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Glucocorticoids ameliorate periostin-induced tissue remodeling in chronic rhinosinusitis with nasal polyps

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Abstract

Background: Periostin plays an important role in the development of chronic rhinosinusitis (CRS) with nasal polyps (CRSwNP). Glucocorticoids (GCs) are anti-inflammatory drugs used to treat CRS, but the mechanism for inhibiting periostin-induced tissue remodeling is still unclear. We sought to investigate the expression of periostin, α -SMA, and extracellular matrix (ECM) components in sinonasal tissues and to evaluate the inhibitory mechanism of GCs in nasal fibroblasts and mucosa.

Methods: We measured the expression of periostin, α -SMA and ECM components in sinonasal tissues. Correlation of CRS severity and periostin was evaluated by the Lund-Mackey score. Fibroblasts and *ex vivo* culture of the inferior turbinate were used to investigate the effects of GCs on periostin-induced alterations using real-time PCR, western blot, and immunostaining. Wound healing, transwell invasion, and collagen gel contraction were performed to evaluate migration and collagen contraction.

Results: Periostin was highly expressed in eosinophilic CRSwNP and correlated with the Lund-Mackay score. In nasal fibroblasts, periostin increased tissue remodeling involved protein. GCs suppressed the alterations of periostin. In addition, periostin induced activation of Src/AKT/mTOR, which was inhibited by GCs. GCs also inhibited periostin-induced migration, invasion, and collagen gel contraction.

Conclusion: We suggest that GCs are therapeutic agents for CRSwNP by inhibiting tissue remodeling through their inhibitory effect on Src/Akt/mTOR signaling pathway.

INTRODUCTION

Chronic rhinosinusitis (CRS) is a multifactorial disease associated with chronic inflammation of the nose and paranasal sinus. CRS lasts for more than 12 weeks, usually accompanied by nasal symptoms such as nasal discharge, obstruction, congestion, and facial pain.[1, 2] Around 10% of the world's population has CRS, which reduces the quality of life and productivity. CRS is heterogeneous and is divided into CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP) depending on the presence or absence of nasal polyps.[3] However, CRSwNP also exhibits a heterogeneous phenotype depending on region and race. In western countries, CRSwNP is characterized by a Th2-based immune response and abundant eosinophil infiltration and edema. In Asia, it is characterized by a mixed T cell immune response and infiltration of neutrophils. Eosinophilic CRSwNP is characterized by basement membrane thickness and goblet cell hyperplasia.[4, 5]

CRSwNP is currently treated using antibiotics, glucocorticoids, or by surgical removal of inflamed tissue, but the possibility of recurrence is high. To effectively treat CRSwNP, it is necessary to understand the precise mechanism underlying the formation of nasal polyps; however, the pathology and etiology of CRSwNP have not been fully elucidated.[6]

ECM accumulation plays important roles in airway remodeling and CRS development. Fibroblast induces the production of ECM and attributes to structurally maintaining the morphology of the tissue. In the process of early nasal polyp development, fibroblasts differentiate into myofibroblasts that express α -SMA and overproduce components of the extracellular matrix (ECM) components such as collagen type I, IV, VI, and fibronectin. Therefore, differentiation of fibroblasts into myofibroblasts and ECM accumulation play an important role in the pathogenesis of CRSwNP.[7]

Periostin, which is a matricellular protein, interacts with the ECM and plays an important role in tissue remodeling and the ECM in the airway. Periostin induces hypersecretion of type 2 inflammatory factors, mucus production, and elevated serum periostin levels. Overexpression of periostin are associated with asthma, allergic rhinitis, and CRS [8]. In addition, the periostin level is correlated with eosinophilic airway inflammation and clinical severity. Periostin is present in the sinonasal mucosa and nasal polyps and is associated with the pathogenesis of CRSwNP.[9-11] Overexpression of periostin also associated with nasal polyps.[12, 13]

Dexamethasone (DEX) and fluticasone propionate (FP), which are synthetic glucocorticoids (GCs), possess potent anti-inflammatory and immunosuppressive properties and are used to treat CRSwNP.[14] However, the effect of GCs on periostin-induced ECM production remains unclear. Therefore, we investigated the relationship between periostin and CRSwNP and how GCs affected ECM production, cell migration, invasion, and collagen gel contraction induced by periostin in CRSwNP.

