Int J Clin Exp Pathol 2019;12(3):933-940
www.ijcep.com /ISSN:1936-2625/IJCEP0087498

Original Article

The balance between HGF and TGF-β1 acts as a switch in the tissue remodeling of chronic rhinosinusitis

Jing Li[1], Xiang Liu[1], Min Sha[2], Yong Li[1]

[1]Department of Otolaryngology, Affiliated Hangzhou First People's Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang Province, China; [2]Department of Otolaryngology, Hangzhou Children's Hospital, Hangzhou, Zhejiang Province, China

Received October 29, 2018; Accepted December 21, 2018; Epub March 1, 2019; Published March 15, 2019

Abstract: Objective: The remodeling patterns in different types of chronic rhinosinusitis (CRS) are controversial. This study aimed to investigate the roles of transforming growth factor-β1 (TGF-β1) and hepatocyte growth factor (HGF) in the CRS remodeling process. Methods: Surgical samples were obtained from CRSwNP patients (n=29), CRSsNP patients (n=34), and controls (n=21). Collagen deposition was detected via Masson trichrome (MT) staining. Immunohistochemical staining was performed to examine the protein expression of α-smooth muscle actin (α-SMA). The expression of TGF-β1 and HGF was measured by ELISA. The relationship between the rate of TGF-β1/HGF and the expression of Collagen III and α-SMA was analyzed using a Pearson correlation test. Primary nasal epithelial cells (NECs) were cultured and divided into four groups. Collagen III secretion was measured in the supernatants by ELISA. The expression of α-SMA was studied by immunofluorescence. Results: Reduced collagen deposition and α-SMA expression were detected in the CRSwNP group (P=0.033). The expression of collagen deposition and α-SMA was increased in the CRSwNP group (P=0.001). The ELISA tests indicated that TGF-β1 levels were significantly increased in the CRSsNP compared with the controls. The expression of HGF was higher in the CRSwNP than in the other two groups. The ratio of TGF-β1/HGF was upregulated in CRSsNP and was correlated positively with collagen and α-SMA expression (P<0.05, R=0.762). TGF-β1 can increase collagen and α-SMA expression in NECs, and HGF can antagonize the remodeling action of TGF-β1. Conclusion: Distinct remodeling patterns are revealed for different types of CRS. The balance of TGF-β1 and HGF is important in the CRS remodeling process.

Keywords: Chronic rhinosinusitis, nasal polyp, tissue remodeling, transforming growth factor-β1, HGF

Introduction

Chronic rhinosinusitis (CRS) is one of the most common chronic diseases in adults. It is associated with impaired quality of life and substantial societal costs [1]. While it is sometimes associated with asthma, allergy, or nasal polyposis, many cases present without an apparent underlying cause. Despite a lack of etiology and the inflammatory nature of the condition, treatment protocols dictate intervention with antibiotics, anti-inflammatory drugs, or surgery [1, 2], often with poor long-term effects. CRS is typically classified into two types: chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP). The development of phenotype-orientated therapeutic strategies is critical for improving CRS treatment. Clinically, CRSwNP is associated more closely with clinical complaints of nasal obstruction and olfactory loss, and more frequently linked to comorbidities such as asthma and aspirin hypersensitivity, than CRSsNP. Immunologically, CRSwNP tissues are characterized by more intense eosinophilic infiltration and a Th2-based cytokine profile, whereas sinonasal tissues from CRSsNP have been reported to display a predominant infiltration of leukocytes and Th1 cytokines [3]. However, there are still some controversies about the distinct role of Th1/Th2 profiles in CRS subtypes.

CRS is an inflammatory disease with distinct cytokine patterns; it is characterized by chronic inflammation of the nasal and paranasal mucosa, and accompanied by tissue remodeling, which includes changes in extracellular matrix

(ECM) protein deposition and tissue structure. Distinct disease entities can be distinguished within a group of chronic sinus diseases on the basis of different inflammation and remodeling patterns. CRSsNP is characterized by nasal epithelial injury, myofibroblast proliferation, and extracellular matrix remodeling with high levels of interferon receptor (IFN-r) and transforming growth factor-β1 (TGF-β1) [4]. In contrast, CRSwNP is characterized by predominant albumin accumulation and edema formation within the ECM, and low levels of myofibroblast proliferation and TGF-β1-3 [5]. TGF-β1 is implicated as a key protein in the tissue remodeling process of CRSsNP. It stimulates fibrosis by attracting stromal cells and the transdifferentiation of primary nasal epithelial cells (NECs), angiogenesis, and the accumulation of extracellular matrix [6]. Some studies have reported lower TGF-β1 expression in CRSwNP than in CRSsNP and healthy controls [5, 7]. There is still a controversy in the literature as to TGF-β1 expression in nasal polyposis [8].

