associated DEGs.

(KEGG) functional enrichment analyses, with a significance threshold of P<0.05. The results indicated that these differential genes were significantly enriched in biological processes such as DNA replication (GO:0006260), cellular component disassembly (GO:0022411), protein-containing complex disassembly (GO:0032984), and mitochondrial gene expression (GO:0140053), among others (Figure 1c). No substantial enrichment pathway for differential genes was identified in KEGG.

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Modified Immunological Microenvironment in Individuals with Allergic Rhinitis

The etiology of allergic rhinitis (AR) encompasses inflammation and immunological responses, with numerous immune cells and proteins participating in its pathophysiological pathways. To investigate the mechanisms of its immune response, we employed ssGSEA to evaluate the disparities in the abundance of various immune cells between AR patients and healthy individuals, subsequently comparing the discrepancies in component samples. Marked disparities were observed between AR patients and the healthy control group in cell types such as Effector memory CD8 T cells, Monocytes, and Natural killer cells (Figure 2). This aligns with earlier data indicating that NK cells may play a role in the pathophysiology of allergic disorders by elevating type 2 cytokines and facilitating eosinophil migration, and that NK cell levels are heightened in individuals with allergic rhinitis.[11,12]

Identification of Pivotal Genes Associated with Histone Modification by WGCNA

To identify essential genes associated with the pathophysiological mechanisms of allergic rhinitis (AR) patients, we employed the expression profile data from GSE50223 to develop a weighted gene co-expression network analysis (WGCNA) for investigating the co-expression regulatory network pertinent to AR. Based on the association between modules and sample types, we identified the modules with considerable strong correlation as essential modules. Upon thorough investigation, we discovered a substantial negative correlation between the log-log(k) of nodes with connectivity k and the log-log(P(k)) of the corresponding node's occurrence probability. The intensity of this negative correlation is quantified by a correlation coefficient exceeding 0.8, as illustrated in Figure 3a. According to the assessment to ascertain the power=12, the log10(P(k)) and log10(k) were By plotting and squaring the correlation coefficient, which is 0.89—indicative of a value approaching 1—it is evident that the two variables exhibit a strong linear relationship, suggesting the construction of a scale-free network in this instance (Figure 3b).

The tom matrix ultimately classified these genes into six modules (Figure 3c), with each row of Figure 3d depicting the eigenvector gene of each module, while the columns display the classification information of the samples. The figure in each cell represents the correlation coefficient of the gene module with the categorization of the respective sample, whilst the figure in parentheses denotes the significance level p-value. The findings revealed that the brown module exhibited the most substantial significant positive connection with AR, leading us to identify the brown module as the principal module associated with AR, comprising 985 genes. We subsequently identified the histone modification genes

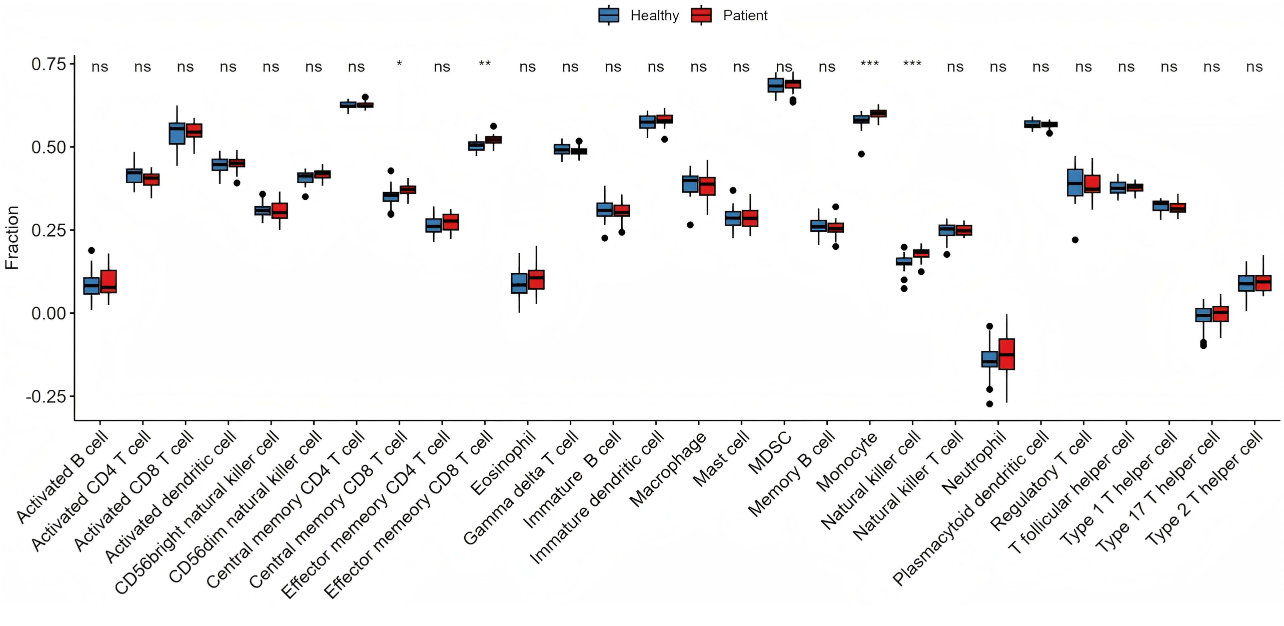

Figure 2 Immune microenvironment analysis. Non-allergic rhinitis group in blue and allergic rhinitis group in red. "*" indicates p<0.05, "**" indicates p<0.01, and "***" indicates p<0.001, "ns" indicates 'not significant'.

