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ORIGINAL RESEARCH

Case-Control Study: Evaluating the Role and Therapeutic Potential of FSTL1 in Type 2 Inflammation of Chronic Rhinosinusitis

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Purpose: Follicle suppressor-like protein 1 (FSTL1) plays a contributory role in chronic inflammation, and it currently been demonstrated in studies of asthma and chronic obstructive pulmonary disease (COPD), but studies in chronic sinusitis with nasal polyps have not been addressed. The aim of this study was to explore the expression of follicle suppressor-like protein 1 (FSTL1) in chronic sinusitis and its possible association with type 2 inflammation.

Patients and Methods: This study included 34 CRS patients (24 ECRS, 10 non-ECRS) and 10 controls. Hematoxylin-eosin (HE) staining to observe the pathomorphological changes of these three groups of tissue specimens in detail, Immunohistochemistry (IHC), real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) technology and Western blotting (WB) were used to evaluate the expression levels of FSTL1 and type 2 inflammatory cytokines IL-4, IL-5, and IL-13 in the three groups.

Results: In the ECRS group, there was thickening of the tissue mucosa and hyperplasia of glands, along with a significant infiltration of inflammatory cells and eosinophils. The experimental results showed that the expression levels of FSTL1 and type 2 inflammatory cytokines IL - 4, IL - 5, and IL - 13 were higher in the ECRS and non - ECRS groups than in the control group ($P<0.05$). Additionally, FSTL1 and IL-4, IL-5, IL-13 were positively correlated in the ECRS group($P<0.05$).

Conclusion: FSTL1, IL-4, IL-5 and IL-13 were highly expressed in chronic sinusitis, and FSTL1 was positively correlated with IL-4, IL-5 and IL-13 in the ECRS group, suggesting that FSTL1 contributes to the development of type 2 inflammation in ECRS, and FSTL1 may serve as a potential therapeutic target for ECRS.

Keywords: chronic sinusitis, follicle suppressor-like protein 1, type 2 inflammation

Introduction

Chronic rhinosinusitis (CRS) is a chronic inflammation of the mucous membrane of the sinuses that lasts for more than 12 weeks, which can cause nasal congestion, mucopurulent mucus, decreased sense of smell, and stuffiness and pain in the head and face, which seriously affects patients' daily life and work.[1,2] EPOS2020[3] classified it into CRS with nasal polyps based on phenotype (CRSwNP) and without nasal polyps (CRSsNP), type 1, type 2 and type 3 based on inflammatory endotypes, and eosinophilic chronic rhinosinusitis (ECRS) and non-eosinophilic chronic rhinosinusitis (non-ECRS) based on eosinophil counts. ECRS and non-ECRS exhibit significant differences in inflammatory cell infiltration, type of inflammation, clinical manifestations, and treatment. The primary characteristic of ECRS is the extensive infiltration of eosinophils in the nasal mucosa and polyps. This degree of infiltration can be assessed through histological examination, typically manifesting as a significant increase in the number of eosinophils per high-power field (HPF). Additionally, patients with ECRS often experience more severe symptoms, including severe nasal congestion, rhinorrhea, hyposmia, and facial pain.[4] Compared to non-ECRS, the prominent features of ECRS are extensive eosinophilic infiltration and a TH2 response. Type 2 inflammation is driven by TH2 cells and ILC2, producing type 2

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cytokines, including IL-4, IL-5, IL-13, and other inflammatory mediators. IL-5 promotes eosinophilia, and IL-4, IL-13 promote the production of eosinophil chemotaxis and activation of B-cells, macrophages, fibroblasts, epithelial cells, and cuvette cells, the activation of which can induce an IgE-mediated inflammatory response.[5,6] Type 2 inflammation drives inflammatory responses in ECRS through various mechanisms, leading to the exacerbation of clinical symptoms and an increased recurrence rate. Controlling the development of type 2 inflammation may be helpful in alleviating the condition of patients with ECRS.

Follistatin-like Protein 1 (FSTL1) is a glycoprotein that plays a pro-inflammatory role in chronic inflammation.[7,8] It has been demonstrated that FSTL1 is involved in the development of a variety of systemic diseases, particularly in respiratory inflammatory disorders having an impact on the course of a variety of diseases such as asthma, pulmonary fibrosis, and infectious diseases of the lungs.[8,9] In the macrophages of the lungs of asthma patients, FSTL1 is highly expressed[10] and can regulate airway remodeling and inflammatory damage in asthma patients through the lncRNA PVT1/miR-590-5p/FSTL1 axis or the miR-200b-3p/FSTL1 axis in asthma airway epithelium. Additionally, it can participate in the pathogenesis of asthma by activating type 2 innate lymphoid cells through the MEK-JAK-STAT-GATA2 pathway.[11–13] These findings regarding FSTL1-induced pathways all indicate a close association between FSTL1 and asthma. Furthermore, researchers have analyzed genotype data extracted from the COPDGene database and found that single-nucleotide polymorphisms near the FSTL1 locus are associated with chronic obstructive pulmonary disease (COPD), and genetic polymorphisms at the FSTL1 locus may affect the COPD and lung function of some individuals.[14] All of the above confirm that FSTL1 plays a role in patients with asthma and COPD, However, there is still a significant knowledge gap regarding FSTL1 in the field of CRS, which greatly limits our comprehensive understanding of the pathogenesis of CRS. Therefore, this study primarily investigates the role of FSTL1 in CRS, especially in type 2 inflammation, which is of great significance for gaining an in-depth understanding of the pathogenesis of CRS and developing new therapeutic targets. Particularly for patients who exhibit suboptimal responses to conventional therapies, the development of novel drugs or therapeutic modalities targeting FSTL1, such as monoclonal antibodies and small molecule inhibitors, holds promise. We can also screen out patient groups that are more sensitive to FSTL1-targeted therapy by detecting the expression level of FSTL1, to achieve personalized treatment and bring more treatment options for ECRS patients.