Hepatocyte growth factor (HGF), a pleiotropic growth factor, demonstrates antifibrotic properties in experimental models of lung, kidney, heart, skin, and liver fibrosis. HGF protein attenuates collagen accumulation and the extent of fibrosis. In addition, HGF affects fibroblast activation and transdifferentiation indirectly by inhibiting the epithelial-to-mesenchymal cell transition (EMT) [9]. HGF acts as an antifibrotic agent that protects the host against TGF-β1-mediated profibrotic effects [10]. Some studies suggest that the HGF complex may play a role in the pathogenesis of nasal polyps [11]. Therefore, the aim of this study was to investigate the roles of TGF-β1/HGF in the remodeling preprocess in CRS.

Materials and methods

Patients and study experimental design

This study was approved by the medical ethics committee of Affiliated Hangzhou First People's Hospital of Zhejiang University, and a written informed consent was obtained from each patient in the study. The work has been carried out in accordance with the declaration of Helsinki. Samples of the ethmoid bulla mucosa from patients without sinus disease who underwent endoscopic hypophysectomy or septoplasty were used as controls. Samples from patients with CRSwNP or CRSsNP were obtained during functional endoscopic sinus surgery (FESS) procedures. A total of 21 controls, 29 CRSwNP patients, and 34 CRSsNP patients were recruited into this study at the Department of Otorhinolaryngology of Hangzhou First People's Hospital, Hangzhou, China, from September 2015 to December 2017. Sinus disease diagnosis was based on history, clinical examination, nasal endoscopy, and a computed tomography (CT) scan of the paranasal cavities according to the standard criteria issued in the European Position Paper on Rhinosinusitis and Nasal Polyps guidelines [1]. All subjects underwent a skin prick test for common inhalant allergens and all tests had to be negative for the subject to be included in the experiment. Other exclusion criteria were a diagnosis of asthma (based on a ventilatory test), aspirin intolerance (based on history), or cystic fibrosis. All patients stopped oral corticosteroids for at least 1 month and topical application for at least 2 weeks before surgery. Patients did not take any other relevant medications. Biopsy specimens were obtained for the assessment of nasal tissue remodeling.

Histological analysis

The tissues were first fixed in 10% acetaldehyde for 24 h at room temperature immediately after surgical removal. The specimens were then decalcified in an EDTA solution and embedded in paraffin. Paraffin-embedded tissues were stained routinely with Masson trichrome (MT) staining to assess collagen deposition. The expression levels were estimated by integrated OD (IOD) of the positive cells. IOD/area was the average cumulative OD of the positive staining area of each group determined by Image Pro Plus software version 6.0. For immunohistochemistry, the sections were washed in running water for an hour, and after this, they were submitted to antigen retrieval using a sodium citrate buffer pH6.0 treatment at 95°C for 30 min. Bovine serum albumin (BSA) at 2% and hydrogen peroxide at 3% were utilized to block nonspecific reactions and remove endogenous peroxidase, respectively. Subsequently, the Sections were also incubated overnight at 4°C in a humidified chamber with the primary antibody to α-smooth muscle actin (α-SMA) diluted 1:100 (Santa Cruz Biotechnology, USA). The sections were rinsed again with PBS and

then incubated with a biotinylated goat anti-rabbit and mouse antibody (Thermo Scientific, USA) for 10 min. The sections were rinsed again with PBS. Finally, the sections were incubated with Streptavidin peroxidase (Thermo Scientific, USA). The slides were incubated for 10 min with 3,3'-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, USA) to visualize the reaction. The slides were counterstained with hematoxylin and then dehydrated and mounted. Primary antibodies were omitted and replaced with PBS for the negative controls. Ten random images from each sample were recorded on a digital camera (Olympus C-5050) without overlapping zones, and were assessed by two independent observers blinded to the tissue staining protocol. Images of the stained sections were analyzed by Image Pro Plus software in 10 microscopic fields.

