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Eupatilin alleviates inflammation and epithelial-to-mesenchymal transition in chronic rhinosinusitis with nasal polyps by upregulating TFF1 and inhibiting the Wnt/β-catenin signaling pathway

Hongxia Su and Yulin Zhao

Department of Rhinology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Summary. Background. Chronic rhinosinusitis with nasal polyps (CRSwNP) is a multifactorial inflammatory disease characterized by high prevalence and morbidity. However, its pathogenesis is still obscure. This work focuses on the effects of Eupatilin (EUP) on inflammation reaction and the epithelial-to-mesenchymal transition (EMT) process in CRSwNP.

Methods. *In vivo* and *in vitro* CRSwNP models were established based on BALB/c mice and human nasal epithelial cells (hNECs) to investigate the effects of EUP on EMT and inflammation in CRSwNP. Protein levels of TFF1, EMT-related factors (E-cadherin, N-cadherin, and Vimentin), and Wnt/β-catenin signaling-related proteins (Wnt3α and β-catenin) were assayed via western blotting. Pro-inflammatory factors (TNF-α, IL-6, and IL-8) were assessed via ELISA assay.

Results. EUP treatment significantly reduced the number of polyps, epithelial thickness, and mucosal thickness in CRSwNP mice. Besides, EUP treatment also suppressed inflammation reaction and EMT events in CRSwNP mice and SEB-challenged hNECs in a dose-dependent manner. Also, EUP treatment dose-dependently upregulated TFF1 expression and inhibited Wnt/β-catenin activation in CRSwNP mice and SEB-challenged hNECs. In addition, TFF1 inhibition or Wnt/β-catenin activation partially abated EUP-mediated protection against SEB-induced inflammation reaction and EMT events in hNECs.

Conclusions. Taken together, our findings highlighted the inhibitory role of EUP on the inflammation and EMT processes in CRSwNP *in vivo* and *in vitro* via upregulating TFF1 and inhibiting the Wnt/β-catenin signaling, suggesting EUP could be a promising therapeutic agent for CRSwNP.

Corresponding Author: Yulin Zhao, Department of Rhinology, The First Affiliated Hospital of Zhengzhou University, No.1 East Jianshe Rd, Zhengzhou, 450052, China. e-mail: yulin_zhao88@163.com

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Introduction

Chronic rhinosinusitis (CRS) is a highly prevalent inflammatory disease of the nasal cavity and paranasal sinuses (Zou et al., 2016). Based on the absence or presence of nasal polyps (NPs), CRS can be typically divided into CRS without NPs (CRSsNP) and CRS with NPs (CRSwNP) (Soyka et al., 2012). Even though CRSwNP only accounts for about 30% of CRS cases (Kim et al., 2019), it often causes nasal stuffiness and olfactory dysfunction, thereby consequently leading to a decline in the quality of life (Yilmaz et al., 2020). CRSwNP is characterized by persistent inflammation and tissue remodeling in the nasal mucosa lesions (Tomassen et al., 2016). As a critical process of tissue remodeling, epithelial-mesenchymal transition (EMT) is also deeply implicated in CRSwNP development (Zhou et al., 2020). Furthermore, aberrant EMT is correlated to recurrent polyposis and severity of CRSwNP (Takahashi and Schleimer, 2021). To identify novel therapeutic targets and develop effective therapeutic drugs for CRSwNP treatment, it is necessary to elucidate the underlying molecular mechanisms of EMT and inflammation in CRSwNP.

Trefoil factors (TFFs), a group of highly conserved peptides, are expressed and secreted by epithelial cells in mucous membranes (Thim and May, 2005). It has long been recognized that TFF1, a member of the TFF family, plays a critical role in epithelial surface protection and repair (Kjellev, 2009). So far, TFF1 has been identified as an essential regulator of inflammation reaction in gastric neoplasia (Soutto et al., 2011), gastric mucosal injury (Zhang et al., 2020), diabetic retinopathy (Zhang et al., 2022), and pterygium (Buron et al., 2008). Besides, TFF1 also exerts inhibitory effects on EMT

events in pancreatic intraepithelial neoplasm (Yamaguchi et al., 2018). In addition, studies have proven that TFF1 is downregulated in CRSwNP (Mihalj et al., 2019; Yao et al., 2019). However, its role in CRSwNP has not been well elucidated.

Eupatilin (EUP), an ingredient found in Artemisia Asiatica (Nageen et al., 2020), is of diverse biological activities, including anti-inflammation (Song et al., 2018), anti-oxidation (Liu et al., 2019), anti-apoptosis (Lou et al., 2019), and neuroprotective efficacies (Sapkota et al., 2017). It has been reported that EUP suppresses collagen-induced inflammation in a murine model of arthritis (Kim et al., 2015), ELS-induced production of pro-inflammatory cytokines in BV2 cells (Fei et al., 2019b), and NF-κB-mediated inflammatory reaction in intestinal epithelial cells (Kim et al., 2009). Besides, EUP also inhibits the EMT process in TGF-β2-stimulated retinal pigment epithelial cells (Cinar et al., 2021) and glioma cells (Fei et al., 2019a). Despite the known biological effects of EUP, there have been no reports on its role in CRSwNP.

In this study, we postulated that EUP might ameliorate inflammation and EMT processes in CRSwNP by upregulating TFF1 and inhibiting the Wnt/β-catenin signaling pathway. Herein, the murine and cellular models of CRSwNP were established to validate this hypothesis *in vivo* and *in vitro* through experimental methods.

Materials and methods

CRSwNP modeling and drug treatment

This study was permitted by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University. Briefly, 4-week-old male BALB/c mice acquired from Shanghai Laboratory Animal Center (Shanghai, China) were randomly categorized into Control, CRSwNP, CRSwNP+EUP (5 mg/kg), CRSwNP+EUP (10 mg/kg), and CRSwNP+EUP (15 mg/kg) groups. To establish the CRSwNP model, BALB/c mice were challenged with ovalbumin (OVA) and staphylococcus aureus enterotoxin B (SEB) according to a previous study (Kim et al., 2013). As for EUP treatment, mice received intraperitoneal injection of EUP at different concentrations (5, 10, and 15 mg/kg). 24 hours after all treatment, all mice were sacrificed to collect specimens for the following experiments.

