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Original Article

Expression of interleukin-22 and its significance in the pathogenesis of chronic rhinosinusitis

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Abstract: In order to explore the role of IL-22 in the pathogenesis of CRS, we observed the expression of IL-22 and associated factors in chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP). Immunohistochemical staining was applied to detect the expression of IL-22, IL-22R, STAT3, retinoic acid orphan receptor C (RORC) and aryl hydrocarbon recptor (AhR). There was significantly higher expression of IL-22 in CRSsNP than in controls (*P<0.05*). But the expression of IL-22 had no significant difference when comparing CRSwNP with CRSsNP and controls. The expression of IL-22R was significantly lower in CRSwNP compared to controls and CRSsNP (*P<0.05*). The expression of AhR was lower in CRSwNP than in CRSsNP (*P<0.05*). There was no significant difference of RORC and STAT3 among CRSwNP, CRSsNP and controls. IL-22 plays the important role in the pathogenesis of CRS, and further research is needed to understand the complex interactions with other cytokines and the exact mechanism of transcriptional regulation for IL-22.

Keywords: Chronic rhinosinusitis, IL-22, IL-22 receptor, aryl hydrocarbon recptor

Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease of the sinus mucous membrane with a heterogeneous and multifactorial pathogenesis. But the etiology of CRS remains not fully understood. Multiple factors have been demonstrated to involve with the pathogenesis of CRS, such as allergy, bacterial and fungal infection, structural anomalies and genetic predisposition [1]. It is recognized that these various factors can result in the common inflammation mediated by innate and adaptive immune system. The interaction between the immune or inflammatory cells through cytokines, especially the reciprocal regulation and counterbalance of T helper cells (T_H cells) demonstrate the important role of T-cells in the pathogenesis of CRS. T_H cells have been classically divided into T_{H1} cells and T_{H2} cells on the basis of the secreted cytokines [2]. The recent described T_H cell subsets, such as T_{H9}, T_{H17}, T_{H22}, regulatory T (T_{reg}) cells and T follicular helper (T_{FH}) cells, can also contribute to the different types of inflammatory responses.

CRS has been divided into two different phenotypes: CRS without nasal polyp (CRSsNP) and CRS with nasal polyp (CRSwNP). CRSsNP is a T_{H1} dominated inflammation with high levels of IFN-γ [3]. In whites, CRSwNP is a T_{H2} dominated eosinophilic inflammation with high levels of IL-4, IL5 and IL-13 [4]. In Chinese CRSwNP, a T_{H1}/T_{H17} polarized inflammation was observed with high levels of IL-17 [5]. T_{reg} cells inhibit T_{H17} differentiation and regulate T_{H1} and T_{H2} immune responses, with fork-head boxP 3 (Foxp3) characterized as the transcription factor [6, 7]. Decreased expression of Foxp3 has been detected in CRSwNP patients from Europe and China [4, 5, 8], which suggested the impairment of T_{reg} contributes to the inflammation of CRS.

Interleukin-22 (IL-22) is a member of the IL-10 related cytokine family. T_{H22} cells are characterized by particularly high production of IL-22. Moreover T_{H17} and innate lymphoid cells called NK-22 cells can produce IL-22. It has been shown that the expression of IL-22 is dependent on the transcription factors including retinoic acid orphan receptor C (RORC) and aryl

Table 1. Clinical and demographic details

	N	Ages (years) (median, range)	Sex	Lund-Mackay	Lund-Kennedy	VAS scores
			Male:Female	CT scores	Endoscopic scores	
CRSwNP	19	45 (13-67)	13:6	15.28 ± 7.22	7.22 ± 1.63	6.61 ± 1.38
CRSsNP	15	32 (10-61)	9:6	9.73 ± 7.06	4.27 ± 1.62	5.73 ± 1.16
Controls	15	29 (18-60)	13:2	0.33 ± 1.29	2.87 ± 0.92	4.67 ± 1.18

CRSwNP, chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps; VAS, visual analogue scale; CT, computed tomography.

hydrocarbon receptor (AhR). IL-22 binds to IL-22 receptors and activates STAT3 signaling pathway in target cells [9]. The role of T_{H22} in the pathogenesis of CRS has not been clarified clearly. In this study, we detected the expression of these related factors involved with IL-22 in two subtypes of CRS in order to explore the role of IL-22 in the pathogenesis of CRS preliminarily.