Materials and Methods

Research Subjects

This investigation involved a cohort of 44 patients who underwent endoscopic nasal surgery at the Department of Otolaryngology-Head and Neck Surgery at Shanxi Medical University First Hospital, spanning the period from November 2023 to June 2024. Among the 44 patients, 34 were diagnosed with chronic rhinosinusitis (CRS). According to the EPOS 2020 and the Chinese Guidelines for the Diagnosis and Treatment of Chronic Rhinosinusitis (2018), five non-consecutive high-power fields (×400) were randomly selected from the HE-stained pathological sections to count eosinophils, and the average value was taken. Patients with ≥10 eosinophils were included in the ECRS group, while those with <10 eosinophils were included in the non-ECRS group. The remaining 10 patients served as the control group. Subsequent to the acquisition of the specimens, they were promptly bifurcated into two distinct samples. The first sample was preserved at ambient temperature, earmarked for HE staining and IHC. The second sample was conserved in a −80°C freezer, designated for qRT-PCR analysis, with a preservation duration of three months.

Inclusion criteria: (1) Patients were diagnosed with CRS based on a comprehensive assessment that included clinical history, anterior rhinoscopy, nasal endoscopy, and nasal computed tomography (CT) findings, in accordance with the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2020 and the Chinese guidelines for the diagnosis and treatment of chronic rhinosinusitis (2018),[1] (2) All patients met the surgical indications for the disease as defined in the clinical pathway, and postoperative pathology confirmed the diagnosis of nasal polyps; (3) The control group had no history of nasal polyps and no history of atopic diseases, with negative results from skin prick tests for common allergens; (4) All patients underwent surgery performed by the same physician; (5) Age between 18 and 80 years; Exclusion criteria: (1) Failure to sign the informed consent form; (2) History of allergic rhinitis, allergic fungal

rhinosinusitis, bronchial asthma, chronic obstructive pulmonary disease, aspirin triad, cystic fibrosis, etc; (3) Acute upper respiratory tract infection within 2 weeks prior to surgery, or use of glucocorticoids, immunosuppressants, antibiotics, etc; (4) History of previous nasal surgery (excluding nasal polyp surgery); (5) Suffering from tumors, infectious diseases, mental disorders, severe systemic diseases, or autoimmune diseases.

All patients had signed informed consent forms, and the study was approved by the hospital's ethics committee (NO. KYLL-2024-101).

Hematoxylin and Eosin (HE) Staining

The specimens underwent a dehydration process and were subsequently embedded in paraffin. Following the solidification of the paraffin, approximately five sections, each with a thickness of 4 μm, were cut in succession. Two of these sections were chosen for the processes of deparaffinization and rehydration. The sections were then stained with hematoxylin for a duration of 10 minutes, followed by eosin for 5 minutes. After undergoing dehydration and clearing, a mounting medium was applied for coverslipping. The treated nasal mucosa tissue sections were meticulously examined under an optical microscope, and any pathological alterations were documented and analyzed comprehensively. High-resolution images were also captured for subsequent analysis and research purposes.

Immunohistochemical (IHC)

Following the drying of the paraffin sections, deparaffinization and rehydration were conducted, which was succeeded by antigen retrieval using sodium citrate and EDTA(ZSGB Biotechnology Co. Ltd.,Beijing). The sections were then treated to inactivate endogenous peroxidase, followed by serum blocking, and were incubated at room temperature for 30 minutes. Subsequently, 50 microliters of primary antibodies [rabbit anti-human-FSTL1 polyclonal antibody 1:200 (Proteintech,20182-1-AP), rabbit anti-human-IL-4 polyclonal antibody 1:400(HUABIO,ER1706-55), rabbit anti-human-IL-5 polyclonal antibody 1:400(HUABIO,ER1911-38), rabbit anti-human-IL-13 polyclonal antibody 1:4000(HUABIO, ER1911-23)] were applied, and the sections were incubated overnight in a humidified chamber at 4°C. After allowing the sections to return to room temperature for 30 minutes, 50 microliters of secondary antibody were introduced, and the sections were incubated in an oven at 37°C for 30 minutes. A DAB(ZSGB Biotechnology Co. Ltd.,Beijing) chromogenic reaction was performed, and the staining results were monitored under a microscope until the desired intensity was achieved. Hematoxylin counterstaining, dehydration, clearing, and coverslipping were subsequently performed, then the sections were sealed and the distribution of FSTL1, IL-4, IL-5, IL-13 and the expression of the proteins in the positive cells were observed under the microscope. For each section, five non-overlapping areas were randomly selected under a 400x high-power lens, and their average optical density values (IOD/Area) were quantified.

Quantitative Real-Time Polymerase Chain Reaction(qRT-PCR)

Thawed tissue samples were placed in enzyme-free Eppendorf tubes, to which TRIzol reagent(Solarbio,Beijing) was added. The samples were then homogenized thoroughly using a tissue grinder to facilitate RNA extraction. Following a purity assessment, the extracted RNA was subjected to reverse transcription, The resultant complementary DNA (cDNA) was utilized for polymerase chain reaction analysis. ACTIN(Forward primer (5′–3′): CCTGGCACCCAGCACAAT, Reverse primer (5′–3′): GGGCCGGACTCGTCATAC)(Sangon Biotech,Shanghai)served as a control for normalizing the gene expression levels and the results were analyzed using the specific method. The PCR procedure was as follows: An initial denaturation step at 95°C for 5 min, followed by 40 cycles of denaturation at 72°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 20 sec, and a melting curve from 60 to 65°C. The primer sequences employed were as follows: FSTL1 (Forward primer (5′–3′): TCTGTGCCAATGTGTTTTGTGG, Reverse primer (5′–3′): TGAGGTAGGTCTTGCCATTACTG)(Sangon Biotech,Shanghai).

Western Blotting (WB)

Approximately 50mg of the sample was taken and added to an Eppendorf (EP) tube for protein extraction. The extracted protein concentrations were determined using the BCA protein assay kit(Boster,AR0146) based on the standard curve. After preparing the gel, the protein samples were subjected to electrophoresis with parameters set to a constant voltage of

80V for about 30 minutes, followed by an adjustment to a constant voltage of 120V. The samples were then transferred to a membrane using a transfer parameter setting of a constant current of 200mA for 90 minutes. After the transfer was complete, the membrane was incubated with 5% skim milk at room temperature for 2 hours to block non-specific binding. It was then immersed in an incubation box containing the primary antibody [rabbit anti-human-FSTL1 polyclonal antibody at a dilution of 1:5000(Proteintech,20182-1-AP)] or the loading control [Anti-Beta Actin/ACTB Antibody at a dilution of 1:10000(Boster,BM0627)] and incubated overnight at 4°C on a shaker. The next day, the primary antibody was recovered, and the secondary antibody [HRP Conjugated AffiniPure Goat Anti- rabbit IgG (H+L) at a dilution of 1:5000(Boster,BA1054)] was added and incubated at room temperature on a shaker for 2 hours. The membrane was then developed using an super sensitive ECL luminescence reagent(Meilunbio,MA0186) developing solution and photographed. After exporting the images, the concentration of the bands was calculated using Image J software.