METHODS

Human subjects

Sinonasal and nasal polyp tissues were obtained from 48 patients (24 males and 24 females; mean age 34.2 ± 4.5 years). Normal uncinate process tissues were obtained from rhinoplasty. The diagnosis of CRS was based on historical, endoscopic, and radiographic criteria and CT findings of sinuses according to the 2012 European position paper on rhinosinusitis and nasal polyps (EPOS) guidelines [15]. Nasal polyp tissues were obtained from the region of the middle meatus at the beginning of endoscopic surgery in CRSwNP patients. No patients had taken oral

steroids, non-steroidal anti-inflammatory drugs, antihistamines, or antibiotics for at least 2 weeks.

Clinical characteristics of patients are summarized in Table I.

All patients were recruited from the Department of Otorhinolaryngology, Korea University Medical Center, Korea. Informed consent was provided according to the Declaration of Helsinki. This study was approved by the Korea University Medical Center Institutional Review Board, which also authorized the research, and it was carried out in accordance with the guidelines of the Human Ethics Committee of Korea University Guro Hospital (KUGH14065-001).

Nasal fibroblast culture

Nasal fibroblasts were obtained from the inferior turbinates of patients who underwent rhinoplasty. All tissues were from patients with no signs of inflammation, allergies, asthma, or aspirin sensitivity. Inferior turbinate tissues were isolated by enzymatic digestion with collagenase (500 U/mL; Sigma-Aldrich, St. Louis, MO), hyaluronidase (30 U/mL, Sigma-Aldrich), and DNase (10 U/mL, Sigma-Aldrich). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% heat-inactivated fetal bovine serum (Invitrogen, Carlsbad, CA), 1% 10,000 µg/mL streptomycin and 1% 10,000 U/mL penicillin (Invitrogen). The purity of the obtained nasal fibroblasts was confirmed by their characteristic spindle-shaped cell morphology and flow cytometry [16].

Real-time PCR

To evaluate mRNA levels in nasal fibroblasts, real-time polymerase chain reaction (PCR) was used. Total RNA was extracted with TRIzol reagent (Invitrogen). Reverse transcription was performed using MMLV reverse transcriptase (Invitrogen) according to the manufacturer's instructions. PCR

was performed using the following primers: *periostin* (sense sequence 5'-GCTATTCTGACGCCTCAAACT-3' and anti-sense sequence 5'-AGCCTCATTACTCGGTGCAAA-3'); α -*smooth muscle actin* (sense sequence 5'-GGT GCT GTC TCT CTA GCC TCT GGA-3' and anti-sense sequence 5'-CCC ATC AGG CAA CTC GAT ACT CTT C-3'); *fibronectin* (sense sequence 5'-GGA TGC TCC TGC TGT CAC-3' and anti-sense sequence 5'-CTG TTT GAT CTG GAC CTG CAG-3'); *collagen type I* (sense sequence 5'-CAT TCC CAG CTC CCC TCT CT-3' and anti-sense sequence 5'-AGT CTA CGT GGC AGG CAA GG-3'); and *GAPDH* (sense sequence 5'-GTG GAT ATT GTT GCC ATC AAT GAC C-3' and anti-sense sequence 5'-GCC CCA GCC TTC ATG GTG GT-3'). Real-time PCR was performed with Quantstudio3 (Applied Biosystems, Foster City, CA) using Power SYBR Green PCR Master Mix (Applied Biosystems). Analysis of relative gene expression was performed using the delta-delta Ct ($2^{-\Delta\Delta Ct}$) method. Experiments were repeated at least three times and *GAPDH* was used as the internal control.

