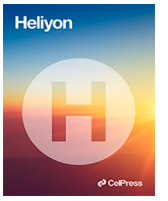
Heliyon 10 (2024) e25653

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

The role of ZEB1 in regulating tight junctions in antrochoanal polyp

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ARTICLE INFO

Keywords:
Antrochoanal polyp
Tight junctions
Epithelial barrier
ZEB1
IL-17A

ABSTRACT

Background: Antrochoanal polyp (ACP) is a benign nasal mass of unknown etiology. Tight junctions (TJs) are essential to the epithelial barrier that protects the body from external damage. However, the phenotype of tight junction in ACP is currently unclear.
Methods: The samples were collected from 20 controls, 37 patients with ACP and 45 patients with chronic rhinosinusitis with nasal polyp (CRSwNP). Quantitative Real-Time PCR (qRT-PCR) and immunofluorescence staining (IF) were performed to analyze the expressions of TJs markers (ZO-1, claudin-3 and occludin) and ZEB1. hNEpCs were transfected with ZEB1 small interfering RNA (si-ZEB1) or ZEB1 over-expression plasmid (OE-ZEB1). qRT-PCR and Western blotting were used to determine the levels of TJs-related markers. Primary human nasal epithelial cells (hNECs) were stimulated with IL-17A and si-ZEB1, and the expression of epithelial barrier markers were measured by qRT-PCR and Western blotting.
Results: Compared to the control group, ACP group showed a significant downregulation in both mRNA and protein levels of ZO-1, occludin, and claudin-3. Furthermore, disease severity correlates positively with the degree of disruption of tight junctions. In addition, higher expression levels of ZEB1, IL-17A, and IFN-γ were observed in the ACP group compared to controls. Overexpression of ZEB1 in hNEpCs led to impairments in the levels of ZO-1, occludin, and claudin-3, while silencing of ZEB1 expression was found to enhance the barrier function of epithelial cells. Finally, IL-17 stimulation of hNECs impaired the expression of TJs-associated molecules (ZO-1, occludin, and claudin-3), which was effectively reversed by the IL-17A + si-ZEB1 group.
Conclusions: The tight junctions in ACP were extremely damaged and were correlated with the severity of the disease. ZEB1 was involved in the pathogenesis of ACP mediated by IL-17A through regulating tight junctions.

1. Introduction

Antrochoanal polyp (ACP) is a chronic inflammatory condition of the upper respiratory tract. It usually originates from the maxillary sinus and enters the nasal cavity through the maxillary sinus natural or accessory foramen. Larger polyps even have

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https://doi.org/10.1016/j.heliyon.2024.e25653
Received 9 August 2023; Received in revised form 30 January 2024; Accepted 31 January 2024
Available online 7 February 2024

extension into the nasopharynx [1,2]. ACP accounted for approximately 5% of all chronic rhinosinusitis with nasal polyp (CRSwNP) and 33% of CRSwNP in children, with adolescents predominating [1]. Patients clinically complain of increasing unilateral nasal congestion, rhinorrhoea and runny nose. Pathology of ACP is not well known. Pathogenic microbial infections such as viruses [human papilloma virus (HPV) and Epstein-Barr virus (EBV)] may be involved in the development of ACP [3], and congenital anatomical abnormalities of the nasal cavity is also a cause of the disease [4,5]. ACP belonged to type 1/3 (T1/3) inflammation according to recent study [6]. The epithelial layer of the tissue in ACP showed a slight thickening with a moderate proliferation of goblet cells, marked squamous metaplasia and a disorganised single layer of pseudostratified ciliated columnar epithelium on the surface of the epithelium [7]. Several external stimuli activate the process of epithelial remodeling in tissue. However, there are no reports on barrier expression in ACP.

In addition to providing a timely immune response to harmful stimuli, the nasal mucosa plays an important role as a natural defensive barrier against the invasion of exogenous pathogens [8]. The epithelium serves as the first physical barrier to the inhalation of allergens and other pathogens [9]. The epithelium is a highly permeable barrier with three main types of junctions, including tight junctions (TJs), adherens junction and desmosomes. Of these, tight junctions play key role in the defence of barrier [10]. Tight junctions regulate the homeostasis of ions, water and certain macromolecules and locate at the top of the epithelium, forming a barrier to selective permeation [11]. Tight junctions are mainly composed of three transmembrane proteins, occludin (OCLN), claudin (CLDN) and junctional adhesion molecules (JAMs) [12]. Zonula-occludens (ZOs) are cytoplasmic linker proteins that link transmembrane proteins to cytoskeletal proteins [13]. Epithelium tight junctions of the nasal mucous are affected by inhaled allergens, pathogens and environmental pollutants. Subsequently, immune cells beneath the epithelium are activated which promotes the onset and development of inflammation [14]. Previous studies have primarily investigated the impact of type 2 (T2) inflammatory factors, such as IL-4 and IL-13, on the disruption of the epithelial barrier. However, our research has found that type 3 (T3) inflammation, mediated by IL-17A, significantly reduces the expression of occludin and ZO-1 through the nuclear factor-κB-p65/tumour necrosis factor-α signalling pathway when stimulating bronchial mucosal epithelial cells in vitro [15]. Our study aims to further explore the molecular mechanism of IL-17A damage to tight junctions.

