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Differential expression of periostin in the nasal polyp may represent distinct histological features of chronic rhinosinusitis

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ABSTRACT

Objective: Chronic rhinosinusitis (CRS) is thought to be a multifactorial disease, and it is classified into a number of subtypes according to clinicohistological features. Periostin, a 90-kDa secreted protein, was reported to exist in nasal polyps (NPs) associated with CRS. We compared the expression of periostin with the degree of eosinophilic infiltration as well as tissue remodeling.

Materials and methods: Tissue samples were collected from 28 patients of CRS with NPs, and clinicohistological features were evaluated. The pattern of periostin expression was assessed immunohistochemically.

Result: Two patterns of periostin expression was observed in nasal polyps: "diffuse type", in which periostin was expressed throughout the lamina propria starting just below the basement membrane, and "superficial type", in which the protein was detected only in the subepithelial layers between the basement membrane and the nasal gland. The average infiltrated eosinophil count in the diffuse type was significantly higher than that in the superficial type (diffuse type 360.5 ± 393.0 vs. superficial type 8.46 ± 13.81, $p = 0.001$). Tissue remodeling was observed in 17 (85.0%) of the 20 diffuse-type nasal polyps, but only in one (12.5%) of the eight superficial-type nasal polyps ($p < 0.001$).

Conclusion: At least two distinct patterns of periostin expression were observed in the nasal polyps associated with CRS in accordance with the heterogeneous mechanisms underlying the pathogenesis of CRS with NPs.

1. Introduction

Chronic rhinosinusitis (CRS) is thought to be a multifactorial disease. According to the Guidelines of Rhinosinusitis in North America and Europe [1,2], CRS is classified into two subgroups, one is chronic rhinosinusitis with nasal polyps (CRSwNP), and the other is chronic rhinosinusitis without nasal polyps (CRSsNP). The histological features of the nasal polyps in cases of CRSwNP are massive infiltration of eosinophils, increased basement membrane thickness, epithelial damage, and goblet cell hyperplasia. These histological features of the nasal polyps are similar to those of the bronchial mucosa in asthmatic patients known as mucosal remodeling. The clinical features of CRSwNP include concomitant bronchial asthma and responsiveness to systemic steroid administration. In East Asia, including Japan, CRSwNP is further classified into two subgroups: one is characterized by eosinophilic inflammation (eosinophilic rhinosinusitis: ECRS) and the other is characterized by neutrophilic inflammation [3–8]. ECRS has features similar to CRSwNP as defined in North America and Europe [3,4]. On the other hand, the latter is characterized by infiltration of neutrophils and lymphocytes into nasal polyps, responsiveness to macrolide therapy, which was proven to be effective for diffuse pan-bronchitis in Japan [9,10], and a low rate of nasal polyp recurrence after ESS [11]. In East Asia, more than 50% of the CRS patients have been reported to have non-ECRS [5], suggesting that at least two different mechanisms are involved in CRSwNP pathogenesis.

Periostin, a 90-kDa secreted protein, was reported to play an important role in remodeling after tissue injury in various organs [12], including the lower airway [13,14]. This protein was reported

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to exist in intact nasal mucosa as well as in nasal polyps associated with CRSwNP [15]. There have been no reports on the differential expression of periostin between the intact nasal mucosa and the nasal polyps, and the role of periostin in the CRSwNP pathogenesis remains to be elucidated.

In this study, we examined the expression pattern of periostin in nasal polyps from CRS patients and sought to clarify the relationship between the expression pattern and a number of clinicohistological parameters.

2. Materials and methods

2.1. Tissue samples

Tissue samples of nasal polyp were collected from 28 patients with CRSwNP who had undergone ESS from January 2002 to December 2005 at the Department of Otorhinolaryngology, Yokohama City University Medical Center. Clinical parameters including patients' age, sex, blood eosinophil ratio, serum Immunoglobulin (Ig) E level, and the presence of seasonal or perennial allergic rhinitis were collected by a retrospective chart review. The presence of bronchial asthma was assessed by pulmonary physicians. The diagnosis of ECRS was obtained according to the previously reported clinical criteria [8]. Briefly, the criteria include three features: (1) blood eosinophilia (>6% of total WBC count), (2) the presence of soft tissue density in the olfactory cleft on computed tomography, and (3) the presence of soft tissue density in the posterior ethmoid sinus. This study was approved by the Internal Review Board of the Yokohama City University Medical Center.

