p120 regulates E-cadherin expression in nasal epithelial cells in chronic rhinosinusitis*

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Abstract

Background: The epithelial barrier plays an important role in the regulation of immune homeostasis. The effect of the immune environment on E-cadherin has been demonstrated in previous studies. This discovery prompted new research on the targeting mechanism of E-cadherin in chronic rhinosinusitis (CRS).

Methods: E-cadherin and p120 expression was determined by quantitative RT–PCR, and western blot. The interaction between E-cadherin and p120 was assessed by immunofluorescence staining and coimmunoprecipitation assays. Human nasal epithelial cells (HNECs) were cultured with submerged methods and transfected with p120-specific small interfering RNA. In other experiments, HNECs differentiated with the air-liquid interface (ALI) method were stimulated with various cytokines and Toll-like receptor (TLR) agonists. The barrier properties of differentiated HNECs were determined by assessing fluorescent dextran permeability.

Results: E-cadherin and p120 expression was decreased in HNECs from patients with CRS, and the p120 protein expression level was positively correlated with that of E-cadherin. Two isoforms of p120 (p120-1 and p120-3) were expressed in HNECs, with p120-3 being the main isoform. Knocking down p120 in HNECs cultured under submerged conditions significantly reduced the E-cadherin protein expression. The Rac1 inhibitor NSC23766 reversed the protein expression of E-cadherin in p120 knockdown experiments. Inflammatory mediators, including IL-4, TNF-α, TGF-β1, LPS and IFN-γ, reduced E-cadherin and p120 protein expression and increased paracellular permeability. Dexamethasone abolished the downregulation of E-cadherin and p120 caused by inflammatory mediators.

Conclusions: p120 is involved in regulating E-cadherin protein expression in CRS. Dexamethasone may alleviate the reduction in E-cadherin and p120 protein expression caused by inflammatory mediators.

Key words: adherens junctions, epithelial barrier, inflammatory mediators, nasal polyps, tight junctions

Introduction

Chronic rhinosinusitis (CRS), characterized by inflammation of the nasal cavity and paranasal sinuses, occurs in approximately 8% of the population in Chinese mainland[1-3]. The integrity of the sinonasal epithelial barrier has an important role in the pathogenesis of CRS, not only as a physical barrier to protect the host from inhaled respiratory pathogens and particulates but also as a key factor in mucociliary clearance and host defense[1,4,5]. Epithelial cell junctions, including tight junctions (TJs), adherens junctions (AJs), and desmosomes, are of considerable importance in maintaining this physical barrier[5,6]. Therefore, understanding the mechanisms underlying cell junctions in the epithelial barrier may be helpful for elucidating the pathogenesis of CRS.

E-cadherin is a widely identified protein associated with maintaining epithelial barrier integrity and immune homeostasis[7-9]. Previous studies have shown that the downregulation of E-cadherin in patients with CRS with nasal polyps (CRSwNP) is

associated with epithelial barrier defects[5,10]. Although those studies have shown a reduction in E-cadherin levels in patients with CRS, the related regulatory mechanism remains unclear. Accumulating evidence has shown that TJs and closely related AJs are dependent on the stability of E-cadherin, which is influenced by p120 catenin (p120) binding[9,11,12]. An early study showed that p120 controls the degradation of vascular endothelial-cadherin in cultured primary human dermal microvascular endothelial cells[13]. In A431 cells expressing p120-specific siRNA, E-cadherin (along with α- and β-catenin proteins) is rapidly internalized, and intercellular adhesion is reduced and defective[11]. However, whether p120 is involved in the regulation of E-cadherin in patients with CRS is still an open question.
In this study, we investigated the expression of E-cadherin and p120 in patients with CRS and explored the possible mechanism associating E-cadherin and p120. Furthermore, we detected the effects of inflammatory mediators and dexamethasone on E-cadherin and p120 in vitro.

Material and Methods

Subjects

The study protocol was approved by the Ethics Committee of Tongji Hospital affiliated with Huazhong University of Science and Technology (Wuhan, China). Each patient provided written informed consent. Fifty-three control subjects, 32 patients with eosinophilic CRSwNP, 34 patients with noneosinophilic CRSwNP, and 31 patients with CRS without nasal polyps (CRSsNP), were recruited. CRS was diagnosed according to the European Position Paper on Rhinosinusitis and Nasal Polyps 2020 guidelines[1]. As defined in our previous study, CRSwNP was classified as eosinophilic when the percentage of tissue eosinophils exceeded 10% of the total infiltrating cells. This cutoff was calculated as twice the standard deviation of the mean eosinophil percentage in controls[14]. Polyp tissues from patients with CRSwNP and diseased sinus mucosa from patients with CRSsNP were obtained during surgery. Normal control sinus mucosa tissues were obtained from subjects undergoing sinus surgery due to sinus cysts or tumors. Human nasal epithelial cells (HNECs) were scraped from nasal polyps in patients with CRSwNP and from the middle meatus mucosa in control patients and CRSsNP patients during surgery, as described previously[15]. Allergic rhinitis was diagnosed based on the concordance between an atopic status and typical allergic symptoms[16]. Asthma was diagnosed based on patient history and a physician's diagnosis according to the Global Initiative for Asthma[17]. Intranasal corticosteroid sprays and oral glucocorticoids were discontinued ≥1 month and ≥3 months before surgery, respectively. No participants had received antileukotrienes or immunotherapy. Individuals were excluded if they had immunodeficiency, fungal sinusitis, cystic fibrosis, antrochoanal polyps, primary ciliary dyskinesia, systemic vasculitis, gastroesophageal reflux disease, or an acute infection of the upper respiratory tract. Given the limited number of tissue samples, not all samples were included in every study protocol. The clinical data for the patients are summarized in Table S1.

RNA extraction and quantitative real-time polymerase chain reaction (qRT–PCR)

Total RNA was extracted from HNECs by using TRI reagent (Invitrogen, Carlsbad, CA, USA). A total of 2 μg of RNA was reverse-transcribed into cDNA using a PrimeScript RT reagent kit (TaKaRa Biotechnology, Dalian, China). qRT–PCR was performed as reported previously with specific primers[15]. PCR was performed by using a SYBR Premix Ex Taq kit (TaKaRa Biotechnology) with appropriate primers (Table S2) on a StepOnePlus system (Applied Biosystems, Foster City, CA, USA). The amplification conditions were as follows: 95°C for 2 min, followed by 40 cycles of 95°C for 10 sec, the specific annealing temperature for 10 sec, and 72°C for 15 sec. Following PCR, a melting curve was constructed by increasing the temperature from 65°C to 95°C with a temperature transition rate of 0.1°C/s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a housekeeping gene for normalization, and relative gene expression was calculated using the 2-ΔΔCT method.

