

Lipopolysaccharide regulates thymic stromal lymphopoietin expression via TLR4/MAPK/Akt/NF- κ B signaling pathways in nasal fibroblasts: differential inhibitory effects of macrolide and corticosteroid

Ju-Hyung Kang

Korea University College of Medicine and School of Medicine

Hyun-Woo Yang

Korea University College of Medicine and School of Medicine

Joo-Hoo Park

Korea University College of Medicine and School of Medicine

Jae-Min Shin

Korea University College of Medicine and School of Medicine

Tae-Hoon Kim

Korea University College of Medicine and School of Medicine

Seung Hoon Lee

Korea University College of Medicine and School of Medicine

Heung-Man Lee (✉ lhman146@gmail.com)

Korea University College of Medicine and School of Medicine

Il-Ho Park (✉ parkil5@korea.ac.kr)

Korea University College of Medicine and School of Medicine <https://orcid.org/0000-0002-7011-6071>

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Abstract

Background

Chronic rhinosinusitis (CRS) is inflammatory disease of sinonasal mucosa. Thymic stromal lymphopoietin (TSLP) is associated with Th-2 response and induced by pathogen, allergen, Toll-like receptor (TLR) ligands, and cytokines. Fibroblasts have known to modulators of wound healing, from inflammation to tissue remodeling. We examined effect of lipopolysaccharide (LPS) on TSLP production and underlying mechanisms. We aimed to determine whether effects of commonly used medications in CRS, corticosteroids and macrolides, are related to LPS-induced TSLP in nasal fibroblasts.

Results

Fibroblasts were isolated from inferior turbinate tissues of CRS patients. TSLP and TLR4 expression was determined by RT-PCR, western blot, ELISA, and immunofluorescence staining. MAPK, Akt, and NF- κ B phosphorylation was determined by western blot and/or luciferase assay.

LPS increased TSLP expression in a dose- and time-dependent manner. LPS antagonist and corticosteroids inhibited TLR4 expression in LPS-stimulated fibroblasts. LPS-RS, macrolides, corticosteroids, and specific inhibitors suppressed LPS-induced alterations. *Ex vivo* culture showed similar results.

Conclusions

LPS induces TSLP production via TLR4, MAPK, Akt, and NF- κ B pathways. Effects of corticosteroids and macrolides are related to LPS-induced TSLP expression. We would explore new treatment modalities targeting LPS-induced TSLP production that could replace current usage of corticosteroid and macrolides in treatment of CRS.

Background

Chronic rhinosinusitis (CRS) is chronic inflammation of the nasal and paranasal sinuses persisting for more than 12 weeks. It is accompanied by symptoms such as nasal obstruction, congestion, discharge, cough and facial pain. Furthermore, it can reduce the sense of smell and taste. These symptoms can result in poor quality of life and low productivity[1, 2]. Approximately 10% of the world's population has CRS. CRS is classified into CRS without nasal polyp (CRSsNP) and CRS with nasal polyp (CRSwNP) according to the presence of nasal polyp. To uncover the pathogenesis of CRS, a number of studies have been conducted, including infection by organisms such as fungal species or *Staphylococcus aureus*, disturbance of the nasal microbiome, and tissue remodeling of the upper respiratory tract. Recently, several reviews have focused on the roles of mucosal immunity, inflammation, cytokines, and T and B lymphocytes in CRS pathogenesis[3, 4].

When a rapid local immune response fails to prevent invasion of the pathogen, adaptive immune T cells and B cell are activated and enhance the immune response. The damaged epithelial layer produces Th-2 promoting cytokine, IL-25, IL-33, and thymic stromal lymphopoietin (TSLP). These cytokines are known to be associated with the development of CRS[5-7]. TSLP is a key factor in the maturation of T cells and in the Th2-inflammatory response. It is a cytokine which is mainly expressed by epithelial cells and stromal cells, including fibroblasts. The expression of TSLP is induced by pathogens, allergens, toll-like receptor (TLR) ligands, and pro-inflammatory and Th2 cytokines[8-10]. A pathogenetic role of TSLP in some patients has been well described in Th2-driven diseases like asthma and atopic dermatitis (AD). TSLP is now receiving attention in CRS research, as the importance of Th2-driven inflammation in the pathogenesis of CRS is growing, especially in Western countries[11, 12].

Fibroblasts are traditionally recognized for their limited role in producing the extracellular matrix in wounded tissues. However, recent studies have indicated that fibroblasts modify the entire process of wound healing, from inflammation to tissue remodeling, so the immune cascades related with those processes are orchestrated by fibroblasts[13, 14]. A close relationship between fibroblasts and inflammation has also been shown in many chronic inflammatory diseases that make micro-wounds persistently by continuous insults to the tissue[15, 16].

Based on the literature, we considered that fibroblasts were shown to express several TLRs, and TSLP is produced not only by epithelial cells but also stromal cells including fibroblasts. We therefore hypothesized that LPS penetrating the stroma through disrupted epithelia would enable fibroblasts to act as a bridge between innate immunity and active inflammation by producing cytokines, including TSLP. In the present study, we examined the effect of LPS on TSLP production in nasal fibroblasts and tissue and investigated its underlying mechanisms. We also aimed to determine whether the effects of commonly used medication in CRS, such as corticosteroids and macrolides, are related to LPS-induced TSLP in nasal fibroblasts and inferior turbinate tissues.