H&E staining

H&E staining was applied for histological analyses. The number of polyp-like lesions (mucosal elevations with inflammatory cell infiltration and microcavity formation) was counted in high-power fields (×400). The epithelium thickness of nasal mucosa (distance between the apices of epithelial cells and the upper border of the subepithelial glands zone) and mucosal thickness were measured via NIS-Elements BR 3.0 system (Nikon Eclipse, Japan).

ELISA

ELISA assay was applied to detect levels of pro-inflammatory cytokines and chemokines (TNF-α, IL-6, and IL-8) in nasal mucosa lysate and cell supernatant from each group with commercial ELISA kits (MyBioSource) as per the manufacturer's instructions.

Western blotting

RIPA lysis buffer was used to extract the total proteins from nasal mucosa and hNECs. Then, the proteins were respectively separated by SDS-PAGE and transferred onto PVDF membranes (Beyotime). Next, PVDF membranes were incubated in the primary antibodies overnight, rinsed with TBST, and cultured with secondary antibody for another 2h. Finally, ECL Western Blotting Substrate Kit (Abcam) was adopted to react with the HRP on the blots. Relative levels of protein bands were analyzed via ImageJ2X software (NCBI).

Cell culture and treatment

Human nasal epithelial cells (hNECs) were acquired from BeNa Culture Collection (Beijing, China) and cultured in airway epithelial cell growth media in a humidified atmosphere (37°C; 5% CO_2).

Briefly, hNECs were divided into Control, SEB, SEB+EUP (10 μM), SEB+EUP (25 μM), SEB+EUP (50 μM), SEB+EUP (50 μM)+si-TFF1, and SEB+EUP (50 μM)+BML-284 (0.1 μM) (Wei et al., 2022). To simulate CRSwNP *in vitro*, hNECs were exposed to SEB (5 μg/mL) for 48 hours (Yoon et al., 2020). For EUP treatment, hNECs were pretreated with at different concentrations (10, 25, 50, 100 μM) (Choi et al., 2011). For BML-284 treatment, treated hNECs were finally treated with BML-284 at 0.1 μM.

Statistical analysis

All experiments were repeated 3 times. The acquired data were expressed as means±SD. All statistical analyses were performed on GraphPad 6.0 software, with comparison evaluated via one-way ANOVA or students' t-test. P-value<0.05 indicates statistical significance.

Results

EUP inhibits polyp formation in CRSwNP mice

To investigate the functional role of EUP in CRSwNP, an *in vivo* model of CRSwNP was established based on BALB/c mice via OVA and SEB challenges. Then, CRSwNP mice were treated with EUP at different concentrations (5, 10, and 15 mg/kg). The histopatho-

logical changes of the mice were evaluated via H&E staining (Fig. 1A). It was found that EUP treatment dramatically reduced the number of polyps in CRSwNP mice (Fig. 1B). CRSwNP mice exhibited greater epithelium thickness of nasal mucosa than Control group, while EUP treatment markedly reduced epithelium thickness of nasal mucosa in a concentration-dependent manner (Fig. 1C). In addition, EUP treatment also abated the increase in mucosal thickness of CRSwNP mice (Fig. 1D). These results suggested EUP treatment suppressed NP formation in CRSwNP mice.

EUP inhibits mucosal inflammation and EMT process in CRSwNP mice

To further explore the effects of EUP on mucosal inflammation and EMT events in CRSwNP mice, pro-inflammatory cytokines and chemokines (TNF-α, IL-6, and IL-8) and EMT-related proteins (N-cadherin, Vimentin, and E-cadherin) were detected in nasal mucosa of mice from each group by means of ELISA and Western blotting assays. It was shown that TNF-α, IL-6, and IL-8 levels were upregulated in CRSwNP mice compared with mice in Control group, which was abated by EUP in a dose-dependent manner (Fig. 2A-C). In addition, CRSwNP mice exhibited a significant increase in N-cadherin and Vimentin levels but an obvious decline in E-cadherin level; however, EUP treatment concertation-dependently reduced N-cadherin and Vimentin levels and increased E-cadherin level (Fig. 2D). Therefore, EUP might mitigate inflammation and EMT process in CRSwNP mice.

EUP inhibits inflammatory reaction and EMT process in SEB-treated hNECs

To explore the underlying molecular mechanism of EUP in treating CRSwNP *in vitro*, a cellular model of CRSwNP was established by exposing hNECs to SEB. Then, SEB-treated hNECs were subject to EUP at different concentrations (10, 25, and 50 μM). As illustrated by ELISA results, EUP treatment substantially abrogated SEB-induced increase of TNF-α, IL-6, and IL-8 levels in hNECs in a dose-dependent manner (Fig. 3A-C). Besides, SEB-triggered changes in N-cadherin, Vimentin, and E-cadherin protein levels in hNECs were also abrogated by EUP (Fig. 3D). Altogether, EUP alleviated SEB-induced inflammation response and EMT event in hNECs.

EUP inhibits inflammation and EMT by upregulating TFF1

Studies have proven that TFF1 is lowly expressed in CRSwNP (Mihalj et al., 2019; Yao et al., 2019). Besides, EUP can also upregulate TFF1 expression (Choi et al., 2009). Therefore, EUP might upregulate TFF1 expression in CRSwNP. To confirm the above hypotheses, Western blotting was employed to determine protein levels of TFF1 in nasal mucosa and hNECs. As