Western blotting

Total cellular protein was extracted from HNECs or cultured HNECs in radioimmunoprecipitation assay (RIPA) lysis buffer containing a 2% protease inhibitor cocktail (Guge Biotechnology, Wuhan, China). The cell lysates were centrifuged at 12,000 rpm and 4°C for 20 min, and the supernatants were harvested. The protein concentrations of the extracts were measured with a bicinchoninic acid (BCA) protein assay kit (Guge Biotechnology). Western blotting analysis was performed as previously reported[15]. Samples containing 40 μg of proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS/PAGE) on 10% gels and transferred to polyvinylidene difluoride (PVDF) membranes (Merck Millipore, Billerica, MA, USA). After blocking in Tris-buffered saline with Tween-20 (TBS-T) containing 5% skim milk powder for 1 h at room temperature, the PVDF membranes were incubated with primary antibodies directed against E-cadherin (1:3000; Abcam, Cambridge, MA, USA), p120 (1:3000; BD, Poway, CA, USA) and β-actin (1:4000; Guge Biotechnology) overnight at 4°C. After washing 3 times in TBS-T buffer, PVDF membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (goat anti-rabbit and goat anti-mouse,1:4000 Guge Biotechnology) for 1 hour at room temperature. The PVDF membranes were treated with ECL reagents (Guge Biotechnology), and the proteins were visualized using a chemiluminescence system (Bio–Rad Laboratories, Hercules, CA, USA). β-Actin was used as an internal standard to correct for variations in total protein loading. E-cadherin was

identified as a single band at approximately 128 kDa. p120 was identified as two bands at approximately 100-120 kDa, with the positions of 100 kDa and 120 kDa representing isoform 3 and isoform 1, respectively.

Histology and immunofluorescence staining of tissue sections

Fresh nasal tissue samples were fixed in a formaldehyde solution and embedded in paraffin. Paraffin sections (4 μm) were prepared from the tissue blocks. After deparaffinization and rehydration, sections were stained with hematoxylin and eosin to determine general pathologic features and count the numbers of eosinophils and total inflammatory cells, as previously reported[14]. For immunofluorescence staining, dewaxed and hydrated paraffin-embedded sections were subjected to heat-induced antigen retrieval (Dako, Carpinteria, CA, USA). The sections were blocked with 5% normal donkey serum for 40 min at room temperature and incubated with primary antibodies directed against E-cadherin (1:200; CST, Trask Lane Danvers, MA, USA) and p120 (1:200; BD) overnight at 4°C. The Alexa Fluor 488-conjugated goat anti-rabbit (1:100; Abbkine, CA, USA) or Cy3-conjugated goat anti-mouse (1:100; Abbkine) secondary antibody was incubated for 1 hour at room temperature. Subsequently, the sections were incubated with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI; Guge Biotechnology, Wuhan, China) for 5 min. The tissue sections were imaged with a confocal microscope (Nikon, Tokyo, Japan). Species- and subtype-matched antibodies were used as controls.

Coimmunoprecipitation (CoIP)

The coimmunoprecipitation assay has been described previously[18]. Briefly, cells were lysed in RIPA buffer supplemented with protease and phosphatase inhibitors in an ice–water bath. After centrifugation at 4°C, the supernatant was collected, and total protein concentrations were determined by a BCA assay (Guge Biotechnology). The specified antibody was added to clarified lysates for 2 hours at 4°C with rotation, followed by incubation with Sepharose-Protein G (GE Healthcare, Little Chalfont, Buckinghamshire, UK) for an additional hour at 4°C. The beads were washed five times with cold immunoprecipitation buffer, resuspended in 1× loading buffer, and heated for 5 min at 95°C. Western blotting was used to analyze the cell lysates and coimmunoprecipitates, as described above.

Cell culture and treatment

HNECs from control subjects were cultured as described elsewhere[15]. The concentrations of these reagents were selected based on previous reports and dose titration experiments[19-21]. Briefly, HNECs obtained by nasal scraping were washed twice in Dulbecco's modified Eagle's medium (Gibco-BRL, Invitrogen, Carlsbad, CA, USA) supplemented with penicillin/streptomycin and then grown submerged in bronchial epithelial cell basal medium (BEPiGM; ScienCell, Carlsbad, CA, USA) supplemented with SingleQuot Kit Suppl (ScienCell) in 6-well plates coated with rat tail collagen type I (Vitrogen, Collagen Biomaterials, Palo Alto, CA, USA) in a 5% CO_2-humidified atmosphere at 37°C. Upon confluence, they were shifted to air-liquid interface (ALI) culture by removing all of the apical medium, and the medium in the basal chamber was replaced with ALI medium consisting of BEBM/Dulbecco's modified Eagle's medium H (50:50; Invitrogen). HNECs were maintained in ALI culture for 21 days to induce differentiation. After HNECs differentiated, they were stimulated with 10 ng/mL interleukin (IL)-4, IL-5, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, IL-17A (all from R&D Systems, Minneapolis, MN, USA), transforming growth factor (TGF)-β1 (PeproTech, Rocky Hill, NJ, USA) or 1 μg/mL LPS (Sigma–Aldrich, St. Louis, MO, USA). In addition, IL-4, TNF-α, TGF-β1, LPS and IFN-γ were separately added to cultures with mixed dexamethasone at 10^{-7} mol/L (Sigma–Aldrich, St Louis, MO, USA). After 6 hours or 36 hours of stimulation, the cells were harvested for further analysis.

Transfection of HNECs

siRNA transfection was performed as previously described[22]. A human p120-specific small interfering RNA (p120 siRNA) oligonucleotide was obtained from Invitrogen Company (Carlsbad, CA, USA). The sense sequence of the p120 siRNA was 5′-GGCUAGAGGAUGACCAGCGUAGUAU-3′ (ID: HSS102463), and scrambled siRNA (siNC) was used as a negative control. siNC or siRNA (100 nM) and Lipofectamine 3000 (Invitrogen) were diluted in a reaction solution and incubated for 5 min at room temperature. Then, they were mixed, incubated for an additional 20 min and finally added to cultures to completely cover the HNECs. After an incubation for 8 hours, the transfection medium was replaced with bronchial epithelial cell medium to allow the cells to recover for 24 hours. After NSC23766 (MedChemExpress (MCE); Princeton, NJ, USA) treatment for 24 hours, the cells were harvested and subjected to qRT–PCR, immunofluorescence staining and western blotting.

Assessment of epithelial barrier integrity

Paracellular permeability was measured using 4 kDa fluorescein isothiocyanate–dextran (FD4; MCE, St. Louis, MO, USA) as previously described[19]. FD4 (2 mg/mL) was added apically to the ALI cultures on day 21, and the fluorescein isothiocyanate fluorescence intensity of the basolateral fluid was measured with a fluorescence reader (FLUOstar Omega; BMG Labtech, Ortenberg, Germany).

Immunofluorescence staining of cultured cells

Cultured cells were processed for immunofluorescence staining as previously described[18,19,23]. Cells were seeded on glass