Materials and methods

Subjects

All nasal tissues for experiments were obtained from inpatients in the department of Otorhinolaryngology-Head and Neck surgery at the second affiliated hospital to Harbin medical university. 19 patients with CRSwNP, 15 patients with CRSsNP, and 15 controls were enrolled for the study. The diagnosis of CRS was based on history, endoscopic examination and CT scan by meeting the criteria of the European Position Paper on Rhinosinusitis and Nasal Polyps 2012. NP tissues in CRSwNP and diseased ethmoid sinus mucosa tissues in CRSsNP were collected from the middle nasal meatus during surgery. The inferior turbinate mucosa was collected from the control patients undergoing septoplasty because of anatomic variations and without sinus diseases. All the tissues were removed during the normal course of endoscopic surgery. Freshly obtained tissue was fixed in 10% formaldehyde solution for hematoxylin/eosin and immunohistochemical staining. Patients were excluded if they had used a course of antibiotics or systemic corticosteroids in the 4 weeks prior to the surgery. Patients with immune deficiencies and other genetic disorders such as cystic fibrosis or primary ciliary dyskinesia were also excluded. Each patient had a CT scan that was graded for a Lund-Mackay score. The visual analogue scale (VAS) was evaluated for all patients with CRS. The demographic and clinical characteristics of all subjects were shown in **Table 1**. This study was approved by the ethics committee of our institution and written informed consent was obtained from all patients.

Immunohistochemistry

Paraffin-embedded tissue blocks were cut into 5-μm-thick sections, mounted on the slides and heated at 64°C for ten minutes. Serial sections from each block were deparaffinized, hydrated. Citrate antigen retrieval solution was heated to 80°C in the microwave stove with high power for 3 minutes; the slides were put into the solution with low power for 5-10 minutes and then frozen at room temperature for 1 hour. All proteins except RORC were treated by the antigen retrieval. After washing by PBS, sections were treated with 3% hydrogen peroxide for 10 minutes to quench the endogenous peroxidase activity. 10% goat serum was used to block the sections for 10 minutes. Each section was incubated by the primary antibodies respectively at 4°C overnight. Primary antibodies were anti-IL-22 (1:300), anti-IL22R (1:300), anti-RORC (1:100), anti-STAT3 (1:30) and anti-AhR (1:50). Negative controls consisted of PBS instead of primary antibodies. Species-matched secondary antibodies were incubated at 37°C for 30 minutes. The slides were washed three times with PBS, and then were visualized by diaminobenzidine (DAB). Following by a final wash, the slides were mounted, coverslipped, and sealed. The number of positive cells per HP field and grading sores were analyzed. Ten fields per sample were randomly selected and scored by 2 independent observers under the microscope.

Statistical analysis

Data are expressed as medians and interquartile ranges, or in box-and-whisker plots. When