Western blot

Nasal fibroblasts were seeded into 60 mm culture dish at a density of 5×10^5 cells/mL. Fibroblasts were lysed in RIPA buffer (Sigma-Aldrich) with protease inhibitors (Sigma-Aldrich) and phosphatase inhibitors (Sigma-Aldrich). Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinyl difluoride membranes (Merck Millipore, Billerica, MA). Membranes were blocked with 5% skim milk. Blots were incubated with primary antibodies against fibronectin, phospho-c-Src, total-c-Src, phospho-Akt, total-Akt, phospho-mTOR, total-mTOR, β -actin (Santa Cruz Biotechnology, Inc., Dallas, TX), α -SMA (Millipore Inc., Billerica, MA), and collagen type I (Abcam, Cambridge, UK). Blots were visualized with HRP-conjugated secondary antibodies and an ECL system (Pierce, Rockford, IL). Images were analyzed using ImageJ software (NIH, Rockville, MD) and protein expression was normalized to β -actin or total protein in the case of phosphorylated proteins.

Immunocytochemical staining

Nasal fibroblasts were seeded on 8well culture slides (SPL Life Sciences, Korea) at 5×10^4 cells/ml per well and grown to 60% - 70% confluence. Fibroblasts were fixed with 4% paraformaldehyde for 10 minutes and treated with 0.01% Triton X-100 (Sigma-Aldrich) for permeabilization. Blocking was performed by adding 3% bovine serum albumin for 1 hour and fibroblasts were stained with the following primary antibodies: anti- α SMA (1:1,000, Millipore), anti-fibronectin (1:1,000, Santa Cruz Biotechnology, Inc.), or anti-collagen type 1 (1:500, Abcam) overnight at 4°C after which they were incubated with anti-mouse Alexa 488 (Invitrogen) or anti-rabbit Alexa 555 (Invitrogen) secondary antibodies for 1 hour. Counterstaining was performed using 4'-6-diamidino-2-phenylindole (Sigma-Aldrich). Image acquisition and processing were performed using a confocal laser scanning microscope LSM700 (Zeiss, Oberkochen, Germany).

Immunohistochemical staining

Sinonasal and inferior turbinate tissues section were deparaffinized and rehydrated. Sections were stained with primary antibodies: anti- α SMA (1:1,000, Millipore), anti-fibronectin (1:1,000, Santa Cruz Biotechnology, Inc.), anti-collagen type 1 (1:500, Abcam) and anti-periostin (1:1,000, Santa Cruz Biotechnology, Inc.) overnight at 4°C after which they were incubated with anti-mouse Alexa 488 (Invitrogen) or anti-rabbit Alexa 555 (Invitrogen) secondary antibodies or 3,3'-Diaminobenzidine (DAB) substrate (zytomed systems). Sections were counterstained with haematoxylin or 4'-6-diamidino-2-phenylindole (Sigma-Aldrich).

Wound scratch assay and Transwell® migration assay

Nasal fibroblasts were seeded into 6-well culture plates at 3×10^5 cells/mL per well and cells were grown until they reached 90% confluence as a monolayer in DMEM supplemented with 10% fetal bovine serum and 1% 10,000 units/mL penicillin, 1% 10,000 µg/mL streptomycin solution (Invitrogen). Fibroblasts were then starved in serum-free DMEM medium for 24 hours. After this period, the medium was replaced with medium containing mitomycin C (5 µg/mL, R&D Systems, Minneapolis, MN) for 15 minutes. Cell monolayer was scratched to mimic a wound using a 200 µl pipette tip. Fibroblasts were pretreated with dexamethasone (DEX, 1 µM) or fluticasone propionate (FP, 1 µM) for 1 hour and then stimulated with periostin (100 ng/ml) for up to 48 hours. Fluorescence and bright field microscopy (Olympus BX51; Olympus, Tokyo, Japan) images were analyzed using ImageJ software (NIH) to determine wound closure.