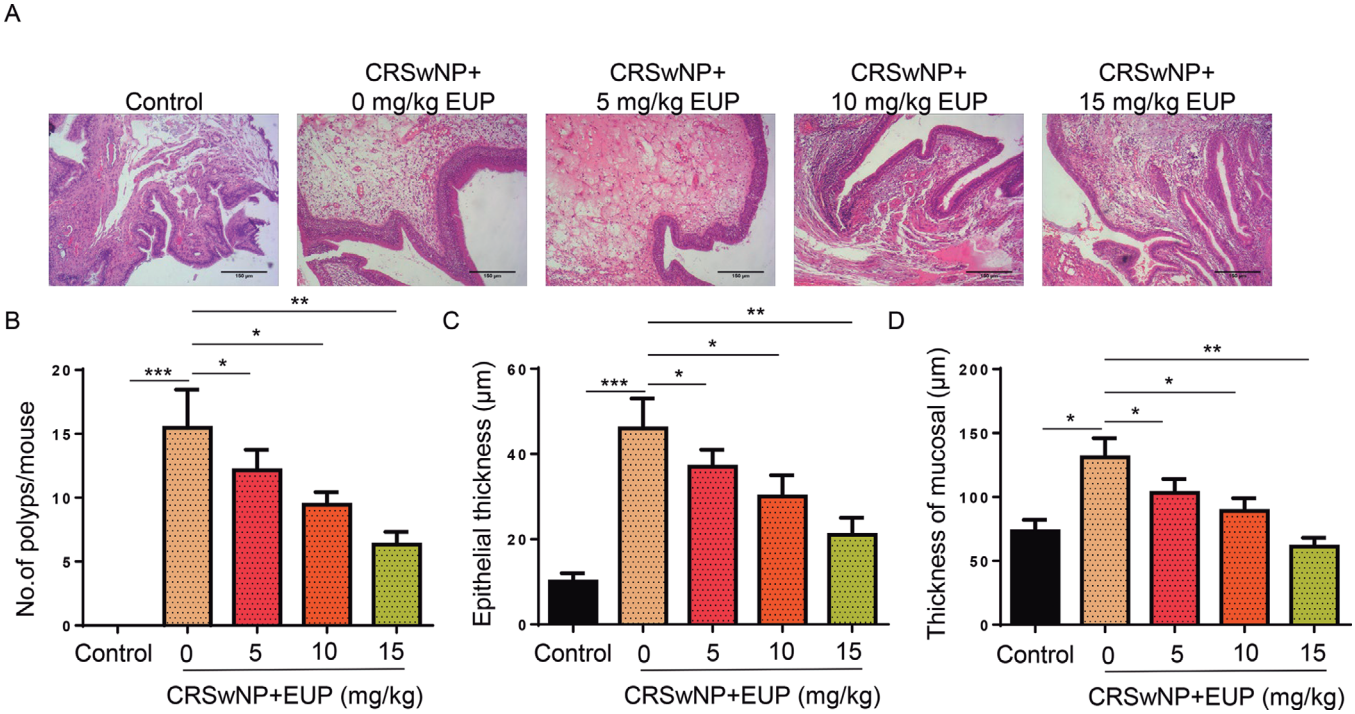

Fig. 1. EUP inhibits polyp formation in CRSwNP mice. Mice were induced with CRSwNP and treated with EUP at different concentrations (5, 10, and 15 mg/kg). **A.** H&E staining of nasal mucosa of mice. **B.** The number of polyps in each group. **C.** Epithelium thickness of nasal mucosa in each group. **D.** Mucosal thickness in each group. *P<0.05; ** P<0.001; ***P<0.005. Scale bar: 150 μm.

shown in Fig. 4A,B, TFF1 expression was down-regulated in CRSwNP mice and SEB-treated hNECs, which was partly abated by EUP treatment in a concentration-dependent manner. To further assess the effects of TFF1 on EUP-mediated inhibition of inflammation and EMT *in vitro*, TFF1 was knocked down in hNECs with si-TFF1. The silencing efficiency was confirmed by Western blotting analysis (Fig. 4C).

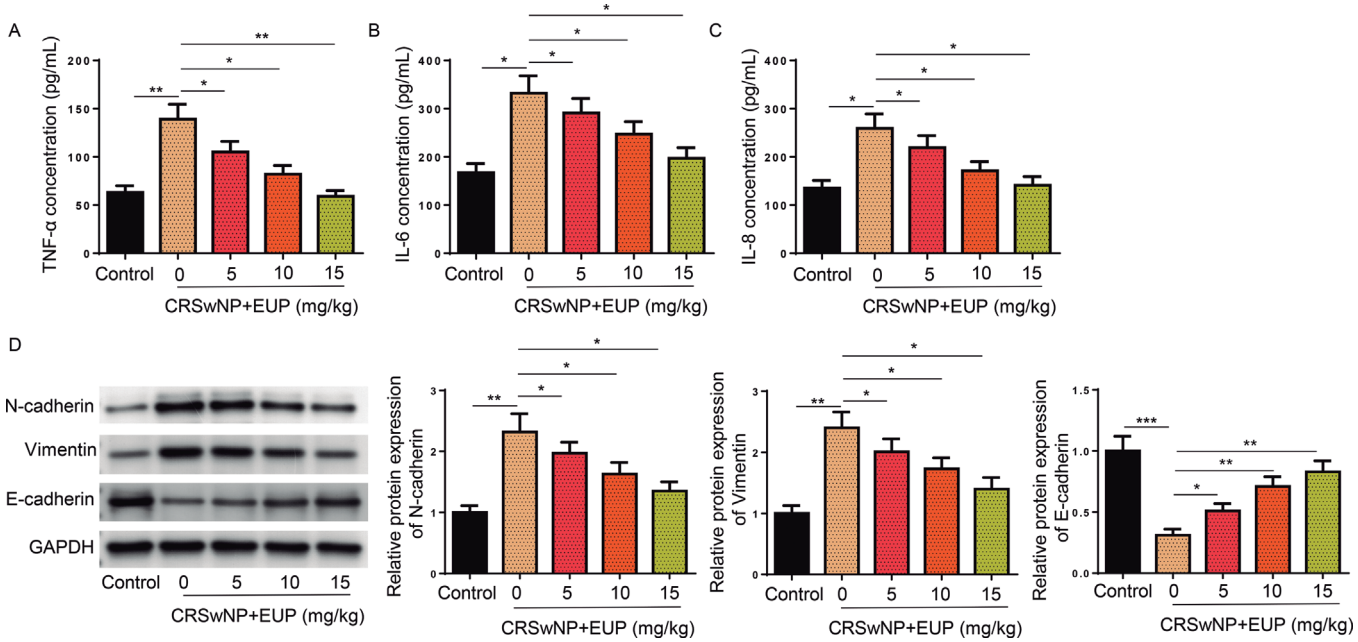

Fig. 2. EUP inhibits mucosal inflammation and EMT process in CRSwNP mice. Mice were induced with CRSwNP and treated with EUP at different concentrations (5, 10, and 15 mg/kg). **A-C.** TNF-α, IL-6, and IL-8 levels in nasal mucosa from each group were assessed by ELISA. **D.** N-cadherin, Vimentin, and E-cadherin protein levels in nasal mucosa from each group were assessed by Western blotting. *P<0.05; ** P<0.001; ***P<0.005.