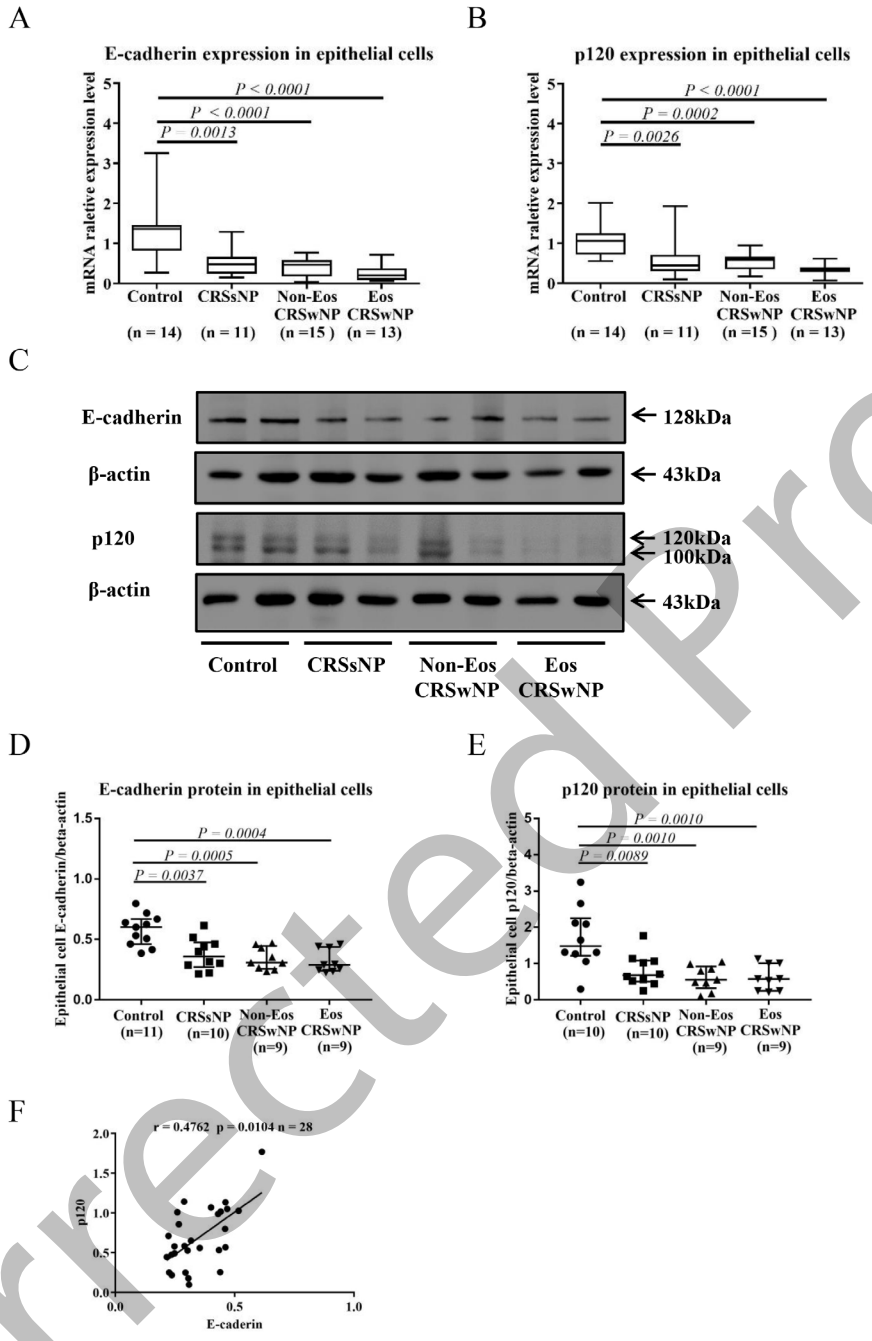

Figure 1. Aberrant expression of E-cadherin and p120 in nasal epithelial cells from patients with CRS. (A-B) The mRNA expression of E-cadherin and p120 from different study groups, as detected by using quantitative RT–PCR. (C–E) The protein expression levels of E-cadherin and p120 in whole-cell lysates of nasal epithelial cells, as detected by western blotting, and densitometric analysis of blots was performed. (F) The correlations between the levels of p120 protein and E-cadherin protein in HNECs from patients with CRS. Images show representative blots of target proteins. CRSsNP, chronic rhinosinusitis without nasal polyps; Eos CRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; Non-Eos CRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyps.

coverslips or Transwell and processed for immunofluorescence staining. Briefly, cells were fixed in 4% paraformaldehyde for 20 min and permeabilized in PBS/0.2% Triton X-100 for 5 min before blocking with 5% donkey serum. Specimens were incubated with primary antibodies (Table S3) at 4°C overnight and then incubated with a 1:100 dilution of an Alexa Fluor 488 or 594 conjugated secondary antibody (Abbkine Scientific Company, Wuhan, China) in the dark for 1 hour at room temperature, followed by mounting with antifade reagent containing DAPI. The samples were imaged with a fluorescence microscope (Olympus,

