The effect of MG53 for modulating TGF-β1/Smad pathway in the nasal epithelial repair of nasal polyps.

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Abstract

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Τηε εφφεςτ οφ ΜΓ53 φορ μοδυλατινγ ΤΓΦ-β1/Σμαδ πατηωαψ ιν τηε νασαλ επιτηελιαλ ρεπαιρ οφ νασαλ πολψπς

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Conclusion: MG53 inhibited the TGF-β1/Smad pathway and fibrosis, enhanced the proliferation of nasal epithelial cells, and supported nasal epithelial repair. The results of this study provide a new therapeutic regimen for the treatment of nasal polyps.

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1. INTRODUCTION

Chronic rhinosinusitis (CRS) is a common disease, having 8% prevalence worldwide, and can occur with (CRSwNP) or without (CRSsNP) nasal polyps.[1, 2] CRS is usually treated via anti-inflammatory drugs and nasal endoscopic surgery. Compared with CRSsNP, CRSwNP has more severe symptoms, such as nasal congestion, anosmia, and headache, and has a higher recurrence rate, with a two-year recurrence rate of 55.3%.[3]However, the pathogenesis of nasal polyps is still unclear. Recent studies have revealed the importance of the nasal mucosal barrier in CRS.[4, 5]

The nasal mucosal barrier is the first line of defence against harmful foreign substances, forming a physical barrier against allergens, pathogens, and irritants. This epithelial barrier is mainly composed of tight junctions (TJs), the topmost intercellular junction complex between adjacent epithelial cells.[6, 7] Abnormal TJs prevent the formation of physical barriers, and increase the vulnerability of the submucosal area to foreign molecules.[8] Patients with CRS have damaged nasal epithelial cells, with decreased cilia count and malfunction, as well as impaired mucosal barrier function, thus, providing the basis for increased permeability, allowing the entry of harmful substances into the sub-epithelial layer. This results in an abnormal immune response in the mucosa, leading to tissue damage, mucosal inflammation, and tissue remodelling changes caused by chronic inflammation.[9, 10]

Zonula occludens (ZO) is a cytoplasmic adhesion protein belonging to the class of membrane-associated guanosine kinases, which contains multiple domains and binds to other TJs proteins. It is involved in the maintenance of the epithelial barrier and the regulation of barrier functions, as well as in distinct cellular processes, such as signal transduction, cell proliferation and differentiation, immune regulation, cancer cell

metastasis, and maintenance of epithelial polarity. There are three subtypes of ZO, viz. ZO-1, ZO-2, and ZO-3, of which ZO-1 is the most characteristic.[11, 12]

Transforming growth factor (TGF)-β1 is associated with tissue remodelling, post-injury repair, wound healing, fibrosis, and tumour invasion. These biological effects are mainly exerted through classical and non-classical Smad-dependent pathways.[13, 14]Studies have shown that in terms of tissue remodelling, CRSwNP shows reduced TGF-β1 and collagen expression, along with mesenchymal oedema.[15, 16] Additionally, TGF-β1 regulates cell aggregation, elevates the expression of tightly connected ZO-1, and accelerates wound repair, suggesting its vital role in mucosal epithelial injury and repair, and regulation of ZO-1.[17, 18]

MG53, a new muscle-specific triplex motif protein (TRIM72), is considered as a "band-aid" in the process of damage repair.[19, 20] MG53 is an intracellular protein that is secreted into systemic circulation in response to physiological activities, and damage to skeletal muscles or the myocardium.[21, 22] Intravenous administration of recombinant human MG53 (rhMG53) has been reported to repair membrane damage in muscle and non-muscle cells,[23, 24] as well as improve the association with muscular dystrophy, myocardial infarction, acute lung injury, and acute kidney injury.[25-27] Moreover, studies on mice have also reported that treatment with recombinant human MG53 (rhMG53) protein accelerated corneal wound healing in rodents, inhibited wound fibrosis, and decreased TGF-β1 secretion.[24, 28, 29]

There is a scarcity of reports addressing the role of MG53 in the nasal mucosal barrier, and thus, the mechanism of nasal mucosal barrier regulation is unclear. This study aimed to explore the role of MG53 in mucosal epithelial repair in CRS, especially the modulating role of the TGF-β1/Smad pathway and fibrosis in CRSwNP. Further studies on the role of MG53 in epithelial barrier may provide an effective way to prevent and treat nasal polyps.

