The role of ZEB1 in regulating Tight junctions in Antrochoanal polyp

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Abstract

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. The samples were collected from 49 controls, 37 patients with ACP and 45 patients with nasal polyp (NP). qRT-PCR and immunofluorescence staining were performed to analyze the expressions of TJs markers (ZO-1, Claudin-3 and Occludin) and ZEB1. The expression levels of TJs markers were analyzed in primary human nasal epithelial cells (hNECs) transfected with IL-17A and ZEB1 small interfering RNA (si-ZEB1). The levels of ZO-1 and Occludin mRNA were significantly downregulated in the ACP compared with the control and NP groups, and the expression of Claudin-3 was markedly lower in the ACP than control group. Immunofluorescence staining showed that the staining intensity of TJs markers was significantly decreased in the ACP and NP groups, and there was no difference between NP and ACP groups. Meanwhile, ZEB1 expression was significantly upregulated in ACP group. In hNECs, The IL-17A group showed much weaker expression of ZO-1, Claudin3 and Occludin compared to the control group. IL-17A and si-ZEB1 group reversed the down-regulation of the expression of TJs-related molecules. In addition, we observed that the degree of disruption of tight junctions correlated with the severity of the disease. The tight junctions in the ACP were extremely damaged and were correlated with the severity of the disease. In conclusion, ZEB1 was involved in the pathogenesis of ACP mediated by IL-17A though regulating tight junctions.

Introduction

Antrochoanal polyp (ACP) is a chronic inflammatory condition of the upper respiratory tract. It usually originates from the maxillary sinus mucosa and enters the nasal cavity through the maxillary sinus natural or accessory foramen. Larger polyps may even have extension into the nasopharynx[1, 2]. ACP accounts for approximately 5% of all nasal polyps and 33% of nasal polyps in children, with adolescents predominating[1]. The cause of ACP is still unclear and may be associated with microbial (e.g. Mycoplasma pneumoniae) and viral (human papillomavirus, Epstein-Barr virus) infections, as well as lymphovascular obstruction[3–5]. ACP belongs to the Th1/Th3 type of inflammation, according to a recent study[6]. The epithelium of ACP is a single pseudo-complex ciliated columnar epithelium with an instable state that is more vulnerable to epithelial remodelling, suggesting that ACP may easily damage nasal mucosal defences.

In addition to providing a timely immune response to harmful stimuli, the nasal mucosa plays an important role as a natural defence barrier against the invasion of exogenous pathogens[7]. The epithelium serves as the first physical barrier to the inhalation of allergens and other pathogens[8]. The epithelium is a highly permeable barrier with three main types of junctions, including tight junctions (TJs), adherens junctions and desmosomes. Of these, the tight junctions play a key role in the defence of the barrier[9]. Tight junctions regulated the homeostasis of ions, water and certain macromolecules and located at the top of the epithelium, forming a barrier to selective permeation[10]. Tight junctions are mainly composed of three transmembrane proteins, occludin (OCLN), claudin (CLDN) and junctional
adhesion molecules (JAMs)[11]. Zonula-occludens (Zos) are cytoplasmic linker proteins that link transmembrane proteins to cytoskeletal proteins[12].

The tight junctions of the epithelium of the nasal mucous membrane are affected by inhaled allergens, pathogens and environmental pollutants. Subsequently, immune cells beneath the epithelium are activated. This promotes the onset and development of inflammation[13]. In allergic rhinitis, a Th2-related disease, OCLN expression is abnormally expressed, accelerating the passage of allergens and proinflammatory factors[14]. IL-13 has been found to mediate TJs dysfunction and weaken epithelial barrier function in chronic rhinosinusitis with nasal polyps (CRSwNP), the antiviral ability to respond to RSV infection is weakened[15]. Moreover, IL-17A stimulation in vitro significantly reduced occludin and ZO-1 expression in bronchial mucosal epithelial cells via the nuclear factor-κB-p65/tumour necrosis factor-α signalling pathway[16]. In addition, Th1-type inflammatory factor IFN-γ can inhibit the expression of Claudin-1 by regulating IFNGR/JAK/STAT, which is involved in the destruction of barrier function[17]. ACP is a chronic nasal disease characterised by a Th1/Th3 inflammatory response. Therefore, the changes in the epithelial barrier and the underlying mechanisms should be investigated.