Statistical Analysis

The data were processed and analyzed utilizing SPSS version 26.0 and GraphPad Prism software. When the data conform to a normal distribution and homogeneity of variance, they are expressed as the mean ± standard deviation (SD), and differences between groups are analyzed using one-way ANOVA. For data that do not follow a normal distribution or have heterogeneous variances, differences between the three groups are assessed using the Kruskal–Wallis *H*-test. Correlations between FSTL1 and IL-4,IL-5,IL-13 were explored by Spearman's rank correlation analysis. $P<0.05$ will be regarded as statistically significant.

Results

Comparison of Clinical Data of Patients

44 patients were included in this study. The clinical data of all patients are shown in Table 1. Gender and age exhibit no statistical difference among the three groups ($P> 0.05$, Table 1).

HE results Showed the Tissue Characteristics of the ECRS and Non-ECRS Groups

Optical microscopy showed that the nasal mucosal epithelium of the ECRS and non-ECRS groups was obviously thickened, with mesenchymal edema and fibrous tissue hyperplasia, in which there was infiltration of a variety of inflammatory cells, such as plasma cells, lymphocytes, and eosinophils, and the degree of eosinophilic cell infiltration of the ECRS group was higher than that of the non-ECRS group. The mucosal epithelium of the control group was pseudocomplex ciliated columnar epithelium with a small amount of inflammatory cell infiltration. The structure of the nasal mucosa was normal, without significant thickening or edema. Eosinophil infiltration was minimal, and the nuclear and cytoplasmic staining was normal (Figure 1).

Table 1 Comparison of Clinical Data Among Three Groups

	N	Age [Mean±SD (Years)]	Gender [N(%)]	
			Male	Female
ECRS	24	38.8±11.0	8 (33.3)	16 (66.7)
Non-ECRS	10	34.0±11.7	7 (70.0)	3 (30.0)
Control	10	39.5±9.4	3 (30.0)	7 (70.0)
P value		0.244[a],0.871[b],0.264[c]	0.102	

[a]Comparison between the ECRS group and the non-ECRS group. [b]Comparison between the ECRS group and the control group. [c]Comparison between the non-ECRS group and the control group.

Abbreviations: ECRS, eosinophilic chronic rhinosinusitis; non-ECRS, non-eosinophilic chronic rhinosinusitis.

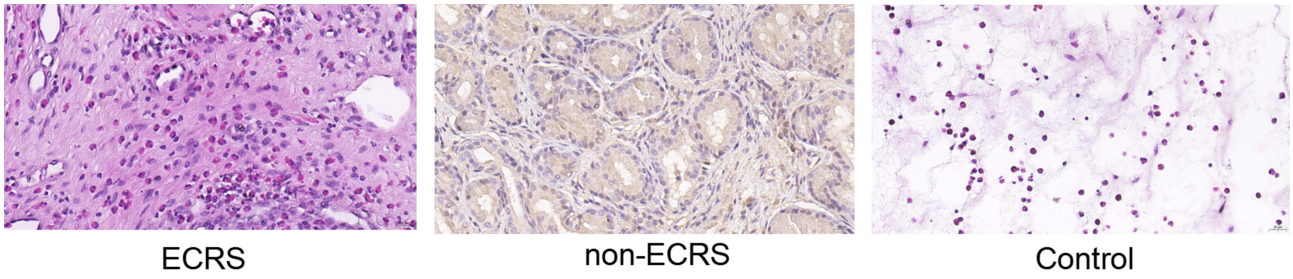

Figure 1 Hematoxylin and eosin staining of nasal mucosa from three groups (400×), In the ECRS sections, there was a high degree of eosinophil infiltration, with clear nuclear and cytoplasmic staining, showing typical blue-violet and pink colors. A large number of eosinophils were visible in the tissue, with their granules appearing bright red under eosin staining. In the non-ECRS stained sections, fewer eosinophils were observed, with nuclear and cytoplasmic staining similar to that in the ECRS group, but the granules of eosinophils were less intensely stained. In the control group stained sections, there was minimal eosinophil infiltration, with normal nuclear and cytoplasmic staining.

FSTL1 Expression Level Was Elevated in the ECRS Group

Optical microscopy revealed that FSTL1 was more stained in the mucosal epithelium of the ECRS group, and the cytosolic staining of its positive cells was brownish-yellow in color, which was located in mucosal epithelium, glandular epithelial cells and inflammatory cells. Comparing the difference in FSTL1 protein expression level among the three groups, the greater the average optical density value (IOD) of FSTL1 protein positive staining, the higher the level of FSTL1 protein expression in the tissues, and the IHC results showed that it was significantly more in the ECRS group (0.239±0.024) and non-ECRS group(0.194±0.017) compared with the control group(0.117 ±0.024), and the level of FSTL1 protein in the ECRS group was higher than that in the non-ECRS group($P < 0.05$, Figure 2A and C). qRT-PCR results showed that specimens from all groups were detected with The expression of FSTL1 was significantly higher in the ECRS(2.899±1.590) and non-ECRS groups(1.439±0.349) compared with the control group(0.565 ±0.161), and the level of FSTL1 mRNA in the ECRS group was higher than that in the non-ECRS group, with statistically significant differences between the two comparisons between the groups ($P < 0.05$, Figure 2E). The relative quantitative detection by WB showed that the levels of FSTL1 protein in the ECRS(1.222±0.796) and non-ECRS groups(0.658±0.233) were significantly higher than those in the control group(0.433 ±0.183), and the differences between the two groups were statistically significant in both comparisons ($P < 0.05$, Figure 2B and D).

