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ORIGINAL RESEARCH

Inhibition of LOXL2 Suppresses Nasal Mucosal Inflammation and Remodeling in Allergic Rhinitis

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Background: Tissue remodeling is a key feature of allergic rhinitis (AR), but its underlying molecular mechanisms remain unclear. Lysyl oxidase-like 2 (LOXL2), a regulator of tissue remodeling, has not been studied in AR.

Methods: Proteomic analysis was performed on nasal mucosal tissues from 8 AR patients and 8 healthy controls (HCs) to identify differentially expressed proteins (DEPs). The top three upregulated DEPs and their association with tissue remodeling markers were validated by immunofluorescence, Western blot, and RT-qPCR in an independent cohort of 30 AR patients and 30 HCs. In vitro, human nasal epithelial cells (HNECs) were treated with IL-4, and the effects of candidate protein inhibitors on remodeling were assessed. An AR mouse model was used to evaluate the impact of these inhibitors on nasal inflammation and remodeling.

Results: Proteomic analysis revealed a disease-specific protein expression profile in the nasal mucosa of AR patients, with the top three upregulated proteins being LOXL2, TGF-β1, and TIRAP. Tissue validation showed that LOXL2 was significantly upregulated in the nasal mucosa of AR patients compared to HCs and was significantly correlated with EMT markers (TGF-β1, α-SMA, and E-cadherin). In vitro, IL-4 stimulation significantly upregulated LOXL2, TGF-β1, and α-SMA, while downregulating E-cadherin in a dose-dependent manner in human nasal epithelial cells. These effects were reversed by inhibition of LOXL2. Further investigations demonstrated that LOXL2 promotes tissue remodeling through activation of the TGF-β1/Smad signaling pathway. In the AR mouse model, LOXL2 inhibitors significantly reduced nasal mucosal inflammation and tissue remodeling.

Conclusion: Our proteomic analysis suggests that LOXL2 may be involved in the pathological remodeling processes of AR, potentially through modulation of the TGF-β1/Smad signaling pathway. These findings provide preliminary evidence that LOXL2 could serve as a candidate biomarker and a possible therapeutic target in AR, warranting further investigation.

Keywords: allergic rhinitis, LOXL2, epithelial-mesenchymal transition, signaling pathway, tissue remodeling

Introduction

Allergic rhinitis (AR) is a highly prevalent chronic inflammatory disease of the upper airway, characterized by recurrent symptoms such as nasal congestion, sneezing, itching, and rhinorrhea.[1,2] It affects approximately 10–30% of the global population, leading to a substantial health and economic burden.[3–5] The pathophysiology of AR is complex and involves immune dysregulation triggered, which activate Th2-dominant inflammatory responses and promote the production of cytokines,[2,6] which can induce Allergic rhinitis (AR) is a highly prevalent chronic inflammatory disease of the upper airway, affected approximately 10–30% of the global population, leading to a substantial health and economic burden.[3–5] The pathophysiology of AR is complex and involves immune dysregulation, which triggers Th2-dominant inflammatory responses and promotes the production of cytokines,[2,6] which can induce inflammatory cell infiltration and disrupt epithelial integrity, contributing to the persistence.[7] Recent findings have identified the nasal mucosal tissue remodeling as a significant pathological feature of AR, characterized by goblet cell hyperplasia, basement membrane thickening, epithelial-to-mesenchymal transition (EMT), and extracellular matrix (ECM) deposition.[8,9] Among these changes, EMT

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has gained attention as a key mechanism that connects epithelial barrier dysfunction to subepithelial fibrosis.[10,11] In the context of AR, EMT contributes to sustained mucosal inflammation, loss of epithelial integrity, and the progression of pathological remodeling. Therefore, investigating the underlying mechanisms of EMT induction in AR may have important clinical implications for preventing chronic mucosal damage and disease progression.

Although airway remodeling is well recognized as a key feature in the pathogenesis of AR, the molecular mechanisms driving epithelial remodeling remain insufficiently understood. Previous studies have explored potential intrinsic mechanisms of epithelial remodeling in AR using serum exosome analysis, single-cell transcriptomics, and metabolomics techniques.[12–14] However, the dynamic changes in proteins involved in this process and their regulatory roles have not been systematically examined. Notably, proteomics analysis offers a powerful approach to identify critical proteins implicated in cellular metabolism, tissue remodeling, and immune regulation in AR.[15] Thus, applying proteomics to AR nasal mucosal tissues not only reveals the complexity and diversity of AR pathomechanisms but also highlights the core molecular drivers of tissue remodeling. This approach opens new avenues for developing targeted therapeutic strategies for AR.

Lysyl oxidase-like 2 (LOXL2) is a member of the lysyl oxidase family of copper-dependent amine oxidases, which has emerged as a multifunctional regulator of tissue remodeling, inflammation, and EMT. LOXL2 has been implicated in the pathogenesis of several remodeling-related diseases, including fibrosis[16] and cancer.[17] Previous study demonstrated that LOXL2 was increased in asthma patients and contributed to asthmatic airway remodeling.[18] In addition, Chien et al[19] found that serum LOXL2 levels were significantly enhanced and associated with increased risk for mortality in idiopathic pulmonary fibrosis. However, its role in AR, particularly in airway remodeling, has not been fully elucidated. Given the importance of tissue remodeling in AR progression, investigating the function of LOXL2 in nasal mucosa may offer new insights into AR pathogenesis and provide potential therapeutic targets.

In this study, we aimed to investigate the expression pattern of LOXL2 in AR patients and to explore the possibility of its involvement in tissue remodeling pathways. Elucidating the role of LOXL2 in AR may provide new mechanistic insights and identify potential molecular targets for therapeutic intervention.