TGF-β1 and HGF expression by ELISA

Samples in each group were homogenized in PBS and stored at -80°C. The levels of human TGF-β1 and HGF protein in the nasal mucosa were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, USA) according to the manufacturer's instructions. The values obtained by ELISA were corrected with a dilution factor and ultimately expressed in pg/mg pro. Correlations of the ratio of TGF-β1/HGF with collagen deposition and α-SMA expression in nasal tissues were investigated. The Pearson correlation test was used, and R values indicate Pearson correlation coefficients.

Primary culture of NECs

The nasal specimens in the control group that did not require histopathologic examination were temporarily placed in a 50 mL conical tube containing 10 mL of DMEM/F-12 medium (Corning), 100 IU/mL penicillin, and 100 mg/mL streptomycin. NECs were cultured according to previously published methods [10]. NECs were cultured to confluence as monolayers in 24-well plates containing BEGM under standard culture conditions. At the end of culturing, the cells were harvested and seeded into 6-well plates at a concentration of 1×10^5 cells / well. The NECs were divided into four groups. The culture media were then replenished with BEGM (control group), BEGM containing 5 ng/mL TGF-β1 (R&D Systems Inc.), BEGM containing 100 ng/mL HGF (R&D Systems Inc.) (HGF group), BEGM containing 5 ng/mL TGF-β1 and 100 ng/mL HGF (TGF-β1+HGF group). The NECs were treated for 48 h at 37°C in a 5% CO_2 in air atmosphere. At the end of culturing, supernatants were collected, and the levels of collagen III were quantified using commercial ELISA kits (eBioscience, USA) according to the manufacturer's instructions. Each experimental condition was tested in six replicate wells, and the mean was taken to represent an individual experiment.

Immunocytochemistry

NECs were fixed with 4% formaldehyde for 30 min and then incubated in 1% BSA/10% normal goat serum/0.3 M glycine in 0.1% PBS-Tween for 1 h to permeabilize the cells and block non-specific protein interactions. The cells were then incubated with the antibody to α-SMA (1:100; Santa Cruz Biotech, USA). Alexa 555-conjugated secondary antibodies (Molecular Probes/Invitrogen) were used. DAPI (Sigma-Aldrich, USA) was used for nuclear staining. Images were obtained via microscopy.

Statistical analysis

The data were statistically analyzed by SPSS version 16.0 statistical package. Data are expressed as the mean ± standard error (SE). Differences between the groups were assessed using one-way analysis of variance (ANOVA) followed by Tukey's test. The Pearson correlation test was also used. Differences were considered significant at $P<0.05$.

Results

Different collagen deposition and fibrotic marker in CRSwNP and CRSsNP patients

A total of 21 controls, 34 CRSwNP patients, and 25 CRSsNP patients were recruited in this study. They ranged in age from 19 to 72 years with the age of 42.8±15.1 years in the control group, from 22 to 75 years with the age of 44.1±13.7 years in the CRSsNP group, and from 21 to 85 with the age of 47.6±18.2 years.

MT staining of the nasal mucosa in different groups was performed to assess collagen fiber distribution, which was evaluated by viewing