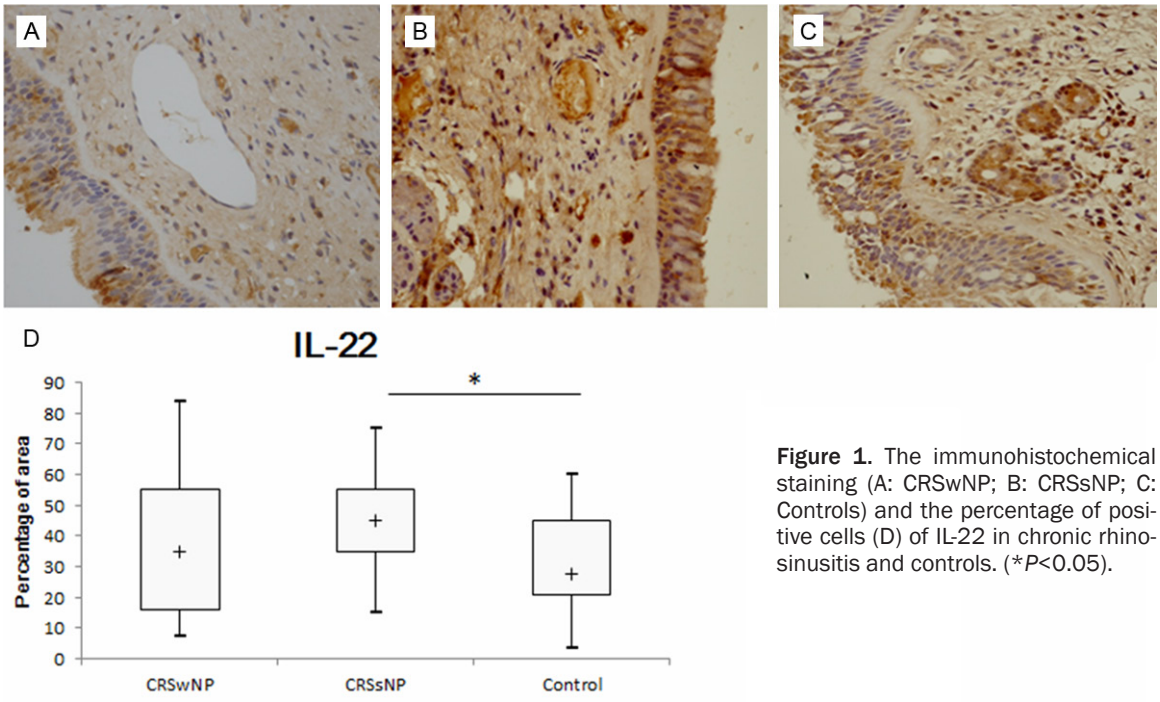

Figure 1. The immunohistochemical staining (A: CRSwNP; B: CRSsNP; C: Controls) and the percentage of positive cells (D) of IL-22 in chronic rhinosinusitis and controls. (*P<0.05).