Methods

Study Design

This study was approved by the Human Ethics Committee of Xiangya Hospital (202302367), and written informed consent was obtained from all participants before recruitment. All human tissue-related investigations complied with the Declaration of Helsinki. Two independent cohorts were recruited between March 1, 2024, and June 1, 2024: a discovery cohort consisting of 8 AR patients and 8 healthy controls (HCs) for proteomic analysis, and a validation cohort with 30 AR patients and 30 HCs. The diagnosis of AR patients was based on the 2016 Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines.[20] All AR participants had not used antileukotrienes, antihistamines, steroids, or undergone immunotherapy in the month before recruitment. The control group had no history of nasal or sinus inflammatory diseases or systemic inflammatory conditions. Those patients who underwent surgical treatment for nasal bone fracture and had no history of nasal or sinus inflammatory diseases or systemic inflammatory conditions were enrolled as the HC group. Nasal inferior turbinate tissues were collected from both AR patients and HCs. All specimens were divided into three parts for different analyses, including immunofluorescence staining, protein extraction, and RNA analysis.

Proteomics Analysis

Inferior turbinate tissues were snap-frozen in liquid nitrogen and pulverized using a Tissue Lyser (QIAGEN, China). Protein extraction was performed with a protease inhibitor-containing reagent (NCMBIO, China), and protein concentrations were quantified using a BCA assay (NCMBIO, China). For proteomic analysis, proteins were digested into peptides (10 ng/μL) and separated on a 15 cm C18 column (Bruker, USA) using an LC-MS system. Protein identification and quantification were conducted using MaxQuant software, with searches against the human Uniprot/SwissProt database. Peptides with ≥2 unique matches and a false discovery rate (FDR) of <1% were considered for further

analysis. Data acquisition was performed in data-dependent acquisition (DDA) mode, and subsequent bioinformatics analysis was carried out using the Spectronaut system (Biognosys, Switzerland).[6]

Bioinformatic Analysis

Differentially expressed proteins (DEPs) between AR and HC groups were identified using stringent criteria (p-value <0.05 and fold change >1.5) and visualized through heatmaps and volcano plots generated by R packages, following an established protocol.[21,22] To elucidate sample variations and reduce data complexity, Principal component analysis (PCA) was performed, effectively preserving essential biological variance while maintaining data integrity. Subsequently, DEPs were subjected to comprehensive functional annotation through gene ontology (GO) analysis using an integrated bioinformatics platform, with pathway enrichment assessed via hypergeometric distribution testing based on Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations. Statistically significant pathways (p-value <0.01) containing ≥2 DEPs were selected for further analyzed.

Immunofluorescence (IF)

Inferior turbinate tissues from AR patients and HCs were collected, fixed, dehydrated, embedded, and sectioned as previously described.[23] Sections were rehydrated by deparaffinization and graded ethanol series. Antigen recovery was performed for 10 minutes using a microwave oven and antigen recovery solution (Servicebio, Wuhan, China). Tissue sections were then incubated in PBS blocking buffer containing 5% goat serum and 0.3% Triton X-100 for 1 hour at room temperature. The sections were then incubated with the primary antibody at 4°C overnight. The sections were washed three times with PBST for 5 min each and then incubated with 488 Alexa Fluor-conjugated secondary antibody for 1 h at room temperature in the dark. Nuclei were counterstained with DAPI (Servicebio, Wuhan, China) for 10 min, and then washed three times with PBST for 5 min each. Finally, the tissue sections were covered with an anti-fluorescence quencher to preserve fluorescence and visualized under a fluorescence microscope for imaging.

Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR)

The expression of mRNA of the target genes in nasal tissues was quantified by qPCR. Total RNA was extracted with 1 mL of TRIzol reagent (Ambion, Austin, Texas), and reverse transcription of RNA was performed with a reverse transcription kit (Servicebio, Wuhan, China) according to the manufacturer's instructions. Expression of mRNAs of related genes was detected by SYBR Premix EX Taq (Servicebio, Wuhan, China). The primer sequences are shown in Table S1. Gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the housekeeping gene, and relative mRNA levels of target genes were calculated using the comparative 2-ΔΔCt method.

Human Nasal Epithelial Cell Culture and Treatment

Fresh inferior turbinate specimens were collected from HCs and immediately transferred to the laboratory, rinsed with PBS (phosphate buffer solution) and cut into 1 mm^3 pellets. HNECs were isolated from the tissue by trypsinization. The pellet was incubated with collagen type I for 3 h at 37 °C, centrifuged for 5 min, and resuspended in PneumaCult™-Ex Plus Medium (Stemcell Technologies Inc., Vancouver, Canada). Finally, HNECs were incubated at 37°C in an incubator with 5% CO2. When the cells reached 80% confluence, they were trypsinized and seeded into 6-well plates. For fluorescence detection experiments, cells were seeded onto coverslips placed in 6-well plates. Upon reaching 70% confluence, the cells were treated with varying concentrations of IL-4 recombinant protein, and a combination of 100nM LOXL2 inhibitor (2-Chloropyridin-4-yl methanamine hydrochloride) (HY-101771A, MCE, New Jersey, USA) and 40 ng/mL IL-4 recombinant protein (MCE, New Jersey, USA) to observe their effects on cell function and related signaling pathways.

Western Blotting (WB) Analysis

Proteins are isolated and extracted from inferior turbinate tissue and cells using RIPA lysis buffer containing protease inhibitors. Homogenize by centrifugation and determine the protein concentration using the BCA assay kit. Approximately 10 μg of protein was separated by SDS-PAGE and subsequently transferred to a PVDF membrane for

transmembrane transfer, at the end of which it was blocked with 5% skimmed milk. The membranes were incubated overnight at 4 °C with primary antibodies against LOXL2, E-cadherin, TGF-β1, α-SMA and the next day with HRP (horseradish peroxidase) conjugated secondary antibodies. Specific proteins were detected in the ECL system.

Enzyme-Linked Immunosorbent Assay (ELISA)

Levels of IL-4 and IL-13 in murine nasal lavage fluid were measured using ELISA kits (MultiSciences, Hangzhou, China) according to the manufacturer's instructions. Concentrations of total IgE and OVA-sIgE in serum samples were detected by ELISA kits provided (CUSABO, Wuhan, China).