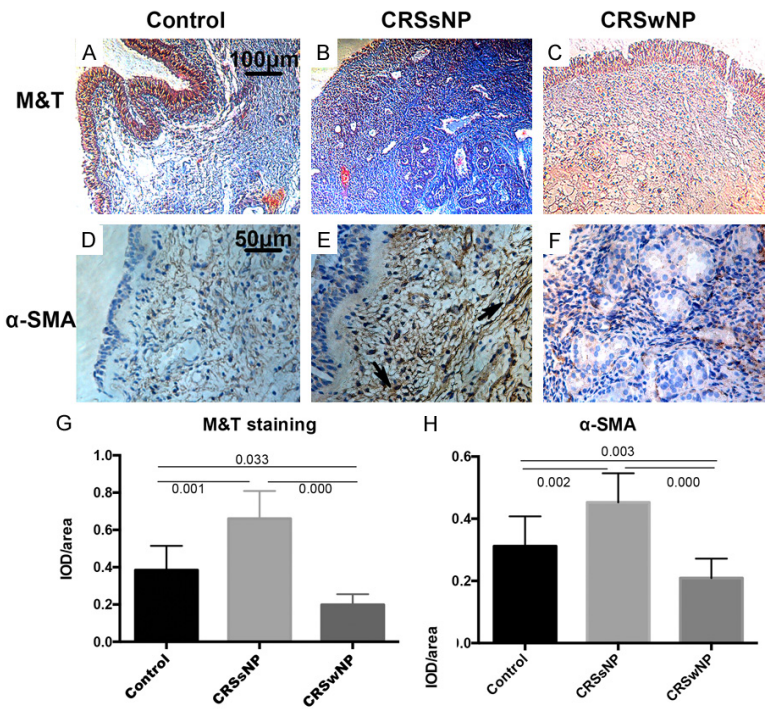

Figure 1. Histopathological examination of nasal mucosa tissues from different groups. (A-C) The MT staining showed that collagen fibers in the CRSwNP groups were thinner and fewer in number than in the CRSsNP and control groups. The CRSsNP group revealed extensive collagen deposition, bridging fibrosis and the collagen fibers were thicker and more. (G) The degree of collagen deposition in the different groups was calculated. (D-F) IHC staining for α-SMA showed normal expression of α-SMA positive staining in control group and α-SMA expression in the CRSwNP group was significant reduced compared to CRSsNP group. Positive staining is indicated by an arrow. The semi-quantitative analysis of IHC is shown in (H). MT, Masson trichrome; CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; IHC, immunohistochemistry.

sections with bright-field microscopy, as shown in **Figure 1A-C**. The control group showed a normal collagen distribution (**Figure 1A**). The CRSsNP group revealed extensive collagen deposition, bridging fibrosis (**Figure 1B**), and thicker and more numerous collagen fibers than collagen in the CRSwNP and control groups. Collagen fibers were reduced in the CRSwNP group compared to the control group (**Figure 1C**). The degree of collagen deposition is calculated in **Figure 1G**. It showed that there are significant differences in collagen deposition among the three groups using ANOVA test (P<0.001). Reduced collagen deposition and α-SMA expression were detected in the CRSwNP group (P=0.033). The expression of collagen deposition and α-SMA was increased in the CRSwNP group (P=0.001) (**Figure 1H**).

Representative sections of immunohistochemical staining for α-SMA were performed in the controls, CRSsNP, and CRSwNP and are shown in **Figure 1D-F**. The expression of α-SMA was significantly higher in CRSsNP than in the controls and was significantly lower in CRSwNP than in the controls. α-SMA immunoreactivity was stained dark brown. The control group showed normal expression of α-SMA-positive staining. α-SMA expression in the CRSwNP group showed a significant reduction compared to the CRSsNP group. **Figure 1H** summarizes the data of the immunohistochemical evaluation of α-SMA expression in the three groups.

TGF-β1/HGF disbalance in CRS groups

TGF-β1 and HGF concentrations in the nasal mucosa are tested by ELISA and the results presented in **Figure 2A** and **2B**. The differences of TGF-β1 and HGF concentrations between the groups were assessed using ANOVA followed by Tukey's test. TGF-β1 levels were significantly increased in the CRSsNP group compared with the controls (P<0.001). However, there was no difference in TGF-β1 between the control and CRSwNP groups (P=1.000). The expression of HGF was higher in the CRSwNP group, and no differences in HGF were found between the control and the CRSsNP groups.

Relation of TGF-β1/HGF with collagen deposition and α-SMA

The ratios of TGF-β1/HGF were different in the three groups, where CRSsNP>control>CRSwNP, as presented in **Figure 2C**. Correlations of the ratio of TGF-β1/HGF with collagen deposition and α-SMA expression in nasal tissues were investigated. The Pearson correlation test was used, and R values indicate Pearson correla-