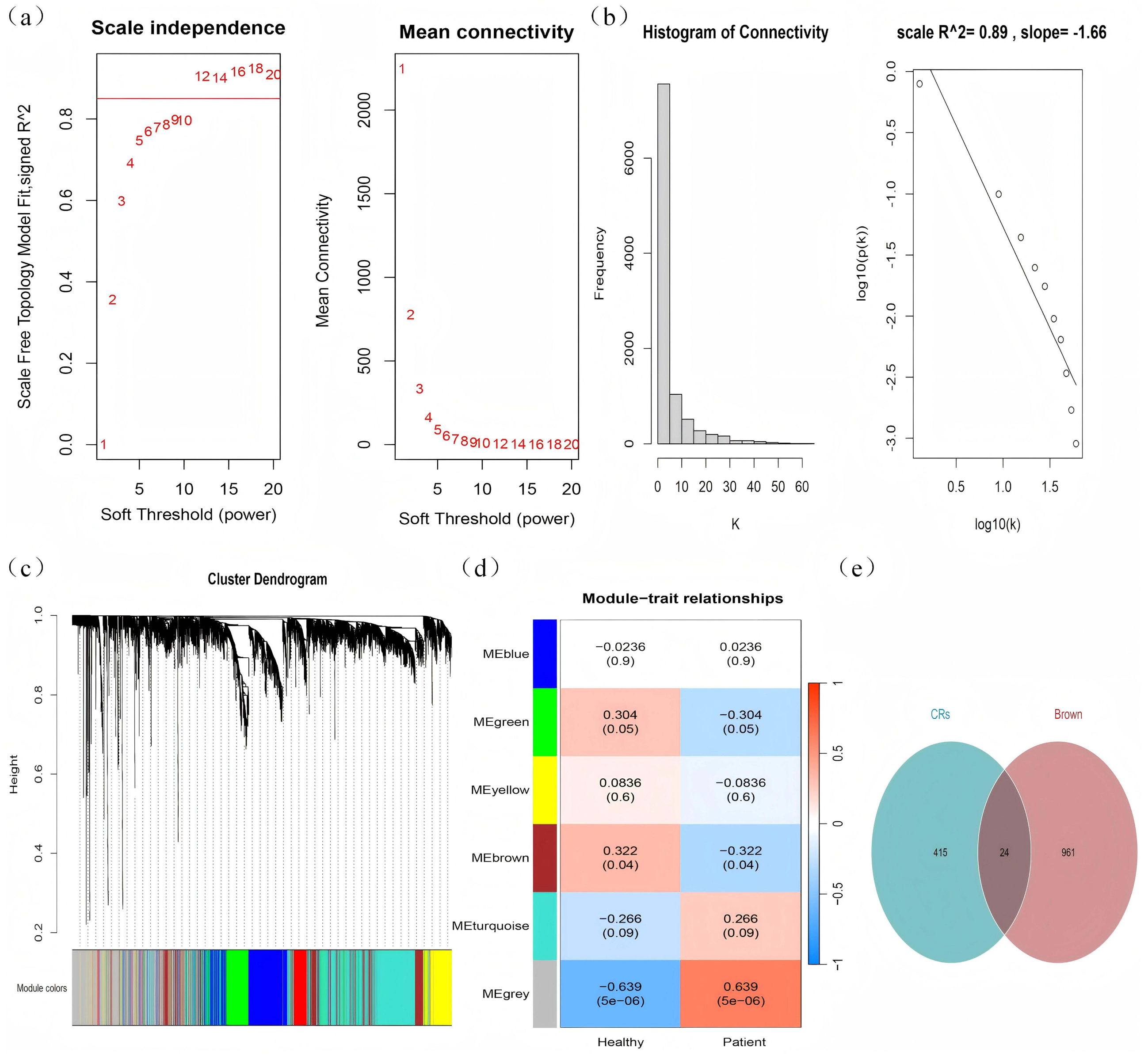

Figure 3 Construction of WGCNA modules. (**a**) Evaluation of optimal soft threshold; (**b**) study of scale-free fit index and average connectivity; (**c**) dendrogram for gene clustering; (**d**) heatmap depicting module-phenotype associations. (**e**) Venn diagram illustrating the intersection of WGCNA brown module genes and histone modification-related genes, with green circles representing the 439 histone modification-related genes expressed in the GSE50223 dataset and brown circles denoting the WGCNA brown module genes.

within the brown module, designating them as histone modification hub genes (Figure 3d), which included *ASF1A*, *BRD2*, *CDK2*, *CHD7*, *SIN3A*, and 24 other genes (Figure 3e). SIN3A was identified as a differentially expressed gene, as previously described.

ROC Analysis of Histone Modification Hub Genes, Associated Medication Analysis, and Immune Cell Infiltration Assessment

Initially, the ROC function was employed to compute the AUC values of the Hub genes relative to the sample categories, revealing that the expression levels of the majority of genes effectively indicated the AR sample categories. Notably, 16 genes exhibited AUC values exceeding 0.6, with *SIN3A*, *SUPT16H*, and *NCOA5* demonstrating AUCs of 0.87, 0.77, and 0.76, respectively (Figure 4a). The findings indicated that *SIN3A* is a crucial element in allergic rhinitis and is