AR Mouse Model

Murine AR models were induced using ovalbumin (OVA, Solarbio, Beijing, China) following previously established protocols.[24] All experimental procedures were approved by the Animal Ethics Committee of Xiangya Hospital of Central South University (No.202305171). Mice were randomly assigned to three groups: the control group (n=10), the AR group (n=10), and the AR + LOXL2 inhibitor group (HY-101771A, MCE, New Jersey, USA) (n=10). On days 0, 7, and 14, mice were sensitized with 200 μL of PBS containing 0.125 mg/mL OVA and 1.5 mg aluminum hydroxide via intraperitoneal injection. Starting on day 21, mice received 20 μL of 25 mg/mL OVA solution intranasally for 7 consecutive days, while controls were given an equal volume of PBS. In the AR + LOXL2 inhibitor group, mice were injected intraperitoneally with 10 mg/kg LOXL2 inhibitor on days 20, 22, 24, and 26 during the challenge period as previously described.[25] Twenty-four hours after the final challenge, mice were euthanized, and their heads were collected. The entire nose of half of the mice was fixed, while the nasal mucosa from the remaining mice was carefully harvested and preserved. Nasal lavage fluid was collected from all mice, centrifuged, and the supernatant was used for cytokine analysis.

Statistical Analysis

All data were analyzed using GraphPad Prism 7 (GraphPad Software, La Jolla, USA) and SPSS 25.0 (IBM, Chicago, IL). Data are presented as mean ± SD. Statistical comparisons between two groups were performed using Student's *t*-test, while one-way ANOVA was used for comparisons among more than two groups. Spearman correlation test was used to assess relationships between variables. A p-value of < 0.05 was considered statistically significant.

Results

Nasal Mucosa in AR Exhibits Disease-Specific Proteomic Profiles

In the discovery cohort, 8 AR patients and 8 HCs were included in tissue proteomic analysis. The clinical characteristics of all subjects are presented in Table 1. There were no statistically significant differences in baseline clinical characteristics between the HC and AR groups. Proteomic analysis identified two distinct clusters via PCA: one predominantly consisting of AR samples and the other primarily composed of HC samples, demonstrating significant global alterations in protein expression profiles within the AR nasal mucosa. (Figure 1A). Further heatmaps and volcano plots illustrated substantial differences in protein expression patterns between the AR and HC groups (Figure 1B and C). Figure 1D presents the GO annotations, revealing several

Table 1 Clinical Data of Subjects in Discover Cohort

Variable	HC	AR	P
Number, n	8	8	
Gender, male/female, n	4/4	5/3	0.614
Age, year	34.4±9.7	35.5±10.6	0.828
BMI, kg/m^2	23.2±0.9	23.0±0.7	0.773
Smoking, Yes/No	3/5	2/6	0.590
Drinking, Yes/No	2/6	1/7	0.522

Abbreviations: HC, healthy control; AR, allergic rhinitis; BMI, body mass index.

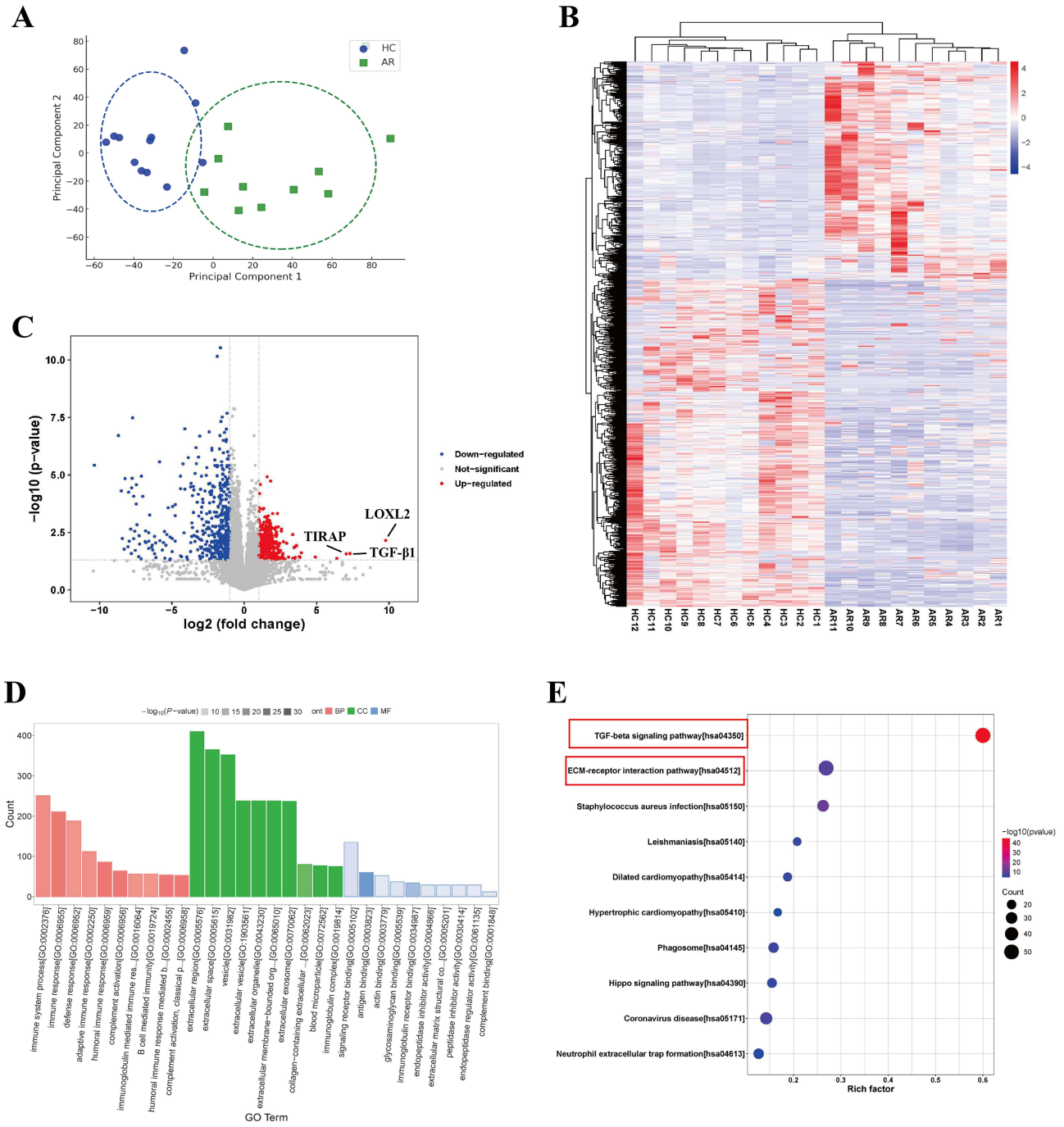

Figure 1 Proteomic analysis reveals a disease-specific protein expression profile in nasal mucosa of AR patients. (**A**) PCA; (**B**) Heat map; (**C**) Volcano; (**D**) GO; (**E**) KEGG, red box highlights TGF-beta signaling pathway and ECM-receptor interaction pathway.