2. METHODS

2.1 | Patients and specimens

Patients admitted at the First Affiliated Hospital of Chongqing Medical University, China, during the same period (from 1/1 2019 to 1/12 2020) were enrolled in the study. Written informed consent was obtained from all subjects. The investigation was approved by the Ethics Committee for Human Study at the First Affiliated Hospital of Chongqing Medical University. We collected specimens from patients in the CRSwNP, CRSsNP, and control groups (n=15 each). Patients were selected based on the Guidelines for Diagnosis and Treatment of CRS formulated by Europe (EPOS2012).[30] The inclusion criteria were as follows: age, 18–60 years; systemic or local glucocorticoids, immunosuppressants, or nasal sprays had not been used by the subject within one month before the study. Patients with the following diseases/conditions were excluded: posterior nostril polyps; cystic fibrosis; fungal sinusitis; varus papilloma and other tumours; ciliary immobility syndrome; lower airway diseases, such as asthma, chronic bronchitis, and emphysema; infectious diseases, such as hepatitis and tuberculosis; acute infectious diseases; and severe systemic diseases.

2.2 | Isolation and identification of primary human nasal epithelial cells (HNECs)

We collected nasal mucosal specimens from patients who met the diagnostic criteria described previously. We isolated HNECs from these specimens using the collagenase digestion method (collagenase IV, Sigma Aldrich, USA). We subsequently performed cytokeratin-19 (CK-19) immunohistochemical and immunofluorescence analyses.[31, 32]

2.3 | HNEC culture

HNECs were cultured in DMEM/F12 medium, containing 10% foetal bovine serum (FBS) (Gibco, USA), at 37 °C in a 5% CO_2incubator. The cells were passaged for less than five generations to reduce the influence of variations in primary cells on the experimental results. All cells used for in vitro stimulation were inoculated in 6-well plates for 24 h, and cultured till 60–80% confluency was achieved. The adenovirus used to overexpress MG53 (Ad-MG53), and the si-RNA (The respective si-RNA sequences are shown in Table 2) used to knock down MG53 were purchased from GenePharma (Shanghai, China). After 6 to 24 h of transfection, the cells

were transferred to fresh DMEM/F12 containing 10% FBS. After 72 h of transfection, the cells were collected separately using cleaning fluid for further tests. For the in vitro drug stimulation experiment, we added 50 nM TGF-β1 inhibitor (screened by western blot; Appendix C) and 10 ng/mL of TGF-β1[33-35] to the cells 24 h after plating. After 72 h of stimulation, the supernatant and cells were collected for further testing.

2.4 | In vitro cell membrane injury assay

We performed an in vitro cell membrane injury repair assay following the protocols described previously.[26, 29] HNECs were treated with TGF-β1/TGF-β1 inhibitor or transfected with Ad-MG53 for 72 h, and then suspended in PBS. Next, 100 μL of the cell suspension (5×10^4 cells/well) was added to a 96-well plate along with acid-washed glass microbeads. The plate was shaken at 200 rpm for 5 min to induce cell membrane injury, followed by centrifugation at 3000 rpm for 10 min, and 80 μL of the supernatant was collected. Lactate dehydrogenase (LDH) activity in the supernatant was determined using the LDH Cytotoxicity Detection Kit (MeiKe, MK0354A, China).

2.5 | ELISA

HNECs were treated with TGF-β1/TGF-β1 inhibitor or transfected with Ad-MG53 for 72 h. The supernatant (100 μL) was used to detect the concentration of soluble Col-a1 using ELISA kits (MeiKe, China) following the manufacturer's instructions. After counting the total number of cells, the concentration of soluble collagen in each group was calculated for 5×10^5 cells.

2.6 | Cell proliferation assay

HNECs were re-suspended in media containing different processing factors (TGF-β1, TGF-β1 inhibitor, or Ad-MG53), at a concentration of 5.0×10^4 cells/mL. Next, 100 μL of this cell suspension (5×10^3 cells/well) was added to each well of a 96-well plate. Cell proliferation was measured at 450 nm via live cell counting at 2, 24, 48, and 72 h after supplementation with 10 μL of CCK-8 reagent (MCE, USA).

2.7 | Apoptosis assay

HNECs were treated with TGF-β1/TGF-β1 inhibitor or transfected with Ad-MG53 for 72 h. After removing the supernatant, the cells were re-suspended in PBS at a concentration of 1.0×10^6/mL, and the rate of apoptosis for each group was determined by flow cytometry.