2.2. Histological analysis

Tissue samples obtained during surgery were immediately fixed with 4% formaldehyde, and then embedded in paraffin, sliced into 5 μm sections, and stained with hematoxylin and eosin (H&E stain). The mean eosinophil count in five regions in high power field (400×) was calculated. To evaluate tissue remodeling, we examined the thickness of the basement membrane. Tissue remodeling was considered positive when the basement membrane was thicker than the size of nucleus of basal cells in the area where the epithelium was cut perpendicularly.

2.3. Immunohistochemical analysis

Immunohistochemical analysis was performed to evaluate the expression of periostin in the nasal polyp using the formaldehyde-fixed paraffin-embedded sections as mentioned above. After deparaffinization and rehydration, the sections were treated by proteinase K for 15 min at room temperature to activate antigen reactions. The sections were placed in 0.1% hydrogen peroxide to quench any endogenous peroxide activity and treated with a blocking reagent. The sections were then incubated with anti-periostin rabbit polyclonal antibody (kindly provided by laboratory of Izuhara, Saga Medical School, Japan [14]) at 1:1000 to 1:3000 dilution for an hour at room temperature. Negative controls for immunohistochemistry were performed by incubation with normal rabbit immunoglobulin G as a primary antibody. Periostin was detected by a standard process using the ENVISION+/HRP (DAB) kit system (Dako Cytomation, Glostrup, Denmark). The expression pattern of periostin was evaluated by comparison with the histological section in low power field (40×).

2.4. Statistical analysis

Statistical analysis was performed by using Welch's *t*-test and Spearman's rank correlation coefficient test. A *p*-value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Patient characteristics

Patient characteristics are summarized in Table 1. The blood eosinophil ratio varied from 0.2% to 28% (median 5.4%). The number of patients with allergic rhinitis, bronchial asthma and ECRS was 13 (48.1%), 6 (21.4%) and 6 (21.4%), respectively. No patient was diagnosed with aspirin intolerance. Systemic steroid were administered after ESS in 13 (46.3%) patients. Ten patients (35.7%) experienced nasal polyp recurrence. Histological analysis revealed that the mean infiltrated eosinophil count in the nasal polyps varied from 0 to 1239/high power field (mean 217.1 ± 292, median 70.0). Tissue remodeling, indicated by basement membrane thickening, was observed in 18 patients (64.3%).

3.2. Immunohistochemical analysis

The expression of periostin was detected in all of the 28 samples. We noticed that there were two patterns of periostin expression in the nasal polyps. In one pattern, the periostin was expressed throughout the lamina propria starting just below the basement membrane (Fig. 1a: immunohistochemistry, low power field, b: H&E stain, low power field, c: immunohistochemistry at

Table 1
Patient characteristics and the relationship between a periostin expression type and clinico-histological factors.

Characteristics or factors	Total (*n* = 28)	Diffuse type (*n* = 20)	Superficial type (*n* = 8)	*p*-Value
Gender				
Male (*n*)	20 (71.4%)	15	5	N.S.
Female (*n*)	8 (28.6%)	5	3	
Mean age (year)	50.9	50.1	54.6	N.S.
Allergic rhinisis (*n*)	13 (48.1%)	11	2	N.S.
Seasonal (*n*)	11 (39.3%)	9	2	N.S.
Perennial (*n*)	7 (25.0%)	6	1	N.S.
Bronchial asthma (*n*)	6 (21.4%)	6	0	0.010
Aspirin intolerant (*n*)	0 (0%)			
ECRS (*n*)	6 (21.4%)	6	0	0.009
Polyp recurrence (*n*)	10 (35.7%)	7 (35.0%)	3 (37.5%)	N.S.
Steroids administration (*n*)	13 (46.4%)	10 (50.0%)	3 (37.5%)	N.S.
Serum IgE (average, IU)	228.2	281.6	90.7	0.033
Blood eosinophil ratio (average, %)	6.9	7.7	4.8	N.S.
Tissue remodeling (*n*)	18 (64.3%)	17 (85.0%)	1 (12.5%)	<0.001

IgE, immunogulobulin E; ECRS, eosinophilic chronic rhinosinusitis; N.S., not significant.