Results

LPS induces expression of TSLP in nasal fibroblasts

We investigated the expression of TSLP in LPS-treated nasal fibroblasts. Nasal fibroblasts were treated with LPS (10 µg/ml) for 8 hours, and the expression of *TSLP* mRNA was assessed by RT-PCR. Stimulation with LPS significantly increased the expression of *TSLP* mRNA at 2 - 4 hours (Fig. 1A). Fibroblasts were then treated with various concentration of LPS (0 - 10 µg/ml) for 2 hours. Treatment with LPS increased the expression of *TSLP* mRNA in dose-dependent manner (Fig. 1B). We then sought to determine whether LPS stimulates TSLP protein expression in nasal fibroblasts. Fibroblasts were treated with LPS (10 µg/ml) for 24 hours, and the protein production of TSLP was measured by ELISA. LPS-induced TSLP production was significantly increased after 8 hours and reached maximum levels at 24 hours (Fig. 1C). TSLP protein levels were then measured after treatment with various concentration of LPS (0 - 10 µg/ml) for 24 hours by ELISA (Fig. 1D). To confirm our findings, immunofluorescence staining

of TSLP protein was also performed in nasal fibroblasts. Expression of TSLP protein was found to be increased by LPS in a dose-dependent manner (Fig. 1E).

LPS induces TSLP expression via TLR4 signaling pathway in nasal fibroblasts and stimulatory effect of LPS on TLR4 and TSLP expression is inhibited by TLR4 inhibitor and glucocorticoid.

Since LPS is recognized by TLR4, we investigated whether TLR4 is involved in LPS-induced TSLP expression. Nasal fibroblasts were pre-treated with TLR4 antagonist (LPS-RS, 10 μ M) and *TLR4* mRNA expression was measured by RT-PCR in 2 hours. We also determined the effect of macrolides and glucocorticoids on LPS-induced TLR4 expression. The expression of *TLR4* mRNA was significantly inhibited by TLR4 antagonist and corticosteroids, but not macrolides (Fig. 2A). The protein expression of TLR4 measured after 24 hours was also decreased in cells treated with TLR4 antagonist and corticosteroids, but not macrolides (Fig. 2B). This finding was confirmed again using immunofluorescence staining (Fig. 2C). We then measured the effect of TLR4 antagonist on LPS-induced TSLP protein expression using ELISA (Fig. 2D). Production of TSLP was inhibited by TLR4 antagonist, glucocorticoids and macrolides after treatment of LPS.

MAPK and Akt regulate LPS-induced TSLP expression in nasal fibroblasts

To verify whether MAPK (ERK, p38, and JNK) and Akt signaling pathways are involved in LPS-induced expression of TSLP in nasal fibroblasts, we investigated the effect of MAPK inhibitor on LPS-induced TSLP expression in nasal fibroblasts. The effect of macrolides and glucocorticoids were also evaluated. Nasal fibroblasts were pre-treated with TLR4 antagonist, clarithromycin, roxithromycin, dexamethasone, or fluticasone propionate for 1 hour, then treated with LPS for 30 minutes. The phosphorylation of MAPK and Akt were significantly increased by LPS. We then confirmed that phosphorylation of MAPK and Akt induced by LPS was inhibited by TLR4 antagonist. MAPK and Akt phosphorylation was decreased by TLR4 antagonist, macrolides, and corticosteroids (Fig. 3A). Additionally, we determined whether LPS-induced TSLP is inhibited by a specific inhibitor of MAPK/Akt activation. Nasal fibroblasts were pre-treated with MAPK inhibitors for 1 hour, then treated with LPS for 24 hours, and the protein production of TSLP was measured by ELISA. LPS-induced TSLP production was significantly decreased by specific inhibitors (U0126, SB203580, SP600125, LY294002) of MAPK and Akt phosphorylation (Fig. 3B).

NF- κ B regulates LPS-induced TSLP in nasal fibroblasts.

We evaluated the contribution of NF- κ B to LPS-induced TSLP expression in nasal fibroblasts. First, we confirmed that phosphorylation of NF- κ B (p-p50) is induced by LPS in nasal fibroblasts and that specific inhibitors (TLR4, MAPK, Akt and NF- κ B), macrolides, and corticosteroids have an inhibitory effect on LPS-induced phosphorylation of p50 (Fig. 4A). The transcriptional activity of NF- κ B was also inhibited in similar manner by the same inhibitors (Fig. 4B). Localization of p-p50 was visualized by immunofluorescence staining and we again confirmed the inhibitory effect of NF- κ B inhibitor on LPS-induced nucleus localization of p-p50 (Fig. 4C). Finally, we checked whether LPS-induced TSLP is inhibited by an NF- κ B inhibitor. LPS-induced TSLP protein production was significantly decreased by a specific

inhibitor of NF- κ B activation (Fig. 4D). These data indicate that NF- κ B activation is involved as a signaling molecule in LPS-induced TSLP expression in nasal fibroblasts.

LPS induces TSLP expression in ex vivo nasal inferior turbinate tissues

To confirm the effect of LPS on TSLP and its underlying signaling pathway shown in the previous experiment, we performed experiments in *ex vivo* nasal inferior turbinate organ culture. Initially, LPS induced TSLP expression in *ex vivo* inferior turbinate tissues as seen in nasal fibroblasts. Nasal inferior turbinate tissues were pre-treated with the same specific inhibitors tested in previous experiments, then treated with LPS for 72 hours. TLR4 antagonist, MAPK inhibitor, Akt inhibitor, NF- κ B inhibitor, macrolides, and corticosteroids all significantly inhibited LPS-induced *TSLP* mRNA expressions in our RT-PCR analysis (Fig. 5A). Furthermore, protein expression of TSLP was increased in LPS-treated nasal inferior turbinate tissues and was reduced by TLR4 antagonist, MAPK inhibitor, Akt inhibitor, NF- κ B inhibitor, macrolide, and corticosteroid treated samples (Fig. 5B).

Discussion

CRS is characterized by chronic inflammation of the sinonasal mucosa. In the era of antibiotics and endoscopic sinus surgery, CRS is not a fatal disease in most cases. However, a high incidence of CRS and its associated symptoms including nasal congestion, nasal discharge, sinus pressure, and olfactory dysfunction cause a huge amount of indirect economic burden in schools, workplaces, and everyday life[1, 17]. Treatment strategies for CRS patients are limited due to its heterogenous pathology. However, recent studies have made advances in uncovering the pathology of CRS. One of the suggested avenues of study is the role of innate immune responses and crosstalk between innate and acquired immune system in chronic sinonasal inflammation.