For Transwell® assays, nasal fibroblasts were seeded into 6.5-mm inserts (with 8.0 µm pores, Sigma-Aldrich) on a 24-well plate with 5×10^4 cells/mL per well and grown to 80% confluence in DMEM supplemented with 10% fetal bovine serum and 1% 10,000 units/mL penicillin, 10,000 µg/mL streptomycin solution (Invitrogen). Fibroblasts were then starved in serum-free DMEM medium for 24 hours and the medium at the bottom of the plate was replaced with medium containing DEX (1 µM) or FP (1 µM) for 1 hour. After this process, periostin (100 ng/ml, R&D Systems) was added to stimulate nasal fibroblasts for up to 72 hours and medium was aspirated from the apical inserts and receiver wells. To remove all medium, three washes with phosphate-buffered saline were performed. Invasion of nasal fibroblasts cells was confirmed by Diff-Quick staining (Sysmex, Kobe, Japan). Images were acquired under a microscope at 200× magnification (Olympus BX51; Olympus, Tokyo, Japan), and stained cells were counted in five fields to determine the average number of cells that had migrated per high power field.

Collagen gel contraction assay

A collagen type I –DMEM solution composed of rat-tail tendon collagen type I was purchased from BD Bioscience (Billerica, MA). Collagen gels were mixed with DMEM and gentamicin, NaOH (0.142 M), and phosphate-buffered saline solution to determine the contractile capacity of fibroblasts. Fibroblasts in the collagen gel were suspended, and aliquots (0.5 mL/well) of a mixture of fibroblasts were incubated in 24-well tissue culture plates at 37°C for 1 hour to allow polymerization. Serum-free DMEM with periostin with or without DEX and FP was added to the wells. Fibroblasts were incubated for 3 days at 37°C in a 5% CO₂ atmosphere. ImageJ software (NIH) was used to individually evaluate gel areas. Results are expressed as the area percentage compared with the initial gel area.

***Ex vivo* organ culture of inferior turbinate tissue**

Ex vivo organ culture of the nasal inferior turbinate was performed as described previously [17]. Inferior turbinate tissues were obtained from rhinoplasty patients (n=6). Nasal inferior turbinate tissues were cut into 2 to 3 mm³ pieces, washed three times with phosphate-buffered saline, and cultured in DMEM supplemented with 2% fetal bovine serum (Invitrogen) and 1% 10,000 units/ml penicillin, 1% 10,000 µg/ml streptomycin solution (Invitrogen). Nasal inferior turbinate tissues were pretreated with or without DEX or FP and stimulated with periostin (100 ng/ml, R&D Systems) for 72 hours.

Statistical analysis

Results were obtained from at least three independent experiments. Statistical significance of differences between control and experimental data was assessed using the unpaired t-test or one-way analysis of variance followed by Tukey's test (GraphPad Prism, version 5, GraphPad

Software, San Diego, CA). Significance was established at the 95% confidence level. P-values <0.05 were accepted as statistically significant.

RESULTS

Periostin expression is increased in patients with CRSwNP

To determine the correlation between periostin and CRS, real-time PCR was performed in sinonasal tissues from each group (Control UP, n=12; CRSsNP-UP, n=12; CRSwNP-UP, n=12; CRSwNP-NP, n=12). Expression of mRNA levels of *periostin* was significantly higher in the CRSwNP group than the other groups (Fig. 1A). The expression of periostin was positively correlated with Lund-Mackay CT score (Fig. 1B). Immunohistochemical images showed that periostin expression was the most significantly increased in CRSwNP-NP when compared to control UP (Fig. 1C). In addition, α -SMA and ECM components, such as fibronectin and collagen type I, were expressed at the highest levels in CRSwNP-NP, and collagen deposition was also higher in this group than the other groups (Fig. 1D). The expression level of periostin was also significantly correlated with eosinophil count (Fig. 1E).