Zinc finger E box binding protein 1 (ZEB1) is a transcriptional repressor mediating a variety of biological effects through downstream factors, including the regulation of JAK-STAT signaling to promote Th1 and Th17 cell differentiation and increased production of IFN-gamma and IL-17A[18]. Therefore, we hypothesized that ZEB1 could regulate epithelial barrier disruption. The aim of this study was to investigate the effect of ZEB1 on the severity and pathogenesis of ACP. In the present study we explored the extent of tight junction disorder in ACP. We identified a role for ZEB1 in the regulation of tight junctions in ACP pathogenesis and disease severity.

**Method**

**Clinical specimens**

The lesion sites of 37 patients with ACPs and 45 patients with nasal polyps (NP) who visited Shandong Provincial ENT Hospital from January 2020 to April 2022 were collected. Forty-nine healthy controls were obtained from uncinate processes of patients who underwent nasal septal deviation correction or skull base tumor resection during the same period. The diagnosis of NP was defined based on the 2020 European position paper on sinusitis and nasal polyps[19]. 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 ENT Hospital, and written informed consent was obtained from patients’ subjects.

**Cell culture and transfection**

Human nasal epithelial cell lines (HNEpCs) 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% CO₂. The cells were stimulated with ZEB1 small interfering RNA (si-ZEB1) or ZEB1 overexpressing plasmid (OE-ZEB1) (GenePharma, China) and transfected after 48h for subsequent experimental analysis.

**Primary cell culture and stimulation**

Primary human nasal epithelial cells (hNECs) were obtained from patients without chronic inflammation 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 have reached 80–90% confluence, the plates are spread out. The cells are stimulated for 48 hours with 100ng/ml IL-17A (R&D, USA) or accompanied by si-ZEB1 and the cells are harvested for subsequent analysis.

**Quantitative real-time PCR (qRT-PCR)**

RNA was extracted from nasal mucosa tissue 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 are 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.

**Western blotting**

Western blotting

Frozen tissues were added to protein lysis solution (Beyotime, China), crushed and allowed to stand on ice for 30 minutes 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 (ZEB1, Abcam; Occludin, Sigma; ZO-1, Abcam; Claudin-3, Abcam; USA, β-actin, ZSJQ; China) were incubated overnight. Incubation with HRP-conjugated goat secondary antibody. For visualisation and analysis, chemiluminescence kits (ECL, Bio-Rad, USA) were used.

**Immunofluorescence staining**

Paraffin sections were baked at 60°C for 2 hours, followed by dewaxing in a gradient of xylene and alcohol, antigen retrieval by microwave, 0.03% Triton X-100 retrieval for 10 minutes, 10% goat serum (ZSJQ, China) blocking for 1 hour, 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 hour. 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.

**Statistical analysis**

All data were statistically analysed using SPSS 26 and GraphPad Prism 8. Data were normally distributed and described as $(x \pm s)$, with t-test for between groups analysis and one-way analysis of variance (ANOVA) for three groups; not normally distributed and described as $M(Q_R)$, with Mann-Whitney test for between groups. The Spearman test was used for correlation analysis and differences were considered statistically significant at $p < 0.05$.

**Results**

1. **Population characteristics**

A total of 131 patients were enrolled in the study, including 49 healthy controls, 37 patients with ACP and 45 patients with NP. Typical pathological pictures of Hematoxylin -Eosin staining in patients with ACP and NP were demonstrated in Fig. 1. It can be seen that ACP was composed of a single layer of pseudostratified columnar ciliated epithelium and highly interstitial fibrosis; and epithelial cell hyperplasia, goblet cell metaplasia and eosinophilic infiltration were seen histologically in NP. Patients with ACP generally had a younger age of onset compared with the healthy and patients with NP, with a median age of 21 years. BMI were significantly lower for ACP compared to controls and NP groups. There were no differences between the three groups in terms of gender, smoking history or presence of allergic rhinitis (Table 2).
Table 1
Primer sequences