The sinonasal mucosa is the first respiratory tissue encountered by environmental agents including many microorganisms[18]. PAMP from those microorganisms are recognized by pattern recognizing receptors, which are best characterized by TLRs. Naturally, there is a close relationship between chronic airway inflammatory diseases and TLRs. [19]. For example, the levels of TLR2 and TLR4 mRNA transcripts in the samples from patients with CRS were significantly higher than those from the controls[20]. The role of the TLR is not simple recognition of microorganisms, but rather to induce infectious inflammatory loading in airway persistent inflammation. Moreover, if we consider that Th2 cytokine production was abolished in TLR4-deficient mice in house dust mite-driven allergic airway inflammation, the role of TLR should be viewed in a wider range[21]. Thus, an analysis of TLR expression and its role in immune response is important for understanding the CRS pathology.

Epithelial cells play crucial roles in the immune system. In most cases, airway epithelium plays a defensive role, especially in innate immune system[22]. When the host immune systems are unable to relieve chronic persistent inflammation, inevitable deformation of the airway epithelia in CRS patients causes damage to the primary defense of the respiratory tract, followed by more severe and persistent inflammation[23, 24]. Traditionally, epithelial cells have received attention, mainly for the aforementioned

process, and fibroblasts have been recognized mainly for their role in extracellular matrix production. However, fibroblasts are becoming increasingly appreciated as an important player in the immune response. Recent evidence shows that structural cells such as fibroblasts also contribute to amplification of the immune response by participating in innate immunity and bridging the innate and adaptive immune systems. For example, fibroblasts have been shown to express several TLRs, and their subsequent activation can activate fibroblasts and promote their transformations into an active form, myofibroblasts[25-27]. Moreover, fibroblasts can participate in the maintenance of an inflammatory response via expression of a variety of chemokines and cytokines[28, 29].

TSLP, well known as a key cytokine in type 2 immune response, is one of the cytokines expressed by fibroblasts[9, 30]. In the present study, we showed that TSLP is produced in nasal fibroblasts in time- and dose-dependent manners in response to LPS stimulation. Originally, TSLP first received attention as a proallergic cytokine released from epithelial cells. Recently, it has been found that there is a correlation between TSLP and the level of airway inflammation in several diseases including asthma and CRS, and that TSLP contributes to tissue remodeling in those diseases.

TLR signaling pathways plays crucial roles in the immune system. TLR4 is one member of the TLR family; activated TLR4 leads to the NF- κ B intracellular signaling pathway and inflammatory cytokine secretion. These processes stimulate innate immune response and induce inflammation in the host. LPS is a known ligand of TLR4[31, 32]. In present study, we evaluated each step known to be related to TLR signaling and found that LPS-TLR4 binding activated the MAPK, Akt and NF- κ B signaling pathways. Furthermore, those pathways contributed to increased TSLP expression in nasal fibroblasts.

In the present study, we identified the effects of LPS on TSLP production in nasal fibroblasts and tissues and the underlying mechanism of LPS-induced TSLP production. We showed that LPS induces TSLP production in dose- and time-dependent manners in nasal fibroblasts. The increase of TSLP by LPS occurred through TLR4, MAPK, Akt and NF- κ B signaling pathway activation. We also demonstrated that macrolides and corticosteroids have inhibitory effects on TSLP expression and its underlying mechanism. Corticosteroids and macrolides are commonly prescribed to treat inflammatory diseases including CRS. We found that corticosteroids suppress TSLP by regulating the TLR4 expression and their downstream pathways. Macrolides exert their inhibitory effects on LPS-induced TSLP production not by regulating the TLR4 expression like corticosteroids, but by suppressing MAPK, Akt and NF- κ B signaling pathways.

Conclusions

In summary, our study showed that LPS induces TSLP production in nasal cells and tissue via TLR4, MAPK, Akt and NF- κ B signaling pathways. We also showed that the effects of corticosteroids and macrolides in CRS treatment are related to LPS-induced TSLP. We are interested in further studies to find new treatment modalities targeting LPS-induced TSLP production that can replace the current usage of glucocorticoids and macrolides in the treatment of CRS.

Methods

Reagents and antibodies

Lipopolysaccharide (LPS) from *Pseudomonas aeruginosa*, dimethyl sulfoxide (DMSO), clarithromycin (CAM), Roxithromycin (RXM), dexamethasone (DEX), fluticasone propionate (FP), and inhibitors of ERK (U0126), p38 (SB203580), JNK (SP600125), Akt (LY294002) and nuclear factor- κ B (NF- κ B, BAY 11-7082) were all provided by Sigma-Aldrich Co. (St. Louis, MO). LPS from the photosynthetic bacterium *Rhodobacter sphaeroides* (LPS-RS, LPS inhibitor) was purchased from InvivoGen (Carlsbad, CA). Primary antibodies against TLR4, TSLP, phospho-ERK, total-ERK, phospho-p38, total-p38, phospho-JNK, total-JNK, phospho-Akt, total-Akt, p-p50, p50, and β -actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Secondary antibodies conjugated with horseradish peroxidase against anti-rabbit and anti-mouse were obtained from Vector Laboratories Inc. (Burlingame, CA).

Human subjects

Nasal inferior turbinate tissues were obtained from six patients with CRS (3 males and 3 females; mean age 41.2 ± 4.5 years) during endoscopic sinus surgery. The diagnosis of CRS was based on the history of patients and endoscopic/radiographic criteria, as well as CT findings of sinuses according to the 2012 European position paper on rhinosinusitis and nasal polyps (EPOS) guidelines. Nasal polyp tissues were obtained from the region of the middle meatus at the beginning of endoscopic sinus surgery in CRSwNP patients. None of the patients had taken oral steroids, non-steroidal anti-inflammatory drugs, antihistamines, or antibiotics for at least 4 weeks prior to endoscopic surgery.

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. The study was carried out in accordance with the guidelines of the Human Ethics Committee of Korea University Guro Hospital (KUGH14065-001).