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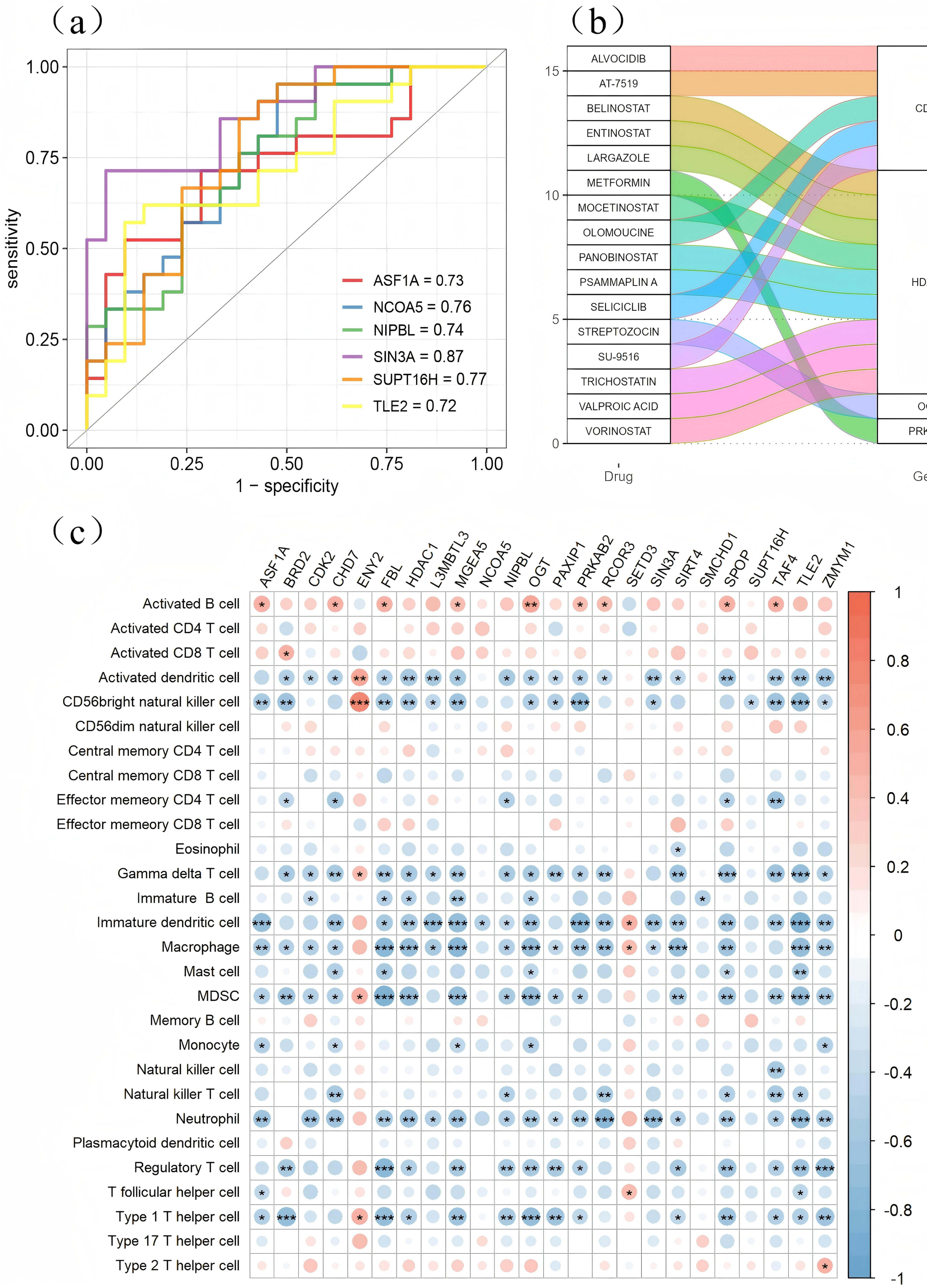

Figure 4 Correlation analysis of hub genes associated with histone modifications. (**a**) The ROC curves for histone modification-associated Hub genes are presented, highlighting the six Hub genes with the highest AUC values in the figure; (**b**) Interaction between histone modification-associated hub genes and pharmaceuticals; (**c**) Correlation between histone modification-associated hub genes and immune cells, with the left side of the figure depicting the types of immune cells and the upper side displaying 24 histone modification-associated hub genes. Red dots signify positive correlations, while blue dots denote negative correlations. "*" indicates p<0.05, "**" indicates p<0.01, and "***" indicates p<0.001, 'ns' indicates 'not significant'.

a differentially expressed gene in Result 2.3.1, potentially serving as a target for elucidating the mechanisms underlying AR pathogenesis.

Subsequently, the DGIdb database was employed to identify drugs that interact with the Hub genes, focusing exclusively on gene-drug interactions documented in a minimum of three publications to ensure credibility. This process yielded four histone-modified genes (*PRKAB2*, *OGA*, *HDAC1*, and *CDK2*) along with their corresponding interacting drugs (Figure 4b), presenting novel candidates for allergic rhinitis treatment. *SIN3A* failed to obtain pertinent drug matches.

We examined the link between histone-modified Hub genes and immunological infiltration, observing a significant and robust correlation between the majority of these histone-modified Hub genes and immune cells (Figure 4c).

SIN3A is the Only Histone Modification Hub Gene with Significant Differential Expression in the AR

We screened seven histone modification-related genes exhibiting significant differential expression in AR and intersected them with the histone modification Hub key genes identified via the WGCNA network. The final results indicated that among these Hub key genes, only *SIN3A* demonstrated significant differential expression between the disease and control groups. Figure 4c illustrates that *SIN3A* exhibits a substantial positive connection with neutrophils, immature dendritic cells, activated dendritic cells, and other immune cells. This suggests that *SIN3A*, a cluster of essential genes for protein modification, may influence the immunological milieu and immune control in AR patients, hence contributing to the pathophysiological mechanisms of AR.

Single-Cell Analysis of *SIN3A* and AR Immune Microenvironment

To further examine the function of *SIN3A* in the AR immune microenvironment and to confirm the expression of *SIN3A* at the single-cell level, the dataset GSE2001074 (peripheral blood samples from patients with allergic rhinitis before and after SLIT treatment) was retrieved from the GEO database, and its scRNA data were utilized for subsequent analysis. Following the filtration of low-quality cells as per the threshold established in Method 2.2.6, the resultant single-cell data were downscaled and clustered utilizing the UMAP method (Figure 5a). Figure 5b illustrates the clustering of various samples prior to and subsequent to de-batching, executed at a resolution of 0.5, resulting in the division into 14 cell clusters (Figure 5c). Four principal cell types were identified through annotation: CD4+ cytotoxic T-cells (CD4+ CTL, marker gene *GZMA*), CD4+ regulatory T cells (CD4+ Treg, marker genes *FOXP3*, *IL2RA*), CD4+ naive T cells (Naive CD4+ T cells, marker gene *SELL*), and CD4+ helper T cells (CD4+ Th17, marker genes *KLRB1*, *CCR6*) (Figure 5). As illustrated in Figure 5d, the expression of *SIN3A* is observed in these four types of immune cells.

The *SIN3A* gene exhibited significant and varied expression in the tissues of patients with allergic rhinitis across distinct cell groups, both prior to and following SLIT treatment. Figure 5e illustrates a significant disparity in *SIN3A* expression between Naive CD4+ T cells and CD4+ Treg cells prior to treatment, as Naive CD4+ T cells can differentiate into CD4+ Treg populations, which play a crucial role in immune regulation in vivo. Conversely, after SLIT treatment, no significant differences were observed among the various cell populations (Figure 5f), indicating that *SIN3A* expression may be a critical factor influencing the condition of patients with allergic rhinitis.

Correlation Study of *SIN3A* with Prevalent Immune Checkpoints and Human Leukocyte Antigens

The findings from the single-cell study indicate that *SIN3A* could be a crucial immunoregulatory gene in allergic rhinitis. To elucidate the function of *SIN3A* in allergic rhinitis immunology, we examined its connection with prevalent immunological checkpoints and human leukocyte antigens. Figure 6 demonstrates a substantial positive correlation between *SIN3A* and *HLA-C*, *HLA-DQB2*, *HLA-F*, and *ADORA2A*.