<table>
<thead>
<tr>
<th>gene</th>
<th>Sequences (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZO-1-F</td>
<td>TTGTCTTCAAAAAACTCCAC</td>
</tr>
<tr>
<td>ZO-1-R</td>
<td>GACTCAGGAGATAGCTTTTAG</td>
</tr>
<tr>
<td>Occludin-F</td>
<td>GGACTGGATCAGGGAATATC</td>
</tr>
<tr>
<td>Occludin-R</td>
<td>ATTCTTTATCCAAACGGGAG</td>
</tr>
<tr>
<td>Cldn3-F</td>
<td>CTGCATGGACTGTGAAAC</td>
</tr>
<tr>
<td>Cldn3-R</td>
<td>AAAATATCAAGTGCCCCTTC</td>
</tr>
<tr>
<td>GADPH-F</td>
<td>GCACCGTCAAGGCTGAGAAC</td>
</tr>
<tr>
<td>GADPH-R</td>
<td>TGGTGAAGACGCCAGTGGA</td>
</tr>
</tbody>
</table>

Table 2
Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 49)</th>
<th>ACP (n = 37)</th>
<th>NP (n = 45)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender/ male (%)</td>
<td>11(55%)</td>
<td>21(56.8%)</td>
<td>31(68.9%)</td>
<td>0.417</td>
</tr>
<tr>
<td>Age/year</td>
<td>53(36, 62)</td>
<td>21(10, 34)</td>
<td>46(34.5, 55.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI³</td>
<td>25.29(20.74, 28.71)</td>
<td>22.61(15.72, 27.25)</td>
<td>26.53(22.90, 28.21)</td>
<td>0.046</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>-</td>
<td>7</td>
<td>10</td>
<td>0.714</td>
</tr>
<tr>
<td>Smoking</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td>0.280</td>
</tr>
</tbody>
</table>

Table 3 compared the clinical parameters of patients with ACP and NP. ACP tended to have a unilateral onset, slightly more on the left than on the right, with 2 patients having bilateral lesions; NP tended 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 NP. Eosinophil counts, neutrophil to lymphocyte ratio (NLR) and eosinophil to lymphocyte ratio (ELR) were significantly higher in NP group than in ACP group, while neutrophil counts were not significantly different, but neutrophil counts were higher in ACP than in NP group.

Body Mass Index = BMI
Table 3
Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>ACP(n = 37)</th>
<th>NP(n = 45)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Side</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left</td>
<td>20</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>15</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>bilateral</td>
<td>2</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Recurrence rate</td>
<td>4</td>
<td>11</td>
<td>0.154</td>
</tr>
<tr>
<td>Lund-Mackay Score</td>
<td>5(4, 10.5)</td>
<td>15(2, 21.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Peripheral blood cell counts

<table>
<thead>
<tr>
<th></th>
<th>ACP</th>
<th>NP</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocyte 10^9/L</td>
<td>0.36(0.28, 0.47)</td>
<td>0.38(0.29, 0.47)</td>
<td>0.734</td>
</tr>
<tr>
<td>Basophil</td>
<td>0.02(0.02, 0.04)</td>
<td>0.02(0.02, 0.05)</td>
<td>0.728</td>
</tr>
<tr>
<td>Platelet</td>
<td>284(241, 330)</td>
<td>243(198, 286)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Neutrophil</td>
<td>3.60(3.01, 4.50)</td>
<td>4.48(2.86, 6.07)</td>
<td>0.138</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>2.09(1.57, 2.61)</td>
<td>1.84(1.35, 2.16)</td>
<td>0.058</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.15(0.07, 0.26)</td>
<td>0.29(0.08, 0.49)</td>
<td><strong>0.032</strong></td>
</tr>
<tr>
<td>NLR</td>
<td>1.76(1.25, 2.26)</td>
<td>2.36(1.41, 3.84)</td>
<td><strong>0.020</strong></td>
</tr>
<tr>
<td>PLR</td>
<td>134.83(108.52, 168.43)</td>
<td>147.95(93.49, 172.59)</td>
<td>0.906</td>
</tr>
<tr>
<td>LMR</td>
<td>0.18(0.14, 0.23)</td>
<td>0.19(0.16, 0.26)</td>
<td>0.131</td>
</tr>
<tr>
<td>ELR</td>
<td>0.06(0.03, 0.10)</td>
<td>0.13(0.06, 0.22)</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>SII</td>
<td>462.35(329.66, 705.75)</td>
<td>441.79(323.47, 1128.10)</td>
<td>0.561</td>
</tr>
</tbody>
</table>