Sinonasal fibroblast culture

Sinonasal 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). Sinonasal fibroblasts were cultured in Dulbecco's Modified Eagle Medium containing 10% heat-inactivated fetal bovine serum (Invitrogen, Carlsbad, CA), 1% 10,000 μ g/mL streptomycin, and 1% 10,000 U/mL penicillin (Invitrogen). The purity of the sinonasal fibroblasts were confirmed by their characteristic spindle-shaped cell morphology and by flow cytometry[33].

Organ culture of nasal inferior turbinate

Nasal inferior turbinates were cut, using scissors, into 2 to 3mm³ pieces under sterile conditions. Tissue fragments were washed three times with phosphate buffered saline. The washed tissue fragments were

placed on a pre-hydrated gelatin sponge (10 mm × 10 mm × 1 mm; Spongostan, Johnson & Johnson, San Angelo, TX) in 6 well plates. Wells were filled with 1.5 ml of culture medium containing Dulbecco's Modified Eagle Medium (DMEM; Invitrogen,) and 2% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA) per well. The plates were placed in maintained at 37°C in 5% CO₂.

Real-time PCR

Real-time polymerase chain reaction was used to evaluate the mRNA levels in nasal fibroblasts and inferior turbinate tissues. Total RNA was extracted using TRIzol reagent (Invitrogen). Reverse transcription was performed using MMLV reverse transcriptase (Invitrogen) according to the manufacturer's instructions. RT-PCR was performed using the following primers: *TSLP* (sense sequence, 5'- TAT GAG TGG GAC CAA AAG TAC CG-3'; anti-sense sequence, 5'- GGG ATT GAA GGT TAG GCT CTG G-3'), *TLR4* (sense sequence, 5'-TGA GCA GTC GTG CTG GTA TC-3'; anti-sense sequence, 5'-CAG GGC TTT TCT GAG TCG TC-3'), *GAPDH* (sense sequence, 5'-GTG GAT ATT GTT GCC ATC AAT GAC C-3'; anti-sense sequence, 5'-GCC CCA GCC TTC TTC ATG GTG GT-3'). RT-PCR was performed with Quantstudio3 (Applied Biosystems, Foster City, CA) using Power SYBR Green PCR Master Mix (Applied Biosystems). The delta-delta Ct ($2^{-\Delta\Delta Ct}$) method was used to analyze relative gene expression levels. 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 dishes at a density of 5×10^5 cells/mL. Sinonasal 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 polyvinylidene difluoride membranes (Merck Millipore, Billerica, MA). Membranes were blocked with 5% skim milk. The blots were incubated with primary antibodies against TLR4, phospho-Akt, total-Akt, β -actin (Santa Cruz Biotechnology, Inc., Dallas, TX), phospho-ERK, ERK, phospho-p38, p38, phospho-JNK, JNK, p-p50, and p50 (Millipore Inc., Billerica, MA). Next, the blots were visualized with HRP-conjugated secondary antibodies and an ECL system (Pierce, Rockford, IL). Images were analyzed using ImageJ software (NIH, Rockville, MD). Protein expression was normalized to β -actin or total protein in the case of phosphorylated proteins.

Enzyme-linked immunosorbent assay (ELISA)

The concentration of TSLP production within the supernatants was determined by using an ELISA kit (R&D Systems, Minneapolis, MN) according to manufacturer's instructions. The optical densities of the standards and samples were measured at 450nm using a microplate reader (Bio-Rad, Hercules, CA).

Immunocytochemical staining

Sinonasal fibroblasts were seeded on 8-well culture slides (SPL Life Sciences, Korea) at 5×10^4 cells/ml per well and grown to 60% - 70% confluence. Sinonasal 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. Sinonasal fibroblasts were stained with the following primary antibodies overnight at 4°C: anti-TSLP, TLR4 (1:1,000, Santa Cruz Biotechnology, Inc.) or p-p50 (Millipore Inc). The sinofibroblasts were then 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).

Luciferase assay

NF- κ B luciferase reporter gene constructs (luc2P/NF- κ B-RE/Hygro and hRluc/TK; Promega Co., Madison, WI) were transiently transfected into nasal fibroblasts by using fetal bovine serum and antibiotic-free DMEM containing 5 μ L of FuGENE transfection reagent (Promega Co). After 5 hours of incubation, the medium was replaced with DMEM containing 10% fetal bovine serum. Luciferase assays were performed to determine the firefly luciferase activity relative to the *Renilla* luciferase activity in the cell lysate using a luminometer (Promega Co.).

Statistical analysis

Statistical analysis of the differences between control and experimental data was performed with unpaired *t*-test or one-way analysis of variance followed by Tukey's test (GraphPad, version 7, GraphPad Software, Inc., La Jolla, CA). Significance was established at the 95% confidence level. *P* values less than 0.05 were accepted as statistically significant. Results were obtained from at least three independent replicate experiments.

Abbreviations

Chronic rhinosinusitis (CRS)

Thymic stromal lymphopoietin (TSLP)

Toll-like receptor (TLR)

Lipopolysaccharide (LPS)

CRS without nasal polyp (CRSsNP)

CRS with nasal polyp (CRSwNP)

Atopic dermatitis (AD)

Dimethyl sulfoxide(DMSO),

Clarithromycin (CAM),

Roxithromycin (RXM),

Dexamethasone (DEX),

Fluticasone propionate (FP)

Declarations

Ethics approval and consent to participate

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. The study was carried out in accordance with the guidelines of the Human Ethics Committee of Korea University Guro Hospital (KUGH14065-001).

Consent for publication

“Not applicable”

Availability of data and materials

“Not applicable”

Competing interests

The authors have no competing financial interests to declare.