Zinc finger E box binding protein 1 (ZEB1) is a zinc finger family of repressive transcription factors. It has been reported to be a key factor in regulating epithelial-mesenchymal transition, mediating a variety of biological effects including epithelial migration, invasion, remodeling and DNA damage repair via downstream factors [16]. ZEB1 modulated JAK/STAT signalling to drive Th1 and Th17 cell differentiation and increased production of IFN-γ and IL-17A [17]. Our study compared the degree of epithelial barrier damage in different subgroups and investigated the potential pathogenesis of ZEB1 in IL-17A-induced antrochoanal polyp.

2. Method and methods

2.1. Study design and population

The lesion sites of 37 patients with ACP and 45 patients with CRSwNP who visited Shandong Provincial ENT Hospital from January 2020 to April 2022 were collected. Twenty healthy controls were obtained from uncinate processes or inferior turbinate of patients who underwent nasal septal deviation correction or excision of maxillary sinus cyst during the same period. The diagnosis of CRSwNP was defined based on the 2020 European position paper on sinusitis and nasal polyps [18]. Atopic status was assessed by specific IgE levels using ImmunoCAP (Phadia250, Uppsala, Sweden) for common inhalants (house dust mites, animal dander, etc.) and foods (proteins, milk and nuts, etc.). Patients with acute and other chronic upper airway infection, immunodeficiency, bronchiectasis, diabetes mellitus, or cystic fibrosis, were excluded. This study was approved by the Ethics Committee of Shandong Provincial ENT Hospital, and written informed consent was obtained from patients.

2.2. Cell culture and transfection

Human nasal epithelial cell lines (HNEpCs) were obtained from Cellbio (Cat. No. CBR-130634), and were cultured in Dulbecco's Modified Eagle medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific; USA) and incubated at 37 °C in an incubator with 5% CO_2. The cells were stimulated with ZEB1 small interfering RNA (si-ZEB1) or ZEB1 overexpressing plasmid (OE-ZEB1) (GenePharma, China) and transfected after 48 h for subsequent experimental analysis.

2.3. Primary cell culture and stimulation

Primary human nasal epithelial cells (hNECs) were obtained from patients without chronic inflammation and undergoing nasal endoscopic septal surgery at Shandong Provincial ENT Hospital. Surgical samples were washed with phosphate-buffered saline (PBS), crushed and digested with Dispase II (Sigma, USA) at 4 °C overnight. The cells were harvested on the following day and cultured in PneumaCult™-Ex Plus medium (Stemcell, Canada). When the cells had reached 80–90% confluence, the plates were spread out. The cells were stimulated for 48 h with IL-17A (R&D, USA) or accompanied by si-ZEB1 and hNECs were harvested for subsequent analysis.

2.4. Quantitative real-time PCR (qRT- PCR)

RNA was extracted from nasal mucosa using RNA extraction kit (Invitrogen, USA). cDNA synthesis was performed according to the instructions of RNA reverse transcriber kit (Takara, Japan) and SYBR Green PCR Premix (Takara, Japan). Primers used in this study

were shown in Supplementary Table 1. All qRT-PCR data were calculated using the $2^{-\Delta\Delta Ct}$ method, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference.

2.5. Western blotting

Frozen tissues were added to protein lysis solution (Beyotime, China), crushed and allowed to stand on ice for 30 min to allow lysis completely. The supernatant was collected for protein concentration using a BCA kit (Beyotime, China), proteins were separated by SDS-PAGE and subsequently transferred to PDVF. 5% skimmed milk was incubated for 2 h at room temperature and antibodies were incubated overnight. Incubation with HRP-conjugated goat secondary antibody. For visualisation and analysis, chemiluminescence kits (ECL, Bio-Rad, USA) were used. See Supplementary Table 2 for the antibodies used.

2.6. Immunofluorescence staining (IF)

Paraffin sections were baked at 60 °C for 2 h, followed by dewaxing in a gradient of xylene and alcohol, antigen retrieval by microwave, 0.03% Triton X-100 retrieval for 10 min, 10% goat serum (ZSJQ, China) blocking for 1 h, antibody incubation overnight at 4 °C. PBS washed 3 times followed by incubation with fluorescent secondary antibody (goat anti-Rabbit IgG, Secondary Antibody, Invitrogen, USA) for 1 h. The sections were sealed with DAPI and then observed under a fluorescence microscope (Olympus, Japan). Three different high magnification fields were randomly selected for each section and the mean optical density values were calculated using Image J processing.