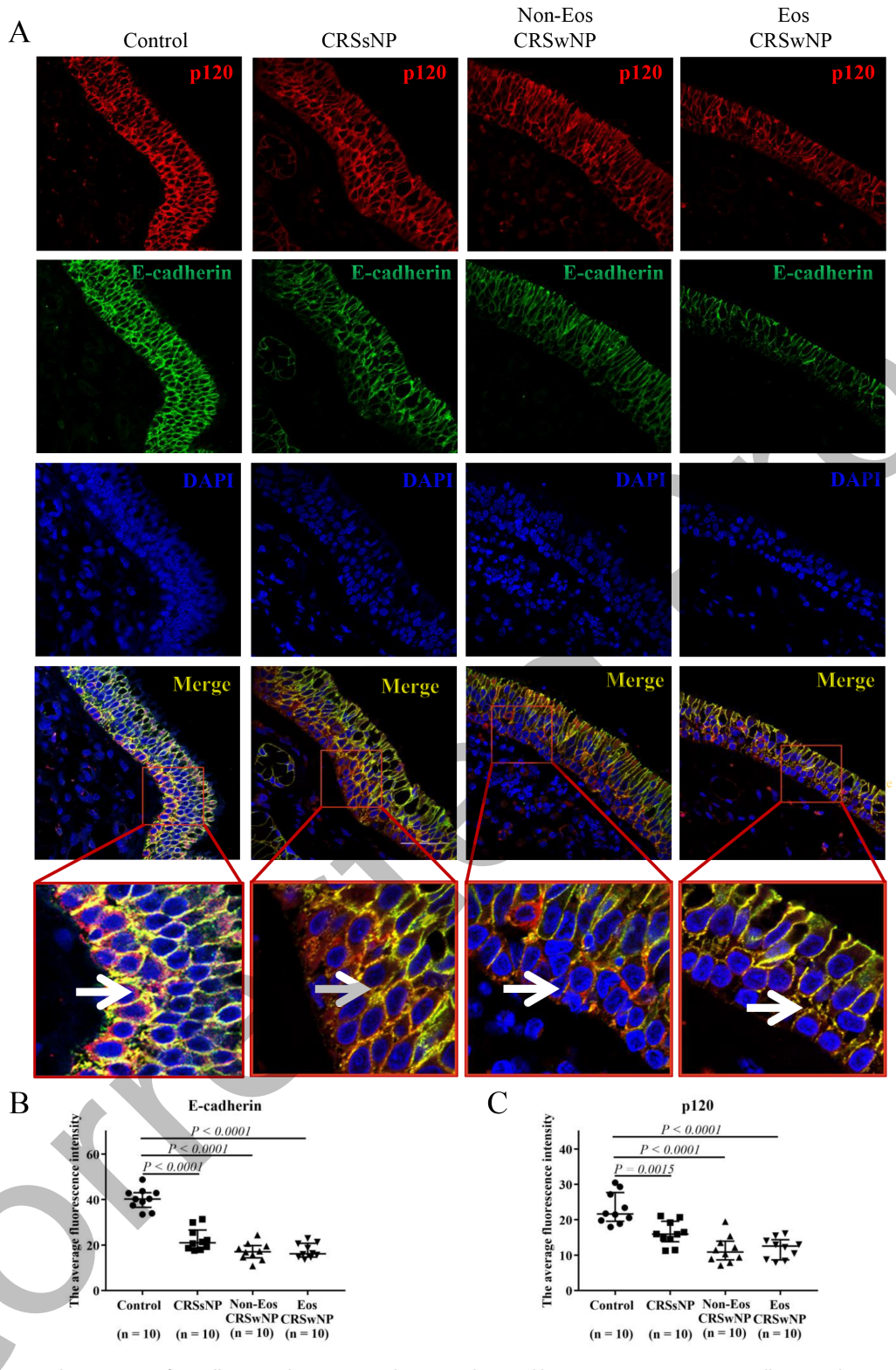

Figure 2. Localization and expression of E-cadherin and p120 in nasal mucosa detected by immunostaining. (A) E-cadherin and p120 expression in the epithelium of the nasal tissue was detected using double immunofluorescence staining. Representative photomicrographs showing double immunofluorescence staining for E-cadherin and p120 in the sinonasal mucosa (original magnification: 600×). In the bottom panel, a higher magnification image of the outlined area is shown. Arrows indicate the expression of E-cadherin and p120. (B–C) The mean fluorescence intensity of E-cadherin (B) and p120 (C) in the epithelium was quantified, and the results are presented as the mean gray values per unit area. Eos CRSwNP = eosinophilic chronic rhinosinusitis with nasal polyps; Non-Eos CRSwNP = noneosinophilic chronic rhinosinusitis with nasal polyps; CRSsNP = chronic rhinosinusitis without nasal polyps.

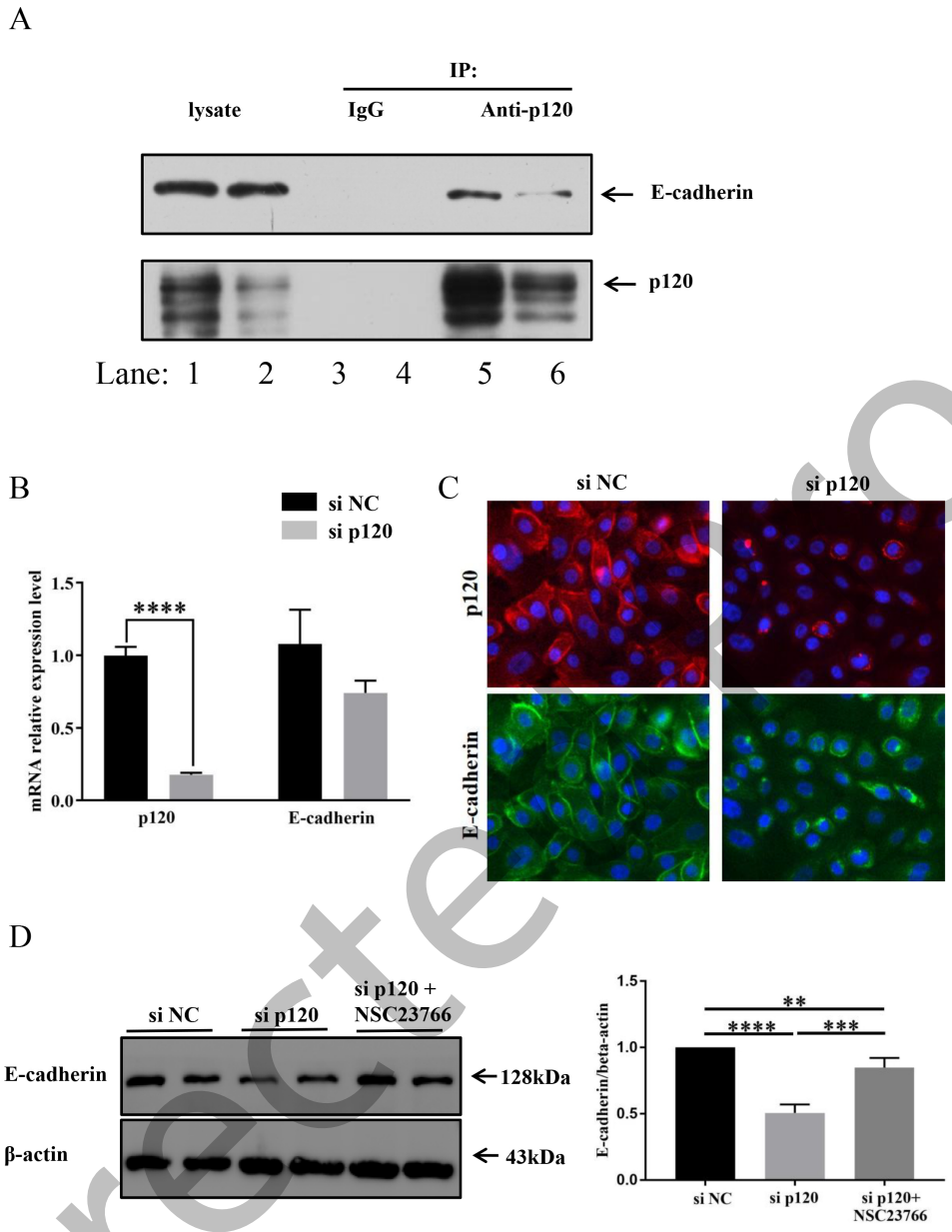

Figure 3. The regulation of E-cadherin expression by p120 in HNECs. (A) Coimmunoprecipitation of E-cadherin and p120 in HNECs. Coimmunoprecipitation of p120 with E-cadherin in HNECs. Lanes 1 and 2: whole-cell lysates. Lanes 3 and 4: immunoprecipitates from HNECs were generated with control antibody (IgG). Lanes 5 and 6: immunoprecipitates from HNECs were generated with anti-p120 antibody. Western blotting of cell lysates and immunoprecipitates was performed with antibody to E-cadherin and p120. HNEC samples of lane 1, 3 and 5 were from one same control subject, and HNEC samples of lane 2, 4 and 6 were from one same patient with CRSwNP (n = 3). (B–C) HNECs were transfected with p120 siRNA and E-cadherin and p120 mRNA (B) and protein (C) expression were detected by qRT–PCR and immunofluorescence staining, respectively (n = 6). (D) Western blotting was used to detect E-cadherin expression levels in HNECs transfected with the p120 siRNA and treated with the Rac1 inhibitor NSC23766 (n = 6). Representative images are shown. *P < 0.05, **P < 0.01, and ***P < 0.001.

Tokyo, Japan).

Statistical analyses

Data distributions were tested for normality using the Kolmogorov–Smirnov test or Shapiro-Wilk test. For continuous variables in the human tissue study, the results are expressed as medians and interquartile ranges or shown as box plots displaying the medians and interquartile ranges. The Kruskal–Wallis H test was used to assess significant intergroup variability, and the 2-tailed Mann–Whitney U test was used for two-group comparisons. For dichotomous variables, the chi-square test or Fisher's exact test was applied to compare the differences in proportions