Characteristics and Results of IL-4 IHC Staining, as Well as Its Expression in the Three Groups

The specimens of all groups were The positively stained cells of IL-4 were mainly concentrated in the cytoplasm of nasal mucosa, vascular endothelial cells and inflammatory cells, which were brown in color, while there was no obvious staining or lighter staining in the control group. The IHC results showed that the mean optical densities for the ECRS group, non-ECRS group, and control group were 0.206±0.014, 0.154±0.019, and 0.113±0.002, respectively. Compared with the control group and the non-ECRS group, the number of IL-4 positively stained cells in the ECRS group was significantly increased, and pairwise comparisons between groups showed statistically significant differences ($P<0.05$, see Figure 3).

The Levels of IL-5 in the Nasal Mucosa of Patients With ECRS are Significantly Elevated

IL-5 was expressed in different degrees in the tissues of each group, which was brown or brownish-yellow, with cytoplasmic coloring, and was negative or weakly positive in the tissues of the normal inferior turbinate.The mean optical density for the ECRS group, non-ECRS group, and control group was 0.198±0.020, 0.148±0.012, 0.097 ±0.020, respectively. Pairwise comparisons between groups showed statistically significant differences ($P<0.05$, see Figure 4).

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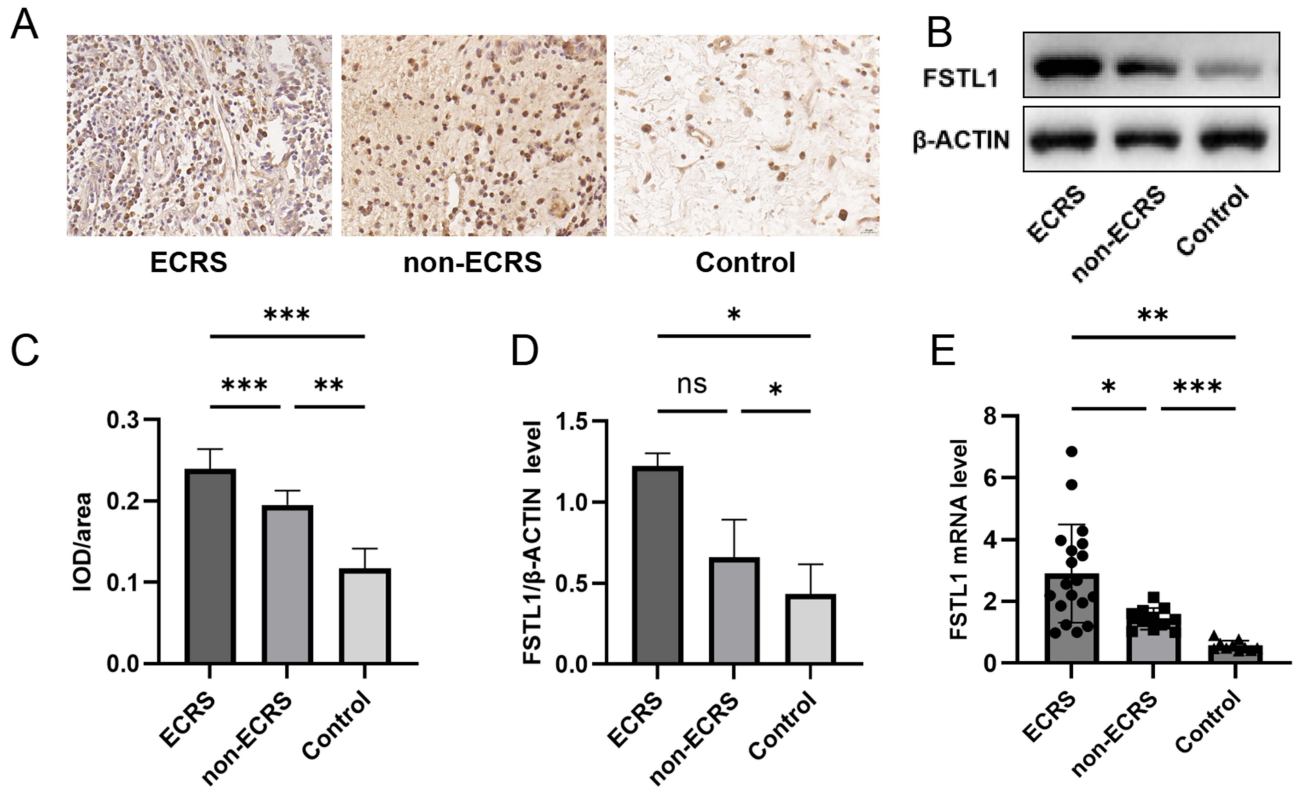

Figure 2 (**A**) IHC staining of FSTL1 in nasal mucosa from the ECRS group, non-ECRS group, and control group (400×). Compared with the control group, the ECRS and non-ECRS groups showed increased inflammatory cells, and FSTL1 staining exhibited strong specificity, clearly localizing to the cytoplasm as brown granular or patchy deposits. (**B**) WB band diagram of FSTL1 in the ECRS group, non-ECRS group, and control group. The grayscale values of FSTL1 decreased progressively from the ECRS group to the non-ECRS group and then to the control group. (**C**) Statistical significance analysis of the mean optical density values of FSTL1 in IHC experiments. Significant differences in mean optical density values were observed among the three groups. Compared with the control group, the mean optical density values in the ECRS and non-ECRS groups were significantly elevated (***P < 0.001, **P < 0.01). Additionally, compared with the non-ECRS group, the mean optical density value in the ECRS group was significantly higher (***P < 0.001). (**D**) Statistical significance analysis of FSTL1/ACTIN levels in WB experiments. Compared with the control group, the mean optical density values in the ECRS and non-ECRS groups were significantly elevated (*P < 0.05). However, no significant difference was observed between the ECRS and non-ECRS groups (ns, no significance). (**E**) Statistical significance analysis of FSTL1 mRNA levels in RT-PCR experiments. Significant differences in mRNA levels were observed among the three groups. Compared with the control group, the mRNA levels in the ECRS and non-ECRS groups were significantly elevated (***P < 0.001, **P < 0.01). Additionally, compared with the non-ECRS group, the mean optical density value in the ECRS group was significantly higher (*P < 0.05).