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

2. Tight junction expression in healthy controls, ACP and NP groups

For clarifying the extent of epithelial barrier dysfunction associated with ACP, we analyzed the mRNA expression levels of ZO-1, Claudin-3 and Occludin. As shown in Fig. 2, Claudin-3 mRNA expression was significantly downregulated in ACP and NP by qRT-PCR and there was no significant difference between ACP and NP groups. Compared to healthy controls, the expression levels of ZO-1 and Occludin were markedly lower in both ACP and NP groups, with the lowest expression levels in the ACP group. We
correlated ZO-1, claudin-3 and occludin expression levels with ACP severity and found a negative correlation between ZO-1, claudin-3, occludin and CT Lund-Mackay scores ($r = -0.425, P = 0.022; r = -0.601, P = 0.001; r = -0.441, P = 0.017$; Fig. 3a-c). In terms of protein expression levels, immunofluorescence staining was shown that ZO-1, Claudin-3 and Occludin were less stained in the ACP and NP groups compared to healthy controls, and quantitative analysis suggested that expression was markedly downregulated in ACP group compared to healthy controls, and there was no change in expression levels either NP or ACP groups (Fig. 2d-g).

3. ZEB1 expression in healthy control and ACP groups

The level of ZEB1 expression in the healthy control and ACP groups was examined with qRT-PCR. As illustrated in Fig. 4a, the mRNA expression of ZEB1 was significantly elevated in ACP when compared to control group. The intensity of ZEB1 was higher in the ACP group than in control group (Fig. 4b). ZEB1 was found to be expressed mainly in the epithelial layer of the nasal mucosa, with a scattered distribution in the sub-epithelium (Fig. 4c).

4. ZEB1 was involved in epithelial barrier breakdown by regulated tight junctions

The knockdown and overexpression potency of ZEB1 after transfection with si-ZEB1 or OE-ZEB1 was determined by qRT-PCR and Western blotting. Compared with the control group, ZEB1 expression was highly increased in the OE-ZEB1 group, whereas it was strongly decreased in the 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 the control group; the levels of tight junction markers were more upregulated in the si-ZEB1 group than in the control group (Fig. 5d).

5. ZEB1 modulates IL-17A-mediated disruption of tight junction

When IL-17A or si-ZEB1 was used to transfect hNECs, as shown in Fig. 6, 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 could reverse the expression levels of ZO-1, Claudin-3 and Occludin. In addition, we analysed 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. 6d-g).

Discussion

In this study, we firstly described the aberrant expression of epithelial tight junctions in ACP. The expression of ZO-1, Claudin-3 and Occludin were downregulated in ACP group compared with healthy controls, and the degree of epithelial barrier disruption correlated with disease severity; there was no
difference in the disruption of tight junctions between ACP and NP. ZEB1 was mainly expressed in the mucosal epithelium and the expression level was statistically higher in the ACP group. Cell experiments revealed that silencing ZEB1 relieve 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 tight junction structures.

ACP is a long-term inflammatory disease of unknown aetiology. The published literature reports that ACP presented in young people and was more common unilateral, slightly more frequent on the left than the right, and rare bilateral[20]. This is in accordance with our data, in which 95% of patients had unilateral lesions, with a 13.5% higher incidence on the left side than on the right. In our cohort, 56.4% of ACP patients were male and the median age of onset was 21 years, significantly lower than in the NP group. NLR, ELR are widely used as major indicators of chronic inflammation in a number of disorders. NLR and ELR were considerably higher in NP than in ACP, suggesting that ACP is more like a limited inflammation of the body compared to NP and does not cause a systemic inflammatory response. As the disease progresses, the increased disruption of the epithelial barrier in ACP is accompanied by greater severity of paranasal sinus disease.