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Authors' contributions

JH Kang and HW Yang (Upper Airway Chronic Inflammatory Diseases Laboratory, Korea University, College of Medicine, Seoul, Korea) conceived the study, designed and performed the experiments, analyzed the data, and wrote the manuscript. JH Park (Upper Airway Chronic Inflammatory Diseases Laboratory, Korea University, College of Medicine, Seoul, Korea), JM Shin, TH Kim, SH Lee (Department of Otorhinolaryngology-Head and Neck Surgery, Korea University, College of Medicine, Seoul, Korea) evaluated the data and discussed this study. HM Lee and IH Park (Department of Otorhinolaryngology-Head and Neck Surgery, Korea University, College of Medicine, Seoul, Korea) supervised the research and reviewed the manuscript. All authors reviewed and approved the manuscript.

Acknowledgement

“Not applicable”

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Figures

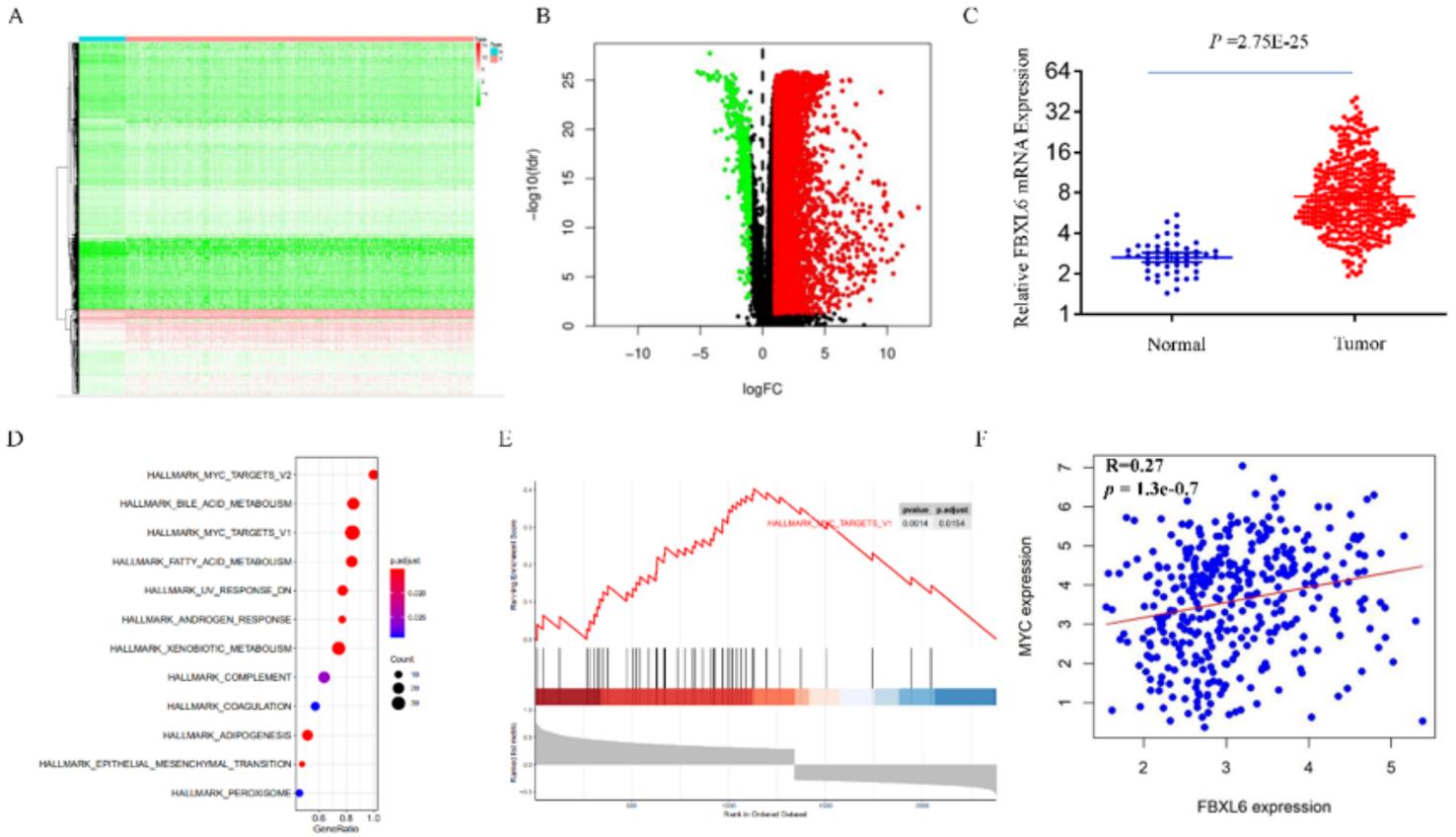


Figure 1

Lipopolysaccharide-induced thymic stromal lymphopoietin (TSLP) expression in nasal fibroblasts. After treatment with LPS (10 $\mu\text{g/ml}$), expression level of TSLP mRNA was determined by RT-PCR (A). After treatment with LPS for 2 hours, level of TSLP mRNA