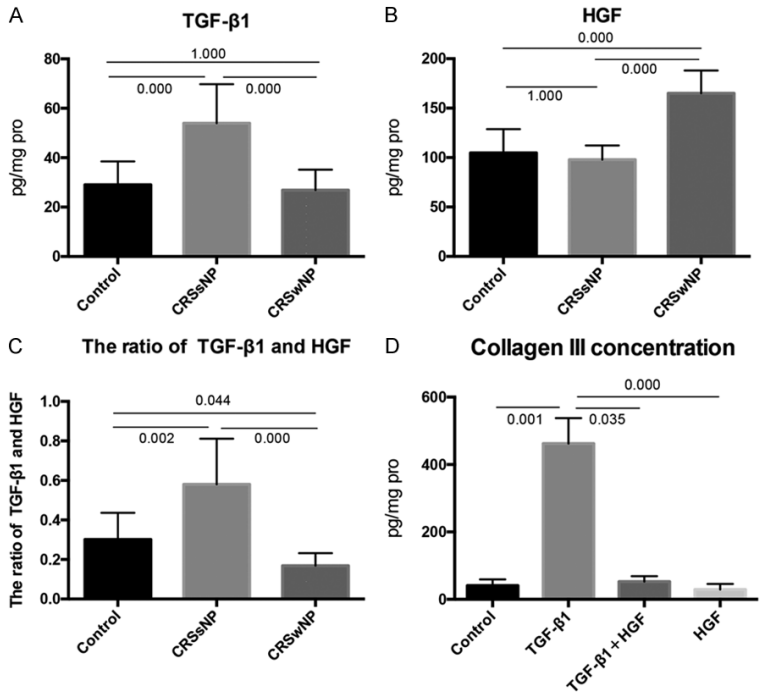

Figure 2. ELISA measurements for TGF-β1 (A), HGF (B), and collagen III, expressed as pg/mg protein. Tissue homogenates were prepared from the control, CRSsNP, and CRSwNP groups. In the CRSsNP group, expression of TGF-β1 was higher than in the other groups, and the HGF level was highest in the CRSwNP group. The ratio of TGF-β1 and HGF is shown in (C). There were significant differences between the three groups ($P<0.05$). The collagen III expression in the culture media of NECs is shown in (D). TGF-β1 can induce the secretion of collagen III in NECs. However, HGF can antagonize this effect and reduce the production of collagen III.

tion coefficients. The result showed that the ratio of TGF-β1/HGF was positively related to the expression of collagen ($P<0.05$, $R=0.889$) and α-SMA ($P<0.05$, $R=0.762$) in the mucosa of patients.

HGF can antagonize the fibrotic effect caused by TGF-β1 in NECs

NECs were treated with different conditions to test whether HGF can antagonize the fibrotic effect of TGF-β1. ELISA was used to quantify collagen III expression in culture media of NECs under different culture conditions. As shown in **Figure 2D**, 100 ng/ml HGF significantly suppressed TGF-β1-induced collagen III release in the culture medium of NECs by ANOVA test ($P=0.035$). We also studied the release of α-SMA in NECs with immunocytochemistry, which plays an important role in the transdifferentiation of epithelial cells. The expression of α-SMA (red) was higher in the TGF-β1 group than in the other groups, which is shown in **Figure 3**. HGF treatment decreased the expression of α-SMA induced by TGF-β1 in NECs.

Discussion

CRS is known to be characterized by tissue remodeling. Studies on the remodeling patterns of CRS might help us understand the pathogenesis of this burdensome disorder. Tissue remodeling in CRS includes changes in tissue structure and ECM deposition. However, the remodeling pattern and histological characteristics in CRSsNP and CRSwNP are different [12]. Traditionally, CRSsNP is characterized by fibrosis, basement membrane thickening, and goblet cell hyperplasia. In contrast, CRSsNP, CRSwNP is histologically characterized by increased numbers of subepithelial cell infiltrate, the formation of pseudocysts, and stromal tissue edema. Collagen levels are reduced in nasal polyps [13]. The aim of this study was to identify the extracellular matrix factors that might play a role in the remodeling of CRS. Our study demonstrated that compared to CRSwNP patients and normal nasal mucosa, CRSsNP patients had a higher level of TGF-β1 and α-SMA expression in the nasal mucosa, which was paralleled by increased collagen deposition. In the polyps, there was a lack of collagen deposition and low α-SMA expression.