Abbreviations: GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; AR, allergic rhinitis; HC, healthy control; PCA, principal component analysis.

extracellular-related biological processes (BP) and cellular components (CC). Figure 1E highlights KEGG pathways, including the TGF-beta signaling pathway and ECM-receptor interaction pathway. These results suggest that AR patients display a disease-specific proteomic profile in the nasal mucosa, with tissue remodeling potentially playing a key role in its pathological mechanisms.

Table 2 Clinical Data of Subjects in Validation Cohort

Variable	HC	AR	P
Number, n	30	30	
Gender, male/female, n	19/11	14/16	0.194
Age, year	35.6±11.8	41.0±12.0	0.088
BMI, kg/m^2	22.5±0.9	22.6±0.8	0.481
Smoking, Yes/No	10/20	13/17	0.426
Drinking, Yes/No	11/19	10/20	0.787

Abbreviations: HC, healthy control; AR, allergic rhinitis; BMI, body mass index.

LOXL2 Was Increased in AR and Correlated with Tissue Remodeling

To further validate the upregulation of the top three proteins, we recruited a validation cohort consisting of 30 AR patients and 30 HCs, with clinical data presented in Table 2. The two groups were comparable in baseline clinical characteristics, including age and sex, with no significant differences observed between them. Immunofluorescence staining revealed significantly higher levels of LOXL2 and TGF-β1 in the AR group, with LOXL2 predominantly localized within the epithelium (Figure 2A and B). Western blot analysis confirmed that LOXL2 and TGF-β1 levels were

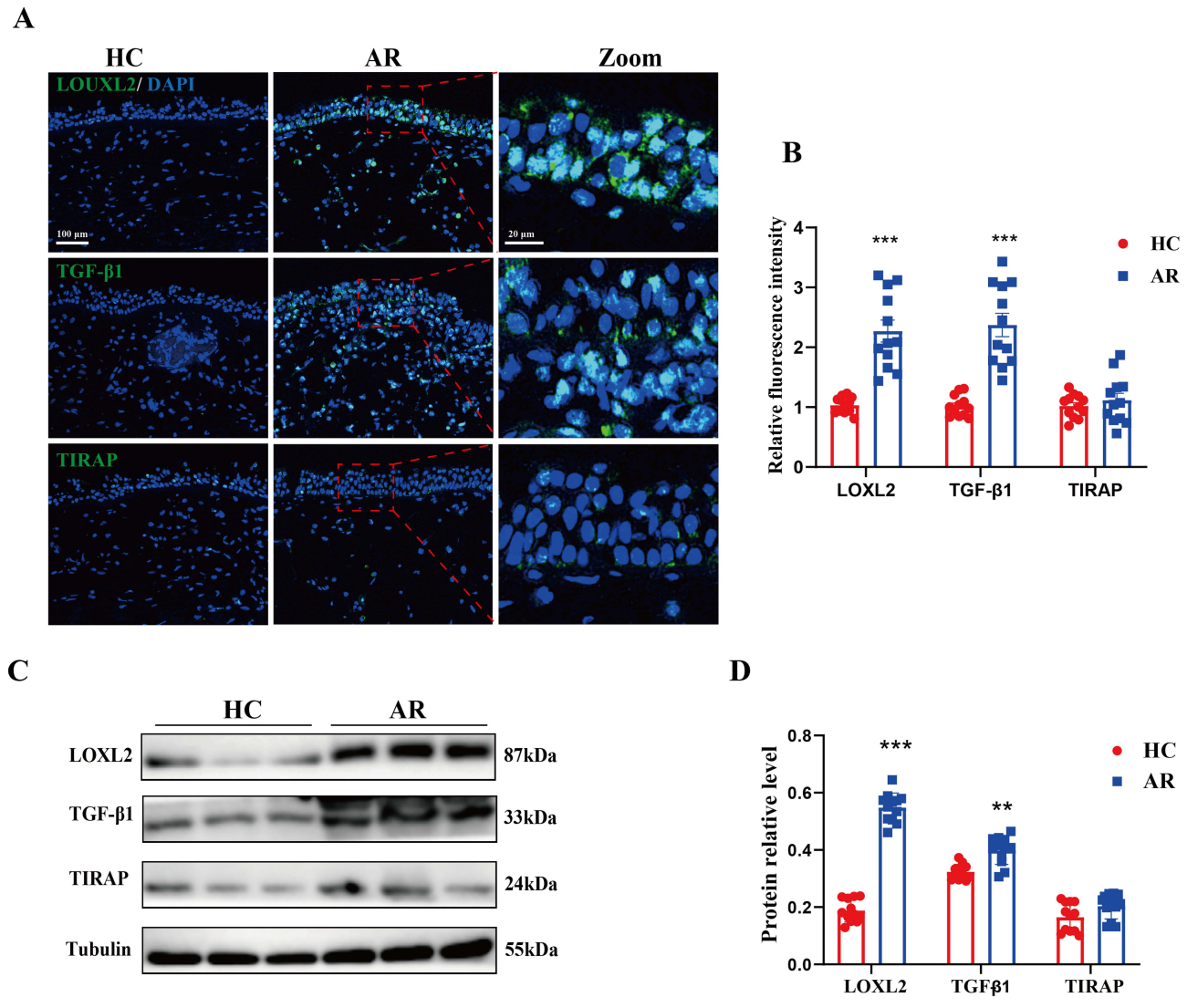

Figure 2 Protein expression of the top three upregulated DEPs was validated in the validation cohort. (**A** and **B**) Immunofluorescence was used to examine the expression of DEPs between the two groups (n=12); (**C** and **D**) WB showing DEPs protein expression between HC and AR groups (n=12). **P<0.01, ***P<0.0001.
Abbreviations: AR, allergic rhinitis; HC, healthy control; DEP, differentially expressed proteins; WB, Western blotting; ns, no significance.

significantly elevated in the nasal mucosa of AR patients compared to the HC group, while no significant difference was observed in TIRAP expression (Figure 2C and D). RT-qPCR further supported these findings, showing higher expression of LOXL2 and TGF-β1 in the AR group compared to HCs (Figure 3A–C). Moreover, correlation analysis revealed a significant positive correlation between LOXL2 and TGF-β1 expression, while no correlation was found with TIRAP (Figure 3D–F). These results suggest that LOXL2 plays a critical role in the pathological mechanisms of AR and may be closely associated with tissue remodeling.

