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# Correlation of the expression of Eotaxin−3 and Foxm1 with the expression of p38MAPK/NF− κ B and inflammatory factors in mucosa tissue of chronic sinusitis

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ABSTRACT

**Objective:** To study the correlation of the expression of Eotaxin-3 and Foxm1 with the expression of p38MAPK/NF- κ B and inflammatory factors in mucosa tissue of chronic sinusitis. **Methods:** 57 patients with chronic sinusitis and 38 patients with nasal septum deviation who received nasal endoscopic surgery in our hospital between March 2015 and August 2016 were selected and included in sinusitis group and control group respectively. Sinus mucosa tissues were collected to test the expression of Eotaxin-3, Foxm1, p38MAPK, NF- κ B and inflammatory factors, and serum samples were collected to test the levels of inflammatory factors. **Results:** Eotaxin-3, Foxm1, p38MAPK and NF- κ B protein expression in mucosa tissue of sinusitis group were significantly higher than those of control group, and the Eotaxin-3 and Foxm1 protein expression were positively correlated with p38MAPK and NF- κ B protein expression; TGF- β 1, IL-1 β , IL-25, IL-33 and YKL-40 levels in nasal mucosa and serum of sinusitis group were significantly higher than those of control group, and TGF- β 1, IL-1 β , IL-25, IL-33 and YKL-40 levels in nasal mucosa and serum were positively correlated with p38MAPK and NF- κ B protein expression. **Conclusion:** Eotaxin-3 and Foxm1 are expressed in mucosa tissue of chronic sinusitis, and can start the expression of inflammatory factors and induce the cascade amplification of inflammatory reaction through p38MAPK/ NF- κ B signaling pathway.

## 1. Introduction

Chronic sinusitis is one of the most common diseases of otolaryngology department, its pathologic nature is the chronic inflammation of sinus mucosa, and persistent chronic inflammation can cause goblet cell hyperplasia, increased mucus secretion and sinus drainage barriers in nasal mucosa, and therefore, the mucus in sinus cavity cannot be discharged, further stimulates the cascade amplification activation of nasal mucosa inflammation and secretes purulent secretion. At present, it is still not entirely clear about the regulatory mechanism of inflammatory response in the development and change of chronic sinusitis, and it is believed that bacterial colonization, structural variation, trauma, allergy and other theories are associated with the cascade activation of sinus mucosa inflammation in patients with chronic sinusitis[1,2]. Eotaxin-3 and transcription factor Forkhead box m1 (Foxm1) are the new inflammation-regulating molecules discovered in recent years, the former is a member of the chemokine CC family, and it can recruit inflammatory cells in local tissue and start the inflammatory response[3]; the latter is the transcription factor involved in the regulation of inflammation process, and it can start the expression of inflammatory signal molecules and inflammatory mediators and work with the Eotaxin-3 to regulate the cascade amplification of the inflammatory response[4]. In order to define whether Eotaxin-3 and Foxm1 participated in the regulation of inflammatory response in nasal mucosa of patients with chronic sinusitis, the correlation of the expression of Eotaxin-3 and Foxm1 with the expression of p38MAPK/NF- κ B and inflammatory factors in mucosa tissue of chronic sinusitis was analyzed.

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## 2. Subjects and methods

### 2.1 Research subjects

A total of 57 patients with chronic sinusitis who received nasal endoscopic surgery in our hospital between March 2015 and August 2016 were selected as the sinusitis group of the study, and all patients were diagnosed with chronic sinusitis after nasal endoscopy and sinus MRI scanning, were up to the indications of nasal endoscopic surgery, were informed of the operation subjects and received nasal endoscopic surgery after the approval of the hospital ethics committee. 38 patients with nasal septum deviation who received nasal endoscopic surgery in our hospital during the same period were selected as the control group of the study, and all patients received endoscopic surgery and were excluded of chronic nasal mucosal inflammation. Inclusion criteria: (1) the course of disease was 3 months; (2) with complete clinical data and voluntarily participating in the study; (3) without the history of allergic rhinitis, nasal polyps or other allergic diseases; (4) without systemic diseases such as diabetes and tuberculosis; (5) without hepatitis b virus, HIV infection or other immune system diseases and without recent application of immunosuppressor; (6)18-60 years old. Sinusitis group included 35 male cases and 22 female cases that were 25-60 years old; control group included 23 male cases and 15 female cases that were 23-58 years old. The two groups of patients were not significantly different in general data ($P$>0.05).