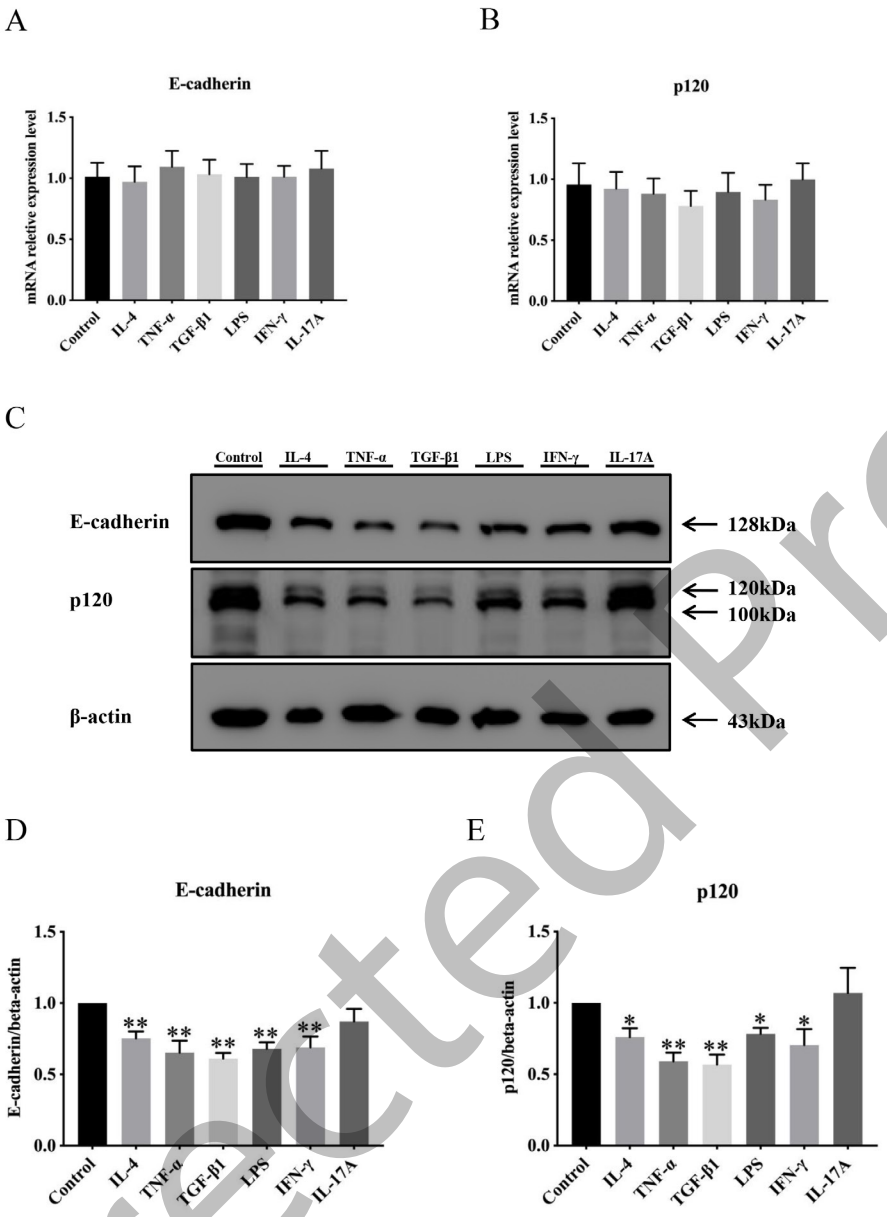

Figure 4. The regulation of p120 and E-cadherin by inflammatory factors in HNECs. The cells were cultured under ALI conditions. (A–B) The mRNA expression of E-cadherin (A) and p120 (B) in HNECs after stimulation with different cytokines for 6 h, as detected by qRT–PCR. (C) Images show representative blots of target proteins after stimulation with inflammatory factors for 36 h. (D–E) Densitometric analysis the expression of E-cadherin (D) and p120 (E) in cultured HNECs in response to IL-4, TNF-α, TGF-β1, LPS, or IFN-γ for 36 h (n = 6). *P < 0.05, **P < 0.01, and ***P < 0.001.

between groups. Spearman's rank correlation analysis was used to analyze correlations. For multiple comparisons among the 4 study groups, Bonferroni's correction was used to adjust the significance level by α = 0.05/6 = 0.008 for each comparison. Cell- culture study data are expressed as the means ± SEMs and were analyzed by using a t test. Data were analyzed with SPSS 22.0 software (SPSS, Chicago, IL, USA), with p<0.05 considered to indicate statistical significance.

Results

E-cadherin and p120 expression is decreased in patients with CRS

We investigated E-cadherin and p120 expression in HNECs by qRT–PCR. We observed a substantial decrease in E-cadherin and p120 mRNA expression in HNECs from patients with CRS, including CRSsNP, noneosinophilic CRSwNP, and eosinophilic CRSwNP, compared with those from control subjects (Figure 1A and B). The mRNA expression of E-cadherin and p120 did not differ among the CRSsNP, noneosinophilic CRSwNP, and eosinophilic CRSwNP groups.

We further measured the protein expression of E-cadherin and p120 in HNECs by western blotting (Figure 1C). The protein

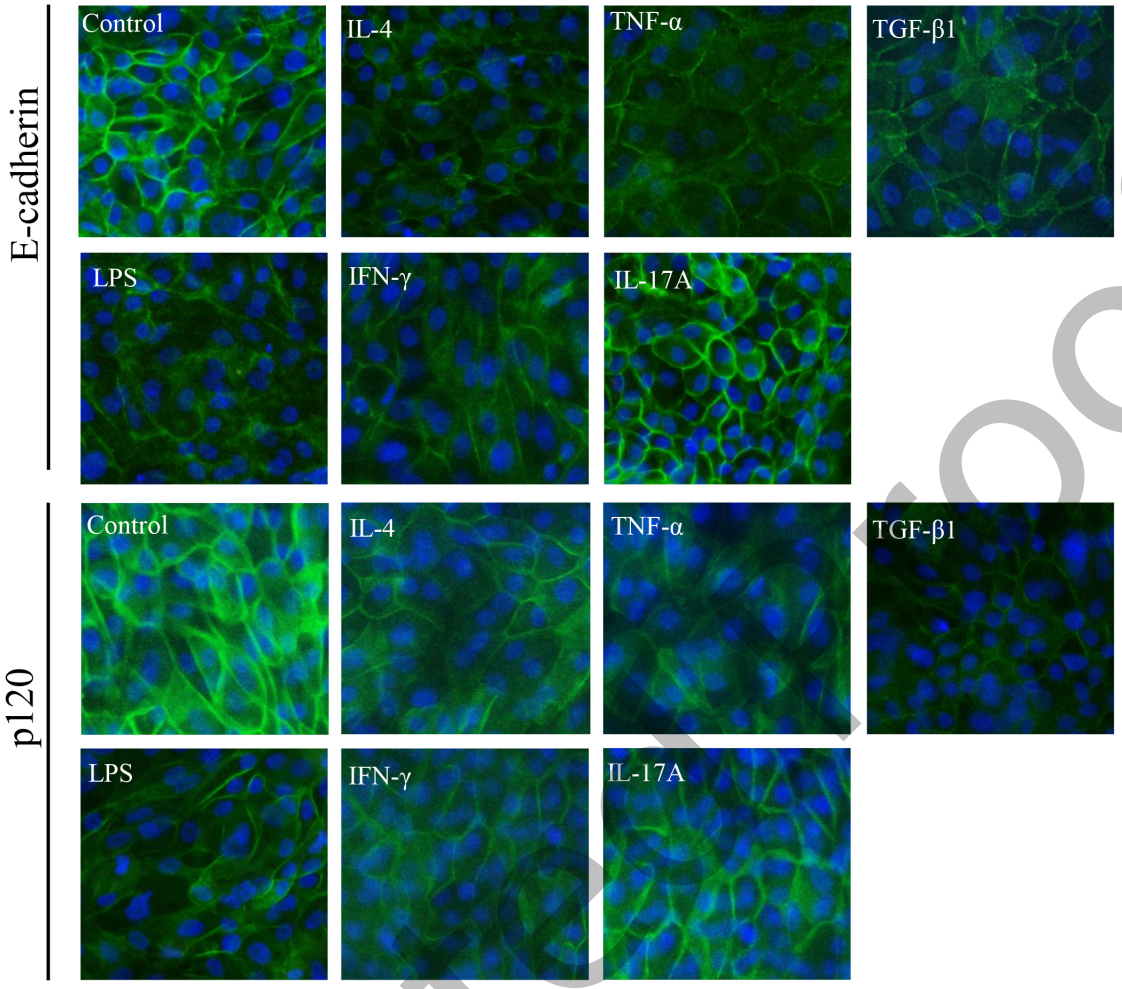

Figure 5. Inflammatory mediators disrupted the expression of the E-cadherin and p120 in HNECs as detected by immunostaining. HNECs were cultured under ALI conditions and treated with inflammatory factors, including IL-4, TNF-α, TGF-β1, LPS, IFN-γ and IL-17A, for 36 h. E-cadherin and p120 protein were detected by immunofluorescence staining (n = 6). Representative images of target proteins are shown (original magnification: 400×).