2.7. Statistical analysis

All data were statistically analyzed using and GraphPad Prism 8.0. Data were normally distributed and described as ($x \pm s$), with *t-test* and *ANOVA* for groups analysis; not normally distributed and described as $M(Q_R)$, with *Mann-Whitney* test and *Kruskal-Wallis* test for groups. The *Spearman* test was used for correlation analysis and differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Population characteristics

A total of 102 patients were enrolled in the study, including 20 healthy controls, 37 patients with ACP and 45 patients with CRSwNP. Typical pathological pictures of hematoxylin & eosin staining in patients with ACP (Fig. 1A–B) and CRSwNP (Fig. 1C–D) were demonstrated in Fig. 1. It could be seen that ACP was composed of a single layer of pseudostratified columnar ciliated epithelium and highly interstitial fibrosis. Epithelial cell hyperplasia, goblet cell metaplasia and eosinophilic infiltration were seen histologically in CRSwNP. Patients with ACP generally had a younger age of onset compared with controls and patients with CRSwNP. BMI were significantly lower for ACP compared to controls and CRSwNP groups. There were no differences among three groups in terms of gender, smoking history or presence of allergic rhinitis (**Table 1**).

Table 2 compared the clinical parameters of patients with ACP and CRSwNP. ACP tended to have a unilateral onset, slightly more on the left than on the right, with 2 patients having bilateral lesions; CRSwNP was more likely to have a bilateral onset. Analysis of peripheral blood counts revealed that platelet counts were significantly higher in patients with ACP than in those with CRSwNP. And

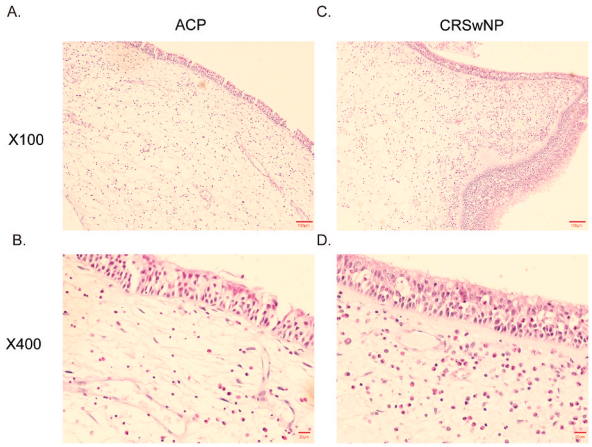

Fig. 1. Pathological features of Chronic rhinosinusitis with nasal polyps (CRSwNP) and Antrochoanal polyp (ACP). A. HE staining of ACP (×100); B. HE staining of ACP (×400); C. HE staining of CRSwNP (×100); D. HE staining of CRSwNP (×400). ×100: scale bar = 100 μm, ×400: scale bar = 20 μm.

we further compared the eosinophil count, neutrophil count, neutrophil-to-lymphocyte ratio (NLR), and eosinophil-to-lymphocyte ratio (ELR) in the two groups, and the eosinophil count, NLR, and ELR in the CRSwNP group were significantly higher than those in the ACP group, whereas there was no significant difference in the neutrophil count between two groups.

3.2. Tight junctions are damaged in ACP

For clarifying the extent of epithelial barrier dysfunction in ACP, the mRNA expression levels of ZO-1, claudin-3 and occludin were analyzed. As shown in Fig. 2, compared to healthy controls and CRSwNP groups, the expression of ZO-1 were markedly ($P = 0.014$; $P = 0.001$) downregulated inACP groups, with the lowest expression levels in ACP groups. Occludin mRNA expression was significantly ($P = 0.001$; $P = 0.001$) decreased in ACP group than health controls and CRSwNP groups. The expression of claudin-3 was significantly downregulated in both ACP ($P < 0.001$) and CRSwNP ($P < 0.001$) groups in comparison with controls, and there was no significant difference between ACP and CRSwNP groups. We performed correlations between ZO-1, occludin and claudin-3 expression levels and ACP severity and found a negative correlation between ZO-1, claudin-3, occludin and Lund-Mackay scores ($r = -0.425$, $P = 0.022$; $r = -0.601$, $P = 0.001$; $r = -0.441$, $P = 0.017$; Fig. 2D–F). In terms of protein expression levels, immunofluorescence staining was shown that ZO-1, claudin-3, and occludin were less stained in the ACP and CRSwNP groups compared to healthy controls (Fig. 3D), and semi-quantitative analysis suggested that expression was markedly ($P = 0.004$; $P = 0.004$; $P = 0.005$) downregulated in ACP groups compared to healthy controls, and there was no change in expression levels between CRSwNP and ACP groups (Fig. 3A–C).

3.3. ZEB1 expression is upregulated in ACP

The level of ZEB1 expression in healthy controls and ACP groups were examined with qRT-PCR. As illustrated in Fig. 4A, the mRNA levels of ZEB1 were significantly ($P < 0.001$) elevated in ACP groups when compared to controls. The intensity of ZEB1 was higher ($P < 0.001$) in ACP group than in control group (Fig. 4B). ZEB1 was found to be expressed mainly in the epithelial layer of the nasal mucosa, and a significantly higher fluorescence intensity was observed in ACP than in control group (Fig. 4C).

3.4. ZEB1 is involved in epithelial barrier breakdown by regulated tight junctions

The knockdown and overexpression potencies of ZEB1 after transfection with si-ZEB1 or OE-ZEB1 were determined by qRT-PCR and Western blotting. Compared with control group, ZEB1 expression was highly increased in OE-ZEB1 group, whereas it was strongly decreased in si-ZEB1 group (Fig. 5A–C). The qRT-PCR showed that the mRNA expression of ZO-1, claudin-3 and occludin were considerably lower in the OE-ZEB1 group compared to control group; the levels of tight junction markers were more upregulated in si-ZEB1 group than in control group (Fig. 5D). Western blotting indicated that the expression of tight junction markers was greatly reduced in the OE-ZEB1 group compared with control group; the levels of ZO-1, claudin-3 and occludin were more strongly up-regulated in the si-ZEB1 group (Fig. 5C–E).