Validation of *SIN3A* in Nasal Mucosal Tissues

This study recruited 12 patients with allergic rhinitis (AR), aged 18–52 years, and 12 control patients with nasal septum deviations, aged 17–59 years (Table 1), to compare the expression levels of *SIN3A* in the nasal mucosal tissues of AR

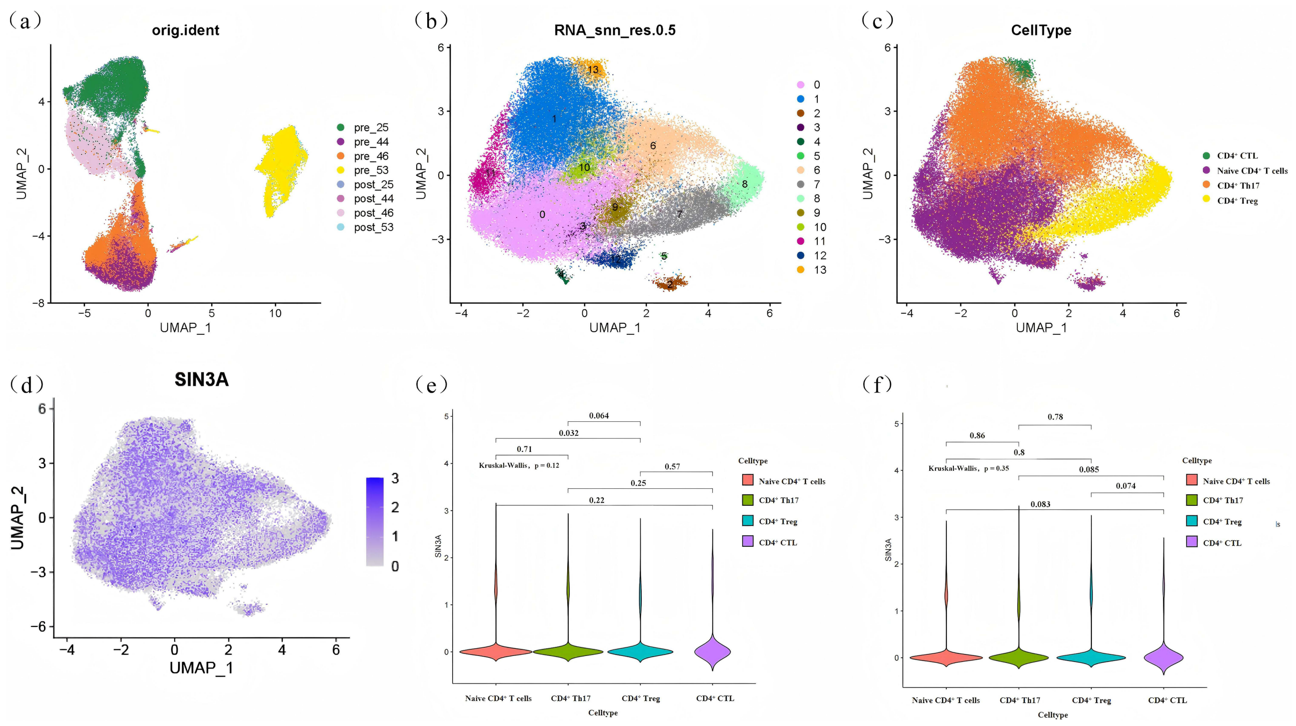

Figure 5 Single-cell data analysis of *SIN3A* in AR. (**a**–**d**) UMAP clustering results: (**a**) "pre" on the right signifies the period prior to SLIT treatment, while "post" denotes the period following SLIT treatment; (**b**) illustrates the clustering of various samples before and after de-batching; (**c**) on the right, the four principal cell types identified through annotation are: CD4+ cytotoxic T cells, CD4+ regulatory T cells, CD4+ naïve T cells, and CD4+ helper T cells17; (**d**) depicts SIN3A expression across each immune cell type. (**e**) Differential expression of the *SIN3A* gene across various immune cell types in allergic rhinitis patients prior to sublingual immunotherapy treatment. (**f**) Differential expression of the *SIN3A* gene in various immune cell types in allergic rhinitis patients following sublingual immunotherapy treatment.