expression levels of both E-cadherin and p120 were significantly reduced in HNECs from patients with CRS compared with control subjects (Figure 1D and E). There was no difference in E-cadherin and p120 protein expression among CRSsNP, noneosinophilic CRSwNP, and eosinophilic CRSwNP patients (Figure 1D and E). p120 was expressed mainly in two forms, the p120-3 (100 kDa) and p120-1 (120 kDa) isoforms, and p120-3 was the main isoform in the nasal epithelium (Figure 1C). Intriguingly, E-cadherin was positively correlated with p120 at the protein level in nasal epithelial cells (Figure 1F).

p120 binds with E-cadherin and regulates E-cadherin protein expression in HNECs

To determine whether p120 physically associates with the E-cadherin protein, immunofluorescence staining and coimmunoprecipitation were performed with HNECs. Using double immunofluorescence staining, the E-cadherin and p120 proteins were consistently expressed and colocalized in the nasal epithelium (Figure 2A), and their levels were significantly reduced in the epithelium of patients with CRS (Figure 2B and C). p120 coimmunoprecipitated with E-cadherin from nasal epithelial cells using anti-p120 monoclonal antibodies but not a negative control antibody, and a moderate amount of E-cadherin consistently coimmunoprecipitated with a relatively large amount of p120 (Figure 3A). To study the regulation of E-cadherin by p120, we knocked down p120 in submerged cultured HNECs in vitro. Although knockdown of the p120 gene had no significant effect on E-cadherin mRNA expression in HNECs ($p = 0.2935$; Figure 3B), a significant reduction in E-cadherin protein levels was observed after p120 knockdown by immunofluorescence staining and western blotting (Figure 3 C and D).

Previous studies reported that downregulation of p120 protein may activate Rac1, which promotes E-cadherin protein degradation [24-26] To explore the mechanism underlying the effects of p120 on E-cadherin, we evaluated the effects of the Rac1 inhibitor NSC23766 in p120 knockdown experiments. After p120

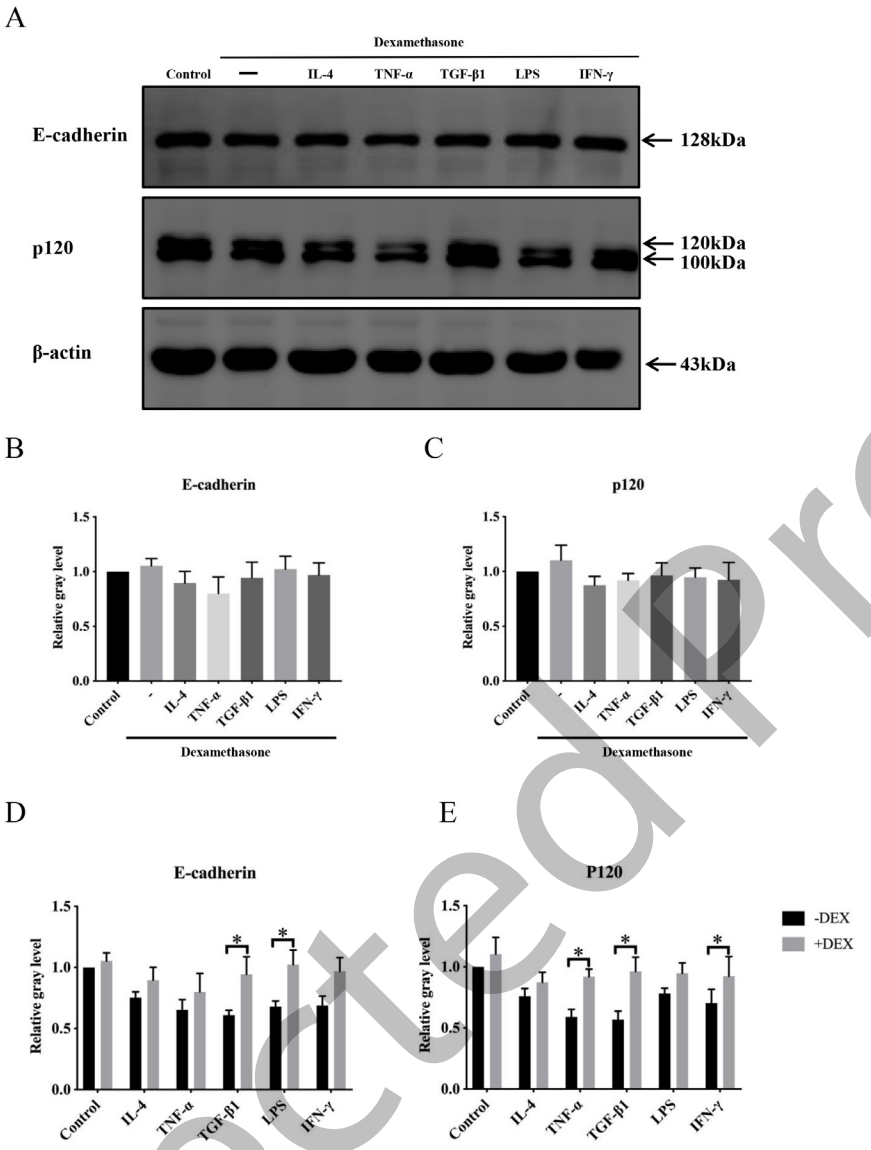

Figure 6. Dexamethasone alleviated the reductions in E-cadherin and p120 protein expression induced by inflammatory mediators. (A–C) The HNECs were cultured with ALI methods. E-cadherin and p120 protein expression were measured after dexamethasone treatment alone or in combination with different inflammatory factors, including IL-4, TNF-α, TGF-β1, LPS, and IFN-γ (n = 6). Images show representative blots of target proteins (A). Densitometric analysis of the E-cadherin (B) and p120 (C) protein levels in HNECs treated with dexamethasone alone or in combination with different inflammatory factors. (D–E) Comparison of the E-cadherin (D) and p120 (E) protein levels in HNECs treated with different inflammatory factors alone, or in combination with dexamethasone. *P < 0.05 and **P < 0.01.

knockdown in HNECs, E-cadherin protein expression was down-regulated, whereas NSC23766 treatment inhibited this response (Figure 3D).