TGF-β1 has been implicated as an important factor in the remodeling processes involved in chronic sinus disease and serves as a main switch for different remodeling patterns in CRS [14]. TGF-β1 also affects fibrosis by increasing ECM deposition, such as increasing collagen and fibronectin [15]. Van Bruaene *et al*. reported in a Belgian population that TGF-β1 and collagen were elevated in CRSsNP [16]. Li *et al*. reported that TGF-β1 and collagen were elevated in CRSsNP in a Chinese population [17]. In

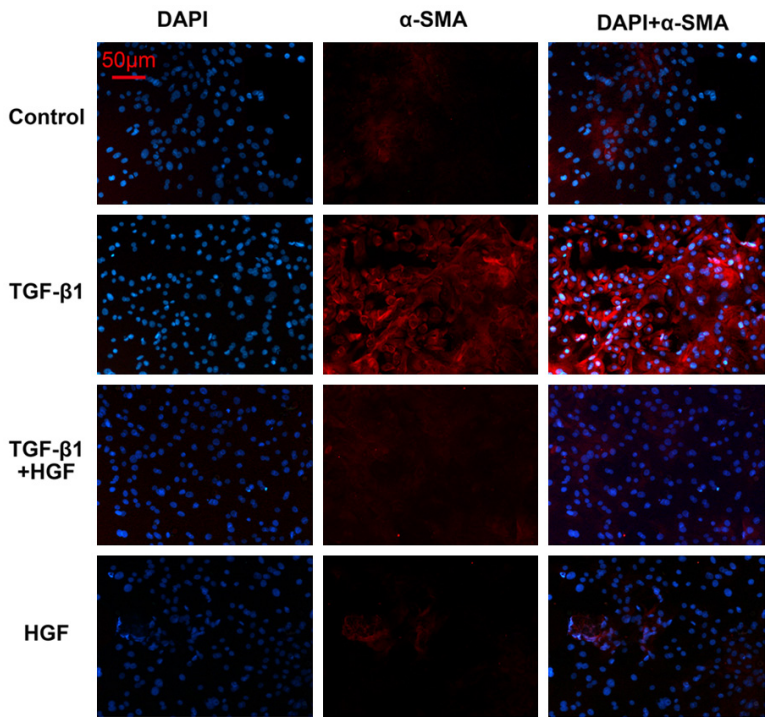

Figure 3. Immunocytochemistry for α-SMA in NECs. The expression of α-SMA (Red) in the TGF-β1 group was significantly higher than in the HGF therapeutic group and controls. HGF treatment decreased the expression of α-SMA induced by TGF-β1 in NECs.

contrast, Shi *et al*. found in a Chinese population that TGF-β1 was reduced, but collagen was elevated in CRSsNP [12]. Although there are some discrepancies, our study suggests that TGF-β1 and collagen are elevated in CRSsNP and that they may contribute to the remodeling process in this form of the disease. As mentioned above, the pattern of remodeling in CRSwNP is known to be different than that in CRSsNP. Indeed, collagen was reduced in CRSwNP and TGF-β1 was also reduced but showed no difference compared to the control group.

Histopathological analysis provided initial evidence of TGF-β1-induced fibrosis in CRS. Of note, α-SMA expression plays a key role in the pathophysiological mechanism of CRS remodeling. α-SMA is a reliable unique marker of EMT. In this study, immunohistochemical analysis showed excessive α-SMA expression in CRSsNP compared to limited expression in controls, confirming that TGF-β1 stimulated the activation of myofibroblasts in the CRSsN and that there was a marked reduction of α-SMA immunoreactivity in CRSwNP.

Importantly, we further found that HGF was significantly increased in CRSwNP patients compared with the control subjects and the CRSsNP group. HGF, a pleiotropic cytokine demonstrates antifibrotic properties in experimental models of liver, kidney, heart, skin, and lung fibrosis [18-20]. In addition, HGF acts as an antifibrotic agent that protects the host against TGF-β1-mediated profibrotic effects. HGF exerts anti-fibrotic effects by antagonizing TGF-β1-mediated ECM accumulation [21]. Therefore, HGF levels may be related to the reduced expression of α-SMA and collagen. Rho *et al.* confirmed the presence of c-MET and HGF in the submucosal glands and epithelium of nasal polyps [22], and this finding was also demonstrated in another study conducted in two geographically distinct populations [23]. These data suggest that polymorphisms in the MET gene may play a role in susceptibility to developing CRS. The MET gene encodes the c-MET receptor of HGF, a transmembrane protein receptor tyrosine kinase. The biological effects of HGF are mediated by binding the c-MET receptor and activating the tyrosine kinase signaling pathways. These results suggest that the MET/HGF pathway may have a role in the pathogenesis of nasal polyps [11].