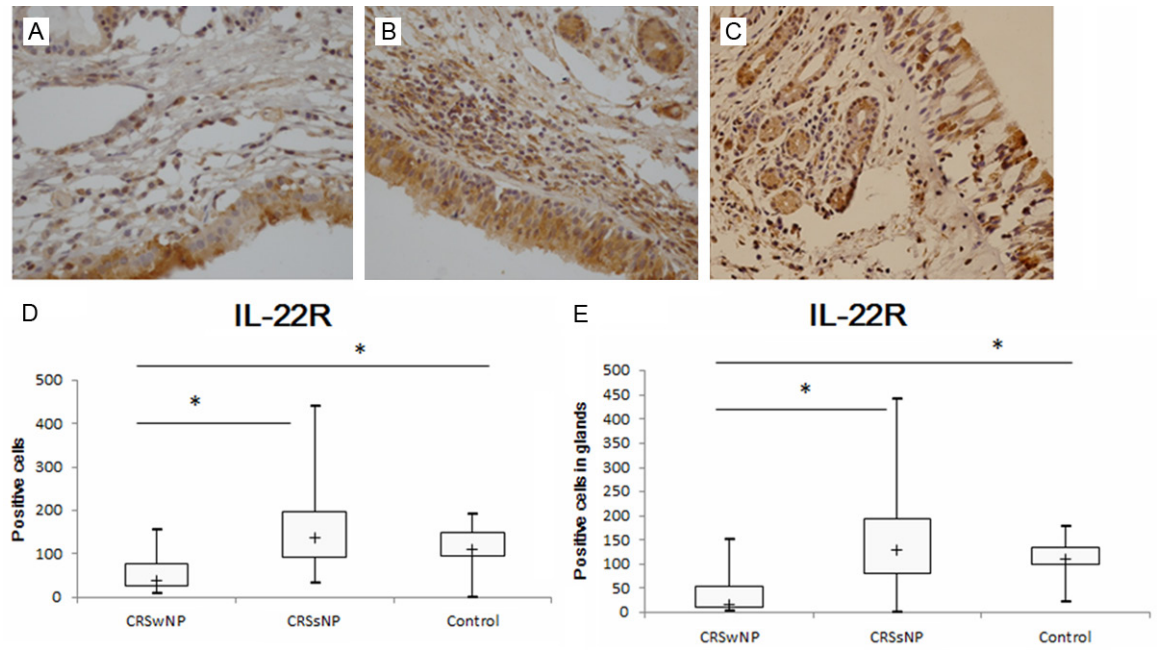

Figure 2. The immunohistochemical staining (A: CRSwNP; B: CRSsNP; C: Controls) and the positive cells (D, E) of IL-22R in chronic rhinosinusitis and controls. (*P<0.05).

comparisons were made between groups, the Kruskal-Wallis H-test was used to assess significant intergroup variability. The Mann-Whitney U 2-tailed test was used for between-group comparison and significance was accepted for P<0.05. Statistical analysis was performed with SPSS 13.0 (SPSS, Inc, Chicago).

Results

Expression of IL-22 and IL-22R

Immunohistochemical staining showed that IL-22 expressed mainly in the cytoplasm, and localized extensively in the superficial epitheli-

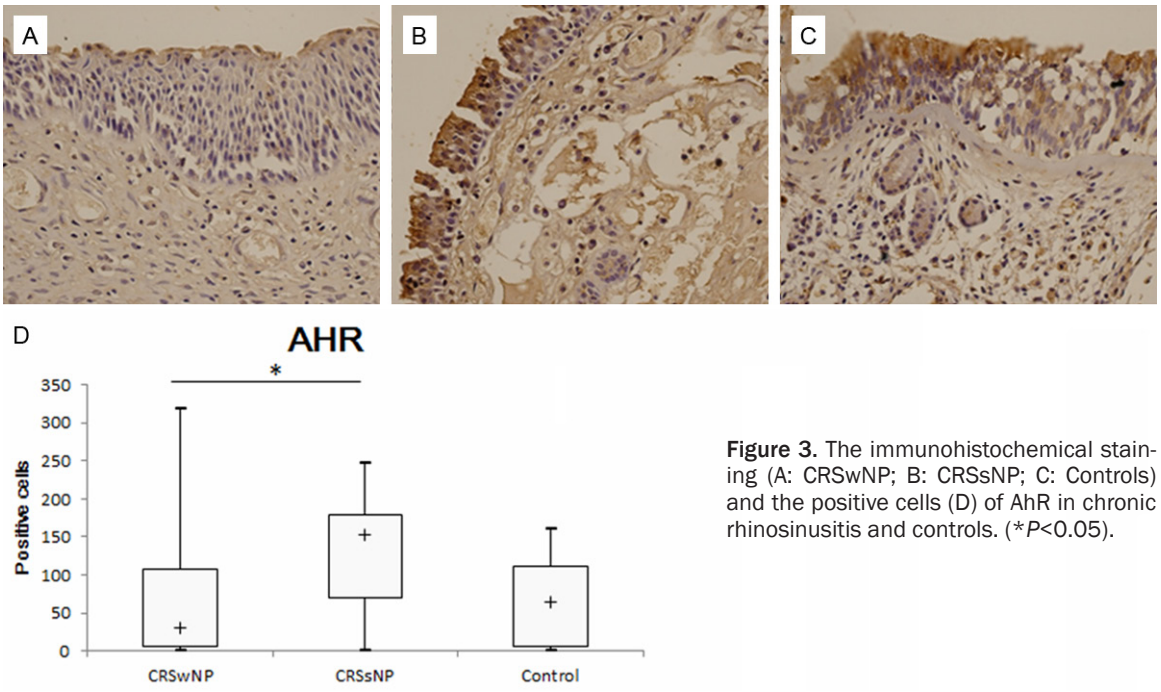

Figure 3. The immunohistochemical staining (A: CRSwNP; B: CRSsNP; C: Controls) and the positive cells (D) of AhR in chronic rhinosinusitis and controls. (*$P<0.05$).

um, submucosal glands and vascular endothelium. There was significantly higher expression of IL-22 in CRSsNP than in controls ($P<0.05$). But the expression of IL-22 had no significant difference between CRSwNP and controls. Moreover there was no significant difference between CRSwNP and CRSsNP (**Figure 1**).