Inflammation "tuned" the protein expression of E-cadherin and p120 but not the mRNA expression

The reduced E-cadherin and p120 expression in patients with CRS prompted us to explore the regulation of E-cadherin and p120 expression in ALI cultured HNECs by cytokines and LPS, which are implicated in the pathogenesis of CRS. We found that the TLR4 agonist LPS, proinflammatory mediators (TNF-α and TGF-β1), type 1 cytokine IFN-γ, type 2 cytokine IL-4, and type 3 cytokine IL-17A alone did not change the mRNA expression of E-cadherin and p120 after 6 hours of treatment (Figure 4A and B). However, LPS, TNF-α, TGF-β1, IL-4, and IFN-γ reduced the E-cadherin and p120 protein expression levels after 36 hours of treatment (Figure 4C and E). Consistently, immunostaining for E-cadherin and p120 became dimmer and more obscure in response to IL-4, TNF-α, TGF-β1, LPS, and IFN-γ treatments, with an apparent decrease in the staining area along the plasma membrane (Figure 5). E-cadherin and p120 protein expression were not significantly different in IL-5-stimulated cultures (Figure

S2). In addition, ALI cultured HNECs basolaterally treated for 36 hours with 10 ng/mL IL-4, TNF-α, TGF-β1, IFN-γ and 1 μg/mL LPS, showed a significant increase in macromolecular permeability to FITC-labeled 4-kDa dextran compared with that of the untreated controls, but no effect was observed for IL-17A (Figure S3).

Dexamethasone alleviates the destructive effects of inflammatory factors

Glucocorticoid has been reported to inhibit inflammatory cytokines released by inflammatory cells and protect epithelial barriers[5,27,28]. We investigated the effects of dexamethasone on E-cadherin and p120 expression in ALI cultured HNECs by western blotting (Figure 6A). We did not observe significant differences in E-cadherin and p120 protein expression in ALI cultures treated with dexamethasone compared with the control group (Figure 6B and C). Compared with the control group, dexamethasone combined with LPS, proinflammatory cytokines (TNF-α and TGF-β1), type 1 cytokine (IFN-γ) or type 2 cytokine (IL-4) alone did not significantly alter E-cadherin and p120 protein expression (Figure 6B and C). These data suggested that dexamethasone may alleviate the disruption of AJs caused by inflammatory mediators. Thus, we compared the effect of inflammatory mediators and dexamethasone combined with inflammatory mediators on E-cadherin and p120 expression. The E-cadherin protein level was significantly increased in differentiated HNECs treated with dexamethasone combined with TGF-β1 or LPS, compared with those treated with TGF-β1 or LPS alone (Figure 6D). Moreover, p120 protein expression was increased in differentiated HNECs treated with dexamethasone combined with TNF-α, TGF-β1 or IFN-γ compared with those treated with TNF-α, TGF-β1 or IFN-γ alone (Figure 6E).

Discussion

E-cadherin, the main AJ protein, is a crucial regulator of epithelial cell–cell contacts and immunity in the airway epithelium[7-9]. Limited studies have reported the potential pathological roles and regulatory mechanism of E-cadherin in CRS. Consistent with a previous study[10], we observed decreased E-cadherin expression in patients with CRS. Furthermore, we revealed that p120 was downregulated in patients with CRS and showed positive correlation with E-cadherin at the protein level. p120 exists as four isotypes in humans (p120-1, p120-2, p120-3 and p120-4), and bronchial epithelial cells express p120-1 and p120-3[12,29]. In our study, we found that HNECs expressed two isoforms (p120-1 and p120-3), with p120-3 being the predominant type. Meaningfully, p120 combined with E-cadherin in HNECs was detected by double immunofluorescence staining and coimmunoprecipitation, suggesting an interaction between E-cadherin protein and p120 protein.

E-cadherin is internalized from plasma membrane with degradation or recycling as potential fates[30]. p120 binds to the cytoplasmic domain in juxtamembrane regions of E-cadherin protein, stabilizes it, and prevents it from degradation[11,31]. Our data showed that knockdown of p120 in HNECs reduced the expression of E-cadherin protein. This prompted us to explore the regulation mechanism by which p120 regulates E-cadherin. Previous studies indicated that Rac1, a small GTP -binding protein, participates in regulating E-cadherin degradation[24-26]. Liu et al. found that knockdown of p120 significantly increased the Rac1 activity and decreased the E-cadherin protein expression in lung cancer cell lines[32]. The facts that knockdown of p120 expression promotes E-cadherin protein degradation and inhibiting Rac1 activity rescues E-cadherin protein expression suggest that p120 regulates the expression of the E-cadherin protein through Rac1 in HNECs.

The inflammatory mediators released from inflammatory cells disrupt the nasal mucosal epithelial barrier[5,21,33]. Wise et al. demonstrated that IL-4 and IL-13 decreased the transepithelial electrical resistance (TEER) of ALI cultures and reduced the levels of TJ proteins in HNECs[33]. Hardyman et al.[19] found that TNF-α reduced TEER and increased fluorescent dextran permeability, accompanied by a loss of TJ proteins and redistribution of p120 catenin and E-cadherin in differentiated human bronchial epithelial cells. Jiao et al.[20] proved that TGF-β1 decreased epithelial TJ integrity in ALI-cultured HNECs. Although these studies indicated that inflammatory mediators participate in the destruction of the epithelial barrier in airway diseases, the mechanism involved in the regulation of p120 and E-cadherin in CRS has rarely been studied. Our data showed that inflammatory mediators (IL-4, TNF-α, TGF-β1, IFN-γ and LPS) inhibited p120 and E-cadherin protein expression and disrupted paracellular permeability in differentiated HNECs, which might weaken the nasal epithelial barrier.

The role of IL-17A in the epithelial barrier of patients with CRS remains controversial. Ramezanpour et al. identified IL-17A as a cytokine impairing the epithelial barriers of HNECs under ALI conditions[34]. While Soyka et al.[21] reported that IL-17A has no effect on the epithelial barrier in ALI cultures[43,44]. Our data showed that p120 and E-cadherin protein expression were unaffected in IL-17A-stimulated ALI cultures, and paracellular permeability was not significantly altered. More studies concerning the effects of IL-17A on AJ proteins and the epithelial barrier are needed in the future.

Glucocorticoid is a first-line drug for CRS that exerts broad anti-inflammatory effects[1]. Previous research has demonstrated that dexamethasone reverses the degradation of E-cadherin caused by TNF-α in normal human bronchial epithelial cell lines[27]. We found that dexamethasone rescued the downregulation of p120 and E-cadherin protein levels induced by inflammatory mediators such as IL-4, TNF-α, TGF-β1, IFN-γ and LPS, indicating that dexamethasone may alleviate the reduction of p120 and E-cadherin caused by inflammatory mediators.

In this study, one limitation is that we did not detect the TEER of differentiated HNECs because of a lack of experimental facilities. Instead, we used 4 kDa fluorescein isothiocyanate–dextran to examine the paracellular permeability of differentiated HNECs. Previous studies have shown that TEER and paracellular permeability are comparable in evaluating the epithelial barrier[21]. Another limitation is that we did not detect the effects of the innate defence cytokines TSLP and IL-33 on E-cadherin and p120, which have been reported to play an important role in epithelial integrity. Their effects on E-cadherin and p120 in CRS deserve further study. Last, the hydrocortisone in cell culture medium may skew the effect of inflammatory mediators. However, given the low concentrations of hydrocortisone (7×10^{-7} mol/L) in medium and the setting of control group with only medium treatment, we do not think that hydrocortisone in cell culture medium will change our conclusion.

Conclusion

Together, our findings underscore the regulation of E-cadherin by p120 in CRS. Our study not only improves the understanding of the regulation of E-cadherin in CRS but also provides a new idea regarding the protective mechanism of the epithelial barrier in CRS.