3.5. ZEB1 modulates IL-17A-mediated disruption of tight junctions

We compared the expression of IL-17A and IFN-γ in the healthy control and ACP groups. The expression of IFN-γ was significantly higher in ACP patients than in healthy controls (Fig. 6A), and the expression level of IL-17A was markedly elevated in the ACP group than in healthy controls (Fig. 6B). Using the 90% median of the healthy controls as the cut-off value, the expression levels of IFN-γ were all higher than the cut-off value in 56.8% of the ACP patients, and the expression levels of IL-17A were higher than the cut-off value in 91.9% of the ACP patients. In addition, our team compared the expression of IFN-γ and IL-17A in healthy controls and CRSwNP in the same period and found that 59% of patients of CRSwNP had IFN-γ expression above the cut-off value and 60% had IL-17A expression above the cut-off value [19]. There was a significant predominance of IL-17A expression in ACP.

IL-17A or si-ZEB1 was used to transfect hNECs, as shown in Fig. 6D, the mRNA expression of ZO-1, claudin-3 and occludin were clearly suppressed stimulated with IL-17A alone compared with the control group; the IL-17A + si-ZEB1 group reversed the expression levels of ZO-1, claudin-3 and occludin. In addition, we analyzed the protein expression levels of ZO-1, claudin-3 and occludin among those groups, and the protein expression levels of tight junction markers showed a consistent trend with the mRNA levels (Fig. 6C–E).

Table 1
Patient characteristics.

	Control (n = 20)	ACP (n = 37)	NP (n = 45)	P value
Gender/male (%)	11 (55%)	21 (56.8%)	31 (68.9%)	0.417
Age/year[a]	53 (36, 62)	21 (10, 34)	46 (34.5, 55.5)	**<0.001**
BMI[a]	25.29 (20.74, 28.71)	22.61 (15.72, 27.25)	26.53 (22.90, 28.21)	**0.046**
Allergic rhinitis	–	7	10	0.714
Smoking	1	5	9	0.280

Body Mass Index = BMI.
[a] Median (range).

Table 2
Patient characteristics.

	ACP(n = 37)	NP(n = 45)	P value
ide			**<0.001**
Left	20	3	
Right	15	1	
bilateral	2	41	
Recurrence rate	4	11	0.154
Lund-Mackay Score	5 (4, 10.5)	15 (2, 21.5)	**<0.001**
Peripheral blood cell counts			
Monocyte 10^9/L	0.36 (0.28, 0.47)	0.38 (0.29, 0.47)	0.734
Basophil	0.02 (0.02, 0.04)	0.02 (0.02, 0.05)	0.728
Platelet	284 (241, 330)	243 (198, 286)	**0.001**
Neutrophil	3.60 (3.01, 4.50)	4.48 (2.86, 6.07)	0.138
Lymphocytes	2.09 (1.57, 2.61)	1.84 (1.35, 2.16)	0.058
Eosinophils	0.15 (0.07, 0.26)	0.29 (0.08, 0.49)	**0.032**
NLR	1.76 (1.25, 2.26)	2.36 (1.41, 3.84)	**0.020**
PLR	134.83 (108.52, 168.43)	147.95 (93.49, 172.59)	0.906
LMR	0.18 (0.14, 0.23)	0.19 (0.16, 0.26)	0.131
ELR	0.06 (0.03, 0.10)	0.13 (0.06, 0.22)	**0.004**
SII	462.35 (329.66, 705.75)	441.79 (323.47, 1128.10)	0.561

Neutrophil to Lymphocyte ratio = NLR; Platelet to Lymphocyte ratio = PLR; Lymphocyte to Monocyte ratio = LMR; Eosinophil to Lymphocyte ratio = ELR; SII: Systemic immune-inflammation index = Neutrophil × Platelet/Lymphocytes.