Periostin induces differentiation of nasal fibroblasts into myofibroblasts and ECM production

To determine the effect of periostin on differentiation of nasal fibroblasts into myofibroblast and ECM production, fibroblasts were treated with periostin (0 – 100 ng/ml) for 72 hours. Total collagen production increased significantly in a dose-dependent manner (Fig. 2A). Protein levels of fibronectin and α -SMA, markers of myofibroblasts, also increased in a dose-dependent manner (Fig. 2B). Gene expression of *fibronectin*, *α -SMA*, and *collagen type I* increased in response to periostin, with peak levels observed at 48 hours (Fig. 2C).

Effect of periostin on nasal fibroblasts is dependent on Src/Akt phosphorylation

Periostin treatment significantly induced phosphorylation of Src and Akt at 7.5 minutes and 15 minutes, respectively. PP2 and LY294002 decreased the periostin-induced phosphorylation of Src and Akt (Fig. 3A). Furthermore, blocking of c-Src and Akt/PI3K signaling ameliorated periostin-induced ECM production and myofibroblast differentiation (Fig. 3B-D). Immunofluorescence microscopy revealed that expression of fibronectin, α -SMA, and collagen type I was decreased by PP2 and LY294002 treatment (Fig. 3C).

Glucocorticoids have an inhibitory effect on periostin-induced differentiation of nasal fibroblasts to myofibroblasts and ECM production

To determine whether GCs inhibited periostin-induced ECM production, fibroblasts were pretreated with DEX or FP for 1 hour and then stimulated with periostin for 72 hours. GCs significantly inhibited periostin-induced α -SMA, fibronectin, and collagen type I mRNA and protein levels (Fig. 4A). Periostin induced phosphorylation of Src/Akt/mTOR signaling components in nasal fibroblasts while GCs inhibited periostin-induced phosphorylation of Src/Akt/mTOR components (Fig. 4B). Immunofluorescence revealed that periostin increased the expression of α -SMA, fibronectin, and collagen type I in the cytoplasm and that these effects were inhibited by GCs (Fig. 4C).

Effects of glucocorticoids on periostin-induced migration and collagen gel contraction in nasal fibroblasts

To investigate the effect of periostin and GCs on migration and collagen gel contraction, wound scratch assays, Transwell® invasion assays, and collagen gel contraction assays were performed. Periostin treatment significantly increased migration of fibroblasts compared to the

control group. PP2, LY294002, rapamycin, and GCs inhibited periostin-induced migration (Fig. 5A). In the Transwell® invasion assay, the number of migrated nasal fibroblasts was significantly higher in the periostin-treated group than the control group. Pretreatment with PP2, LY294002, rapamycin, and GCs prevented periostin-induced migration (Fig. 5B). Periostin treatment significantly decreased the surface area of collagen gels while pretreatment with PP2, LY294002, rapamycin, and GCs prevented periostin-induced collagen contraction (Fig. 5C).

Glucocorticoids inhibit periostin-induced myofibroblast differentiation and ECM production in *ex vivo* organ cultures of the inferior turbinate

To evaluate the effects of GCs on the nasal inferior turbinate tissues, air-liquid interface cultures were performed. Periostin increased the mRNA and protein expression of α -SMA, fibronectin, and collagen type I (Figs. 6A and 6B). However, GCs inhibited the periostin-induced increase in expression of ECM components. Immunohistochemical staining revealed that periostin increased α -SMA, fibronectin, and collagen type I expression in the inferior turbinate tissues. GCs prevented periostin-induced ECM production (Fig. 6C).

DISCUSSION

In this study, we investigated the correlation between periostin and CRSwNP, confirmed that GCs had an inhibitory effect on ECM production, migration, and collagen gel contraction induced by periostin, and determined the mechanism through which GCs exerted their effects. Periostin induced differentiation of nasal fibroblasts into myofibroblasts and increased the expression of ECM components. In addition, periostin induced the migration and invasion of nasal fibroblasts and their collagen gel contraction ability; all of these processes were inhibited by GCs.