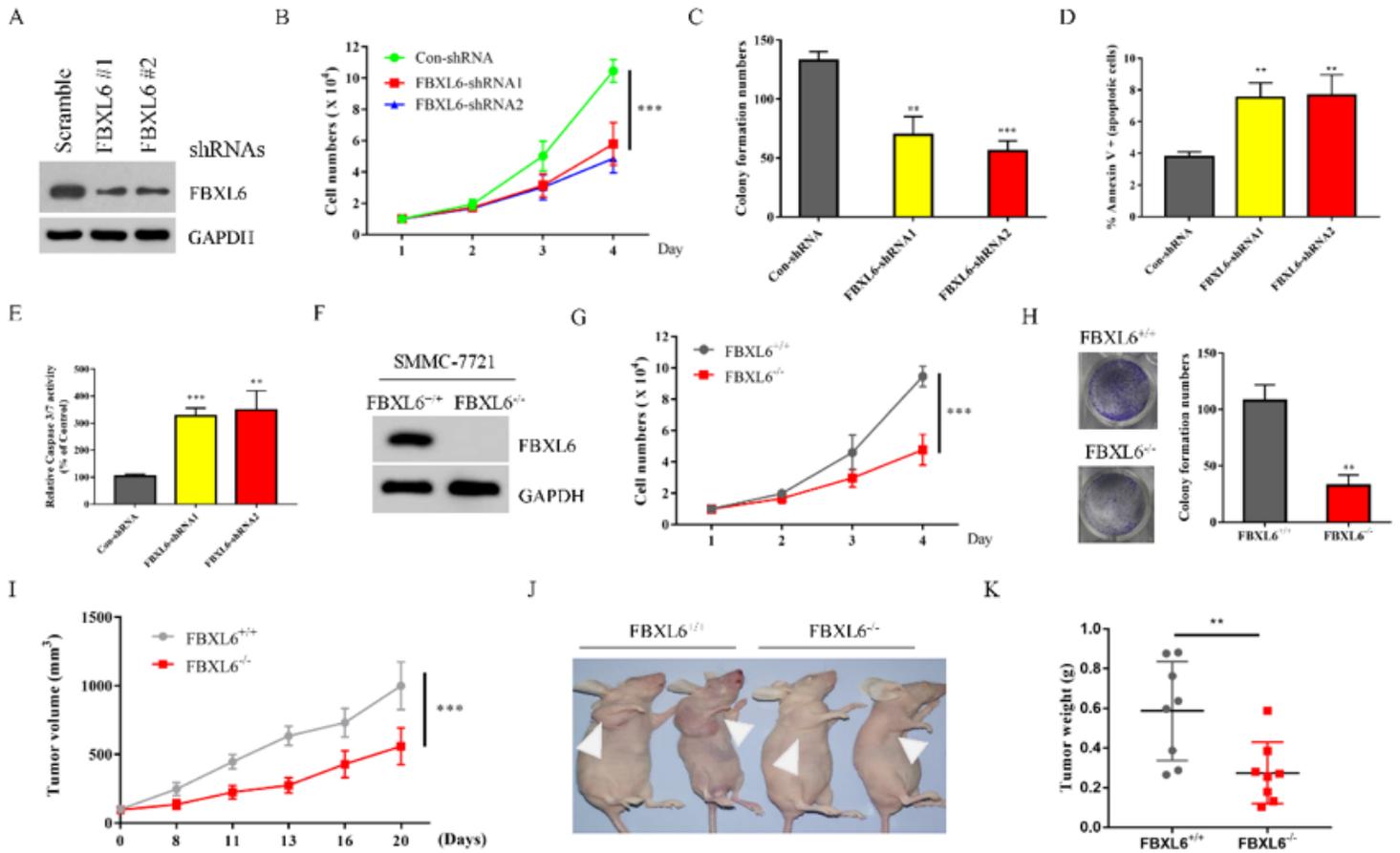


Figure 2

Effect of macrolides and corticosteroids on TLR4 expression induced by lipopolysaccharide (LPS) in nasal fibroblasts. Nasal fibroblasts were stimulated with LPS (10 µg/ml) with or without TLR4 antagonist (LPS-RS, 10 µM), clarithromycin (CAM, 10 µg/m)