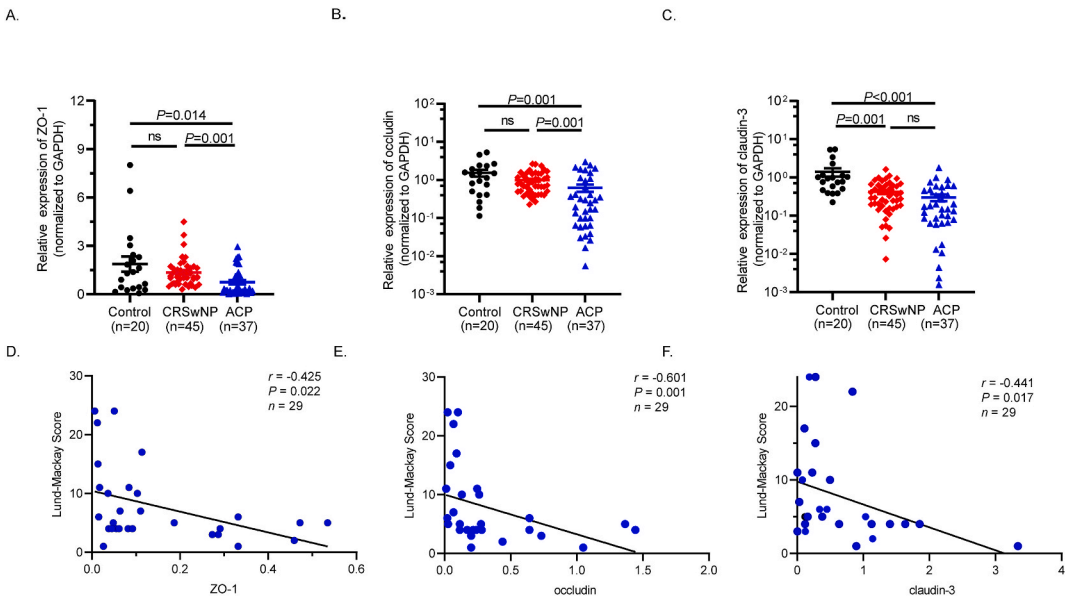

Fig. 2. Expression of TJs markers among Controls, CRSwNP and ACP and correlation with Lund-Mackay score in ACP. A-C. qRT-PCR examined ZO-1, occludin and claudin-3 mRNA expression in healthy control, CRSwNP and ACP groups; D-F. Spearman analysis showed the correlation between ZO-1, occludin and claudin-3 and Lund-Mackay scores in patients with ACP.

4. Discussion

In this study, we preliminary described the aberrant expression of epithelial tight junctions in ACP and CRSwNP. The expression of ZO-1, occludin and claudin-3 were downregulated in ACP or CRSwNP groups compared with controls, and the degree of epithelial barrier disruption were correlated with disease severity of ACP; there was no difference in the disruption of tight junctions between ACP and CRSwNP. ZEB1 was statistically higher in ACP group. Validation at the cellular level revealed that silencing ZEB1 relieved IL-17A-mediated downregulation of ZO-1, claudin-3 and occludin expression. In conclusion, ZEB1 may participate in the pathogenesis of ACP through the regulation of IL-17A-mediated destruction of epithelial barrier.

ACP is a long-term inflammatory disease of unknown etiology. The published literature reports that ACP presents in young people and is more common unilateral, slightly more frequent on the left than right, and rarely bilateral [20]. This was in accordance with our data, in which 90% of patients had unilateral lesions, with 13.5% higher incidence on left side than on right. In our study, 56.4% of ACP patients were male and the median age of onset was 21, significantly lower than CRSwNP group. One study finds that adults

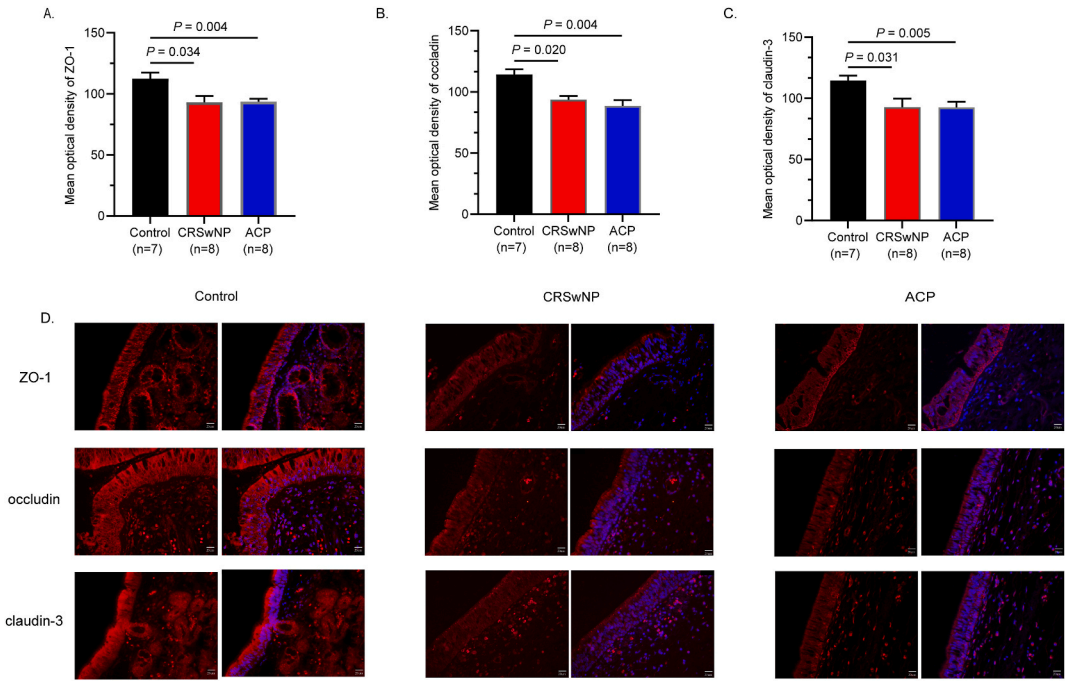

Fig. 3. Abnormal tight junction expression among Controls, CRSwNP and ACP. A-C. Immunofluorescence staining analyzed mean fluorescence intensity of ZO-1, occludin and claudin-3; D. Representative immunofluorescence staining for ZO-1, occludin and claudin-3 in Control, CRSwNP and ACP, scale bar = 20 μm.