CRS is a chronic inflammatory disease of the paranasal sinus and nose. It is accompanied by symptoms such as nasal obstruction, nasal airway inflammation, and discharge.[1, 2] However, the pathogenesis of the CRS has yet to be fully elucidated. Several recent studies have demonstrated that differentiation of nasal fibroblasts into myofibroblasts expressing α -SMA can be induced by a variety of factors, and that myofibroblasts deposit ECM, which can cause tissue remodeling of the airway and contribute to CRS.[16, 18, 19]

Stankovic *et al.* reported that periostin was present in intact nasal mucosa and that the expression of periostin was related to the pathogenesis of CRSwNP. They also reported that periostin is expressed specifically in the lower part of the basement membrane in normal mucosa, but is expressed entirely in the lamina propria as well as in the lower part of the basement membrane in nasal polyps.[10]

Shiono *et al.* classified the expression pattern of periostin into two types: diffuse type and superficial type. They found a significant correlation between the pattern of periostin expression and eosinophil infiltration; the number of eosinophils in diffuse type was significantly greater than that in superficial type.[20] Nobuo ohta *et al.* reported that overexpression of periostin was detected in the basal lamina portion of sinonasal tissue with CRSwNP. The concentration of periostin in serum and nasal lavage fluid of patients with CRSwNP also significantly increased in nasal mucosal tissues of CRS patients than healthy control [8].

We compared the expression pattern of periostin by dividing sinonasal tissues into four types: normal-UP, CRSsNP-UP, CRSwNP-UP, and CRSwNP-NP. Periostin was partially expressed only in the lower part of the basement membrane in the normal uncinat process, and the expression level was low. Periostin expression gradually increased in the order of CRSsNP-UP, CRSwNP-UP, and finally CRSwNP-NP. CRSwNP can be classified into eosinophilic and non-eosinophilic groups. In 12

CRSwNP patients, 7 patients were eosinophilic CRSwNP patients and 5 patients were non-eosinophilic CRSwNP patients. Eosinophilic CRSwNP defined as having an eosinophil count of more than 10% of the inflammatory cell population per high power field (HPF). To confirm the correlation between periostin expression and the number of eosinophils, we determined that in sinonasal tissues. Fig 1E showed that periostin expression and eosinophil infiltration have a positive correlation in sinonasal tissues. Especially, the expression of periostin and the number of eosinophils was highly increased and correlated in eosinophilic CRSwNP-NP group. In case of non-eosinophilic CRSwNP-NP group also showed correlation, but periostin expression and eosinophil numbers were lower than CRSwNP-NP group.

Periostin, a matricellular protein, is induced by Th2 cytokine; IL-4, IL-13 in airway epithelial cells [8]. Periostin is known to interact with ECM components and play an important role in ECM production and tissue remodeling.[9, 11] Therefore, we performed immunohistochemical staining and *in vitro*, *ex vivo* experiments to determine if periostin expression also affected nasal fibroblasts and the nasal mucosa. Although eosinophilic CRSwNP had an edematous phenotype, tissues overexpressing periostin expressed high levels of ECM components such as α -SMA, fibronectin, and collagen type I.

Periostin induces myofibroblast differentiation and involved in cell growth, proliferation, and motility [21]. We found that periostin induced myofibroblast differentiation of nasal fibroblasts. Myofibroblasts are activated fibroblasts, and myofibroblasts produce ECM components such as fibronectin and collagen and have the ability to migrate to and invade tissues.[22-24] Differentiation of fibroblasts into myofibroblasts and ECM accumulation are important factors in the formation of nasal polyps and are associated with nasal polyp pathogenesis. In normal condition, periostin

attribute to the wound healing process. However, persistent expression of periostin can lead to airway inflammation and tissue reconstruction [8]. We found that periostin mediated migration of fibroblasts and involved with wound healing process. Also, the specific inhibitors of c-Src/Akt/mTOR and GCs suppressed periostin-induced fibroblast migration and collagen contraction.