### 2.2 Research methods

#### 2.2.1 Clinical sample collection methods

During nasal endoscopic surgery, diseased sinus mucous tissue was collected from sinusitis group, normal sinus mucosa tissue was collected from control group, and the tissues were cleaned with saline for 3-5 times, then frozen in liquid nitrogen and preserved at -80 ℃; 5 mL of preoperative peripheral blood was collected from the sinusitis group and control group and then centrifuged to separate serum and preserve it at -80 ℃.

#### 2.2.2 Clinical index detection methods

Proper amount of sinus mucosa tissue was taken, added in RIPA lysis buffer and then fully grinded, the obtained tissue suspension was centrifuged in 4 ℃ centrifuge for 20 min to get the supernatant, and enzyme-linked immunosorbent assay kits were used to determine Eotaxin-3, Foxm1, p38MAPK, NF-κB, TGF-β1, IL-1β, IL-25, IL-33 and YKL-40 levels; Serum specimens were taken to determine TGF-β1, IL-1β, IL-25, IL-33 and YKL-40 levels with enzyme-linked immunosorbent assay kits.

### 2.3 Statistical methods

SPSS 21.0 software was used to input and statistically analyze data, measurement analysis between two groups was by t test, correlation between two measurement data was by Pearson test and $P$<0.05 indicated statistical significance in differences.

## 3. Results

### 3.1 Eotaxin-3, Foxm1, p38MAPK and NF-κB expression in mucosa tissue and correlation

Analysis of Eotaxin-3 (ng/mL), Foxm1 (pg/mL), p38MAPK (ng/mL) and NF-κB (pg/mL) expression in mucosa tissue between sinusitis group and control group was as follows: Eotaxin-3, Foxm1, p38MAPK and NF-κB protein expression in mucosa tissue of sinusitis group were significantly higher than those of control group. Differences in Eotaxin-3, Foxm1, p38MAPK and NF-κB expression in mucosa tissue were statistically significant between two groups of patients ($P$<0.05); Pearson correlation analysis showed that Eotaxin-3 and Foxm1 protein expression in mucosa tissue of sinusitis group were positively correlated with p38MAPK and NF-κB protein expression.

**Table 1.**

Eotaxin-3, Foxm1, p38MAPK and NF-κB expression in mucosa tissue.

| Groups | $n$ | Eotaxin-3 | Foxm1 | p38MAPK | NF-κB |
|---|---|---|---|---|---|
| Sinusitis group | 57 | 4.57±0.62 | 365.67±41.26 | 2.78±0.35 | 552.36±67.41 |
| Control group | 38 | 2.26±0.31 | 203.41±24.67 | 1.32±0.16 | 246.75±32.31 |
| $T$ | | 10.589 | 8.274 | 11.752 | 14.275 |
| $P$ | | <0.05 | <0.05 | <0.05 | <0.05 |

### 3.2 Inflammatory factor expression in mucosa tissue and the correlation with p38MAPK and NF-κB expression

Analysis of inflammatory factors TGF-β1 (ng/mL), IL-1β (ng/mL), IL-25 (pg/mL), IL-33 (pg/mL) and YKL-40 (ng/mL) expression in mucosa tissue between sinusitis group and control group was as follows: TGF-β1, IL-1β, IL-25, IL-33 and YKL-40 protein expression in mucosa tissue of sinusitis group were significantly higher than those of control group. Differences in TGF-β1, IL-1β, IL-25, IL-33 and YKL-40 expression in mucosa tissue were statistically significant between two groups of patients ($P$<0.05); Pearson correlation analysis showed that TGF-β1, IL-1β, IL-25, IL-33 and YKL-40 protein content in mucosa tissue of sinusitis group were positively correlated with p38MAPK and NF-κB protein expression.