To further investigate the relationship between LOXL2 and tissue remodeling, WB and RT-qPCR analysis revealed a decrease in E-cadherin expression and an increase in α-SMA expression in AR tissues (Figure 4A–D). Correlation analysis further demonstrated a positive association between LOXL2 and α-SMA expression, and a negative correlation between LOXL2 and E-cadherin expression (Figure 4E and F). These findings collectively suggest that aberrant LOXL2 expression may contribute to tissue remodeling in the nasal mucosa of AR patients.

LOXL2 Mediates IL-4-Induced Remodeling via the TGF-β1/Smad Pathway

To further explore the role of LOXL2 in epithelial remodeling, we isolated nasal epithelial cells from HCs and treated them with IL-4. Western blot analysis revealed that 48 hours of IL-4 treatment significantly increased the expression of LOXL2, TGF-β1, and α-SMA, while also causing a dose-dependent reduction in E-cadherin levels (Figure 5A). When cells were co-treated with LOXL2 inhibitor and 20 ng/mL IL-4 for 48 hours, LOXL2 inhibition significantly reduced the levels of TGF-β1 and α-SMA and reversed the downregulation of E-cadherin (Figure 5B). These results suggest that LOXL2 plays a key role in IL-4-induced epithelial-to-mesenchymal transition disruption. A previous study has shown that the TGF-β1/Smad signaling pathway is critical for tissue remodeling.[26] To examine whether LOXL2 mediates IL-4-induced remodeling through this pathway, we assessed the phosphorylation levels of Smad2 and Smad3. Our results demonstrated that IL-4 stimulation increased the phosphorylation of Smad2 and Smad3, while LOXL2 inhibition significantly suppressed this effect (Figure 5C and D). Collectively, these findings suggest that LOXL2 contributes to IL-4-induced pathological remodeling in HNECs via the TGF-β1/Smad signaling pathway.

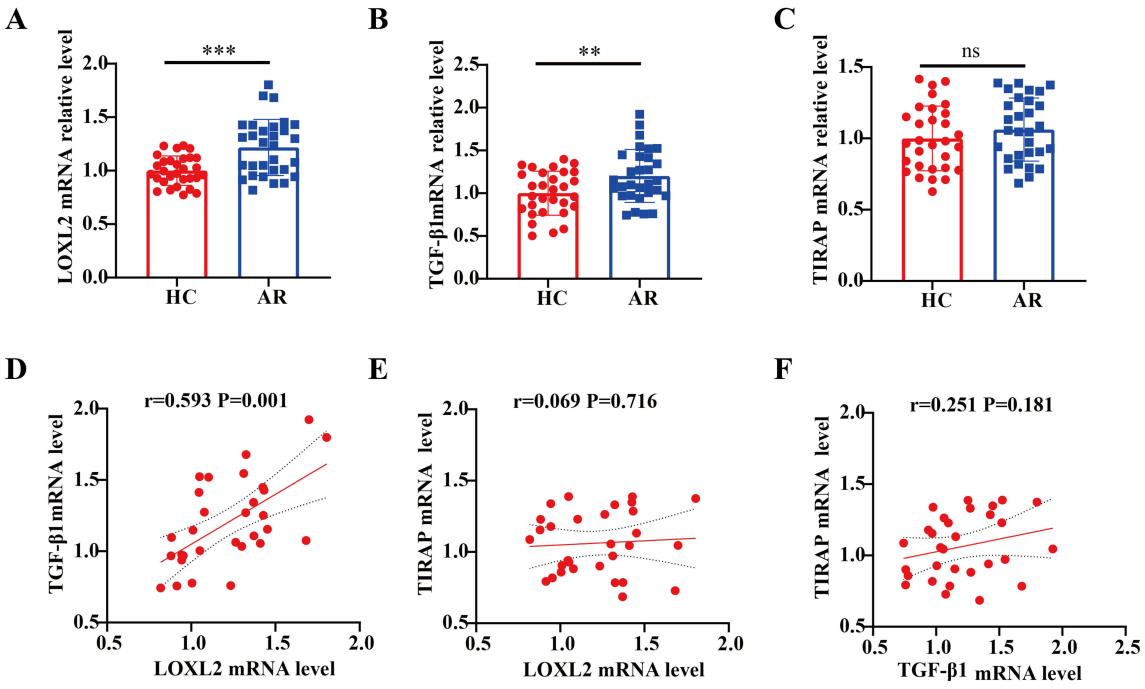

Figure 3 Validation of mRNA expression levels of DEPs in tissues and correlation analysis in AR patients. (**A–C**) RT-qPCR analysis between the two groups (n=30); (**D–F**) The correlations of mRNA expression levels among three DEPs in AR patients (n=30). **P<0.01, ***P<0.0001.

Abbreviations: AR, allergic rhinitis; HC, healthy control; DEP, differentially expressed proteins; RT-qPCR, quantitative reverse transcription polymerase chain reaction; ns, no significance.