Abbreviations: IHC, immunohistochemistry; WB, Western blotting; RT-PCR, reverse transcription-polymerase chain reaction; ECRS, eosinophilic chronic rhinosinusitis; non-ECRS, non-eosinophilic chronic rhinosinusitis.

The Expression Differences of IL-13 Among Different Groups

Under the light microscope, IL-13 positive staining is concentrated in the mucosa and around the blood vessels of the glands, localized in the cytoplasm or nucleus. Positive cells appear granular under high magnification, with typical staining appearing as brownish-black. The control group shows lighter staining. Through IHC staining, the expression differences of IL-13 in different tissues can be clearly observed. The mean optical densities for the ECRS group, non-ECRS group, and control group were 0.180±0.015, 0.144±0.011, and 0.113±0.005, respectively. Pairwise comparisons between groups showed statistically significant differences($P<0.05$, see Figure 5).

The Correlation Between FSTL1 Levels and IL-4,5,13 Levels

There was a significant positive correlation between FSTL1 and IL-4,5,13 in ECRS group, the correlation between FSTL1 and IL-5,13 was not statistically significant in the non-ECRS group($P<0.05$, see Figure 6). It is further suggested that high FSTL1 expression may inhibit the development of type 2 inflammation in ECRS group.

Discussion

Chronic sinusitis, as a serious public health problem that has long plagued the general public, is far from enough to solve this disease more effectively by relying solely on existing treatments. FSTL1 plays an important role in the inflammatory

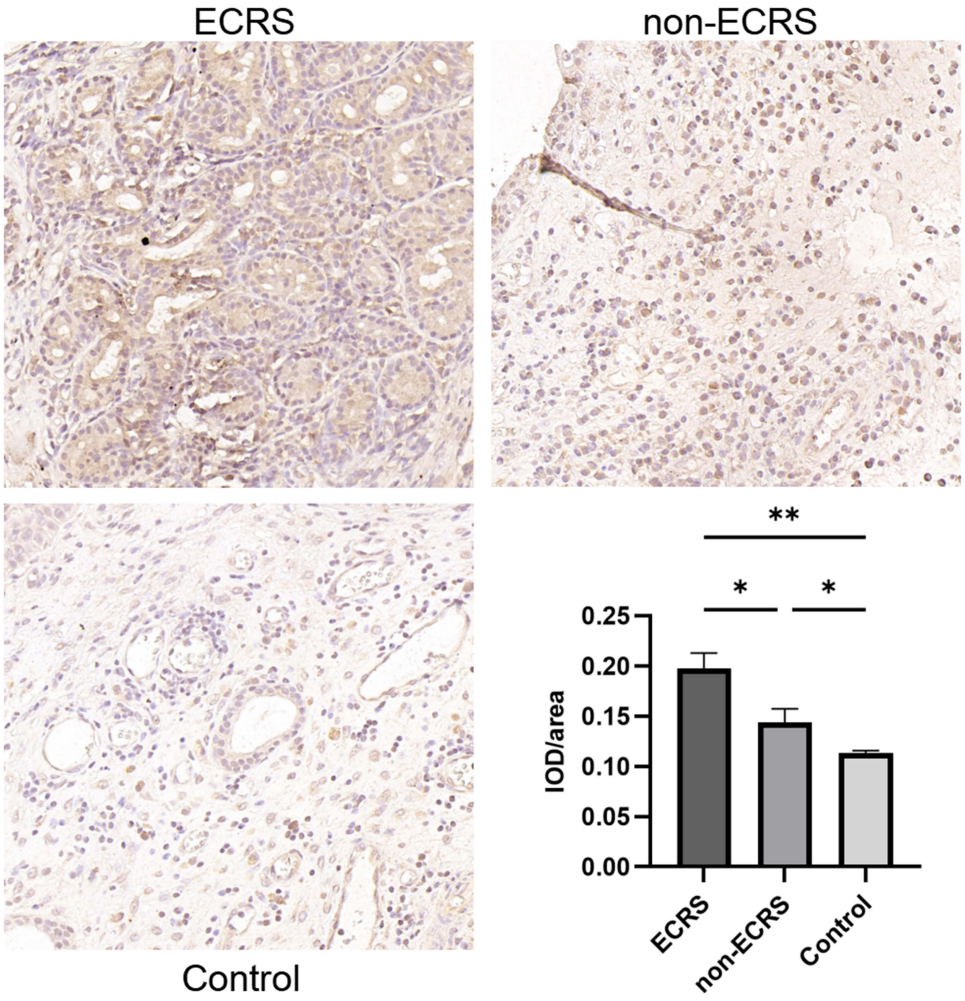

Figure 3 IHC staining of IL-4 in different groups (400 ×). In ECRS, the IL-4-stained sections appear brownish-black with strong positive staining intensity. In non-ECRS, the IL-4-stained sections appear brownish-yellow or light brown with weak positive staining intensity. In the control group, IL-4 distribution is minimal, and the staining intensity is negative. Significant differences in mean optical density values were observed among the three groups. Compared with the control group, the mean optical density values in the ECRS and non-ECRS groups were significantly elevated (**P < 0.01, *P < 0.05). Additionally, compared with the non-ECRS group, the mean optical density value of IL-4 in the ECRS group was significantly higher (*P < 0.05).

Abbreviations: IHC, immunohistochemistry; ECRS, eosinophilic chronic rhinosinusitis; non-ECRS, non-eosinophilic chronic rhinosinusitis; IOD/area, average optical density.

response[7,15] and is not only involved in inflammatory diseases of the lungs leading to pulmonary fibrosis and lung failure,[16,17] but also in the development of airway inflammation through the MEK-JAK-STAT- GATA2 pathway to promote the development of airway inflammation and participate in the pathogenesis of asthma.[13] It has been reported that FSTL1 inhibits myocardial fibrosis and apoptosis through the USP10/Notch1 signaling pathway,[11] and that many cancer-associated signaling cascades (TGF-β/BMP/Smad signaling, AKT, NF-κB, and Wnt-β-catenin signaling pathways) are regulated by FSTL1.[18] In addition, FSTL1 is also an important pro-fibrotic glycoprotein, and TGF-β1 (transforming growth factor β1, a central mediator of tissue fibrosis) up-regulates FSTL1 protein and mRNA expression in mouse lung fibroblasts.[19] These findings suggest that FSTL1 is regulated by multiple factors and signaling pathways during disease development and is likely to be a key target for disease treatment. Although extensive research has been conducted on FSTL1, its role in CRS has not been explored. Studying how FSTL1 is expressed and regulated can help us gain a more comprehensive understanding of the nature of CRS and provide a solid theoretical foundation for subsequent precision treatment.