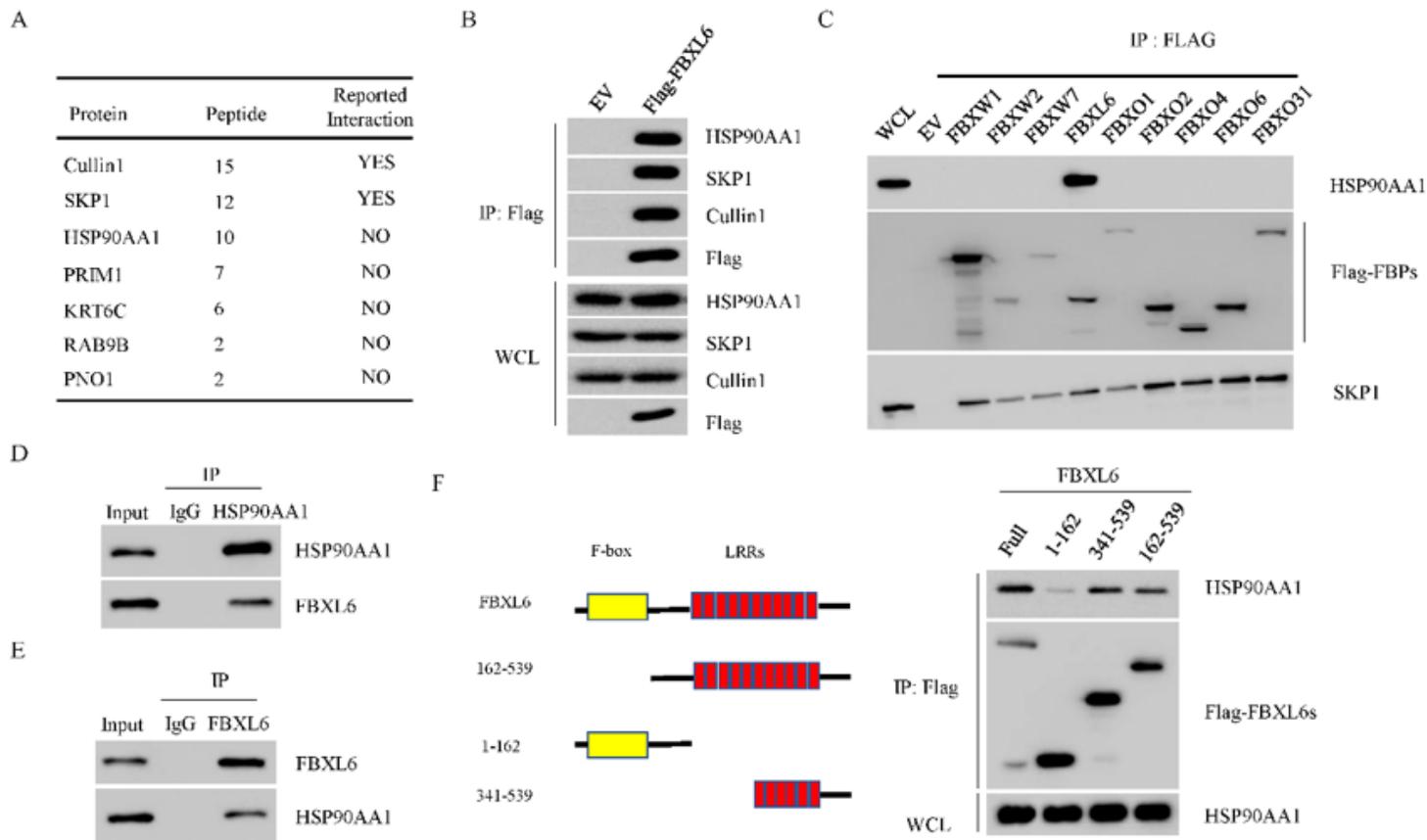


Figure 3

Effect of macrolides and corticosteroids on lipopolysaccharide-induced MAPKs and Akt phosphorylation in nasal fibroblast

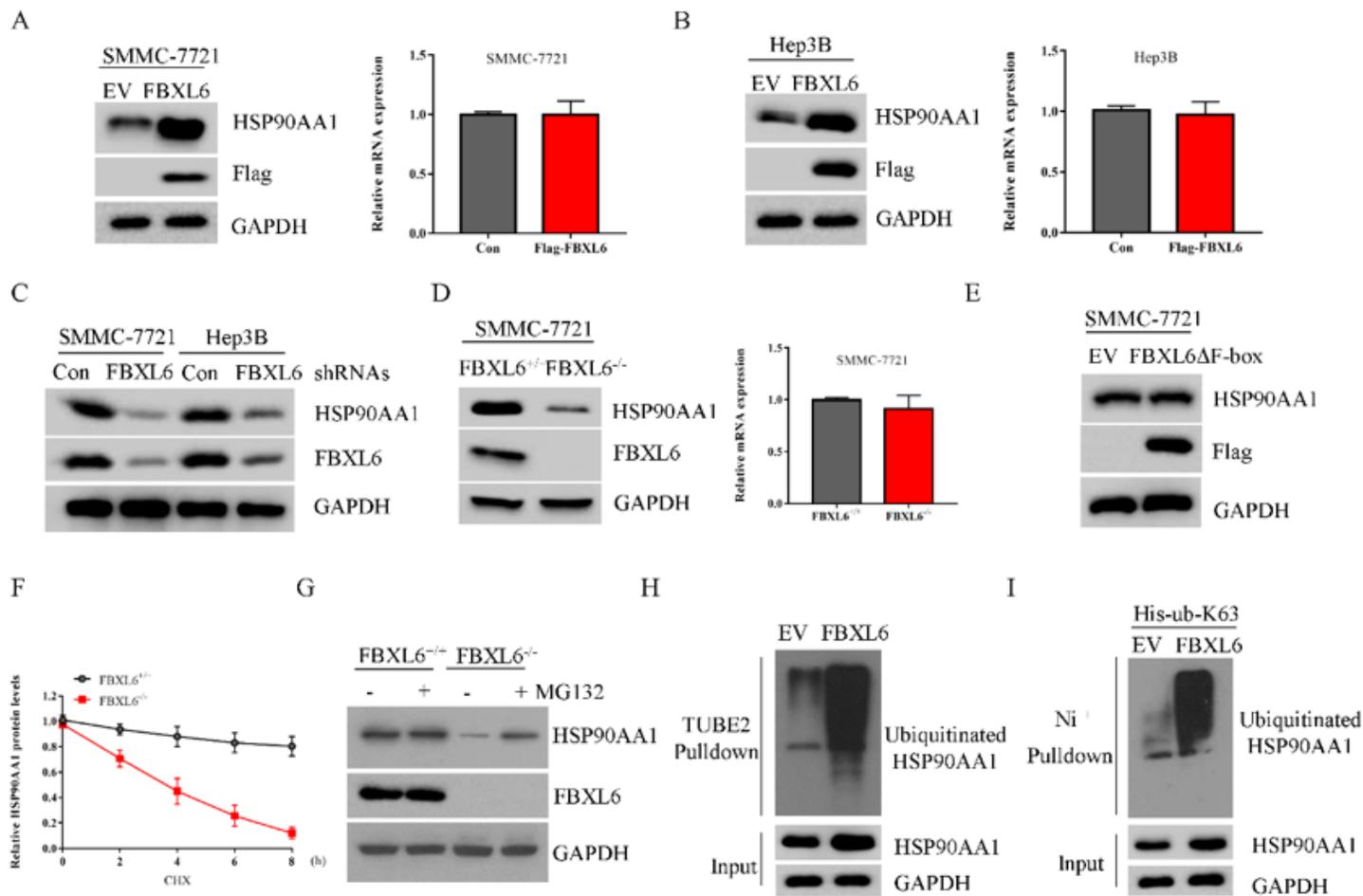


Figure 4

Effect of macrolide and corticosteroid on lipopolysaccharide-induced NF- κ B activation in nasal fibroblasts.