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None.

Authorship contribution

Conceptualization: XLL, QMF. Data curation: XLL QMF. Formal analysis: XLL. Funding acquisition: XL, ZL, YHC. Methodology: HNY, JWR, YFK, ZEY, JXL, ANC. Supervision: XL, ZL. Writing - original draft: XLL. Writing - review & editing: XLL, ZL.

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Conflict of interest

There is no conflict of interest in relation to this study and the results described in the manuscript.

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This manuscript contains online supplementary material

SUPPLEMENTARY MATERIAL

Table S1. Demographic characteristics of the enrolled subjects.

	Control	CRSsNP	Non-Eos CRSwNP	Eos CRSwNP	P value
Total subjects enrolled	53	31	34	32	
RT-PCR					
Subject no.	14	11	15	13	
Male sex	11 (79%)	8 (73%)	9 (60%)	7 (54%)	0.529
Age (y)	32 (20-45)	38 (31-51)	45 (34-59)	53 (47-58)	0.011
Patients with atopy	0 (0%)	1 (9%)	0 (0%)	2 (15%)	0.189
Patients with AR	0 (0%)	2 (18%)	0 (0%)	2 (15%)	0.103
Patients with asthma	0 (0%)	0 (0%)	0 (0%)	1(8%)	0.453
Immunofluorescence					
Subject no.	10	10	10	10	
Male sex	7 (70%)	5 (50%)	4 (40%)	6 (60%)	0.715
Age (y)	26 (17.75-42)	50 (35-55.)	50 (37-54)	46 (35-61)	0.051
Patients with atopy	0 (0%)	0 (0%)	0 (0%)	2 (20%)	0.231
Patients with AR	0 (0%)	2 (20%)	0 (0%)	1 (10%)	0.595
Patients with asthma	0 (0%)	0 (0%)	0 (0%)	1 (10%)	1.000
Western blotting					
and CoIP					
Subject no.	11	10	9	9	
Male sex	9 (82%)	6 (60%)	4 (44%)	3 (33%)	0.159
Age (y)	20 (18-32)	47 (33-52)	54 (36-62)	50 (34-52)	0.003
Patients with atopy	0 (0%)	1 (10%)	0 (0%)	2 (22%)	0.199
Patients with AR	1 (9%)	3 (30%)	0 (0%)	3 (33%)	0.171
Patients with asthma	0 (0%)	0 (0%)	0 (0%)	2 (22%)	0.097
Cell culture					
Subject no.	18				
Male sex	15 (83%)	-	-	-	-
Age (y)	28 (23-35)	-	-	-	-
Patients with atopy	0 (0%)	-	-	-	-
Patients with AR	0 (0%)	-	-	-	-
Patients with asthma	0 (0%)	-	-	-	-

For continuous variables, the results are expressed as medians and interquartile ranges. Categorical variables are summarized using percentages. AR, allergic rhinitis; CRSsNP, chronic rhinosinusitis without nasal polyps Eos CRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; Non-Eos CRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyps.

Table S2. Primers used for the quantitative PCR analysis.

Target	Primer sequence	Annealing temperature (°C)
p120	(F)5′-GGACACCCTCTGACCCTCG-3′	62
	(R)5′-GCTTGCTAAACTTCCTCGCTC-3′	
E-cadherin	(F)5′-CGAGAGCTACACGTTCACGG-3′	60
	(R)5′-GGGTGTCGAGGGAAAAATAGG-3′	
GAPDH	(F)5′-GGAGCGAGATCCCTCCAAAAT-3′	60
	(R)5′-GGCTGTTGTCATACTTCTCATGG-3′	

Table S3. Primary antibodies used in western blotting, immunofluorescence and coimmunoprecipitation studies.

Specificity	Species	Concentration	Source
p120	Mouse	IF: 1:100 WB: 1: 3000 CoIP: 1:1000	BD, Poway, CA, USA
E-cadherin	Rabbit	IF: 1:100	CST, Trask Lane Danvers, MA, United States
E-cadherin	Mouse	IF: 1:100 WB:1: 3000	Abcam, Cambridge, MA, USA
β-actin	Rabbit	WB:1: 4000	Guge Biotechnology (Wuhan, China)

Table S4. Secondary antibodies used for immunofluorescence studies and western blotting

Specificity	Species	Concentration	Source
Alexa Fluor 568	Donkey	1:100	Abbkine (Wuhan, China)
Alexa Fluor 488	Donkey	1:100	Abbkine (Wuhan, China)
Goat anti-rabbit antibody	Goat	1:4000	Boster Biotechnology, Wuhan, China
Goat anti-mouse antibody	Goat	1:4000	Boster Biotechnology, Wuhan, China

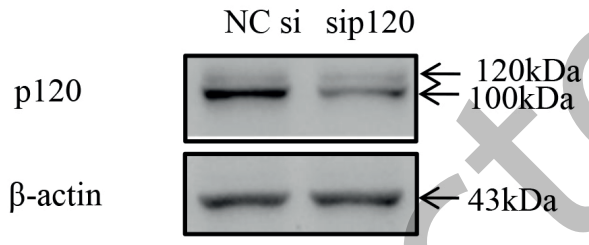

Figure S1. The efficiency of p120 siRNA in cultured HNECs.

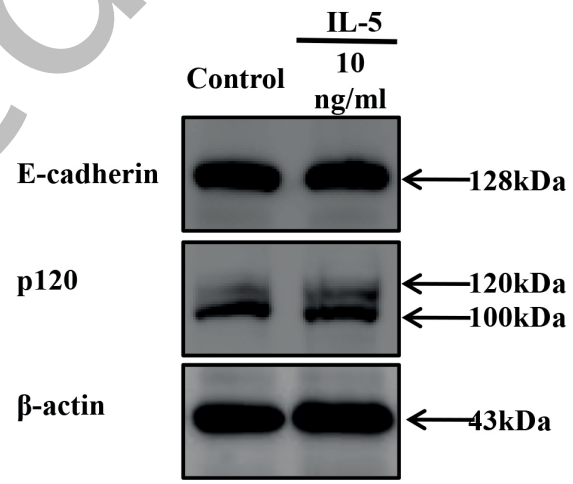

Figure S2. The expression of E-cadherin and p120 proteins in HNECs cultured with the ALI method and treated with IL-5 (10 ng/ml).

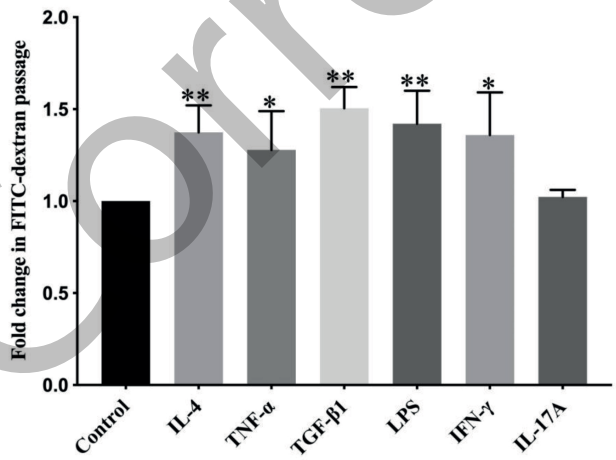

Figure S3. Inflammatory mediators regulated epithelial barrier integrity. The ALI cultures were stimulated with different inflammatory mediators for 36 h, and FITC-dextran permeability was measured (n = 6).