In this experiment, we observed that in the CRS tissue specimens, the nasal mucosal epithelium was significantly thickened, with interstitial edema, fibrous tissue proliferation, and infiltration of various inflammatory cells such as

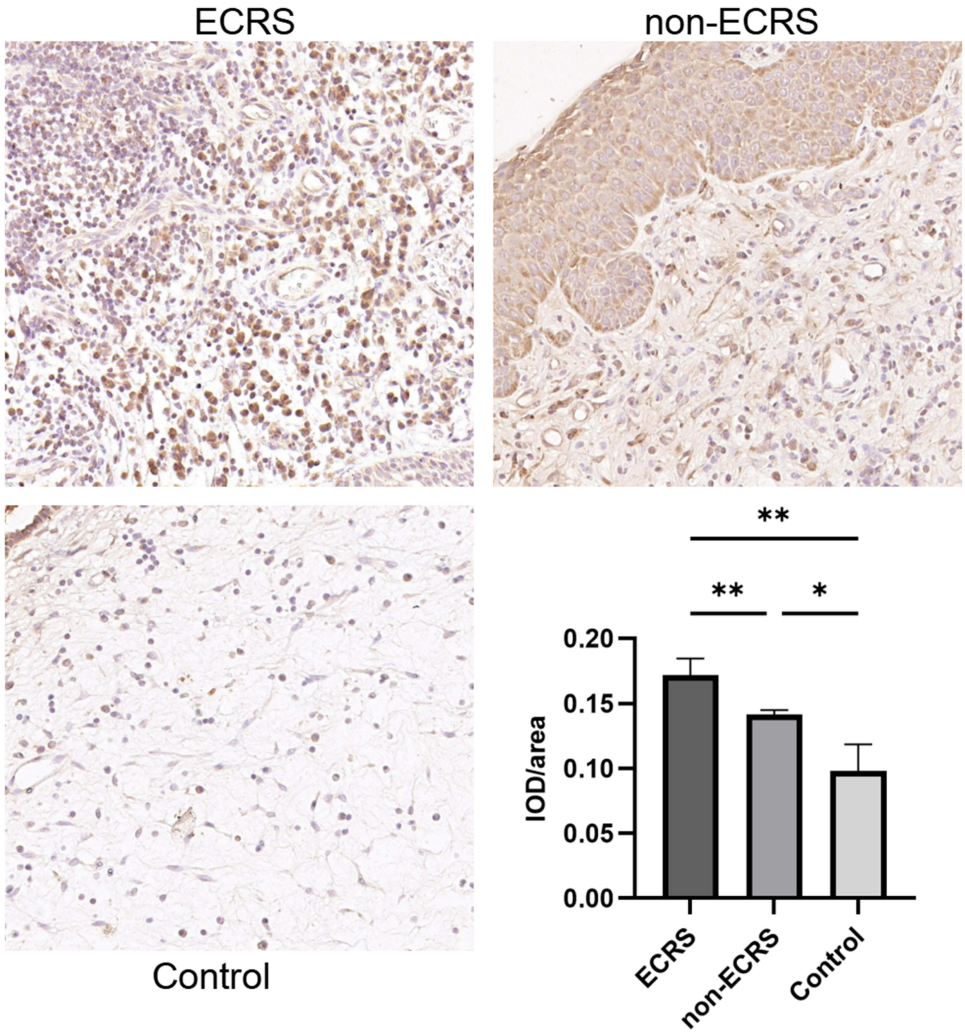

Figure 4 IHC staining of IL-5 in different groups (400 ×). In ECRS, IL-5 staining shows positive staining in brownish-yellow or brownish-black. In the ECRS and control groups, the expression of IL-5 is relatively low. Significant differences in mean optical density values were observed among the three groups. Compared with the control group, the mean optical density values in the ECRS and non-ECRS groups were significantly elevated (**P < 0.01, *P < 0.05). Additionally, compared with the non-ECRS group, the mean optical density value of IL-5 in the ECRS group was significantly higher (**P < 0.01).

Abbreviations: IHC, immunohistochemistry; ECRS, eosinophilic chronic rhinosinusitis; non-ECRS, non-eosinophilic chronic rhinosinusitis; IOD/area, average optical density.

plasma cells, lymphocytes, and eosinophils. By comparing the ECRS group, non-ECRS group, and the control group, we discovered that the expression of FSTL1 protein and mRNA in both the ECRS and non-ECRS groups was significantly upregulated compared to the control group, so we can preliminarily conclude that FSTL1 plays a key role in the pathogenesis of CRS. This results of this experiment further confirmed and complemented the promotional role of FSTL1 in chronic inflammatory diseases and extended our studies on the inflammatory mechanisms of CRS.

Classification of CRS based on inflammatory endotypes can more accurately reflect the underlying pathophysiology and can better guide treatment,[20,21] and HE staining of nasal mucosa remains the most reliable test.[22] The HE staining results in this experiment showed that the nasal mucosa was significantly thickened in the ECRS and non-ECRS groups compared with the control group, and the degree of eosinophilic infiltration was higher in the ECRS group than in the non-ECRS group. In CRS, type 2 immune response was associated with eosinophilic inflammation. Type 2 immunity was mediated by ILC2, macrophages, mast cells, basophils and eosinophils, which produced cytokines such as IL-4, IL-5, and IL-13, among which IL-5 mainly promoted eosinophil differentiation and recruitment to the site of allergic inflammation, and IL-4 and IL-13 were responsible for driving and maintaining the Th2 response, IL-4 and IL-13 mainly exert their biological functions by binding to the IL-4R receptor complex, and IL-4Rα is the common receptor subunit of IL-4 and