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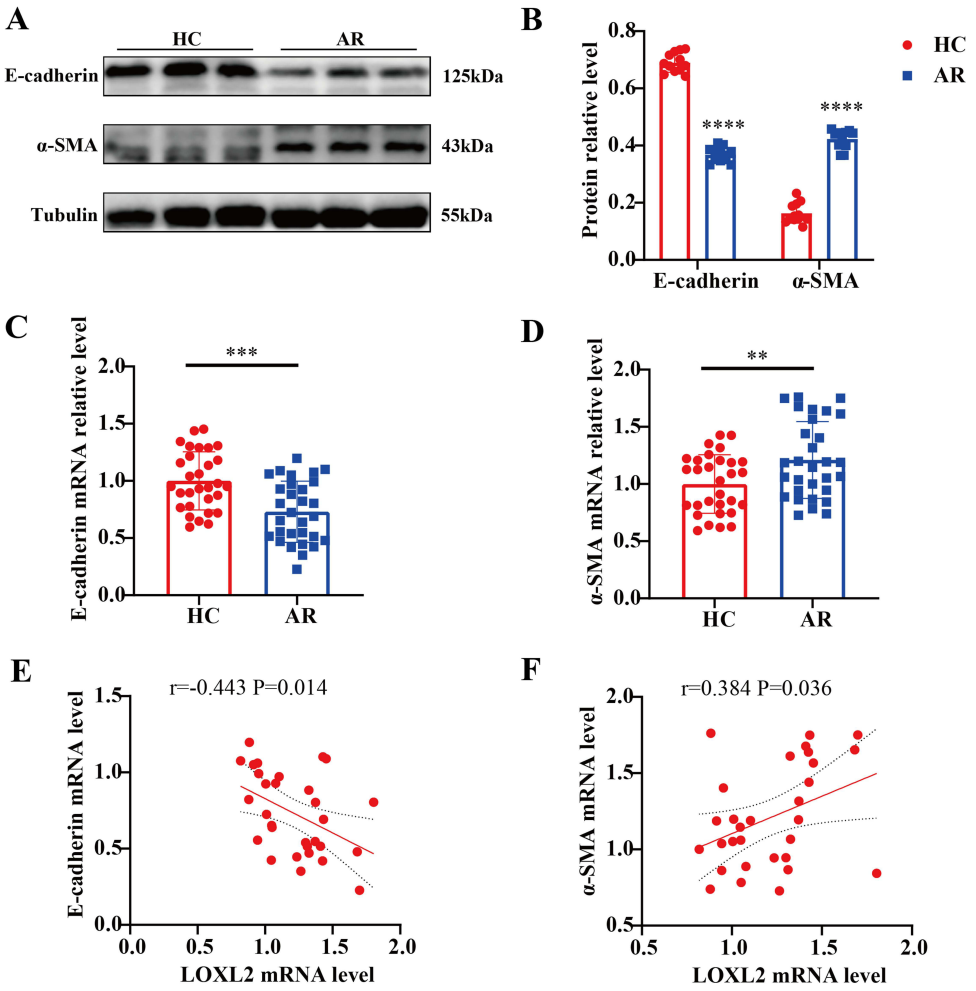

Figure 4 Increased LOXL2 is associated with the expression of tissue remodeling markers in AR patients. (**A** and **B**) WB and (**C** and **D**) RT-qPCR for E-cadherin and α-SMA between the two groups (n=30); (**E** and **F**) The correlations between LOXL2 mRNA, E-cadherin and α-SMA mRNA in AR patients (n=30). **P<0.01, ***P<0.001, ****P<0.0001.

Abbreviations: AR, allergic rhinitis; HC, healthy control; RT-qPCR, quantitative reverse transcription polymerase chain reaction.

LOXL2 Inhibition Reduces Nasal Inflammation and Tissue Remodeling in an AR Murine Model

To assess the effect of LOXL2 inhibition on nasal epithelial remodeling, we performed in vivo experiments using an AR mouse model. ELISA results demonstrated that serum total IgE and OVA-specific IgE levels, as well as IL-4 and IL-13 concentrations in nasal lavage fluid, were significantly elevated in the AR group compared to the control group. These increases were notably attenuated following inhibitor treatment in the AR + inhibitor group (Figure 6A and B). HE staining revealed marked structural disruption and epithelial thickening of the nasal mucosa in AR mice, along with pronounced infiltration of inflammatory cells (Figure 6C). Further tissue IF and RT-qPCR analyses confirmed that LOXL2 expression was significantly elevated, while E-cadherin levels were decreased, and α-SMA levels were increased in the AR group. These pathological alterations were markedly alleviated in the AR + inhibitor group (Figure 6C–E). Collectively, these results suggest that LOXL2 plays a critical role in AR pathogenesis by promoting nasal mucosal tissue remodeling and inflammation.