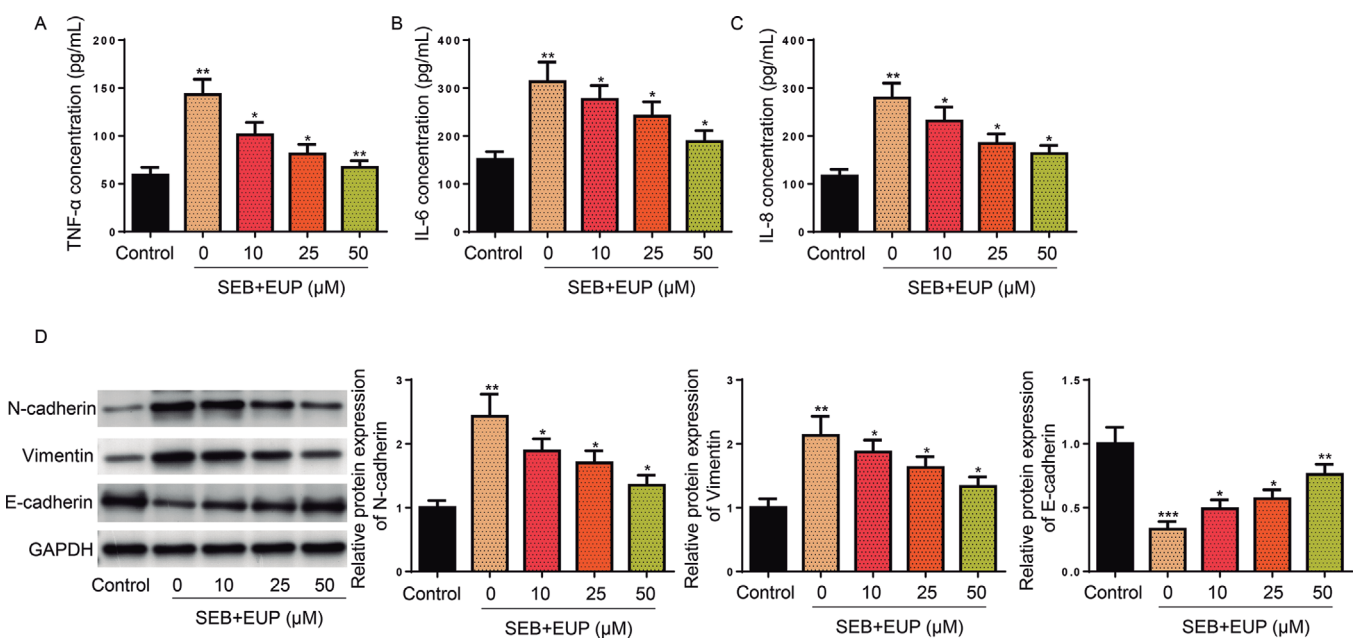

Fig. 3. EUP inhibits inflammatory reaction and EMT process in SEB-treated hNECs. hNECs were stimulated with SEB and treated with EUP at different concentrations (10, 25, and 50 μM). **A-C.** TNF-α, IL-6, and IL-8 levels in hNECs from each group. **D.** N-cadherin, Vimentin, and E-cadherin protein levels in hNECs from each group. *P<0.05; ** P<0.001.

Then, hNECs were divided into Control, SEB, SEB+EUP (50 μM), and SEB+EUP (50 μM)+si-TFF1 groups. It was shown that TFF1 inhibition partly eliminated the inhibitory effects of EUP on TNF-α, IL-6, and IL-8 levels in SEB-challenged hNECs (Fig. 4D-F). In addition, TFF1 knockdown also reversed the impact of EUP on N-cadherin, Vimentin, and E-cadherin protein levels in SEB-challenged hNECs (Fig. 4G). Thus, EUP might impair CRSwNP-related inflammation and EMT processes via upregulation of TFF1 expression.

Activation of Wnt/β-catenin signaling partly reverses the suppressive effects of EUP on inflammation and EMT events

Wnt signaling is upregulated in CRSwNP and is positively related to inflammation and EMT events (Boscke et al., 2017). A former report has demonstrated that TFF1 can deactivate the Wnt/β-catenin signaling (Hasebe et al., 2022). From all the above, it was speculated that TFF1-dependent Wnt/β-catenin may be also involved in EUP-mediated protection against CRSwNP. Consistently, EUP dose-dependently reversed the upregulation of Wnt3α and β-catenin levels in both CRSwNP mice and SEB-treated hNECs (Fig. 5A,B). However, TFF1 silencing evidently abrogated the EUP-caused inhibition on Wnt3α and β-catenin levels in hNECs (Fig. 5C).

To further confirm the above hypothesis, a Wnt agonist, BML-284, was applied (Song et al., 2020). Then, hNECs were divided into Control, SEB, SEB+EUP (50 μM), and SEB+EUP (50 μM)+BML-284 groups. As shown in Fig. 5E,F, BML-284 substantially abolished EUP-triggered inhibition on Wnt/β-catenin activation. In addition, BML-284 remarkably eliminated EUP-induced suppression of inflammation and EMT in SEB-challenged hNECs (Fig. 5D,E). Collectively, Wnt/β-catenin activation might abate the inhibitory effects of EUP on inflammation and EMT processes in CRSwNP.