2.8 | Immunohistochemical staining

We collected nasal mucosal specimens from patients who met the diagnostic criteria described previously. During surgery, the nasal mucous was removed, fixed in 4% paraformaldehyde for 48 h, and then embedded in paraffin. The antigens were extracted and incubated overnight with ZO-1 antibody (Proteintech, China) and Col-a1 antibody (CST, USA) at 4 °C. On the next day, the slices were washed with PBS, followed by incubation with secondary antibodies at room temperature for 1 h. Microscopically, positive expression of Col-a1 and ZO-1 was confirmed if the particles turned brownish yellow or tan. The positive field of vision under 400× magnification was considered from each stained section. After image collection, we used ImageJ software to analyse the total area and cumulative optical density of the positive cells. The average optical density (AOD) was used for statistical analysis.

2.9 | Immunofluorescent staining

The HNECs were seeded in 6-well plates to perform immunofluorescent staining of the TJ of CRSwNP, CRSsNP, and control. When the degree of cell fusion was 100%, the cells were fixed with 4% paraformaldehyde for 15 min, and incubated overnight with ZO-1 antibody at 4 °C. On the next day, the specimens were washed with PBS, and incubated with secondary antibodies in the dark at room temperature for 1 h. After rinsing with PBS, the specimens were incubated with DAPI at room temperature for 10 min. The slices were sealed using nail polish, and the specimens were visualised under a fluorescence microscope (Olympus, Japan).

2.10 | Quantitative RT-PCR (qRT-PCR)

We performed qRT-PCR to determine the expression of MG53, TGF-β1, Smad2/3, ZO-1, and Col-a1 in treated and untreated nasal tissues and epithelial cells. TRIZOL reagent (TAKARA, Japan) was used to extract total RNA; contamination by genomic DNA was removed using gDNA Eraser (TAKARA, Japan), following the manufacturer's instructions. Next, total RNA (1000 ng) was reverse transcribed by cDNA synthesis (TAKARA, Japan). qRT-PCR was performed using a SYBR Premix ExTaq II kit (TAKARA, Japan) with a CFX96 RT-PCR Detection System (Bio-Rad, Hercules, USA). Human GAPDH gene was used as the internal control. The primers used in this study are shown in Table 1.

2.11 | Western blot

Protein lysates from tissue and cell sources were separated by SDS-PAGE, and transferred to PVDF membranes at 4 °C. The blots were washed with TBST (TBS + 0.1% Tween-20), blocked with 7% skim milk in TBST for 1 h, and incubated with the specific primary antibodies overnight at 4 °C on a shaking table. Next, the membranes were incubated with HRP-conjugated anti-mouse or anti-rabbit IgG secondary antibodies (1:5000) in 2% skim milk for 2 h with shaking at room temperature. The immunoblots were visualised with an ECL kit (Zen-Bio, Inc., China).

2.12 | Statistical analysis

All data are presented as the mean ± SEM. Statistical analyses were performed using GraphPad Prism 8 software (San Diego, USA) and SPSS 20 (IBM, USA). Testing for normality and homogeneity of variance was performed on the data from each group. If the data obeyed normal distribution and had equal variance, comparisons between groups were performed by one-way ANOVA. If equal variances were not assumed, the Kruskal–Wallis H test was performed to compare variables among groups. The Mann–Whitney test was used for intergroup comparisons. P -value < 0.05 was considered statistically significant.

3. RESULTS

3.1 | Εξπρεσσιον οφ ΜΓ53, ΤΓΦ-β1, Σμαδ, ΖΟ-1, ανδ ὁλ-α1 ωας ηιγηερ ιν ῞ΡΣσΝΠ, βυτ λοωερ ιν῞ΡΣωΝΠ, ςομπαρεδ ωιτη τηε ςοντρολ

Immunohistochemical and immunofluorescent staining of nasal mucosal specimens revealed significantly enhanced expression of ZO-1 and Col-a1 in the upper cortex in the CRSsNP group, whereas reversed patterns were observed in the CRSwNP group (Fig. 1A). Additionally, results of immunofluorescence showed that the AOD of ZO-1 in the CRSsNP group was significantly higher than that in the CRSwNP group (Fig. 1B). Western blot and qRT-PCR analyses showed that the expression of MG53, TGF-β1, Smad2/3, ZO-1, and Col-a1 was significantly upregulated in the CRSsNP group, but significantly downregulated in the CRSwNP group (Fig. 1C–E).