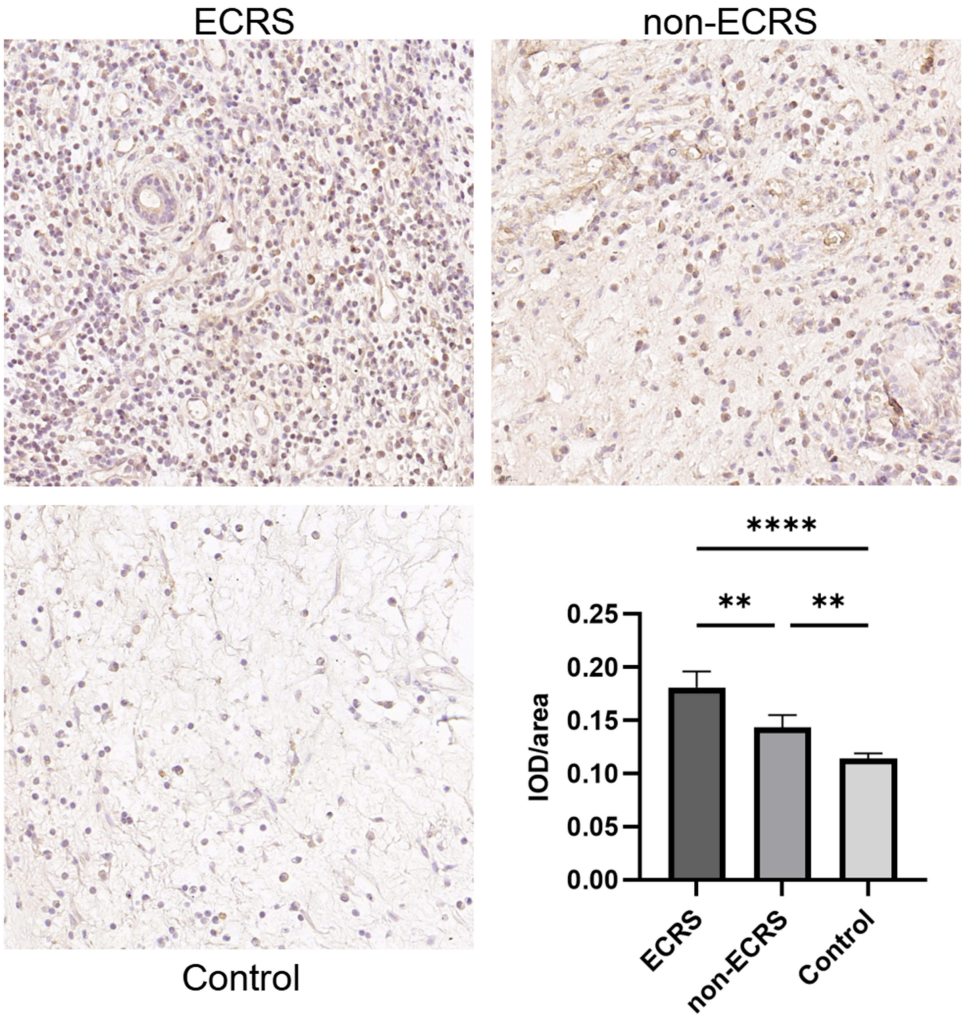

Figure 5 IHC staining of IL-13 in different groups.(400 ×). The expression of IL-13 in ECRS-stained sections was significantly increased, mainly distributed in the cytoplasm. Positive cells were mostly arranged in a patchy or diffuse pattern. In the nasal mucosa of non-ECRS sections, IL-13 expression was lower, and in the control group, IL-13 expression was barely detectable, with negative staining intensity. Significant differences in mean optical density values were observed among the three groups. Compared with the control group, the mean optical density values in the ECRS and non-ECRS groups were significantly elevated (****P < 0.0001, **P < 0.01). Additionally, compared with the non-ECRS group, the mean optical density value of IL-13 in the ECRS group was significantly higher (**P < 0.01).

Abbreviations: IHC, immunohistochemistry; ECRS, eosinophilic chronic rhinosinusitis; non-ECRS, non-eosinophilic chronic rhinosinusitis; IOD/area, average optical density.

IL-13,[6,23] FSTL1 may affect the differentiation and activation of TH2 cells by regulating the expression or function of IL-4Rα, thereby increasing the expression of IL-4, IL-5 and IL-13 in ECRS and exacerbating inflammation. In the present study, we confirmed by IHC experiments that the levels of IL-4, IL-5 and IL-13 were significantly up-regulated in the nasal mucosa tissues of patients in the ECRS group compared with those in the control group, suggesting that ECRS is closely related to type 2 inflammation, and that controlling the development of type 2 inflammation may be helpful in alleviating the conditions of patients with ECRS. To further explore the relationship between FSTL1 and type 2 inflammation in CRS, we conducted a correlation analysis and found that in the ECRS group, FSTL1 is positively correlated with IL-4, IL-5, and IL-13. This suggests that FSTL1 may be involved in the regulation of type 2 inflammation in ECRS, and its increased expression may promote the development of type 2 inflammation in ECRS. Although the experimental results are consistent with our expectations, the small sample size remains a potential limitation of this experiment. We still need larger-scale studies to validate the findings. The study has meticulously recorded patients' comorbidities, allergy histories, and medication usage. However, any unrecorded comorbidities or undiagnosed allergies in patients could also affect the experimental results.