**Table 2.**

Inflammatory factor expression in mucosa tissue.

| Groups | $n$ | TGF-β1 | IL-1β | IL-25 | IL-33 | YKL-40 |
|---|---|---|---|---|---|---|
| Sinusitis group | 57 | 7.31±0.79 | 10.36±1.26 | 356.12±46.75 | 295.25±33.21 | 5.62±0.67 |
| Control group | 38 | 3.34±0.37 | 4.69±0.56 | 178.87±22.14 | 142.56±17.68 | 3.02±0.41 |
| $T$ | | 12.589 | 13.401 | 10.482 | 10.938 | 8.481 |
| $P$ | | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

**Table 3.**

Inflammatory factor levels in serum.

| Groups | *n* | TGF-β | IL-1β | IL-25 | IL-33 | YKL-40 |
|---|---|---|---|---|---|---|
| Sinusitis group | 57 | 32.52±4.27 | 11.38±1.75 | 78.98±9.23 | 121.34±14.53 | 8.49±1.03 |
| Control group | 38 | 17.65±2.03 | 5.23±0.76 | 33.41±3.58 | 60.63±7.68 | 4.41±0.65 |
| *T* | | 8.398 | 11.247 | 13.038 | 10.937 | 9.184 |
| *P* | | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

### *3.3 Inflammatory factor levels in serum and the correlation with p38MAPK and NF-κB expression*

Analysis of inflammatory factors TGF-β1, IL-1β, IL-25, IL-33 and YKL-40 levels in serum between sinusitis group and control group was as follows: serum TGF-β1, IL-1β, IL-25, IL-33 and YKL-40 levels of sinusitis group were significantly higher than those of control group. Differences in serum TGF-β1, IL-1β, IL-25, IL-33 and YKL-40 levels were statistically significant between two groups of patients ($P$<0.05); Pearson correlation analysis showed that serum TGF-β1, IL-1β, IL-25, IL-33 and YKL-40 levels of sinusitis group were positively correlated with p38MAPK and NF-κB protein expression.

## 4. Discussion

Chronic nasal mucosa inflammation is the basic pathological change of local tissue in patients with chronic sinusitis, and persistent inflammation can cause increased secretions and poor drainage in nasal sinus cavity, and result in the cascade amplification of inflammatory response in the form similar to positive feedback[5,6]. At present, the regulatory mechanism of inflammation in nasal mucosa of patients with sinusitis is not yet clear. Foxm1 and Eotaxin-3 are the new inflammation regulators discovered in recent years, the former can trigger the expression of inflammatory signal molecules and inflammatory mediators, and then regulate the cascade amplification of inflammatory response under the synergy of Eotaxin-3. Eotaxin-3 is an important member of the Eotaxin family, and its combination with CC chemokine receptor-3 can prompt the infiltration of eosinophils, basophils, neutrophils, lymphocytes and other inflammation-related cells in local tissue, induce inflammatory cell activation and promote the cascade activation of inflammatory reaction[7,8]; Foxm1 is a member of forkhead box transcription factor family, has winged helix DNA structure, and can regulate the expression of a variety of signaling molecules and cytokines in the process of inflammation[9,10]. In order to define whether the Eotaxin-3 and Foxm1 participated in the regulation of the inflammatory response in nasal mucosa of patients with chronic sinusitis, the Eotaxin-3 and Foxm1 expression in nasal mucosa tissue were analyzed in the study, and the results showed that Eotaxin-3 and Foxm1 protein expression in mucosa tissue of sinusitis group were significantly higher than those of control group. This confirms that the highly expressed Eotaxin-3 and Foxm1 in nasal mucosa are closely related to the nasal mucosa inflammation in patients with sinusitis.