3.2 | Εξπρεσσιον οφ ΜΓ53, ΤΓΦ-β1, Σμαδ2/3, ΖΟ-1, ανδ ὁλ-α1 ωας ελεατεδ ιν ΗΝΕ῞ς ιν τηε ῞ΡΣσΝΠ γρουπ, βυτ ρεδυςεδ ιν τηε ῞ΡΣωΝΠ γρουπ

Results of HNEC immunofluorescence staining showed elevated ZO-1 expression in the CRSsNP group, whereas reduced expression was found in the CRSwNP group compared with the control group (Fig. 2A). Western blot and qRT-PCR analyses showed that the expression of MG53, TGF-β1, Smad2/3, ZO-1, and Col-a1 was significantly enhanced in the CRSsNP group, whereas it was significantly reduced in the CRSwNP group (Fig. 2B–D), consistent with the results of nasal mucosa specimens.

3.3 | ΜΓ53 ινηιβιτεδ τηε ΤΓΦ-β1/Σμαδ πατηωαψ ας ωελλ ας τηε εξπρεσσιον οφ ΖΟ-1 ανδ ὁλ-α1

MG53 were overexpressed in HNECs from the specimens of the CRSwNP group by transfection with adenovirus to understand the regulatory relationship between MG53 and TGF-β1/Smad Pathway. The transfection efficiency of adenovirus is shown in appendices A and B. Western blot and qRT-PCR analyses showed that overexpression of MG53 downregulated the expression of TGF-β1, Smad2/3, ZO-1, and Col-a1 (Fig. 3A–C).

3.4 | Κνοςκδοων οφ ΜΓ53 προμοτεδ τηε ΤΓΦ-β1/Σμαδ πατηωαψ ας ωελλ ας τηε εξπρεσσιον οφ ZO-1

HNECs from the specimens of the CRSwNP group were transfected by si-RNA for MG53 knockdown to further understand the regulatory relationship between MG53 and the TGF-β1/Smad pathway. Fluorescence after siRNA transfection is shown in Fig. 4A. Based on the results of qRT-PCR analysis, we observed that the expression of TGF-β1, Smad2/3, ZO-1, and Col-a1 genes was upregulated after MG53 knockdown (Fig. 4B), consistent with the results of western blot analysis (Fig. 4C–D).

3.5 | Τρεατμεντ ωιτη ΤΓΦ-β1 ινςρεασεδ ΜΓ53, ΤΓΦ-β1, ὃλ-α1, ανδ ZO-1 εξπρεσσιον, ωηερεας τρεατμεντ ωιτη ΤΓΦ-β1 ινηιβιτορ δεςρεασεδ τηε εξπρεσσιον

In vitro cytokine stimulation experiments were conducted to verify the regulatory relationship between MG53 and the TGF-β1/Smad pathway. We found that TGF-β1 stimulation increased the expression of MG53, TGF-β1, Col-a1, and ZO-1, whereas TGF-β1 inhibitor treatment decreased the expression in HNECs (Fig. 5A). The results of western blot and qRT-PCR analyses showed that MG53, TGF-β1, Smad2/3, ZO-1, and Col-a1 were significantly increased after TGF-β1 stimulation, but decreased after treatment with TGF-β1 inhibitor in HNECs of CRSwNP (Fig. 5B–C). The results of immunofluorescence analysis of ZO-1 showed that the mean fluorescence intensity was significantly enhanced by TGF-β1, but decreased upon TGF-β1 inhibitor treatment. Ad-MG53 did not cause significant enhancement in the mean fluorescence intensity of ZO-1 (Fig. 5D).

3.6 | ΤΓΦ-β1 προμοτεδ ZO-1 ανδ ὃλ-α1 εξπρεσσιον, βυτ ινηιβιτεδ τηε προλιφερατιον οφ επιτηελιαλ ςελλς, ωηερεας ΜΓ53 ψιελδεδ ρεερσεδ τρενδς

A series of experiments, including cell proliferation and apoptosis assays, as well as ELISA-based detection of the secretion of soluble Col-a1 in the culture supernatant and the epithelial injury marker LDH, were performed to further understand the effects of MG53 and TGF-β1 on the epithelial barrier. We found that MG53 significantly promoted epithelial cell proliferation, whereas TGF-β1 inhibited this activity (Fig. 6A). ELISA-based analysis of soluble Col-a1 in the supernatant of the culture maintained for 72 h showed that TGF-β1 promoted the secretion of soluble Col-a1, whereas TGF-β1 inhibitors and Ad-MG53 reduced the secretion of soluble Col-a1 (Fig. 6B). Results of the in vitro cell membrane injury test and ELISA showed that both TGF-β1 and Ad-MG53 reduced LDH release (Fig. 6C). Results of flow cytometry indicated that Ad-MG53 inhibited cell apoptosis compared with negative control-MG53 (NC-MG53), (Fig. 6D).