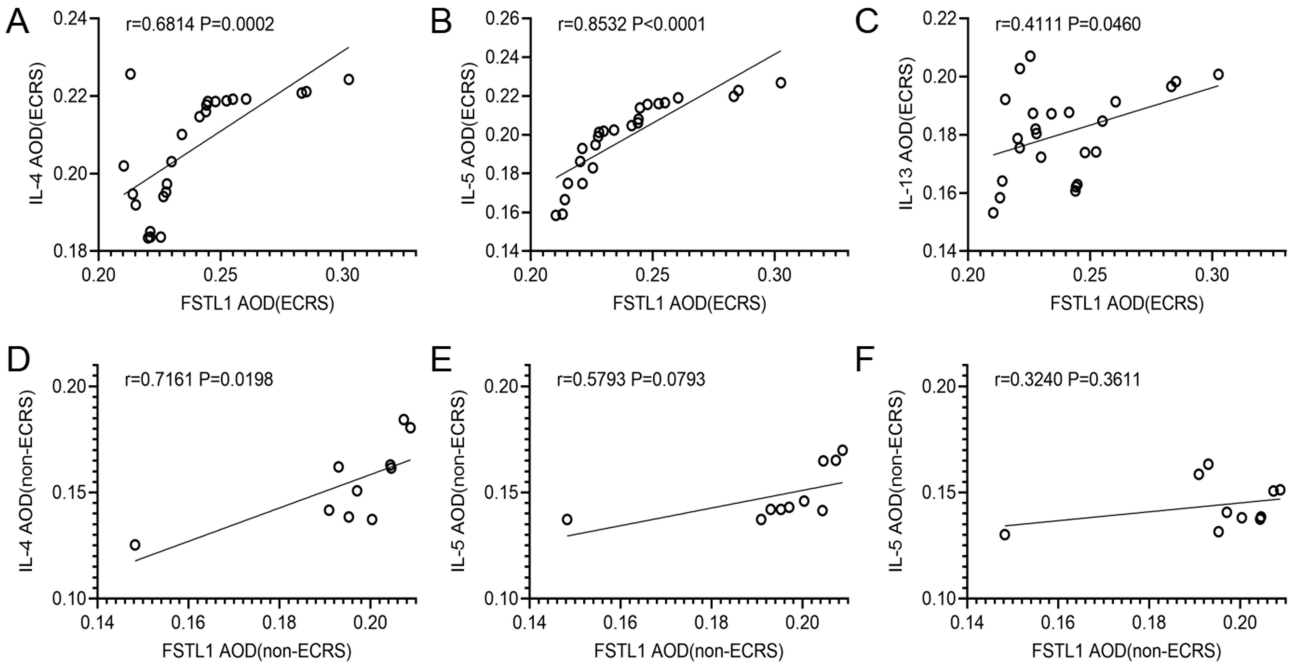

Figure 6 Correlation between FSTL1 levels and IL-4,5,13 levels. (**A**) Correlation between FSTL1 levels and IL-4 levels in ECRS group(r=0.6814 p=0.0002),In the ECRS group, there was a significant positive correlation between FSTL1 and IL-4, which was statistically significant. (**B**) Correlation between FSTL1 levels and IL-5 levels in ECRS group(r=0.8532 p<0.0001), In the ECRS group, there was a significant positive correlation between FSTL1 and IL-5, which was statistically significant. (**C**) Correlation between FSTL1 levels and IL-13 levels in ECRS group(r=0.4111 p=0.0460), In the ECRS group, there was a significant positive correlation between FSTL1 and IL-13, which was statistically significant. (**D**) Correlation between FSTL1 levels and IL-4 levels in non-ECRS group (r=0.7161 p=0.0198), In the non-ECRS group, there was a positive correlation between FSTL1 and IL-4, which was statistically significant. (**E**) Correlation between FSTL1 levels and IL-5 levels in non-ECRS group (r=0.5793 p=0.0793), In the non-ECRS group, the correlation between FSTL1 and IL-5 was not statistically significant. (**F**) Correlation between FSTL1 levels and IL-13 levels in non-ECRS group (r=0.3240 p=0.3611), In the non-ECRS group, the correlation between FSTL1 and IL-13 was not statistically significant.

FSTL1 is highly expressed in the nasal mucosa of patients with ECRS and positively correlates with the type 2 inflammatory cytokines IL-4, IL-5, and IL-13, suggesting that FSTL1 promotes the occurrence and development of type 2 inflammation in ECRS and may be a potential therapeutic target. There are many biologics targeting the mechanism of type 2 inflammation, such as omalizumab (anti-IgE), mepolizumab (anti-IL-5), benralizumab (anti-IL-5R), and dupilumab,[24] but these targeted biologics do not completely relieve the symptoms of the majority of patients, and even have the potential to produce adverse effects.[25] Therefore, exploring new targets to block inflammatory factors and related signal transduction pathways is essential to alleviate patient suffering. CRS is a chronic inflammation that has been extensively studied by researchers, but most of these studies are broad in scope. Our research focuses on type 2 inflammation in ECRS to achieve precision in ECRS treatment. Our previous research confirmed that the prostacyclin receptor is underexpressed in the nasal mucosa of ECRS patients and negatively correlated with IL-4, IL-5, and IL-13.[26] The current experiments have further validated that FSTL1 is highly expressed in ECRS and positively correlated with IL-4, IL-5, and IL-13. Next, we will conduct cellular and animal experiments, such as in vitro cell culture and analysis of cell signaling pathways. Alternatively, we will induce disease models to construct mouse models, followed by specific FSTL1 gene knockout, to study the functional role of FSTL1 in different disease mouse models. Through larger-scale studies, we will further verify their specific roles in ECRS and explore the feasibility of FSTL1 as a therapeutic target.

Conclusion

This study explored the differential expression of FSTL1 in eosinophilic chronic rhinosinusitis (ECRS) and non-eosinophilic chronic rhinosinusitis (non-ECRS) groups compared to the control group, as well as its relationship with type 2 inflammation. It was found that FSTL1, IL-4, IL-5, and IL-13 are highly expressed in chronic rhinosinusitis (CRS), with a positive correlation between FSTL1 and IL-4, IL-5, and IL-13 specifically in the ECRS group, indicating that FSTL1 may play a promotional role in the occurrence and development of type 2 inflammation in ECRS. Based on

the aforementioned findings, FSTL1 may represent a potential therapeutic target for ECRS. Targeting FSTL1 could offer ECRS patients more options, such as combining it with existing treatments to achieve better outcomes, or providing personalized precision therapy for patients who are sensitive to FSTL1. In summary, this study has unveiled the possible mechanisms of action of FSTL1 in ECRS and has provided new molecular markers for the diagnosis and treatment of ECRS. However, Future studies involving larger sample sizes and functional experiments are needed to confirm FSTL1's role in ECRS and its therapeutic potential.

Data Sharing Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Statement of Ethics

Study approval statement: Our study complies with the Declaration of Helsinki and has been reviewed and approved by the Ethics Committee of Shanxi Medical University, with the approval number [NO.KYLL-2024-101].

Consent to Participate Statement

The study was conducted with the informed consent of the patients themselves.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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