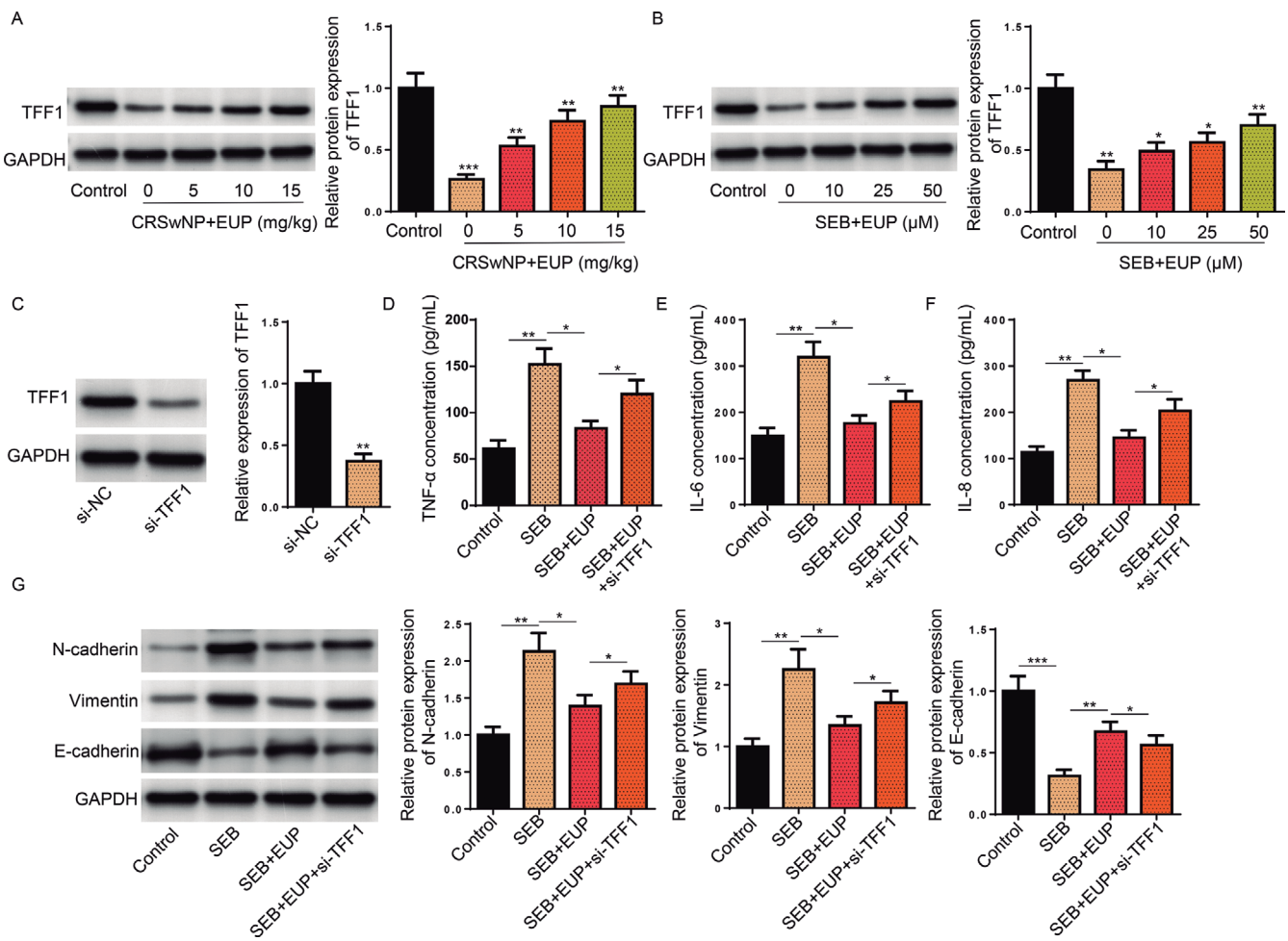

Fig. 4. EUP inhibits inflammation and EMT by upregulating TFF1. **A, B.** TFF1 protein level in nasal mucosa and hNECs from each group. **C.** TFF1 protein level in hNECs transfected with si-NC or si-TFF1. Then, transfected hNECs were stimulated with SEB and treated with EUP. Briefly, hNECs were divided into Control, SEB, SEB+EUP (50 μM), and SEB+EUP (50 μM)+si-TFF1 groups. **D-F.** TNF-α, IL-6, and IL-8 levels in hNECs from each group. **G.** N-cadherin, Vimentin, and E-cadherin protein levels in hNECs from each group. *P<0.05; ** P<0.001; ***P<0.005.