In the current investigation, we found that the ratio of TGF-β1/HGF had a positive relationship with the expression of collagen and α-SMA in CRS patients. Higher TGF-β1/HGF ratios resulted in greater collagen and α-SMA expression in the CRSsNP group; lower TGF-β1/HGF ratios reduced collagen deposition and α-SMA expression, which may result in edema in the nasal mucosa of CRSwNP patients. In vitro studies have found that HGF specifically counteracts the profibrotic actions of TGF-β1 and reduces collagen secretion in NECs. The EMT maker α-SMA was also lower in the HGF-treated group than in the TGF-β1 group. The results demon-

strated that the balance between HGF and TGF-β1 may play a decisive role in the pathogenesis of CRSsNP and CRSwNP. Hence, the balance of HGF and TGF-β1 acted as two sides of the same coin in the tissue fibrotic signals [24].

Above all, HGF effectively blocks TGF-β1-mediated cellular transdifferentiation into α-SMA-producing cells both in vivo and in vitro. The balance between HGF and TGF-β1 acts as a switch in the pathogenesis of CRSsNP and CRSwNP.

Acknowledgements

This paper was supported by projects of the Medical and Health Technology Development Program in Zhejiang Province (No. 2016KYB227 and 2019RC066) and the Medical and Health Technology Development Program in Hangzhou City (No. 2017A10).

Disclosure of conflict of interest

None.

Abbreviations

ANOVA, assessed using one-way analysis of variance; BSA, Bovine serum albumin; CRS, chronic rhinosinusitis; CRSwNP, chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps; CT, computed tomography; ELISA, enzyme-linked immunosorbent assay; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal cell transition; FESS, functional endoscopic sinus surgery; HGF, hepatocyte growth factor; IFN-r, interferon receptor; IOD, integrated OD; MT, Masson trichrome; NECs, nasal epithelial cells; TGF-β1, transforming growth factor-β1; SE, standard error; α-SMA, α-smooth muscle actin.

Address correspondence to: Yong Li, Department of Otolaryngology, Affiliated Hangzhou First People's Hospital, School of Medicine, Zhejiang University, 261 Huansha Road, Shangcheng District, Hangzhou 310006, Zhejiang Province, China. Tel: +86-13806527172; Fax: +86-571-87914773; E-mail: leeyung828@163.com

References

[1] Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F, Cohen N, Cervin A, Douglas R, Gevaert P, Georgalas C, Goossens H, Harvey R, Hellings P, Hopkins C, Jones N, Joos G, Kalogjera L, Kern B, Kowalski M, Price D, Riechelmann H, Schlosser R, Senior B, Thomas M, Toskala E, Voegels R, Wang de Y and Wormald PJ. European position paper on rhinosinusitis and nasal polyps 2012. Rhinol Suppl 2012; 23: 3 p preceding table of contents, 1-298.

[2] Cope EK and Lynch SV. Novel microbiome-based therapeutics for chronic rhinosinusitis. Curr Allergy Asthma Rep 2015; 15: 504.

[3] Van Zele T, Claeys S, Gevaert P, Van Maele G, Holtappels G, Van Cauwenberge P and Bachert C. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. Allergy 2006; 61: 1280-1289.

[4] Van Bruaene N, Perez-Novo CA, Basinski TM, Van Zele T, Holtappels G, De Ruyck N, Schmidt-Weber C, Akdis C, Van Cauwenberge P, Bachert C and Gevaert P. T-cell regulation in chronic paranasal sinus disease. J Allergy Clin Immunol 2008; 121: 1435-1441.

[5] Bachert C, Gevaert P, Holtappels G, Cuvelier C and van Cauwenberge P. Nasal polyposis: from cytokines to growth. Am J Rhinol 2000; 14: 279-290.

[6] Little SC, Early SB, Woodard CR, Shonka DC Jr, Han JK, Borish L and Steinke JW. Dual action of TGF-beta1 on nasal-polyp derived fibroblasts. Laryngoscope 2008; 118: 320-324.

[7] Figueiredo CR, Santos RP, Silva ID and Weckx LL. Microarray cDNA to identify inflammatory genes in nasal polyposis. Am J Rhinol 2007; 21: 231-235.

[8] Zaravinos A, Soufla G, Bizakis J and Spandidos DA. Expression analysis of VEGFA, FGF2, TGF-beta1, EGF and IGF1 in human nasal polyposis. Oncol Rep 2008; 19: 385-391.