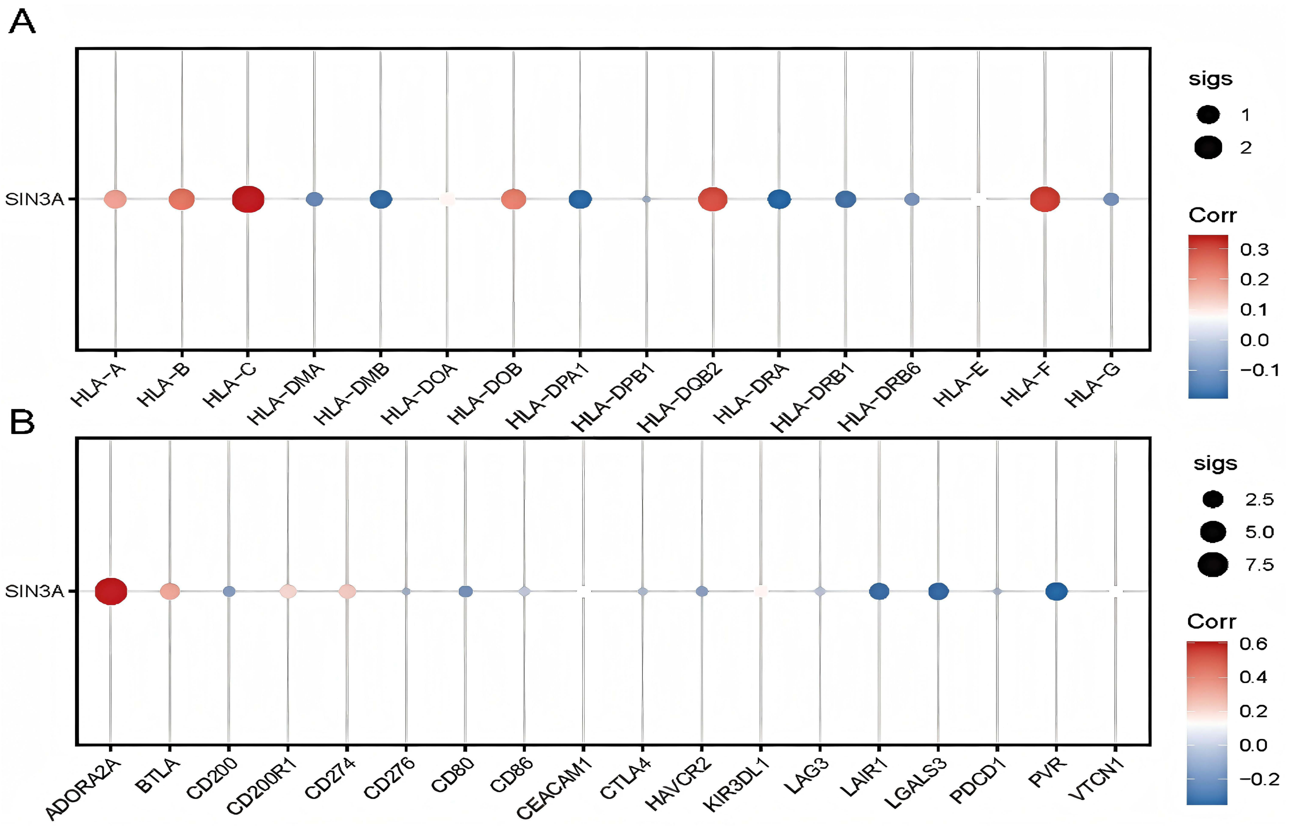

Figure 6 Correlation of *SIN3A* with common immune checkpoints and human leukocyte antigens. (**A**) Correlation of *SIN3A* with common immune checkpoints; (**B**) Correlation of *SIN3A* with common immune checkpoints.

Table 1 Basic Clinical Information and Laboratory Findings of Recruits

Characteristics	AR	Control	P value
Age (years)	34.16±12.85	32.08±9.57	0.621
Sex (Male vs Female)	8:4	9:3	0.699
VAS score (0–10)			
Nasal congestion	6.75±1.36	7.58±1.16	0.218
Runny nose	5.28±2.35	3.91±1.83	0.042
Sneezing	6.02±1.13	2.58±1.08	<0.001
Itchy nose	5.83±1.74	2.67±2.02	<0.001
Blood eosinophil count (×10^9/L)	0.391±0.235	0.195±0.121	0.013
Serum IgE levels (IU/mL)	231.83±157.63	51.35±23.75	<0.001

patients against those of patients with simple deviated nasal septum. The expression level of *SIN3A* in the nasal mucosal tissues of allergic rhinitis patients was significantly elevated compared to the control group, with the difference being statistically significant (Figure 7a–c).

The Effect of *SIN3A* on the Differentiation of Th17/Treg in HDM-Sensitized Nasal Epithelial Cells

To clarify the role of *SIN3A* in regulating T cell differentiation in nasal mucosal epithelial cells, we first established an in vitro allergic rhinitis (AR) model by treating HNEpCs with house dust mite (HDM). Western Blot results showed that compared with the Control group, the protein expression of key inflammatory factors such as IL-25, IL-33, and TSLP in HDM-treated nasal mucosal epithelial cells was significantly increased (Figure 8a and b), indicating the successful establishment of the AR cell model.