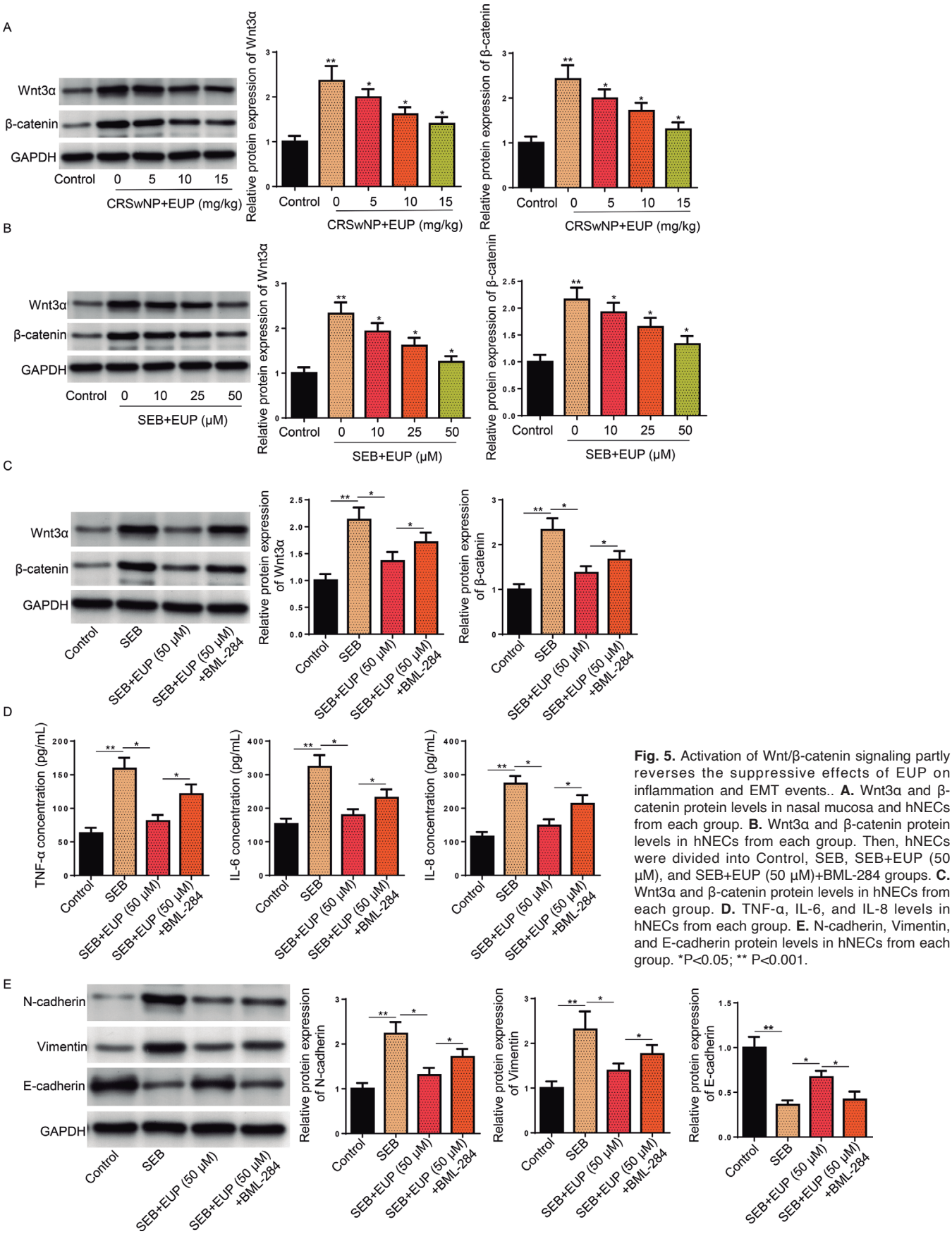

Fig. 5. Activation of Wnt/β-catenin signaling partly reverses the suppressive effects of EUP on inflammation and EMT events.. **A.** Wnt3α and β-catenin protein levels in nasal mucosa and hNECs from each group. **B.** Wnt3α and β-catenin protein levels in hNECs from each group. Then, hNECs were divided into Control, SEB, SEB+EUP (50 μM), and SEB+EUP (50 μM)+BML-284 groups. **C.** Wnt3α and β-catenin protein levels in hNECs from each group. **D.** TNF-α, IL-6, and IL-8 levels in hNECs from each group. **E.** N-cadherin, Vimentin, and E-cadherin protein levels in hNECs from each group. *P<0.05; ** P<0.001.

Discussion

As a refractory disease of the upper airway (Fernandez-Bertolin et al., 2015), CRSwNP is mainly featured with inflammation and dysregulated EMT (Bae et al., 2020). Clinically, CRSwNP often causes symptoms such as nasal discharge, stuffiness, and olfactory dysfunction, which remarkably affects the quality of life of CRSwNP patients (Yilmaz et al., 2020). Therefore, it is extremely important to discover novel therapeutic agents for CRSwNP treatment.

The anti-inflammatory and anti-EMT properties of EUP have been identified in previous studies. It was reported that EUP inhibited inflammatory responses to hepatic ischemia-reperfusion injury (Lee et al., 2016), DSS-induced colitis (Zhou et al., 2018), and bronchial epithelial inflammation (Jung et al., 2012). In addition, EUP also suppressed EMT events in prostate cancer cells (Serttas et al., 2021), glioma cells (Fei et al., 2019a) and TGF-β2-challenged retinal pigment epithelial cells (Cinar et al., 2021). However, the exact effects of EUP in CRSwNP remain to be elucidated. In this study, EUP significantly attenuated the number of nasal polyps, epithelium thickness of nasal mucosa, and mucosal thickness in CRSwNP mice in a concentration-dependent manner. Consistent with previous studies, EUP dose-dependently ameliorated inflammation and EMT process in CRSwNP mice and SEB-treated hNECs. Taken together, EUP alleviated CRSwNP severity, inflammatory response and EMT *in vitro* and *in vivo* in a dose-dependent manner.

TFF1, a member of the TFFs, has been found to protect mucosa and promote epithelial healing (Liang et al., 2022). Besides, TFF1 also exerts suppressive effects on inflammation and/or EMT in gastric mucosal injury (Zhang et al., 2020), pterygium (Buron et al., 2008), diabetic retinopathy (Zhang et al., 2022), and pancreatic intraepithelial neoplasm (Yamaguchi et al., 2018). Additionally, it has been demonstrated that TFF1 is downregulated in CRSwNP (Mihalj et al., 2019; Yao et al., 2019). Besides, TFF1 expression can be upregulated by EUP (Choi et al., 2009). Similarly, we observed significant TFF1 downregulation in CRSwNP mice and SEB-treated hNECs. It was also shown that TFF1 expression in CRSwNP mice and SEB-treated hNECs was upregulated with the increase of EUP concentration. In addition, TFF1 inhibition substantially abrogated the inhibitory effects of EUP on SEB-induced inflammation and EMT in hNECs. Our results implied that EUP diminished inflammation and EMT in CRSwNP by upregulating TFF1.

A previous study has reported that TFF1 can inhibit the activation of the Wnt/β-catenin signaling pathway (Hasebe et al., 2022). The Wnt signaling pathway is abnormally activated and positively associated with inflammation and EMT events in CRSwNP (Boscke et al., 2017). Therefore, it was conjectured that TFF1 upregulation by EUP might ameliorate inflammation and EMT in CRSwNP via inactivating the Wnt/β-catenin signaling. Our results showed that EUP treatment dose-dependently abated the increase of Wnt3α and β-catenin levels in CRSwNP mice and SEB-treated hNECs; however, TFF1 inhibition considerably reversed EUP-induced deactivation of Wnt/β-catenin signaling in hNECs. Furthermore, it was also illustrated that BML-284, a Wnt activator, partially abolished EUP-induced suppression on the inflammation and EMT of SEB-challenged hNECs.

Conclusion

To sum up, our study is the first to elucidate the inhibitory effects of EUP on the inflammation and EMT processes in CRSwNP. Based on murine and cellular CRSwNP models, our findings demonstrated that EUP ameliorated inflammation and EMT processes in CRSwNP by upregulating TFF1 and inhibiting the Wnt/β-catenin signaling pathway *in vivo* and *in vitro* (Fig. 6), suggesting EUP may be a potential therapeutic agent for CRSwNP patients.

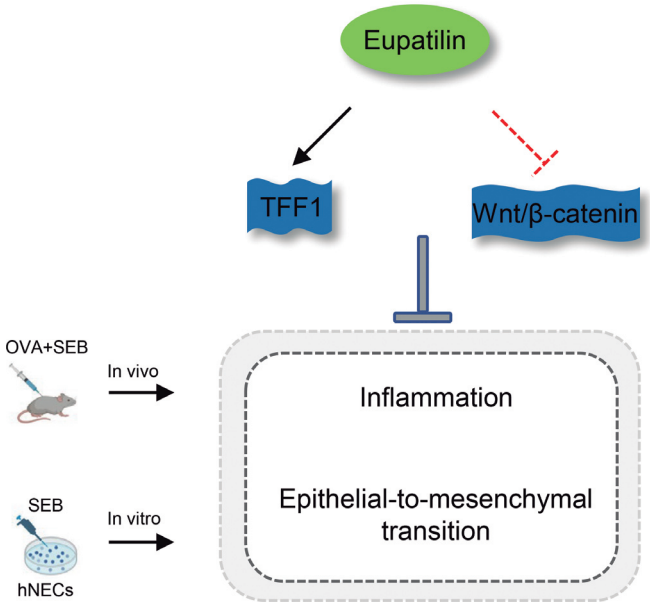

Fig. 6. Graphical abstract.