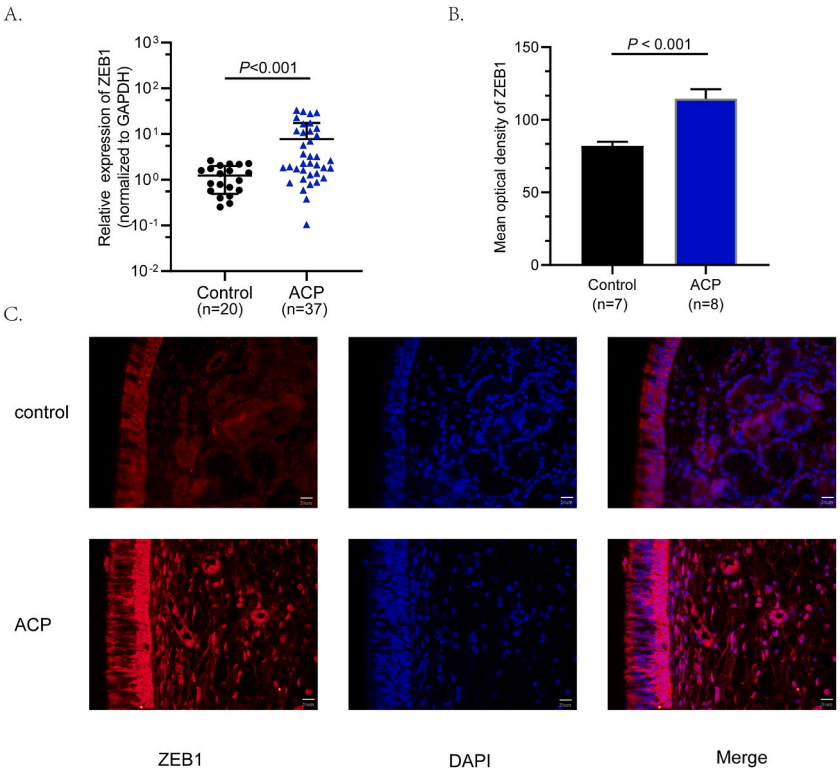

Fig. 4. ZEB1 was upregulated in ACP. A. Relative ZEB1 expression in the control and ACP groups was assessed by qRT-PCR; B. Protein expression levels of ZEB1 were detected by Immunofluorescence; C. Representative immunofluorescence staining for ZEB1 in Control and ACP, scale bar = 20 μm.