Recombinant periostin induced activation of the c-Src/Akt/mTOR signaling pathway in nasal fibroblasts. Activated c-Src signaling induces myofibroblast differentiation and is involved in matrix contraction of collagen gels.[25] In colon and ovarian cancers, periostin induces phosphorylation of PI3K/Akt by binding to integrin.[26, 27] Activation of PI3K/Akt is associated with homeostasis and tissue remodeling.[28] mTOR signaling is associated with wound healing and plays an important role in protein synthesis.[29] We showed here that periostin significantly activated the c-Src/Akt/mTOR signaling pathway in nasal fibroblasts and that activation of this pathway was associated with accumulation of ECM, migration, invasion, and collagen gel contraction by fibroblasts. GCs inhibited these effects of periostin and prevented ECM production.

GCs are widely used to treat inflammatory diseases because of their immunomodulatory effects. In clinical rhinology, it has limited effects on patients with CRSwNP but is considered the first effective treatment method [3]. Although GCs have been reported that have inhibitory effects on nasal mucosal tissue remodeling, one of the features of CRSwNP,[30, 31] the effects on periostin-induced tissue remodeling have not been reported. Our results demonstrate that GCs effectively inhibit periostin-induced myofibroblast differentiation, collagen deposition and also reduce the functional activities of myofibroblasts such as migratory property and collagen contraction. This suppression of nasal mucosal tissue remodeling can prevent or alleviate the development of nasal polyps.

Our results suggest that GCs have an anti-tissue remodeling effect on the sinonasal mucosa.

This activity is due to inhibition of ECM components and cellular motility via regulation of the c-Src/Akt/mTOR signaling pathway (Fig. 7). We demonstrated that DEX and FP had anti-ECM activity on periostin-stimulated ECM processes. Our results indicate that GCs are effective therapeutic agents because they suppress the expression of α -SMA, fibronectin, and collagen type I and subsequently inhibit cellular migration, ultimately reducing tissue remodeling.

Competing interests

The authors have no competing financial interests to declare.

Author contributions

HW Yang (Department of Biomedical Science, Korea University, College of Medicine, Seoul, Korea) conceived the study, designed and performed the experiments, analyzed the data, and wrote the manuscript. JH Park and JM Shin (Department of Otorhinolaryngology-Head and Neck Surgery, Korea University, College of Medicine, Seoul, Korea) confirmed the data and discussed this study. HM Lee (Department of Otorhinolaryngology-Head and Neck Surgery, Korea University, College of Medicine, Seoul, Korea) supervised the research and reviewed the manuscript. All authors reviewed the manuscript.

Abbreviation used

α -SMA: alpha-smooth muscle actin

CRS: chronic rhinosinusitis

CRSSNP: chronic rhinosinusitis without nasal polyp

CRSSNP-UP: chronic rhinosinusitis without nasal polyp uncinat process

CRSwNP: chronic rhinosinusitis with nasal polyp

CRSwNP-UP: chronic rhinosinusitis with nasal polyp uncinat process

CRSwNP-NP: chronic rhinosinusitis with nasal polyp nasal polyp

DEX: dexamethasone

ECM: extracellular matrix

FP: fluticasone propionate

GC: glucocorticoid

UP: uncinat process

NP: nasal polyp

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Table I. Clinical characteristics of patients (N=48).

Characteristic	UP (n=12)	CRSsNP-UP (n=12)	CRSwNP-UP (n=12)	CRSwNP-NP (n=12)
No. women/men	6/6	4/8	8/4	6/6
Age, y, mean (SD)	35.2 (5.7)	32.4 (4.4)	39.5 (6.7)	35.2 (7.8)
Asthma, no.	0	0	0	0
Aspirin sensitivity no.	0	0	0	0
Lund-Mackay CT score, mean (SD)	0.67 (0.47)	8 (2.94)	13.5 (7.95)	18 (4.93)
<i>UP = uncinat process; NP = nasal polyps; CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; SD = standard deviation</i>				