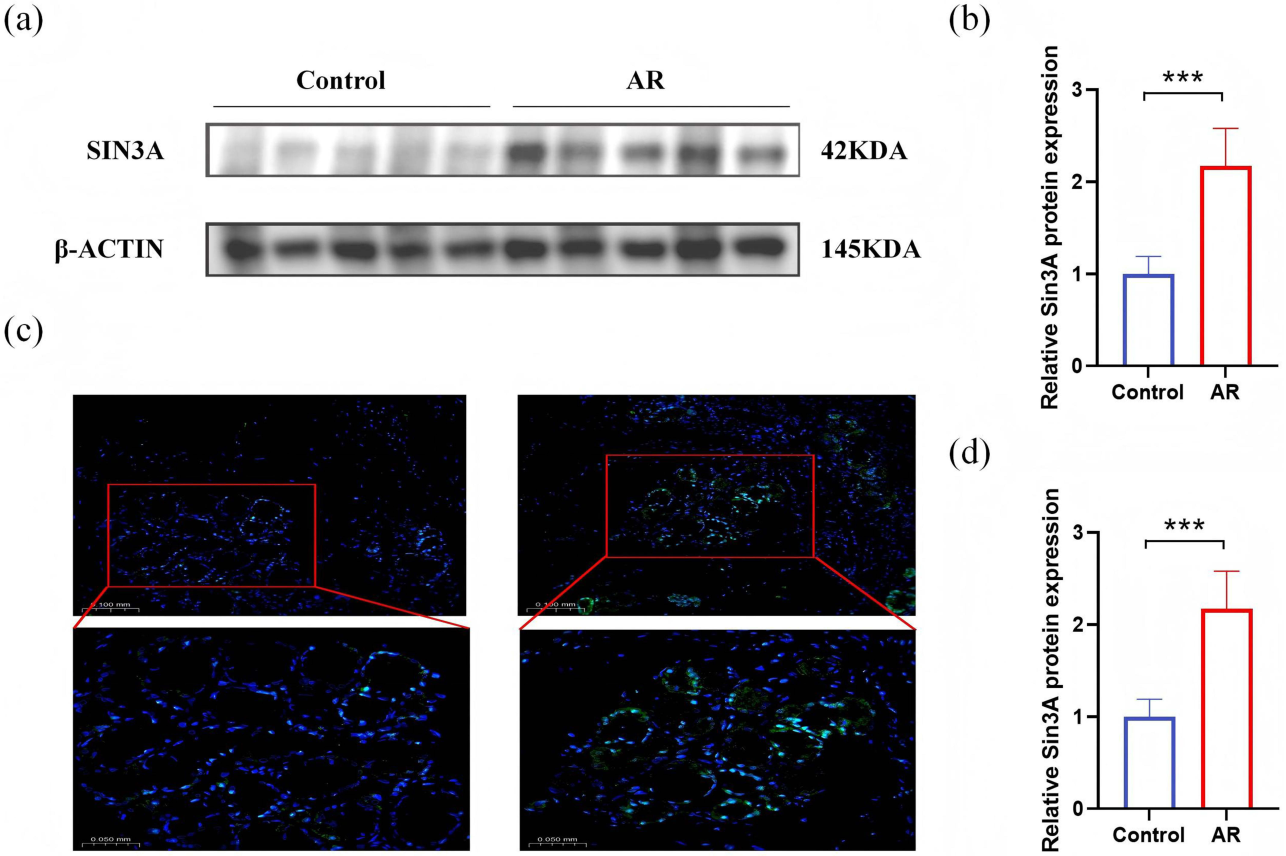

Figure 7 *SIN3A* is differentially expressed in AR patients.(**a** and **b**) Relative protein expression of *SIN3A* in AR and controls. (**c**) *SIN3A* expression in nasal mucosa (immunofluorescence detection 20×; 40×). (**d**) Relative mRNA expression of *SIN3A* in AR and control. “***” indicates p<0.001.

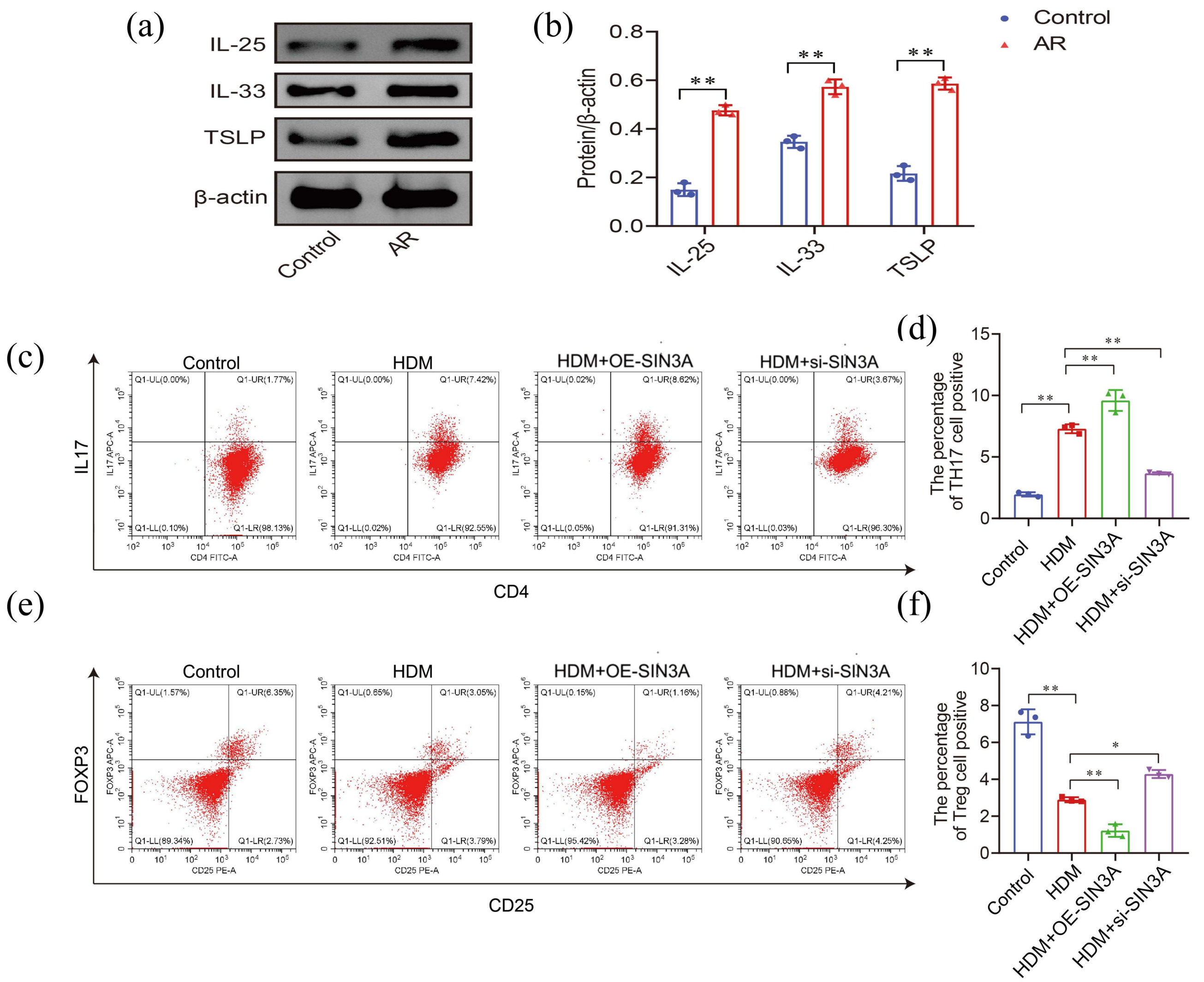

Figure 8 Functional verification of *SIN3A* in in vitro cell experiments. (**a** and **b**) Relative expression levels of IL-25, IL-33 and TSLP proteins in HDM-sensitized nasal mucosal epithelial cells. (**c-f**) Representative flow cytometry plots of IL-17A and Foxp3 expression in naive CD4+T cells co-cultured with nasal mucosal epithelial cells from different treatment groups. Experimental groups were as follows: control group (untreated HNEpCs), HDM group (HDM-sensitized HNEpCs), HDM + si-*SIN3A* group (HDM-sensitized and *SIN3A* knockdown HNEpCs), HDM + OE-*SIN3A* group (HDM-sensitized and *SIN3A* overexpressed HNEpCs). (**d**) Quantitative analysis of Th17 cell proportions in each group; (**f**) Quantitative analysis of Treg cell proportions in each group.

Subsequently, we co-cultured nasal mucosal epithelial cells under different treatment conditions with naive T cells and detected the proportions of Th17 (CD4+IL-17A+) and Treg (CD4+CD25+Foxp3+) cells in CD4+ T cells by flow cytometry. The experimental groups were set up as follows: (i) Control group: naive T cells co-cultured with untreated HNEpCs; (ii) HDM group: naive T cells co-cultured with HDM-sensitized HNEpCs; (iii) HDM + si-*SIN3A* group: naive T cells co-cultured with HDM-sensitized HNEpCs in which *SIN3A* was knocked down; (iv) HDM + OE-*SIN3A* group: naive T cells co-cultured with HDM-sensitized HNEpCs in which *SIN3A* was overexpressed.