4. DISCUSSION

CRSwNP is a complex disease involving various types of cells, inflammatory mediators, and cytokines, and has a high recurrence rate.[4] However, its pathogenesis is still unclear. Recent studies have revealed that nasal mucosal barrier damage plays an important role in CRSwNP. Therefore, studies on mucosal barrier damage repair may reveal new strategies for the treatment of nasal polyps.

Many studies have suggested that MG53 is involved in various physiological and pathological processes[19], including acute cell membrane repair,[29] lung injury,[25] kidney injury,[26] ischemic protection of cardiac fibroblasts,[27] and anti-tissue fibrosis.[29] Thus, it is believed to play an important role in the process of injury repair. TGF-β1 is a pluripotent cytokine that promotes the deposition of extracellular matrix (ECM). Studies have shown that TGF-β1 regulates ECM mainly through epithelial-mesenchymal transition, thereby regulating tissue remodelling and repair, wound healing, fibrosis, and tumour invasion.[15] Thus, TGF-β1 acts as a key factor in tissue repair and fibrosis, reducing tissue damage by inhibiting the release of pro-inflammatory mediators. It is also known to activate myofibroblasts for wound closure, and to cause abnormal collagen deposition. In this study, we found that MG53 exhibited a dual effect, that is, it not only protected the epithelial barrier, but also promoted the proliferation of epithelial cells in the nasal mucosa.

We found that the expression of MG53, TGF-β1, Smad2/3, ZO-1, and Col-a1 in tissues and extracted nasal epithelial cells was higher in the CRSsNP group and lower in the CRSwNP group compared with the control group. This indicated that higher amounts of ZO-1 and Col-a1 were present in CRSsNP, and excessive repair

occurred after epithelial barrier injury, nasal epithelial fibrosis, and consequently, tissue remodelling.[24] The low expression of ZO-1 and Col-a1 in the CRSwNP group resulted in insufficient repair after epithelial injury, leading to nasal mucosal oedema. MG53 showed increasing and decreasing trends similar to TGF-β1 and Smad2/3, indicating that there was a regulatory relationship between the MG53/TGF-β1/Smad pathway and the nasal epithelial barrier process.

Previous studies have suggested that MG53 has a protective effect in cell membrane damage,[23, 24] but its protective effect on nasal epithelial repair remains unclear. In our study, nasal epithelial cells isolated from the CRSwNP group were treated with Ad-MG53, siRNA, TGF-β1, and TGF-β1 inhibitor, and it was observed that MG53 inhibited the TGF-β1/Smad pathway, and both MG53 and TGF-β1 had protective effects on the nasal epithelial barrier. However, they adopted different pathways to repair the nasal epithelial barrier after injury; TGF-β1 promoted the expression of ZO-1 and Col-a1 to repair the nasal epithelial barrier by promoting fibrosis, whereas MG53 inhibited the TGF-β1/Smad pathway, in turn, inhibiting fibrosis repair, and promoting epithelial cell proliferation, thus, playing a protective role in the nasal epithelial barrier.

Our study had various limitations. First, we lacked an animal model of nasal polyps. Second, TGF-β1 had a wide range of effects, likely caused by the different concentrations. The concentrations used in our study were based on previous literature. Therefore, we did not screen for the concentrations used. Finally, MG53 exerted its protective effect on the cell membrane in various ways. However, our study only elaborated on its protective mechanism from the perspective of the epithelial barrier. Thus, further studies are required to understand the possible mechanism of MG53.

Our study demonstrated the importance of repairing the imbalance after nasal epithelial barrier injury in CRSwNP and CRSsNP, and provided significant cues for the role of MG53 in inhibiting fibrosis and promoting epithelial hyperplasia. Thus, MG53 might be an important clinical treatment option for nasal polyps.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Y-C.Y provided the research ideas, X-C.J, X-Y.J, JL collected nasal tissue and extracted cells, XK, YS, J-J.H and G-H.H participated in selection of clinical patients. D-Q.A and YJ performed the experiments and analyzed the data. D-Q.A and MP drafted the paper. D-Q.A, XX and Y-C.Y reviewed the data and finalized the manuscript. All authors reviewed and approved the final version.