IL-22R also expressed in the cytoplasm and the positive expression distributed in the epithelium, mesenchyma, submucosal glands, vascular endothelium and leukocytes. The expression of IL-22R was significantly lower in CRSwNP compared to controls and CRSsNP ($P<0.05$). But there was no significant difference in the expression of IL-22R between CRSsNP and controls (**Figure 2**).

Expression of AhR, RORC and STAT3

AhR expressed in the cytoplasm and mainly located in the glands, monocytes, lymphocytes and macrophages. The expression of AhR was lower in CRSwNP than in CRSsNP ($P<0.05$). But there was no significant difference between CRSwNP, CRSsNP and controls (**Figure 3**).

RORc expressed mainly in the basement membrane and the glands. The expression level was scored on 4-point scale, with 0 representing without staining, 1 representing faint yellow staining, 2 representing yellow staining, and 3 representing brown yellow staining. There was no significant difference among CRSwNP, CRSsNP and controls ($P>0.05$) (**Figure 4**).

STAT3 expressed in the cell nucleus and the positive location was mainly in the vascular endothelium, epithelial cells and glands. There was no significant difference among CRSwNP, CRSsNP and controls ($P>0.05$) (**Figure 5**).

Discussion

IL-22 belongs to IL-10 family cytokines, which additionally includes IL-10, IL-19, IL-20, IL-24, IL-26, IL-28a, IL-18b, and IL-29 [10]. These cytokines elicit diverse host defense mechanisms, especially from epithelial cells, during various infections and inflammations. IL-22 is expressed in several T cell populations that can influence host defense or autoimmunity. Th22 and Th17 are the major T cell subtypes producing IL-22 [11], and $CD8^+$T cells can also express IL-22 [12]. IL-22 is also expressed in other innate lymphocytes, such as innate lymphoid cells (ILCs) called NK-22 cells [13], and $CD11c^+$ cells [14]. IL-22 has a dual function, protective versus inflammatory, in modulating the immune responses. The exact role of IL-22 is dependent on the nature of the affected tissue and local cytokine milieu. The pathogenetic roles of Th22