[9] Lee YH, Suzuki YJ, Griffin AJ and Day RM. Hepatocyte growth factor regulates cyclooxygenase-2 expression via beta-catenin, Akt, and p42/p44 MAPK in human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 2008; 294: L778-786.

[10] Li J, Zheng CQ, Li Y, Yang C, Lin H and Duan HG. Hepatocyte growth factor gene-modified mesenchymal stem cells augment sinonasal wound healing. Stem Cells Dev 2015; 24: 1817-1830.

[11] Castano R, Bosse Y, Endam LM, Filali-Mouhim A and Desrosiers M. c-MET pathway involvement in chronic rhinosinusitis: a genetic association analysis. Otolaryngol Head Neck Surg 2010; 142: 665-671.e661-662.

[12] Shi LL, Xiong P, Zhang L, Cao PP, Liao B, Lu X, Cui YH and Liu Z. Features of airway remodel-

ing in different types of Chinese chronic rhinosinusitis are associated with inflammation patterns. Allergy 2013; 68: 101-109.
[13] Meng J, Zhou P, Liu Y, Liu F, Yi X, Liu S, Holtappels G, Bachert C and Zhang N. The development of nasal polyp disease involves early nasal mucosal inflammation and remodelling. PLoS One 2013; 8: e82373.
[14] Van Bruaene N and Bachert C. Tissue remodeling in chronic rhinosinusitis. Curr Opin Allergy Clin Immunol 2011; 11: 8-11.
[15] Al-Alawi M, Hassan T and Chotirmall SH. Transforming growth factor beta and severe asthma: a perfect storm. Respir Med 2014; 108: 1409-1423.
[16] Van Bruaene N1, Derycke L, Perez-Novo CA, Gevaert P, Holtappels G, De Ruyck N, Cuvelier C, Van Cauwenberge P, Bachert C. TGF-beta signaling and collagen deposition in chronic rhinosinusitis. J Allergy Clin Immunol 2009; 124: 253-259.
[17] Li X, Meng J, Qiao X, Liu Y, Liu F, Zhang N, Zhang J, Holtappels G, Luo B, Zhou P, Zheng Y, Lin P, Liu S and Bachert C. Expression of TGF, matrix metalloproteinases, and tissue inhibitors in Chinese chronic rhinosinusitis. J Allergy Clin Immunol 2010; 125: 1061-1068.
[18] Ogaly HA, Eltablawy NA, El-Behairy AM, El-Hindi H and Abd-Elsalam RM. Hepatocyte growth factor mediates the antifibrogenic action of ocimum bacilicum essential oil against CCl4-Induced liver fibrosis in rats. Molecules 2015; 20: 13518-13535.
[19] Gazdhar A, Temuri A, Knudsen L, Gugger M, Schmid RA, Ochs M and Geiser T. Targeted gene transfer of hepatocyte growth factor to alveolar type II epithelial cells reduces lung fibrosis in rats. Hum Gene Ther 2013; 24: 105-116.
[20] Kajihara I, Jinnin M, Makino T, Masuguchi S, Sakai K, Fukushima S, Maruo K, Inoue Y and Ihn H. Overexpression of hepatocyte growth factor receptor in scleroderma dermal fibroblasts is caused by autocrine transforming growth factor beta signaling. Biosci Trends 2012; 6: 136-142.
[21] Chakraborty S, Chopra P, Hak A, Dastidar SG and Ray A. Hepatocyte growth factor is an attractive target for the treatment of pulmonary fibrosis. Expert Opin Investig Drugs 2013; 22: 499-515.
[22] Rho HS, Lee SH, Lee HM, Lee SH, Jung HH, Choi J, Park MK and Kang SM. Overexpression of hepatocyte growth factor and its receptor c-Met in nasal polyps. Arch Otolaryngol Head Neck Surg 2006; 132: 985-989.
[23] Stankovic KM, Goldsztein H, Reh DD, Platt MP and Metson R. Gene expression profiling of nasal polyps associated with chronic sinusitis and aspirin-sensitive asthma. Laryngoscope 2008; 118: 881-889.
[24] Liu Y. Hepatocyte growth factor in kidney fibrosis: therapeutic potential and mechanisms of action. Am J Physiol Renal Physiol 2004; 287: F7-16.