The regulation of Eotaxin-3, as a chemokine, on the inflammatory response in nasal mucosa tissue depends on recruiting inflammatory cells and activating inflammation-related signaling molecules; the regulation of Foxm1, as a transcription factor, on the inflammatory response in nasal mucosa tissue depends on regulating the expression of inflammation-related signal molecules and inflammation-related mediators. P38MAPK signaling pathway is an important pathway that regulates inflammation in the body[11], p38MAPK belongs to the MAPK family, it can cause NF-κB activation through a series of signal transduction pathways downstream after activated in the form of phosphorylation, and the activated NF-κB can start the expression of a variety of inflammatory mediators after transferring into the nucleus, and the excessively expressed inflammatory mediators mediate the inflammation in local mucosa[12,13]. In order to determine whether the highly expressed Eotaxin-3 and Foxm1 in nasal mucosa tissue regulated inflammation through p38MAPK signaling pathway, the p38MAPK and NF-κB expression in nasal mucosa were analyzed at first in the study, and the results showed that p38MAPK and NF-κB protein expression in mucosa tissue of sinusitis group were significantly higher than those of control group. This means that the highly expressed p38MAPK and NF-κB in nasal mucosa are closely related to the inflammatory response in nasal mucosa of patients with sinusitis. On the basis, further analysis of the correlation of Eotaxin-3 and Foxm1 with p38MAPK and NF-κB expression showed that Eotaxin-3 and Foxm1 protein expression in nasal mucosa tissue of patients with sinusitis were positively correlated with p38MAPK and NF-κB protein expression. It means that Eotaxin-3 and Foxm1 can activate the p38MAPK/NF-κB signaling pathway in nasal mucosa tissue of patients with sinusitis, and then regulate the expression of inflammatory mediators and the activation of inflammatory response through p38MAPK/NF-κB signaling pathway.

Highly expressed Eotaxin-3 and Foxm1in nasal mucosa tissue can activate p38MAPK/NF-κB signaling pathway and then start the expression of a variety of inflammatory mediators, which cause the inflammation of nasal mucosa. The inflammatory mediators regulated by the NF-κB that transfers into the nucleus include TGF-

β 1, IL-1 β , IL-25, IL-33, YKL-40, etc. TGF- β 1 is a cytokine that can regulate fibroblast activity, and it can also promote goblet cells to secrete mucus and aggravate the inflammatory response[14]; IL-1 β is an important member of interleukin family that mediates inflammatory reaction, and it can directly cause the inflammation of mucosa tissue[15]; IL-25 and IL-33 are secreted by T lymphocytes and mediate Th2 immune response, and they can also promote the expression of a variety of pro-inflammatory factors and chemokines[16,17]; YKL-40 is a new inflammation marker discovered in recent years, and plays a regulatory role in inflammatory response and immune response. In order to define whether the p38MAPK/ NF- κ B signaling pathway regulated the expression of above inflammatory mediators in nasal mucosa tissue, the contents of above inflammation mediators in nasal mucosa and serum were analyzed in the study, and the results showed that TGF- β 1, IL-1 β , IL-25, IL-33 and YKL-40 levels in nasal mucosa and serum of sinusitis group were significantly higher than those of control group. This means that the abnormal expression and secretion of inflammatory mediators TGF- β 1, IL-1 β , IL-25, IL-33 and YKL-40 are closely related to the inflammatory response in nasal mucosa of patients with sinusitis. Further analysis of the correlation of these inflammatory mediators with p38MAPK and NF- κ B expression showed that TGF- β 1, IL-1 β , IL-25, IL-33 and YKL-40 levels in nasal mucosa and serum were positively correlated with p38MAPK and NF- κ B expression. It shows that the abnormally activated p38MAPK/NF- κ B signaling pathway in nasal mucosa tissue of patients with sinusitis have promoting effect on the expression of inflammatory mediators TGF- β 1, IL-1 β , IL-25, IL-33 and YKL-40.

To sum up, it is believed that Eotaxin-3 and Foxm1 are expressed in mucosa tissue of chronic sinusitis, and can start the expression of inflammatory factors and induce the cascade amplification of inflammatory reaction through p38MAPK/ NF- κ B signaling pathway.

## Referencess


[1] Sohal M, Tessema B, Brown SM. Medical management of frontal sinusitis. *Otolaryngol Clin North Am* 2016; **49**(4): 927-934.

[2] Fang A, England J, Gausche-Hill M. Pediatric acute bacterial sinusitis: diagnostic and treatment dilemmas. *Pediatr Emerg Care* 2015; **31**(11): 789-794

[3] De Corso E, Baroni S, Battista M, Romanello M, Penitente R, Di Nardo W, et al. Nasal fluid release of eotaxin-3 and eotaxin-2 in persistent sinonasal eosinophilic inflammation. *Int Forum Allergy Rhinol* 2014; **4**(8): 617-624.