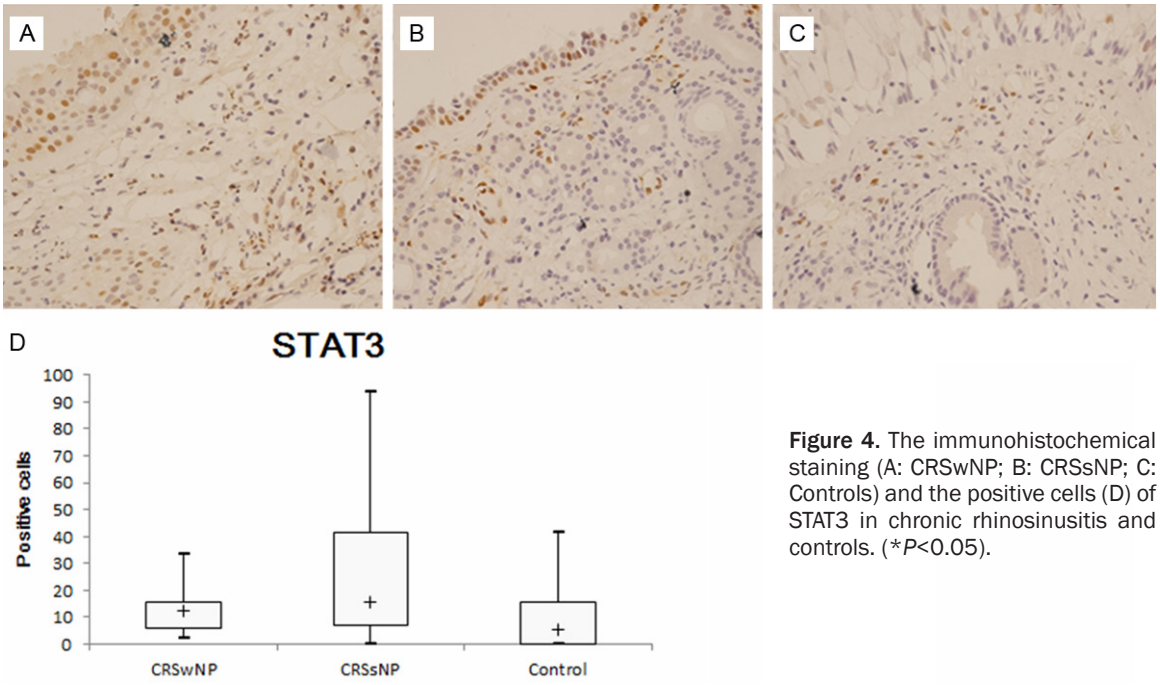

Figure 4. The immunohistochemical staining (A: CRSwNP; B: CRSsNP; C: Controls) and the positive cells (D) of STAT3 in chronic rhinosinusitis and controls. (*P<0.05).

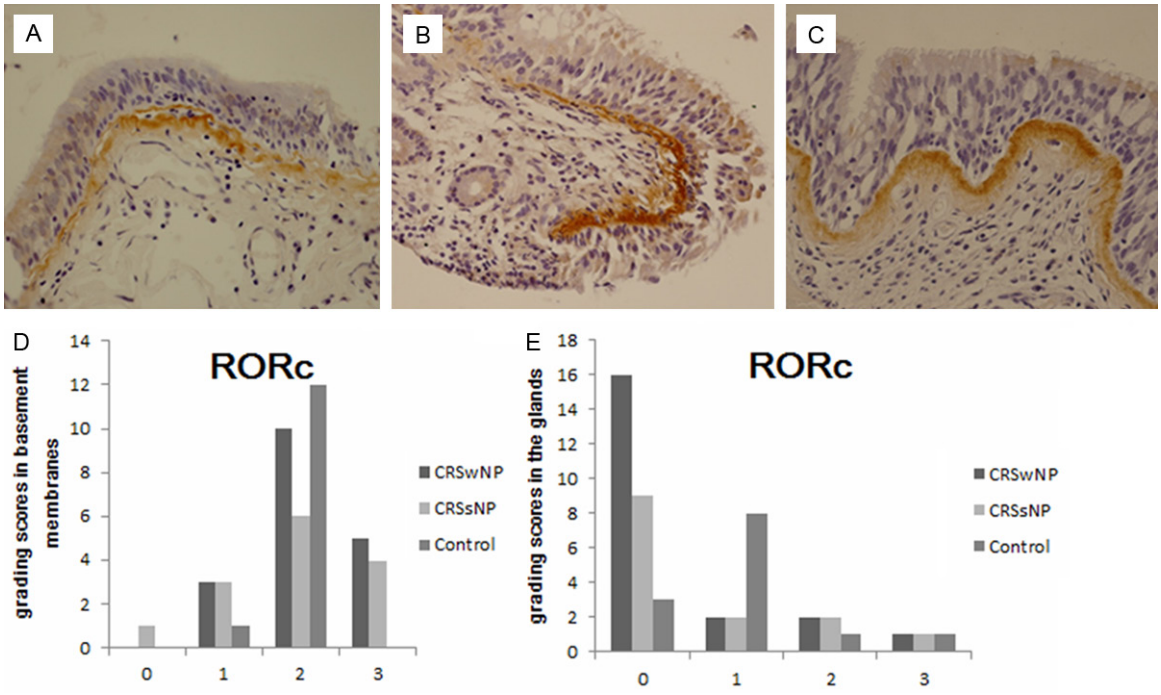

Figure 5. The immunohistochemical staining (A: CRSwNP; B: CRSsNP; C: Controls) and the grading sores (D, E) of RORc in chronic rhinosinusitis and controls. (*P<0.05).