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TABLES

Table 1. Primer sets for PCR amplification.

Gene	Oligonucleotide primer sequen
Human MG53 Human TGF-β1 Human Smad2 Human Smad3 Human Col-a1 Human Zo-1	Forward: CTGTGAGTCCCT

Table 2. Trim72-si-rna sequences.

Trim72-si-rna Negative control Negative control FAM	Sense: 5´-GCAUUGGCCUUUACCUGAGTT-3´ Antisense: 5´-CU

The sequences used in the study were provided by GenePharma (Shanghai, China).

LEGENDS

ΦΙΓ1.Τηε εξπρεσσιον οφ ΜΓ53/ΤΓΦ-β1, ςολ-α1 ανδ ΖΟ-1 ιν νασαλ τισσυες οφ ὀντρολ˚ΡΣσΝΠ˚ΡΣωΝΠ

A. Immunohistochemistry (magnification 400X) measured the expression of Col-a1 and ZO-1 in nasal tissues of Control, CRSsNP and CRSwNP (n=5 per group). B. Immunofluorescence (magnification 800X) measured

the expression of ZO-1 in nasal tissues of Control, CRSsNP and CRSwNP (n=3 per group). C. Western blot analysis for MG53/TGF-β1/Smad/ Col-a1 /ZO-1 in nasal tissues of Control, CRSsNP and CRSwNP, and GAPDH expression in total protein extracted from nasal tissues.D. MG53/TGF-β1/Smad2、3/ Col-a1 /ZO-1 were quantified by densitometric analysis using ImageJ software, and data were expressed as relative values against GAPDH (n=4 per group). E. The expressions of MG53/TGF-β1/Smad/ Col-a1 /ZO-1 in nasal tissues of Control, CRSsNP and CRSwNP were analyzed by RT-qPCR (compared with Control).

Error bars are shown as means ±SEMs. Significance was calculated by using the paired Student t test. *p<0.05 and**p<0.01.

ΦΙΓ2. Τηε εξπρεσσιον οφ ΜΓ53/ΤΓΦ-β1, ςολ-α1 ανδ ΖΟ-1 ιν ΗΝΕ"ς οφ ὃντρολ"ΡΣσΝ-Π"ΡΣωΝΠ.

A. The expression of ZO-1 (magnification 400X) in HNECs of CRSsNP, CRSwNP and CRSwNP by immunofluorescence, and were quantified by Mean fluorescence intensity using ImageJ software (n=3 per group). B. Western blot analysis for MG53/TGF-β1/Smad/ Col-a1 /ZO-1 in nasal epithelial cells of Control, CRSsNP and CRSwNP, and GAPDH expression in total protein extracted from HNECs. C. MG53/TGF-β1/Smad/ Col-a1 /ZO-1 were quantified by densitometric analysis using ImageJ software, and data were expressed as relative values against GAPDH. D. The expressions of MG53/TGF-β1/Smad/ Col-a1 /ZO-1 in HNECs of Control, CRSsNP and CRSwNP were analyzed by RT-qPCR.

Error bars are shown as means ±SEMs. Significance was calculated by using the unpaired Student t test (FIG2 A). Significance was calculated by using the paired Student t test (FIG2 C and D). *p<0.05 and**p<0.01. ns not Significant.

FIG3. Αδενοιρυς τρανσφεςτιον ιν ΗΝΕ"ς ωιτη οερεξπρεσσιον οφ ΜΓ53 δοωνρεγυλατε ΤΓΦ- β1/Σμαδ πατηωαψ.

A and B. The expressions of MG53/TGF- β1/Smad/ Col-a1 /ZO-1 in Adenovirus transfected HNECs of CRSwNP were analyzed by Western blot, and quantified by densitometric analysis using ImageJ software, and data expressed as relative values against GAPDH. C. The expressions of MG53/TGF-β1/Smad/ Col-a1 /ZO-1 in adenovirus transfected HNECs of CRSwNP were analyzed by RT-qPCR.

Error bars are shown as means ±SEMs. Significance was calculated by using the paired Student t test. *p<0.05 and**p<0.01. ns not Significant.