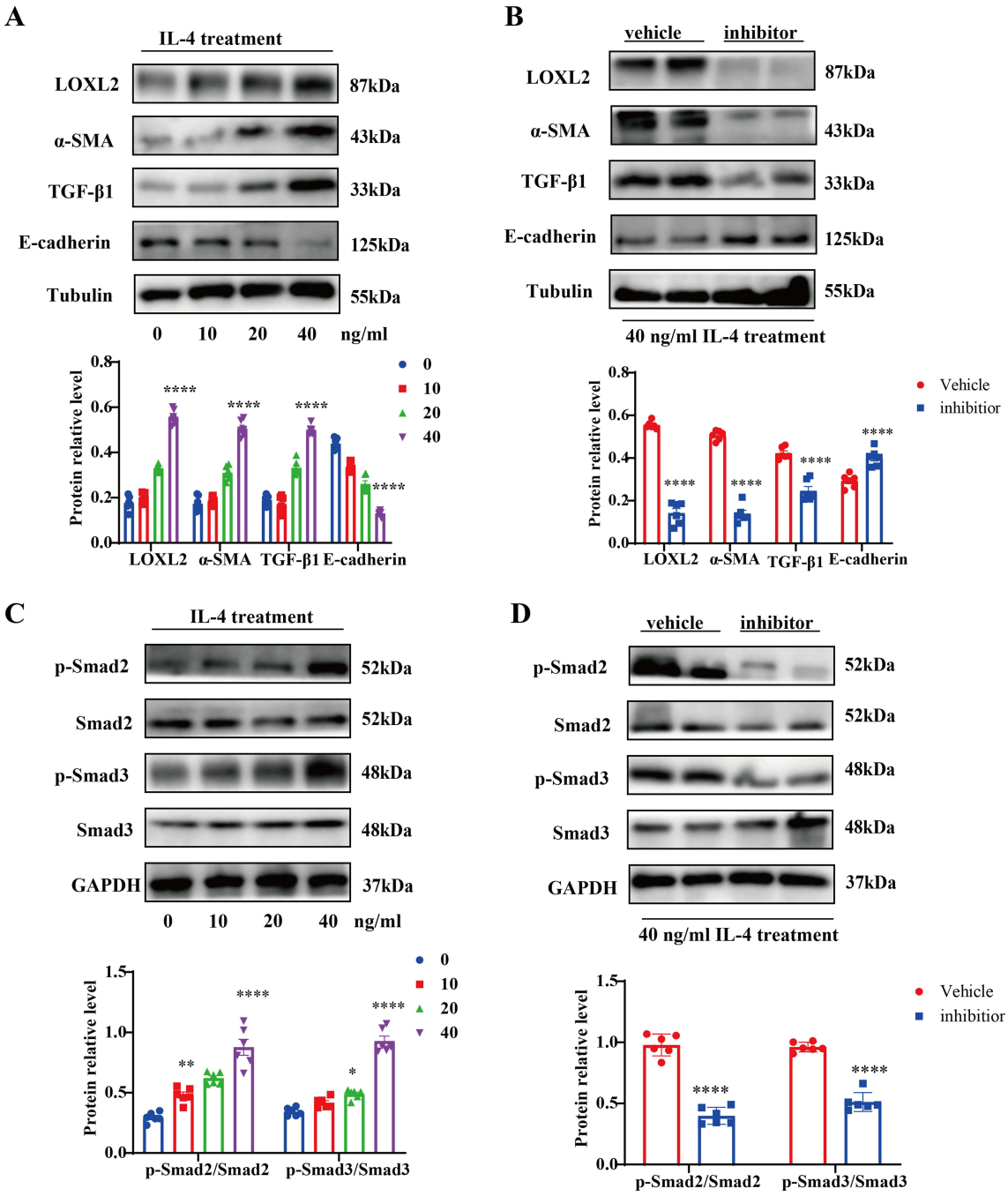

Figure 5 LOXL2 drives IL-4-induced remodeling via TGF-β1/Smad signaling. (**A**) WB showing LOX2 and EMT markers expression in nasal epithelial cells treated with different concentrations of IL-4 (n=6). (**B**) LOXL2 inhibitor reverses IL-4-induced EMT marker expression (n=6); (**C**) IL-4 dose-dependently activates Smad2/3 phosphorylation (n=6). (**D**) LOXL2 inhibition blocks IL-4-induced Smad2/3 phosphorylation (n=6). *P<0.05, **P<0.01, ****P<0.0001.
Abbreviations: WB, Western blotting; EMT, epithelial-mesenchymal transition.

Discussion

Currently, the role of tissue remodeling in the pathological process of AR is gaining increasing attention.[8,27] Epithelial damage and repair processes induced by chronic allergic reactions are primary contributors to significant structural changes in nasal mucosal tissue.[28–30] Previous studies have shown that AR patients exhibit compromised nasal mucosal barrier function, characterized by epithelial cell hyperplasia, thickening of the basement membrane, and local immune cell infiltration.[31–33] As allergic reactions persist, localized inflammation intensifies, leading to dysfunction of the nasal epithelium. This dysfunction facilitates the penetration of allergens into the epithelium, exacerbating immune responses

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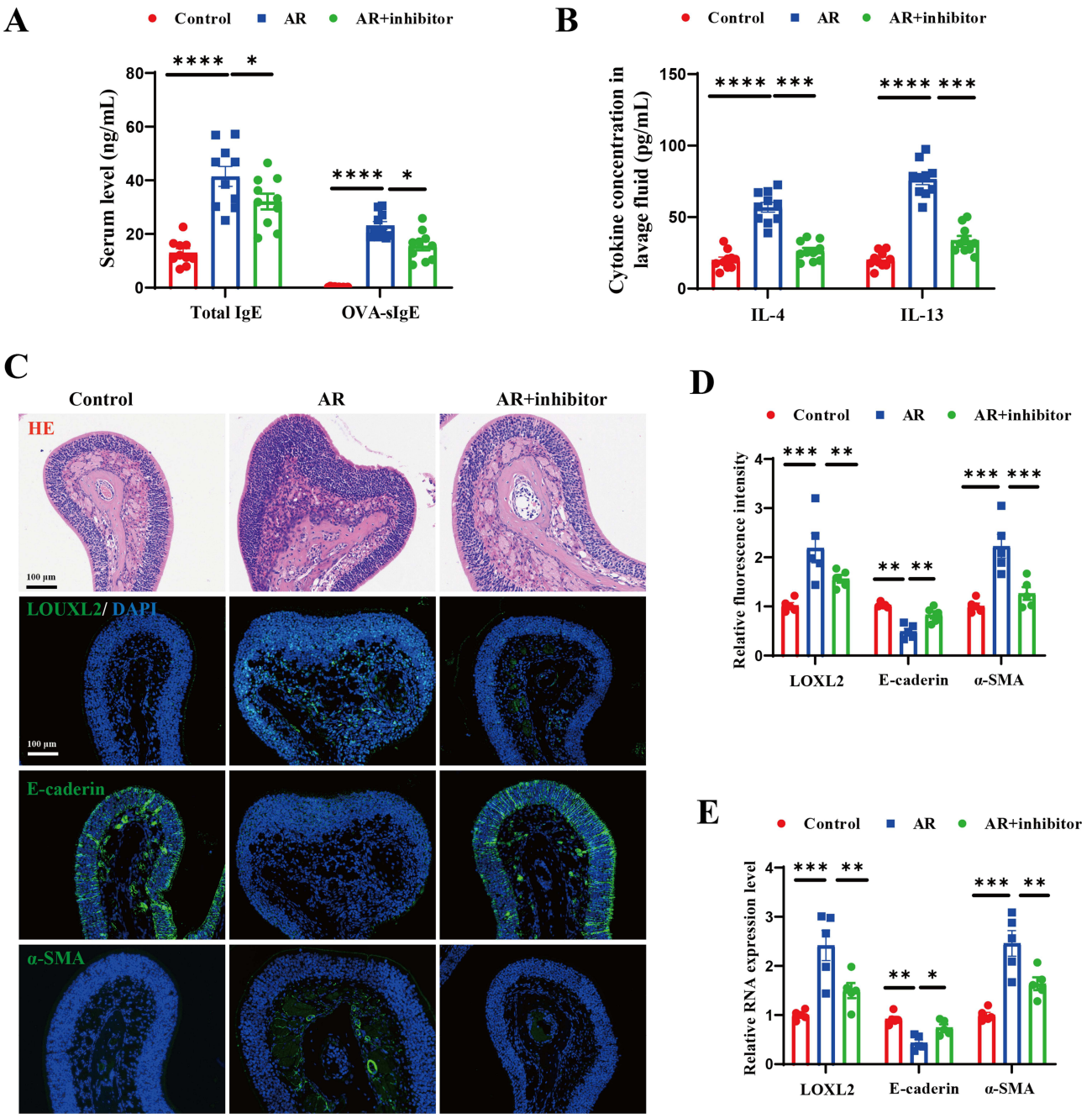