and IL-22 have been observed in many inflammatory and autoimmune diseases, such as psoriasis [15], systemic lupus erythematosus [16], rheumatoid arthritis [17], inflammatory bowel diseases [18], and immune thrombocytopenic purpura [19]. But the role of IL-22 in the pathogenesis of CRS has been rarely investigated. Ramanathan et al [20] demonstrated the expression of IL-22R1 protein on the surface of cultured nasal epithelial cells by flow

cytometry. Moreover levels of IL-22R1 were significantly lower in recalcitrant CRSwNP than compared to controls and CRSsNP. But IL-22 levels had no statistical difference within the three groups. Our results showed that the expression of IL-22R was significantly lower in CRSwNP compared to controls and CRSsNP, which was consistent with the former research. But IL-22 had significantly higher expression in CRSsNP than controls, which suggested IL-22 might play more important roles in the pathogenesis of CRSsNP.

Recently, few reports investigated the role of IL-22 in the allergic airway inflammation. It has shown that IL-22 expression is induced in the lung of antigen-sensitized mice upon antigen inhalation [21-23]. IL-22 inhibits the expression of lung epithelial cell-derived cytokines and chemokines, including IL-25, IL-33, and CCL17, and suppresses eosinophilic airway inflammation and Th2 cytokine production in the airways [24, 25]. In our results, high expression of IL-22 in CRSsNP could contribute to Th1 biased inflammation by inhibiting Th2 cytokines and eosinophilic inflammation. IL-22 interacts with IL-22R complex and primarily activates STAT3 signaling pathway. IL-22 signaling can elicit various innate defensive mechanisms from epithelial cells, such as activation of proliferative and/or anti-apoptosis programs, expression of mucins, antimicrobial peptides and secretions of proinflammatory Th1 cytokines including IL-6, IL-1β and IL-8. In our study, much lower expression of IL-22R in CRSwNP than CRSsNP may result in reduced epithelial barrier and decreased pro-Th1 cytokine production. The imbalance of Th1/Th2 inflammation in CRSwNP would lead to the pathology.

IL-22 expression is dependent on the transcription factors, retinoic acid orphan receptor γt (RORγt) and aryl hydrocarbon receptor (AhR). Although RORγt is absolutely required for IL-17 expression, it is not sufficient for IL-22 production, which can still be driven by other factors in its absence [26]. But the expression of AhR correlates well with IL-22 production across many cell types including Th17 cells, γδT cells and human Th22 cells [26]. AhR is essential for IL-22 expression, but not for expression of other Th17 cytokines [27]. The recent research showed that the expression of AhR was reduced in CRSwNP, and the expression of AhR was lower in the atopic group than in the non-atopic group. But there was a very low level of RORC and IL-17 in the control group compared to atopic and non-atopic CRSwNP groups [28]. Contrasted with the detection of AhR only in CRSwNP in this research, we investigated the expression of AhR in CRSwNP and CRSsNP, which suggested the expression of AhR was lower in CRSwNP than in CRSsNP. Reduced expression of AhR in CRSwNP could lead to the low expression of IL-22 in CRSwNP. Consistent with decreased expression of IL-22R in CRSwNP, our results suggested the potential role of Th22 associated factors in the pathogenesis of CRSwNP.

In general, IL-22 has the dual function, protective or proinflammatory, which depends on a variety of factors, such as the target tissue, the concentration of IL-22 and the cytokine environment. IL-22 and downstream pathway are influenced by cytokines including IL-17, IFN-α, IFN-γ, or TNF-α [26]. The synergistic function of IL-17 and IL-22 are important for host defense and determinant for the pathogenic function of IL-22. Further research is needed to understand the complex of IL-22 with other cytokines in the pathogenesis of CRS. The transcriptional regulation is critically important for the expression of IL-22. The uncontrolled expression of IL-22 can cause the pathology. However, the exact mechanism of these transcriptional factors in promoting IL-22 production remains elusive. Our study discussed preliminarily the expression of IL-22 and associated factor in the pathogenesis of CRS by immunohistochemical method. Although the expression of RORc and STAT3 had no difference between every group, more research is still required for elucidating the interaction among these transcriptional factors from different levels. Further studies to explore the role of IL-22 in the pathogenesis of CRS will provide a new therapeutic target for CRS.

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Disclosure of conflict of interest

None.

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