Flow cytometry analysis results (Figure 8c–f) indicated that compared with the Control group, the proportion of Th17 cells in the co-culture system of the HDM group was significantly increased, while the proportion of Treg cells was significantly decreased, suggesting that in the allergic inflammatory microenvironment, nasal mucosal epithelial cells can disrupt the Th17/Treg immune balance. To further verify the function of *SIN3A* in this process, we conducted *SIN3A* overexpression and knockdown experiments. The results showed that overexpression of *SIN3A* in HDM-sensitized cells could further enhance its regulatory effect on T cell differentiation: the proportion of Th17 cells was significantly higher than that in the HDM group, while the proportion of Treg cells was further decreased. Conversely, knockdown of *SIN3A* partially reversed the effect induced by HDM: the proportion of Th17 cells was significantly lower than that in the HDM group, while the proportion of Treg cells was restored to or even higher than that in the HDM group.

In conclusion, in the context of allergic rhinitis, *SIN3A* in nasal mucosal epithelial cells may be an important molecule regulating the differentiation fate of T cells. Its high expression can promote the differentiation of naive T cells into pro-inflammatory Th17 cells and simultaneously inhibit the differentiation of anti-inflammatory Treg cells.

Discussion

As a chronic condition impacting 10–40% of the global populace, AR currently lacks a comprehensive solution, detrimentally influencing patients' quality of life and imposing significant economic and societal burdens. Consequently, medical specialists worldwide are persistently examining the pathophysiological processes of AR. Numerous studies have indicated that histone modification is intricately linked to the pathogenesis of allergic disorders,[7–9] although the impact of histone modification dysfunction on the pathogenesis of allergic rhinitis remains unexplored. This work uses bioinformatics to identify the principal genes related to AR and histone modification, subsequently investigating the specific pathways linked to immune infiltration, ultimately concluding that *SIN3A* may serve as a crucial immune regulatory protein in AR.

Histone modification is a primary mechanism for regulating epigenetics, which pertains to inheritable epigenetic information distinct from the DNA sequence. Nucleosomes, the fundamental structural units of chromatin, consist of histones and the DNA that coils around them. The post-translational modification processes, including methylation, phosphorylation, and acetylation of the N- and C-termini of histones associated with DNA, can alter the charge and structure of their tails, subsequently affecting chromatin configuration and gene expression. Consequently, histone modification is pivotal in numerous cellular biological processes.[13–15]

Prior research has demonstrated that histone alterations have a role in the progression of several allergy disorders. Increased histone H2 acetylation in airway smooth muscle cells in asthma results in the deregulation of *CXCL8* transcriptional regulation, contributing to the pathophysiology of asthma.[7] Research indicates that airway smooth muscle cells in asthmatics exhibit an elevated secretion of vascular endothelial growth factor (*VEGF*) compared to the general population, and methylation of histone H3 at the H3K9me3 locus may result in the removal of the *VEGF* promoter inhibitory complex.[8] Literature indicates that aberrant histone modifications in peripheral blood mononuclear cells may play a role in the pathophysiological mechanisms of allergic purpura.[9] Consequently, investigations into histone modifications may represent a novel approach to the management of allergic rhinitis. Nonetheless, the function of histone modification in allergic rhinitis remains unexamined. This work aims to investigate the role of histone modification in allergic rhinitis and to examine the interaction between histone modification and the immune system, with the objective of elucidating its significant involvement in the disease mechanism.

Our data indicate that *SIN3A* is the sole histone modification-related gene exhibiting differential expression between AR and healthy populations. *Sin3A* is a constituent of the *SIN3* family of transcriptional regulatory proteins, specifically member A. *Sin3A*, a pivotal component of the multiprotein chromatin modification complex, exhibits co-inhibitor, co-activator, and transcription factor characteristics, interacting with structural domains of histone deacetylases via class I histone deacetylases, thereby influencing chromatin architecture and modulating the expression of functional genes.[16–18] Research has demonstrated that *SIN3A* may play a role in the fibrosis of asthmatic airways by influencing connective tissue growth factors.[19] Kozlik et al[20] discovered that *SIN3A* exhibits variable expression in eosinophilic asthmatics and plays a significant role in most transcriptional responses to hypoxia. Literature indicates that in atopic dermatitis, *SIN3A* and *STAT3* form a signaling pathway crucial in the pathogenesis of *TSLP*-mediated atopic dermatitis.[21] All aforementioned research indicates that *SIN3A* has a role in the pathophysiological mechanisms of allergy-related diseases via histone modification, suggesting that *SIN3A* may be a critical element in the pathogenesis of allergic rhinitis due to histone modification abnormalities.

SIN3A is a primary regulator of *STAT3* transcriptional activity,[22] and *STAT3* is crucial in developing of Th17 cells.[23] Perucho et al[24] discovered that *SIN3A* facilitates Th17 development through *STAT3* in vitro studies, and the inhibition of *SIN3A* attenuates the pro-inflammatory effects of TH17, which is advantageous for immunomodulatory functions. The prevalence of Th17 cells in allergic rhinitis patients is markedly elevated compared to healthy individuals, and the release of pro-inflammatory cytokines by Th17 cells is crucial in the inflammatory response[25]; furthermore, the dysregulation of

Th17 cells constitutes a pathogenic mechanism of allergic rhinitis. Consequently, *SIN3A* is expected to be instrumental in the pathogenesis of AR, particularly concerning the facilitation of Th17 cell development.

This study employed Western blotting, qRT-PCR, and immunofluorescence to confirm the differential expression of *SIN3A* in AR patients using nasal mucosa tissue specimens from two groups. The findings indicated that *SIN3A* exhibited elevated expression levels in the nasal mucosa of patients with allergic rhinitis. The AUC value of 0.87 for the *SIN3A* gene in the aforementioned article indicates a strong differential diagnostic value, presenting a novel potential target for diagnosing and AR patients.