Figure 6 LOXL2 inhibitor alleviates nasal mucosal inflammation and tissue remodeling in murine AR model. (**A**) Serum IgE and OVA-sIgE levels (n=10). (**B**) IL-4 and IL-13 concentrations in nasal lavage fluid (n=10). (**C**) Representative HE and immunofluorescence images of nasal mucosal in control, AR and AR+inhibitor groups (n=5); (**D**) Relative fluorescence intensity of LOXL2 and EMT marker among the three groups (n=5); (**E**) Relative fluorescence intensity of LOXL2 and EMT marker among the three groups (n=5); *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Abbreviations: AR, allergic rhinitis; OVA, ovalbumin; EMT, epithelial-mesenchymal transition.

and contributing to further tissue remodeling.[34,35] However, the mechanisms underlying remodeling in AR nasal epithelium are still not fully understood. In this study, our proteomic analysis identified AR-specific protein expression profiles, revealing that several DEPs are closely associated with tissue remodeling. Among the DEPs, we selected the top three upregulated proteins (LOXL2, TGF-β1, and TIRAP) for validation in a cohort of AR patients. Our analysis revealed that both LOXL2 and TGF-β1 expression were significantly elevated in AR patients. Notably, LOXL2 levels

showed a strong correlation with TGF-β1 expression and other tissue remodeling markers. These findings suggest that the upregulation of LOXL2 may play a pivotal role in driving tissue remodeling within the nasal mucosa of AR patients.

LOXL2, a lysyl oxidase enzyme, plays a crucial role in extracellular matrix (ECM) remodeling by facilitating the crosslinking of collagen and elastin.[36,37] Recent studies have highlighted its significant immunomodulatory function in airway inflammatory diseases. Previous research indicated that elevated LOXL2 levels can promote the release of local inflammatory cytokines, exacerbate inflammation, and drive airway remodeling in asthma.[37] Moreover, in patients with chronic obstructive pulmonary disease (COPD), LOXL2 expression was notably increased in lung tissues and strongly correlated with disease severity. The elevated LOXL2 levels were associated with impaired airway function and worsened airway remodeling through the damage of airway smooth muscle cells.[38] In our study, we found significantly higher LOXL2 expression in the nasal mucosa of AR patients, with LOXL2 predominantly localized in epithelial cells. Significantly, the upregulation of LOXL2 was closely associated with changes in EMT markers, suggesting that LOXL2 may contribute to epithelial remodeling by modulating the EMT process in AR.

Given that the pathological mechanisms of AR are primarily characterized by Th2-driven inflammation, we treated HNECs with recombinant IL-4. Our results showed that IL-4 stimulation significantly upregulated LOXL2 expression and activated the TGF-β1/Smad signaling pathway. TGF-β1/Smad signaling pathway is a well-recognized regulator of tissue remodeling,[39,40] and previous studies demonstrated that TGF-β1 induces EMT by facilitating the phosphorylation of Smad2/3, which leads to transcriptional suppression of epithelial markers and upregulation of mesenchymal markers.[41] Our study demonstrated that inhibiting LOXL2 expression can alleviate IL-4-induced EMT by suppressing the activation of the TGF-β1/Smad signaling pathway. In vivo experiments in AR mice showed significant structural disruption of the nasal epithelium, along with inflammatory cell infiltration and upregulation of LOXL2 in the nasal mucosa. Treatment with LOXL2 inhibitors significantly reduced these pathological alterations, as evidenced by decreased inflammatory cell infiltration, restored epithelial integrity, and lowered α-SMA expression. Moreover, LOXL2 inhibition resulted in a marked reduction in IL-4 and IL-13 levels in nasal lavage fluid, further supporting the role of LOXL2 in immune response modulation and EMT in AR. Together, these findings highlight that LOXL2 plays a crucial role in tissue remodeling through its modulation of immune responses in epithelial cells. Targeting LOXL2 could provide a novel therapeutic approach to address both inflammation and tissue remodeling in AR, offering promising potential for improved disease management.

While this study offers significant insights into the role of LOXL2 in AR, several limitations should be considered. First, since this study is a preliminary study with a small sample size, the results have yet to be externally validated in a larger sample of a multicenter population to assess the stability and prevalence of expression of the candidate proteins in different clinical phenotypes. Second, although we demonstrated the impact of LOXL2 inhibition on epithelial remodeling and inflammation, the long-term efficacy and safety of LOXL2 inhibitors remain unclear. These inhibitors will need to be rigorously tested in future studies to assess their therapeutic potential in AR. Finally, while this study focused on the TGF-β1/Smad signaling axis, emerging evidence suggests that LOXL2 may also interact with other remodeling-related pathways, such as Wnt/β-catenin, which regulates epithelial regeneration and fibrosis, and NF-κB, a key mediator of inflammation and the mucosal immune response. A more comprehensive analysis of these pathways could help to fully elucidate the complex molecular mechanisms underlying tissue remodeling in AR.

In conclusion, our proteomic analysis identified LOXL2 as a protein potentially associated with nasal mucosal remodeling in AR. The data suggest that LOXL2 may participate in epithelial remodeling and inflammatory responses, possibly through involvement of the TGF-β1/Smad signaling pathway. These findings provide preliminary insights into the molecular mechanisms of AR and support further investigation into the role of LOXL2 as a potential contributor to disease pathogenesis and a candidate for therapeutic targeting.

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Disclosure

The authors report no conflicts of interest in this work.

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