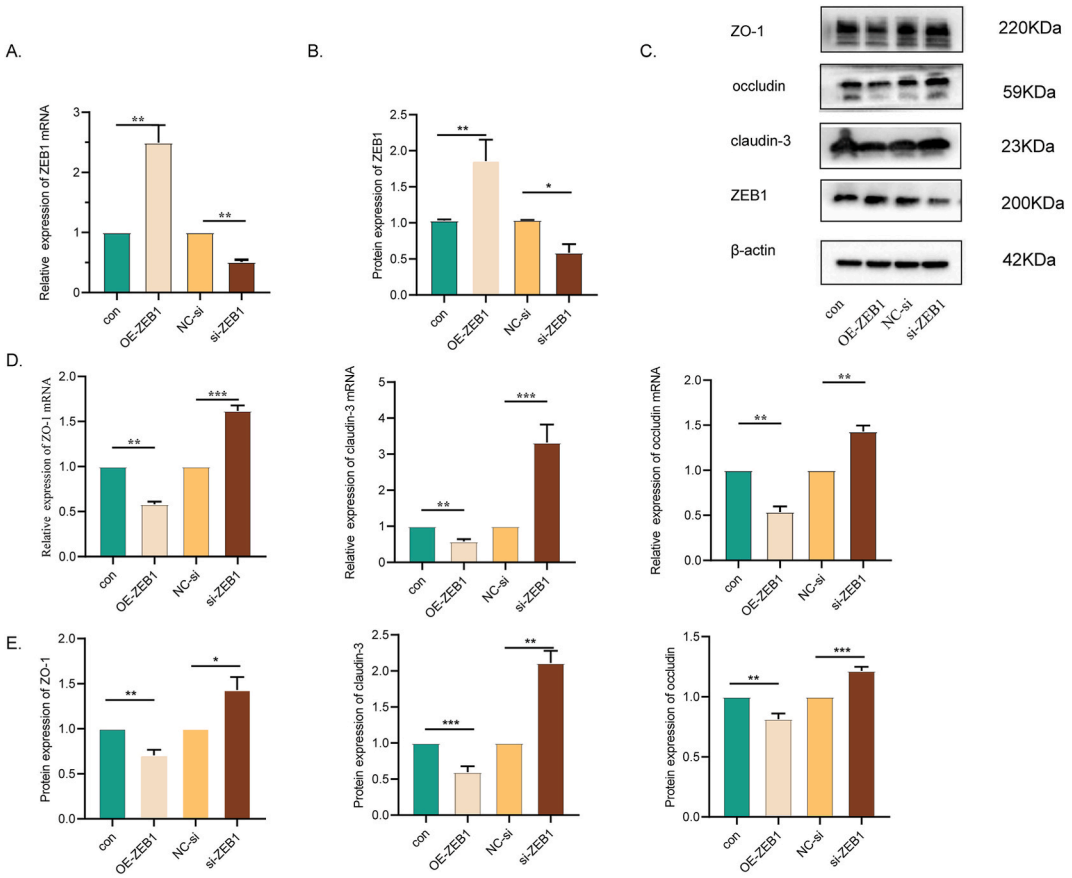

Fig. 5. ZEB1 inhibited cell tight junction in hNEpCs. A-B. Expression of ZEB1 was examined by qRT-PCR and Western bloting in si-ZEB1 or OE-ZEB1 groups; C. Western bloting showed expression of ZO-1 ，claudin-3 and occludin (Full, non-adjusted images are shown in Figure 5C.S); D-E. qRT-PCR and Western bloting analyzed the expression of tight junction markers. *P < 0.05; **P < 0.01; ***P < 0.001.

generally have a higher BMI than adolescents [21]. The significant difference in BMI between the three groups in our study may be due to the younger age of onset in the ACP group. NLR and ELR are widely used as major indicators of chronic inflammation in a number of disorders [22]. Compared to ACP, NLR and ELR were significantly increased in CRSwNP, suggesting that ACP was more like a limited inflammation of body compared to CRSwNP and did not cause a systemic inflammatory response. As the disease progresses, the increased disruption of epithelial barrier in ACP was accompanied by greater severity of sinus disease.

The mucosal surface epithelium forms a protective barrier between the environment and the subcutaneous tissue and is mainly composed of specific intercellular junctions that form the apical junctional complex [23]. Within this structure, tight junctions and adherens junction play a dynamic regulatory role. Changes in epithelial morphology and remodeling processes can be regulated by the assembly and disassembly of the apical junctional complex [24,25]. Our study demonstrated that the expression of TJs-associated protein (occludin, claudin-3 and ZO-1) were significantly downregulated in ACP and CRSwNP. In respiratory diseases, decreased expression of ZO-1, claudins and occludin results in increased airway epithelial barrier permeability and barrier disruption [26–28]. Marked rise in IL-4 in mice with house dust mite-mediated allergic rhinitis impairs occludin and ZO-1 expression, and degree of damage correlates with disease severity [29]. Tight junctions were abnormally expressed in ACP and the abnormality correlated with disease severity. Compared with chronic sinusitis without nasal polyps, CRSwNP has a higher epithelial permeability and more pronounced disruption of the mucosal barrier [30]. Our study showed that occludin, claudin-3 and ZO-1 were significantly reduced in ACP group compared to normal controls, with disrupted tight junctions and disordered epithelial barrier. However, there was no statistical difference of tight junction dysfunction between ACP and CRSwNP.

Chen et al. find that T1/3 inflammation accounts for 45.5% of ACP patients and is the most prevalent inflammatory endotype among all inflammatory subtypes; eosinophilic CRSwNP has the highest frequency of simple T2 and mixed T1/2/3 types, both at 27.6%; while non-eosinophilic CRSwNP has the highest proportion of T1 alone and mixed T1/3 at 24.1% [6]. The levels of IFN-γ and IL-17A expression are significantly enhanced in ACP, with 56.8% of ACP patients showing IFN-γ expression levels above the cut-off, and 91.9% of ACP patients having IL-17A expression levels above the cut-off. Therefore, we speculated that IL-17A may play a key role in the pathogenesis of ACP. Studies have revealed that IL-17A can attack microbes by promoting the recruitment of neutrophils or by stimulating the production of antimicrobial proteins [31,32]. IL-17A induces matrix metalloproteinases for tissue damage, as well as