Ethical Approval. This study was permitted by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University.

Competing interests. The authors declare that they have no competing interests.

Authors' contributions. HS and QW designed this study, performed all the experiments and prepared the figures. YZ and QW drafted the initial manuscript and analyzed the data. QW reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

Bae J.S., Ryu G., Kim J.H., Kim E.H., Rhee Y.H., Chung Y.J., Kim D.W., Lim S., Chung P.S., Shin H.W. and Mo J.H. (2020). Effects of wnt signaling on epithelial to mesenchymal transition in chronic rhinosinusitis with nasal polyp. Thorax 75, 982-993.

Boscke R., Vladar E.K., Konnecke M., Husing B., Linke R., Pries R., Reiling N., Axelrod J.D., Nayak J.V. and Wollenberg B. (2017). Wnt signaling in chronic rhinosinusitis with nasal polyps. Am. J. Respir. Cell Mol. Biol. 56, 575-584.

Buron N., Guery L., Creuzot-Garcher C., Lafontaine P.O., Bron A., Rio M.C. and Solary E. (2008). Trefoil factor TFF1-induced protection of conjunctival cells from apoptosis at premitochondrial and postmitochondrial levels. Invest. Ophthalmol. Vis. Sci. 49, 3790-3798.

Choi E.J., Oh H.M., Wee H., Choi C.S., Choi S.C., Kim K.H., Han W.C., Oh T.Y., Kim S.H. and Jun C.D. (2009). Eupatilin exhibits a novel anti-tumor activity through the induction of cell cycle arrest and differentiation of gastric carcinoma AGS cells. Differentiation 77, 412-423.

Choi E.J., Lee S., Chae J.R., Lee H.S., Jun C.D. and Kim S.H. (2011). Eupatilin inhibits lipopolysaccharide-induced expression of inflammatory mediators in macrophages. Life Sci. 88, 1121-1126.

Cinar A.K., Ozal S.A., Serttas R. and Erdogan S. (2021). Eupatilin attenuates TGF-β2-induced proliferation and epithelial-mesenchymal transition of retinal pigment epithelial cells. Cutan. Ocul. Toxicol. 40, 103-114.

Fei X., Wang J., Chen C., Ding B., Fu X., Chen W., Wang C. and Xu R. (2019a). Eupatilin inhibits glioma proliferation, migration, and invasion by arresting cell cycle at G1/S phase and disrupting the cytoskeletal structure. Cancer Manag. Res. 11, 4781-4796.

Fei X., Chen C., Kai S., Fu X., Man W., Ding B., Wang C. and Xu R. (2019b). Eupatilin attenuates the inflammatory response induced by intracerebral hemorrhage through the TLR4/MyD88 pathway. Int. Immunopharmacol. 76, 105837.

Fernandez-Bertolin L., Mullol J., Fuentes-Prado M., Roca-Ferrer J., Alobid I., Picado C. and Pujols L. (2015). Effect of lipopolysaccharide on glucocorticoid receptor function in control nasal mucosa fibroblasts and in fibroblasts from patients with chronic rhinosinusitis with nasal polyps and asthma. PLoS One 10, e0125443.

Hasebe K., Yamazaki K., Yamaguchi J., Kokuryo T., Yokoyama Y., Miyata K., Fukaya M., Nagino M. and Ebata T. (2022). Trefoil factor 1 inhibits the development of esophageal adenocarcinoma from barrett's epithelium. Lab. Invest. 102, 885-895.

Jung J., Ko S.H., Yoo do Y., Lee J.Y., Kim Y.J., Choi S.M., Kang K.K., Yoon H.J., Kim H., Youn J. and Kim J.M. (2012). 5,7-dihydroxy-3,4,6-trimethoxyflavone inhibits intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 via the Akt and nuclear factor-κB-dependent pathway, leading to suppression of adhesion of monocytes and eosinophils to bronchial epithelial cells. Immunology 137, 98-113.

Kim J.M., Lee D.H., Kim J.S., Lee J.Y., Park H.G., Kim Y.J., Oh Y.K., Jung H.C. and Kim S.I. (2009). 5,7-dihydroxy-3,4,6-trimethoxy-flavone inhibits the inflammatory effects induced by bacteroides fragilis enterotoxin via dissociating the complex of heat shock protein 90 and i kappab alpha and i kappab kinase-gamma in intestinal epithelial cell culture. Clin. Exp. Immunol. 155, 541-551.

Kim S.W., Kim D.W., Khalmuratova R., Kim J.H., Jung M.H., Chang D.Y., Shin E.C., Lee H.K., Shin H.W., Rhee C.S., Jeon S.Y. and Min Y.G. (2013). Resveratrol prevents development of eosinophilic rhinosinusitis with nasal polyps in a mouse model. Allergy 68, 862-869.

Kim J., Kim Y., Yi H., Jung H., Rim Y.A., Park N., Jung S.M., Park S.H. and Ju J.H. (2015). Eupatilin ameliorates collagen induced arthritis. J. Korean Med. Sci. 30, 233-239.

Kim D.K., Eun K.M., Kim M.K., Cho D., Han S.A., Han S.Y., Seo Y., Lee D.H., Cho S.H. and Kim D.W. (2019). Comparison between signature cytokines of nasal tissues in subtypes of chronic rhinosinusitis. Allergy Asthma Immunol. Res. 11, 201-211.

Kjellev S. (2009). The trefoil factor family - small peptides with multiple functionalities. Cell. Mol. Life Sci. 66, 1350-1369.

Lee H.M., Jang H.J., Kim S.S., Kim H.J., Lee S.Y., Oh M.Y., Kwan H.C., Jang D.S. and Eom D.W. (2016). Protective effect of eupatilin pretreatment against hepatic ischemia-reperfusion injury in mice. Transplant. Proc. 48, 1226-1233.

Liang T., Zhao P., Zhang X., Han X., Hong B., Kong L., Chang H. and Liu L. (2022). FOXA1 transcription activates TFF1 to reduce 6-OHDA-induced dopaminergic neuron damage. Exp. Ther. Med. 23, 372.

Liu H., Hao J., Wu C., Liu G., Wang X., Yu J., Liu Y. and Zhao H. (2019). Eupatilin alleviates lipopolysaccharide-induced acute lung injury by inhibiting inflammation and oxidative stress. Med. Sci. Monit. 25, 8289-8296.

Lou Y., Wu J., Liang J., Yang C., Wang K., Wang J. and Guo X. (2019). Eupatilin protects chondrocytes from apoptosis via activating sestrin2-dependent autophagy. Int. Immunopharmacol. 75, 105748.