[4] Zhao YD, Huang X, Yi F, Dai Z, Qian Z, Tiruppathi C, et al. Endothelial FoxM1 mediates bone marrow progenitor cell-induced vascular repair and resolution of inflammation following inflammatory lung injury. *Stem Cells* 2014; **32**(7): 1855-1864.

[5] Guan WJ, Gao YH, Li HM, Yuan JJ, Chen RC, Zhong NS. Impacts of co-existing chronic rhinosinusitis on disease severity and risks of exacerbations in Chinese adults with bronchiectasis. *PLoS One* 2015; **10**(9): e0137348.

[6] Uluyol S, Arslan IB, Demir A, Mercan GC, Dogan O, Çukurova I. The role of the uncinate process in sinusitis aetiology: isolated agenesis versus maxillary sinus hypoplasia. *J Laryngol Otol* 2015; **129**(5): 458-461.

[7] Gentili A, Zaibi MS, Alomar SY, De Vuono S, Ricci MA, Alaeddin A, et al. Circulating levels of the adipokines monocyte chemotactic protein-4 (mcp-4), macrophage inflammatory protein-1 β (mip-1 β ), and eotaxin-3 in severe obesity and following bariatric surgery. *Horm Metab Res* 2016; **48**(12): 847-853.

[8] Park JY, Zhang X, Nguyen N, Souza RF, Spechler SJ, Cheng E. Proton pump inhibitors decrease eotaxin-3 expression in the proximal esophagus of children with esophageal eosinophilia. *PLoS One* 2014; **9**(7): e101391.

[9] Xia H, Ren X, Bolte CS, Ustiyan V, Zhang Y, Shah TA, et al. Foxm1 regulates resolution of hyperoxic lung injury in newborns. *Am J Respir Cell Mol Biol* 2015; **52**(5): 611-621.

[10]Lim R, Barker G, Lappas M. FOXM1 is lower in human fetal membranes after spontaneous preterm labour and delivery. *Reprod Fertil Dev* 2014; **26**(7): 1052-1060.

[11]Subhashini, Chauhan PS, Dash D, Paul BN, Singh R. Intranasal curcumin ameliorates airway inflammation and obstruction by regulating MAPKinase activation (p38, Erk and JNK) and prostaglandin D2 release in murine model of asthma. *Int Immunopharmacol* 2016; **31**: 200-206.

[12]Park IH, Park JH, Shin JM, Lee HM. Tumor necrosis factor- regulates interleukin-33 expression through extracellular signal-regulated kinase, p38, and nuclear factor- κ B pathways in airway epithelial cells. *Int Forum Allergy Rhinol* 2016; **6**(9): 973-980.

[13]Zhang D, Chen B, Zhou J, Zhou L, Li Q, Liu F, et al. Low concentrations of trichosanthin induce apoptosis and cell cycle arrest via c-Jun N-terminal protein kinase/mitogen-activated protein kinase activation. *Mol Med Rep* 2015; **11**(1): 349-356.

[14]Wang M, Ye T, Liang N, Huang Z, Cui S, Li Y, et al. Differing roles for TGF- β /Smad signaling in osteitis in chronic rhinosinusitis with and without nasal polyps. *Am J Rhinol Allergy* 2015; **29**(5): e152-9.

[15]Wang Z, Li P, Zhang Q, Lv H, Liu J, Si J. Interleukin-1 β regulates the expression of glucocorticoid receptor isoforms in nasal polyps in vitro via p38 MAPK and JNK signal transduction pathways. *J Inflamm* 2015; **12**(1): 3.

[16]Endo Y, Hirahara K, Iinuma T, Shinoda K, Tumes DJ, Asou HK, et al. The interleukin-33-p38 kinase axis confers memory T helper 2 cell pathogenicity in the airway. *Immunity* 2015; **42**(2): 294-308.

[17]Lam M, Hull L, Imrie A, Snidvongs K, Chin D, Pratt E, et al. Interleukin-25 and interleukin-33 as mediators of eosinophilic inflammation in chronic rhinosinusitis. *Am J Rhinol Allergy* 2015; **29**(3): 175-181.