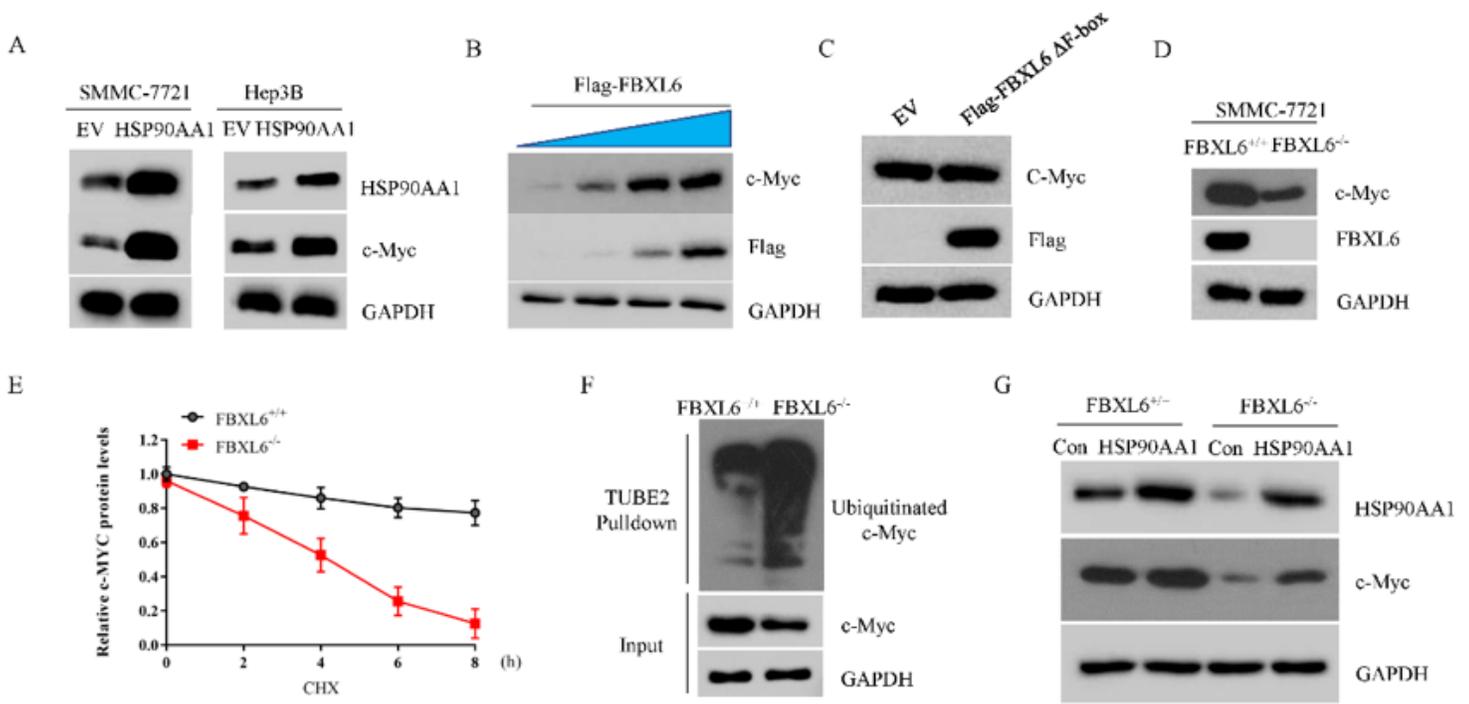


Figure 5

LPS-induced TSLP production in ex vivo nasal inferior turbinate tissues.

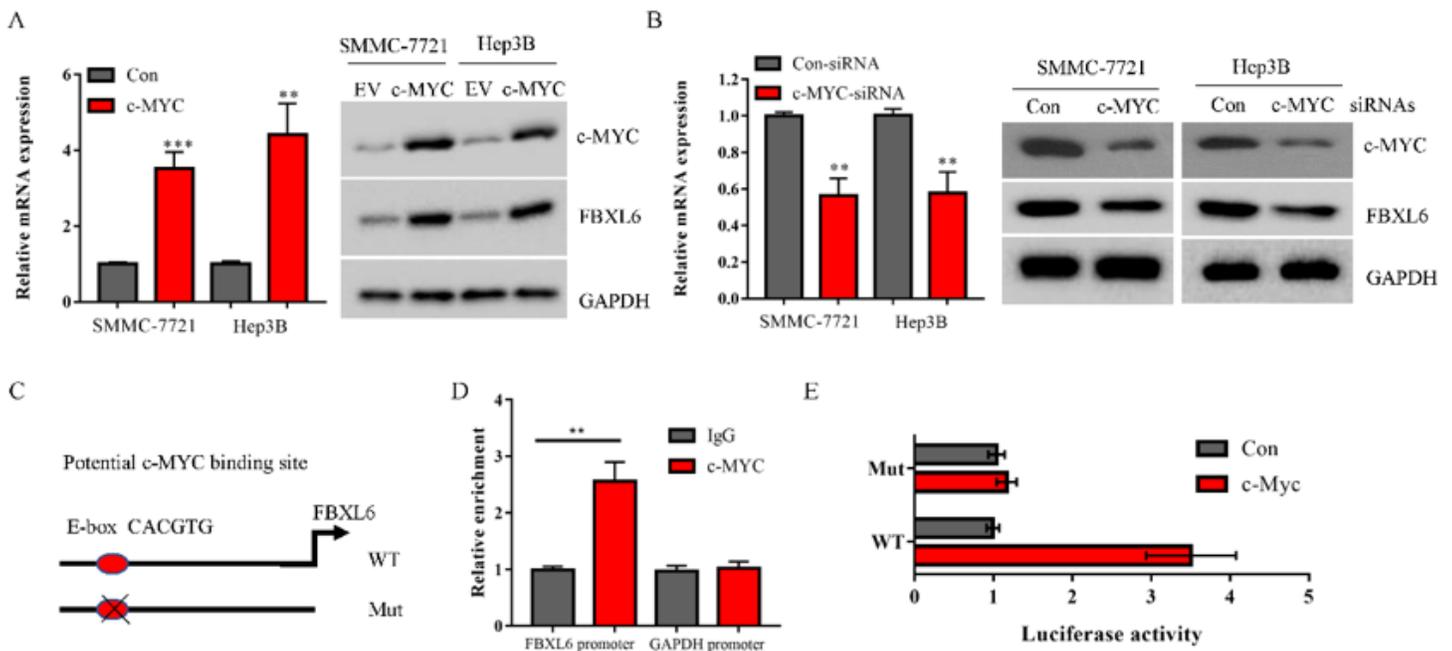


Figure 6

Schematic diagram of the lipopolysaccharide-induced thymic stromal lymphopoietin expression in nasal fibroblasts and ex vivo inferior turbinates.