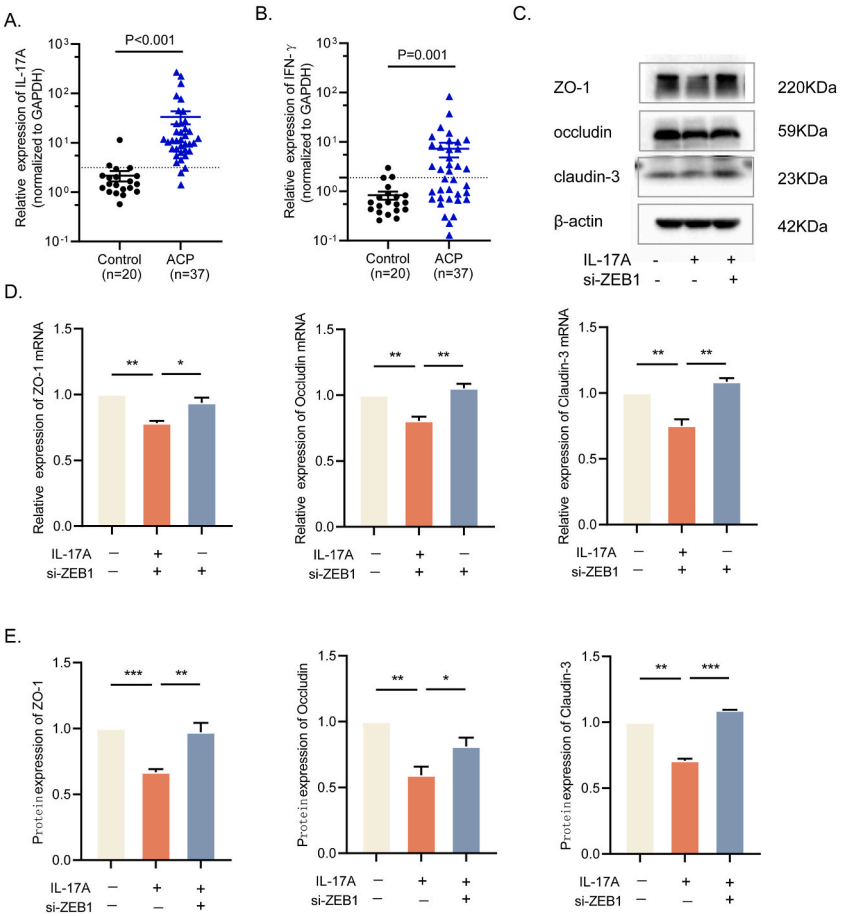

Fig. 6. ZEB1 regulated IL-17A mediated expression of tight junction markers in hNECs. A. qRT-PCR showed the expression levels of IL-17A in control and ACP groups, dotted line indicated 90th percentile normal control group; B. qRT-PCR demonstrated IFN-γ expression in healthy controls and ACP group, dotted line indicated 90th percentile normal control group; C. Representative western bloting of ZO-1, occludin and claudin-3 in groups (Full, non-adjusted images are shown in Figure 6C.S); D. qPCR analysis of mRNAs of ZO-1, occludin and claudin-3 in IL-17A or IL-17A + si-ZEB1group (n = 3); E. Western bloting tested the protein of ZO-1 , claudin-3 and occludin in IL-17A or IL-17A + si-ZEB1 group (n = 3). *P < 0.05; **P < 0.01; ***P < 0.001.

downregulation of ROS production and recombinant TJs-related molecules which leading to blood-brain barrier disruption [33]. In hNECs, IL-17A impaired the expression of ZO-1, occludin and claudin-3, and IL-17A may be involved in the disruption of epithelial barrier integrity in ACP.

ZEB1 is an important regulator of the epithelial mesenchymal transition. In normal epithelia, it causes a weakening of intercellular junctions and a permanent morphological change in cell phenotype from rectangular to spindle-shaped, in a process that is highly susceptible to the loss of tight junctions [34]. Abnormal expression and localization of ZEB1 are found in the beginning stages of idiopathic pulmonary fibrosis, and strong expression of ZEB1 is observed near alveolar epithelial cells with elevated extracellular matrix, supporting the implication of ZEB1 in the disease process of epithelial remodeling in pulmonary fibrosis [35]. We found that in patients with ACP, epithelial tight junctions were impaired and ZEB1 expression was highly upregulated. ZEB1 acted as a disruptive factor that promotes epithelial remodeling and severely damages epithelial tight junctions. Downregulation of ZEB1 expression in ACP alleviated IL-17A-mediated barrier damage.

There are limitations to our study. Firstly, we used a limited samples for the differential comparison of ACP and CRSwNP, thus inconsistent conclusions were obtained at the mRNA and protein level, which will need to be validated with larger samples in the future. However, when based on statistical differences between proteins, conclusions are more convincing. Secondly, although the results in tissue showed that both IFN-γ and IL-17A were significantly increased in ACP, we selected only IL-17A, the most important of these, as the relevant study for the pathological mechanisms of ACP. Thirdly, our study only explored the role of ZEB1 in IL-17A-mediated ACP, but its specific mechanisms and pathways of action are in need of further investigation.

5. Conclusion

Currently, the main treatment for ACP is surgical removal by nasal endoscopy. However, this is due to the higher recurrence rate of ACP and the younger age of onset, among other factors. The remodeling of the epithelium in ACP is a complex process that involves a large number of molecular and regulatory mechanisms. Our study showed that there is a disruption of the tight junctions in ACP. In our opinion, ZEB1 expression is significantly upregulated in ACP and that the modulation of IL-17A affects tight junctions across cells, and provides new insights into the pathogenesis of ACP.

Ethical approval and consent

The study was approved by Shandong Provincial ENT Hospital (China approval number: XYK-20200905). Informed consent was obtained from all individual participants included in the study.

CRediT authorship contribution statement

Yisha Wu: Writing – original draft. **Dingqian Hao:** Writing – original draft. **Yanyi Tu:** Resources. **Lin Chen:** Visualization. **Peng Yu:** Methodology. **Aiping Chen:** Supervision. **Yuzhu Wan:** Project administration. **Li Shi:** Writing – review & editing.

Declaration of competing interest

The authors report no conflicts of interest in relation to this work and declare that the research was conducted without any commercial or financial relationships construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25653.

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