ΦΙΓ4. Σι-ρνα τρανσφεςτιον ιν ΗΝΕ"ς ωιτη κνοςκδοων οφ ΜΓ53 υπρεγυλατε ΤΓΦ-β1/Σμαδ πατηωαψ.

A. The cell morphology and FAM of HNECs transfected with si-rna after cells were transfected si-rna for 48h (magnification 200X). B. The expressions of MG53/TGF-β1/Smad/ Col-a1 /ZO-1 in si-rna transfected HNEC of CRSwNP were analyzed by RT-qPCR. C and D. The expressions of MG53/TGF- β1/Smad/ Col-a1 /ZO-1 in si-rna transfected HNECs of CRSwNP were analyzed by Western blot, and quantified by densitometric analysis using ImageJ software, and data expressed as relative values against GAPDH.

Error bars are shown as means ±SEMs. Significance was calculated by using the paired Student t test. *p<0.05 and**p<0.01. ns not Significant.

ΦΙΓ5. Ιν ιτρο, ΤΓΦ-β1 τρεατεδ ΗΝΕ"ς ινςρεασεδ ΜΓ53/ΤΓΦ-β1, ὃλ-α1 ανδ ΖΟ-1, ον τηε ςοντραρψ, ΤΓΦ-β1 ινηιβιτορ τρεατεδ δεςρεασεδ ΜΓ53/ΤΓΦ-β1, ὃλ-α1 ανδ ΖΟ-1

A. In vitro, TGF-β1(10ng/ml) or/and inhibitor TGF-β1(50nM) treatment HNECs of Control、CRSsNP and CRSwNP for 72h, the expression of MG53/TGF-β1/Smad/ Col-a1 /ZO-1 by Western blot. B. The expressions of MG53/TGF- β1/Smad/ Col-a1 /ZO-1 in treatment by TGF-β1 or/and inhibitor TGF-β1 of nasal epithelial cells of CRSwNP were analyzed by Western blot. C. The expressions of MG53/TGF- β1/Smad/ Col-a1 /ZO-1 in treatment by TGF-β1 or inhibitor TGF-β1 of epithelial cells of the nasal mucosa of CRSwNP were analyzed by RT-qPCR. D. Immunofluorescence was used to detect ZO-1 expression in CRSwNP treated

with TGF-β1/inhibitor TGFβ1/Ad-MG53(Cells were treated by TGF-β1/inhibitor TGFβ1/ Ad-MG53 for 72h).

Error bars are shown as means ±SEMs. Significance was calculated by using the unpaired Student t test (FIG5 E). Significance was calculated by using the paired Student t test (FIG2 C and D). *$p<0.05$ and**$p<0.01$. ns not Significant.

FIG6. The effect of Ad-MG53 on proliferation and apoptosis as well as epithelial repair function.

A. HNECs of CRSwNP proliferation quantified by cell counting. B. The amount of soluble col-a1 in the supernatant of HNECs of CRSwNP culture were determined by ELISA kit. C. HNECs of CRSwNP treatment by TGF-β1/Ad-MG53 prevented LDH release following glass bead damage. D. HNECs of CRSwNP treatment by Ad-MG53 prevented apoptosis compared with NC-MG53.

Error bars are shown as means ±SEMs.Significance was calculated by using the unpaired Student t test (FIG 6 A.B.C). Significance was calculated by using the paired Student t test (FIG 6D). *$p<0.05$ **$p<0.01$ ***$p<0.001$. ns not Significant.

APPENDIX

A. Adenovirus transfection with different gradients, the expressions of MG53/TGF-β1/Col - a1 and ZO-1 were detected by Western blot. The HNECs were transfected adenovirus for 72h. (When Adv-MOI =100, MG53 was significantly overexpressed and showed significant inhibitory effects on TGF-β1, Col-a1 and ZO-1.Therefore, Adv-MOI =100 was selected as the transfection condition).

B. The HNECs were transfected with adenovirus for 48 hours after transfection and photographed under fluorescence microscope(magnification 200X).

C. The HNCES were treated with different concentrations of TGF-β1 inhibitor for 72H.The inhibitory effect of MG53/TGF-β1/Smad/Col - a1 and ZO-1 were analyzed by Western blot. (When TGF-β1 inhibitor =50n M, the expression of MG53 /TGF-β1/ Col-a1 and ZO-1 were significant inhibited .Therefore, TGF-β1 inhibitor =50n M was selected as the optimum condition).