The epithelial barrier is the first defence of the body against the external environment and an intact mucosal barrier provides the host with protection against invading pathogens[7]. Tight junctions are positioned at the most apical point of the epithelium and play an intrinsic protective role in the epithelial barrier[21]. In respiratory diseases, decreased expression of the tight junction proteins ZO-1, claudin-1, claudin-3, claudin-4 and occludin all result in increased airway epithelial barrier permeability, and these molecules play a critical role in nasal mucosal epithelial barrier protection[15, 22, 23]. Marked rise in IL-4 in mice with house dust mite-mediated allergic rhinitis impaired Occludin and ZO-1 expression, and degree of damage correlates with disease severity[14]. Our study demonstrated that tight junctions were abnormally expressed in ACP and this abnormality correlates with disease severity. Compared with chronic sinusitis without nasal polyps, CRSwNP had a higher epithelial permeability and more pronounced disruption of the mucosal barrier[24]. Our study showed that occludin, claudin-3 and ZO-1 mRNA and protein levels were significantly reduced in ACP group when compared to normal controls, with disrupted tight junctions and disordered epithelial barrier. However, there was no statistical difference in the extent of tight junction dysfunction between ACP and NP. The literature confirmed that NP is mostly a type 2 with dominant pathogenesis of IL-13, and ACP is a Th1/3 inflammation, and we were further investigating the mechanisms of tight junction dysfunction in type 3 inflammation. Studies have revealed that IL-17A can attack microbes by promoting the recruitment of neutrophils or by stimulating the production of antimicrobial proteins[25, 26]. IL-17A can induce matrix metalloproteinases for tissue damage, as well as downregulation of ROS production and recombinant TJs-related molecules which lead to blood-brain barrier disruption[27]. In hNEpCS, IL-17A impaired the expression levels of occludin, claudin-3 and ZO-1, and IL-17A may be involved in the disruption of tissue epithelial barrier integrity in ACP.
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[28]. Within this structure, tight junctions and adherens junctions 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[29]. ZEB1 is an important regulator of the epithelial mesenchymal transition. In normal epithelia, it caused 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 cell junctions[30]. Abnormal expression and localization of ZEB1 was found in the beginning stages of idiopathic pulmonary fibrosis, and strong expression of ZEB1 was observed near alveolar epithelial cells with elevated extracellular matrix, supporting the implication of ZEB1 in the disease process of epithelial remodeling in pulmonary fibrosis[31]. We found that in patients with ACP, epithelial tight junctions were impaired and ZEB1 expression was highly upregulated, and cell experiments showed that ZEB1 acts 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.

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. In our opinion, there is a need for a better understanding of the pathogenesis of ACP and the search for new targets for treatment. The remodeling of the epithelium in ACP is a complex process that involves a large number of molecular and regulatory mechanisms. Our study shows that there is a disruption of the tight junctions of the ACP. Providing new insights into the pathogenesis of ACP, we found that ZEB1 expression is significantly upregulated in ACP and that the modulation of IL-17A affects tight junctions across cells.

Declarations

Funding This work was supported by Yu (Grant numbers ZR2021MH136).

Ethical Approval The study was approved by Shandong Provincial ENT Hospital.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent to publish Approved

Data Availability All data is available upon request

References


Figures
**Figure 1**

Pathological features of NP and ACP. HE staining of nasal polyps (NP); HE staining of the Antrochoanal polyp (ACP)
Aberrant expression of Tight junction markers in the ACP. A-C. qRT-PCR examined ZO-1, Claudin-3 and Occludin mRNA expression in healthy control, NP and ACP groups. D-F. Immunofluorescence staining analysis of mean fluorescence intensity of ZO-1, Claudin-3 and Occludin. G. Immunofluorescence staining localised ZO-1, Claudin-3 and Occludin, X400
Figure 3

Correlation analysis of tight junction markers and disease severity. Spearman analysis showing the correlation between ZO-1, Claudin-3 and Occludin and CT Lund-Mackay scores in patients with ACP.

Figure 4
ZEB1 was upregulated in ACP. A. Relative ZEB1 expression in the control, NP and ACP groups was assessed by qRT-PCR. B. Relative protein expression levels of ZEB1 were detected by IF. C. The localization of ZEB1 on nasal tissues from control, NP, ACP group.

**Figure 5**

ZEB1 inhibits cell Tight junction in hNEpCs. A-B. Expression of ZEB1 was examined by qRT-PCR and Western blotting in si-ZEB1 or OE-ZEB1 cells. C. Western blotting showed the expression of ZO-1 Claudin-3 Occludin. D-E. qRT-PCR and Western blotting analyzed the expression of tight junction markers. * P<0.05 ** P<0.01 *** P<0.001
Figure 6

ZEB1 regulates the IL-17A mediated expression of tight junction markers in hNECs. A-C. qPCR analysis of miRNAs of ZO-1 Claudin-3 Occludin in IL-17A or IL-17A+si-ZEB1 cell. D-G. Western blotting tested the protein of ZO-1 Claudin-3 Occludin in IL-17A or IL-17A+si-ZEB1 group.