This study screened out multiple histone modification genes including *HDAC1* and their interacting drugs through bioinformatics analysis (Figure 4b), suggesting that targeting epigenetic regulation might be a new direction for AR treatment. Among them, the broad-spectrum HDAC inhibitor sodium butyrate (NaB) has been confirmed in multiple studies[26] to effectively alleviate allergic symptoms and mucosal inflammation in AR animal models by restoring histone acetylation levels (such as H3-AcK9), reducing *HDAC*1/8 expression, and rebalancing Th1/Th2/Th17 and Treg. However, such pan-HDAC inhibitors lack gene specificity and may cause pleiotropic biological effects and potential side effects. In contrast, *SIN3A*, a specific transcriptional co-repressor identified in this study, may more precisely focus on regulating key immune pathways (such as Th17/Treg differentiation). Although no reported clinical drugs targeting *SIN3A* have been retrieved through public drug databases (such as *DGIdb*) at present, this makes it difficult to directly compare the efficacy of *SIN3A* inhibitors with that of NaB. *SIN3A* inhibitors have been formulated for in vivo application, enhancing the potential for targeting *SIN3A* in forthcoming AR therapy.[27]

To elucidate the function of *SIN3A* in the AR immune milieu, we conducted single-cell annotation analysis of immune cells from AR patients before and following SLIT treatment. The findings indicated that *SIN3A* exhibited considerable differential expression in CD4+ naïve T cells and Treg cells in pre-treatment AR patients. This discovery indicates that *SIN3A* may assume distinct functions in these two cell types, hence influencing the pathophysiology of AR. After SLIT treatment, the disparity in *SIN3A* expression between CD4+ naïve T cells and Treg cells in AR patients diminished. Treg cells differentiate from CD4+ naïve T cells and function to regulate the cessation of immune system overactivity. This indicates that SLIT therapy may have effectively addressed AR by modulating the expression of *SIN3A*, hence facilitating the differentiation of CD4+ naïve T cells into Treg cells.

The imbalance between Treg and Th17 cells constitutes a pathogenic mechanism of allergic rhinitis. However, our study did not reveal any variation in the expression of *SIN3A* in Th17 cells among the allergic rhinitis population pre-and post-treatment. This lack of difference may stem from the limited sample size and the selection criteria, which focused solely on patients undergoing sublingual immunotherapy, without including healthy controls or a broader analysis of the allergic rhinitis population, thereby failing to represent the overall condition of AR patients comprehensively. To investigate the specific mechanism further, common immunological checkpoints and human leukocyte antigens were chosen for correlation analysis, revealing a substantial positive association between *SIN3A* and *ADORA2A*, *HLA-C*, *HLA-F*, and *HLA-DQB2*. Elevated levels of *HLA-C* have been strongly associated with illness duration in AR patients, as indicated in a prior study.[28] Additionally, *HLA-DQB2*, a highly conserved HLA molecule, has been demonstrated in the literature to facilitate antigen presentation and T cell stimulation.[29] Moreover, *HLA-F* serves as a distinct marker on the surface of activated T cells.[30] *ADORA2A* has been confirmed as a crucial element in sustaining CD4+ naïve T cell populations[31] and plays a significant role in the modulation of T cell and NK cell activation by Treg cells.[32]

Although previous studies have implicated *SIN3A* in immune dysregulation in diseases such as asthma and atopic dermatitis, direct functional evidence of its role in AR—particularly in regulating T-cell differentiation—has been lacking. Our study provides initial experimental support for this mechanism in an AR-relevant context: through in vitro knockdown and overexpression experiments in human nasal epithelial cells co-cultured with naïve T cells, we demonstrated that *SIN3A* expression significantly skews CD4+ T cell differentiation toward a Th17 phenotype while suppressing Treg generation. These findings position *SIN3A* as a potential epigenetic regulator of mucosal immune balance in AR.

Although this study confirmed the upregulated expression of *SIN3A* in the nasal mucosa of AR patients and revealed through functional experiments that *SIN3A* participates in the pathological process of AR by promoting the differentiation of naive T cells into Th17 cells while inhibiting the differentiation of Treg cells, this regulatory mechanism has not yet been further verified in animal models. In addition, the specific regulatory mechanisms between *SIN3A* and its positively correlated molecules (such as *ADORA2A*, *HLA-C*, *HLA-F*, and *HLA-DQB2*) have not been deeply explored. The potential functions of these molecules in immune regulation and their synergistic effects with *SIN3A* will be the focus of future research. Subsequent work will be dedicated to clarifying how *SIN3A* regulates the differentiation fate of CD4+ T cells through interactions with these molecules and the detailed molecular mechanisms underlying the occurrence and development of AR.

This study has several shortcomings, including: the fact that Serologic testing is a primary diagnostic tool for AR patients, and *SIN3A*, a crucial gene for histone modification, has not been identified in the serum expression of these patients. The nasal mucosal tissues analyzed in this study were sourced from surgically treated allergic rhinitis patients, whose clinical symptoms are more pronounced than those of outpatients, potentially introducing bias into the experimental outcomes. The sample size of the subgroups in this study (for example, n = 12 in each group of the sublingual immunotherapy response group and non-response group and in the nasal mucosa tissue validation) may limit the generalizability of the research results and the statistical power to detect subtle transcriptomic changes. Although WGCNA has been successfully applied to similar-scale exploratory transcriptomic studies.[33] In future research, we plan to validate the above findings using larger and independent cohorts to further confirm the robustness and clinical relevance of the results of this study.

While the role of histone modification in the pathophysiology of several airway allergic illnesses has been examined and highlighted, its significance in allergic rhinitis remains unexplored and undiscussed. This work concludes that *SIN3A* is a crucial gene associated with histone modification in allergic rhinitis; nevertheless, the understanding of its precise mechanism remains restricted, necessitating further research to elucidate the pathogenesis of *SIN3A* in allergic rhinitis.

Conclusion

In conclusion, we identified *SIN3A* as a pivotal gene associated with histone modification that exhibits differential expression between the AR population and healthy individuals, potentially influencing the progression of AR by modulating the balance of Th17 and Treg cell, Our findings establish *SIN3A* as a potential candidate for future therapeutic strategies against allergic rhinitis.

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Ethics Approval

This study was approved by the human study ethics committees at The Second Xiangya Hospital of Central South University (approval no.LYF2022222). Informed consent was obtained from both adult patients and their legal guardians, who are minors, prior to their participation in the study. lt was conducted in accordance with the principles of the Declaration Helsinki.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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