Mihalj M., Bujak M., Butkovic J., Zubcic Z., Tolusic Levak M., Ces J., Kopic V., Baus Loncar M. and Mihalj H. (2019). Differential expression of TFF1 and TFF3 in patients suffering from chronic rhinosinusitis with nasal polyposis. Int. J. Mol. Sci. 20, 5461.

Nageen B., Sarfraz I., Rasul A., Hussain G., Rukhsar F., Irshad S., Riaz A., Selamoglu Z. and Ali M. (2020). Eupatilin: A natural pharmacologically active flavone compound with its wide range applications. J. Asian Nat. Prod. Res. 22, 1-16.

Sapkota A., Gaire B.P., Cho K.S., Jeon S.J., Kwon O.W., Jang D.S., Kim S.Y., Ryu J.H. and Choi J.W. (2017). Eupatilin exerts neuroprotective effects in mice with transient focal cerebral ischemia by reducing microglial activation. PLoS One 12, e0171479.

Serttas R., Koroglu C. and Erdogan S. (2021). Eupatilin inhibits the proliferation and migration of prostate cancer cells through modulation of PTEN and NF-κB signaling. Anticancer Agents Med. Chem. 21, 372-382.

Song E.H., Chung K.S., Kang Y.M., Lee J.H., Lee M. and An H.J. (2018). Eupatilin suppresses the allergic inflammatory response *in vitro* and *in vivo*. Phytomedicine 42, 1-8.

Song J., Zhu X.M. and Wei Q.Y. (2020). MSCs reduce airway remodeling in the lungs of asthmatic rats through the Wnt/β-catenin signaling pathway. Eur. Rev. Med. Pharmacol. Sci. 24, 11199-11211.

Soutto M., Belkhiri A., Piazuelo M.B., Schneider B.G., Peng D., Jiang A., Washington M.K., Kokoye Y., Crowe S.E., Zaika A., Correa P., Peek R.M., Jr. and El-Rifai W. (2011). Loss of TFF1 is associated with activation of NF-κB-mediated inflammation and gastric neoplasia in mice and humans. J. Clin. Invest. 121, 1753-1767.

Soyka M.B., Wawrzyniak P., Eiwegger T., Holzmann D., Treis A., Wanke K., Kast J.I. and Akdis C.A. (2012). Defective epithelial barrier in chronic rhinosinusitis: The regulation of tight junctions by

IFN-γ and IL-4. J. Allergy Clin. Immunol. 130, 1087-1096.e10.

Takahashi T. and Schleimer R.P. (2021). Epithelial-cell-derived extracellular vesicles in pathophysiology of epithelial injury and repair in chronic rhinosinusitis: Connecting immunology in research lab to biomarkers in clinics. Int. J. Mol. Sci. 22, 11709.

Thim L. and May F.E. (2005). Structure of mammalian trefoil factors and functional insights. Cell. Mol. Life Sci. 62, 2956-2973.

Tomassen P., Vandeplas G., Van Zele T., Cardell L.O., Arebro J., Olze H., Forster-Ruhrmann U., Kowalski M.L., Olszewska-Ziaber A., Holtappels G., De Ruyck N., Wang X., Van Drunen C., Mullol J., Hellings P., Hox V., Toskala E., Scadding G., Lund V., Zhang L., Fokkens W. and Bachert C. (2016). Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers. J. Allergy Clin. Immunol. 137, 1449-1456.e4.

Wei J., Zheng X., Li W., Li X. and Fu Z. (2022). Sestrin2 reduces cancer stemness via Wnt/β-catenin signaling in colorectal cancer. Cancer Cell Int. 22, 75.

Yamaguchi J., Yokoyama Y., Kokuryo T., Ebata T., Enomoto A. and Nagino M. (2018). Trefoil factor 1 inhibits epithelial-mesenchymal transition of pancreatic intraepithelial neoplasm. J. Clin. Invest. 128, 3619-3629.

Yao Y., Xie S. and Wang F. (2019). Identification of key genes and pathways in chronic rhinosinusitis with nasal polyps using bioinformatics analysis. Am. J. Otolaryngol. 40, 191-196.

Yilmaz I., Turk M., Nazik Bahcecioglu S., Tutar N. and Gulmez I. (2020). Efficacy of mepolizumab treatment in oral corticosteroid-dependent severe eosinophilic asthma patients with chronic rhinosinusitis with nasal polyps: Single center, real life study. Turk. J. Med. Sci. 50, 433-441.

Yoon Y.H., Yeon S.H., Choi M.R., Jang Y.S., Kim J.A., Oh H.W., Jun X., Park S.K., Heo J.Y., Rha K.S. and Kim Y.M. (2020). Altered mitochondrial functions and morphologies in epithelial cells are associated with pathogenesis of chronic rhinosinusitis with nasal polyps. Allergy Asthma Immunol. Res. 12, 653-668.

Zhang Y., Xu H., He H., Li X., Feng M., He Y., Jiang W., Wang J., Xu D. and Zou K. (2020). Total triterpenes from the fruits of chaenomeles speciosa (sweet) nakai protects against indomethacin-induced gastric mucosal injury: Involvement of TFF1-mediated EGF/EGFR and apoptotic pathways. J. Pharm. Pharmacol. 72, 409-423.

Zhang W., Zhang D., Cheng Y., Liang X. and Wang J. (2022). Runx1 regulates Tff1 expression to expedite viability of retinal microvascular endothelial cells in mice with diabetic retinopathy. Exp. Eye Res. 217, 108969.

Zhou K., Cheng R., Liu B., Wang L., Xie H. and Zhang C. (2018). Eupatilin ameliorates dextran sulphate sodium-induced colitis in mice partly through promoting AMPK activation. Phytomedicine 46, 46-56.

Zhou X., Cui Z., Liu Y., Yue Z., Xie F., Ding L., Xu S., Han J. and Zhang H. (2020). Correlation of bromodomain protein BRD4 expression with epithelial-mesenchymal transition and disease severity in chronic rhinosinusitis with nasal polyps. Front. Med. (Lausanne) 7, 413.

Zou Y., Wang Y., Wang S.B., Kong Y.G., Xu Y.U., Tao Z.Z. and Chen S.M. (2016). Characteristic expression and significance of CCL19 in different tissue types in chronic rhinosinusitis. Exp. Ther. Med. 11, 140-146.

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