International Archives of Allergy and Immunology

Experimental Allergy – Review Article

Int Arch Allergy Immunol 2022;183:1029–1039
DOI: 10.1159/000524950

Received: January 30, 2022
Accepted: May 3, 2022
Published online: June 23, 2022

The Role of Epithelial-Mesenchymal Transition in Chronic Rhinosinusitis

Yifan Xia[a] Hongtian Wang[b] Jinshu Yin[a]

[a]Department of Otolaryngology/Head and Neck Surgery, Ninth School of Clinical Medicine, Peking University, Beijing, China; [b]Department of Allergy, Capital Medical University Affiliated Beijing Shijitan Hospital, Beijing, China

Keywords
Chronic rhinosinusitis · Epithelial-mesenchymal transition · Transforming growth factor-β signaling · Wnt signaling · Hypoxia-inducible factor-1α signaling

Abstract
Background: Chronic rhinosinusitis (CRS) is a common condition in otorhinolaryngology. It is characterized by chronic inflammation of the nasal cavity and the sinus mucosa. However, its specific pathogenesis remains unclear. Epithelial dysfunction is closely related to inflammatory airway diseases. Various evidences support that epithelial-mesenchymal transition (EMT) plays a key role in the development of CRS. ***Objective:*** The study aimed to explore our understanding of how EMT contributes to the pathogenesis of CRS and to examine the role of several signaling pathways in EMT. ***Methods:*** PubMed database was used to review the literature related to EMT in CRS pathogenesis. The following key words were used for the search strategy: CRS, sinusitis, nasal polyps, epithelial cells, EMT, dysfunction, cytokines, signaling pathways, pathogenesis, and therapy. ***Results:*** EMT is widely present in the nasal mucosa of CRSwNP patients and contributes to the pathogenesis of the disease. However, there is no sufficient evidence for the existence of EMT in CRSsNP. Multiple signaling pathways and molecules, such as transforming growth factor-β signaling, Wnt signaling, and hypoxia-inducible factor-1α signaling, have been found to be involved in the EMT process and promote CRSwNP. ***Conclusion:*** EMT is closely associated with CRS pathogenesis. Our study supports further research on epithelial EMT changes in CRS patients and provides a basis for revealing its pathogenesis and exploring new treatments.

Introduction

Chronic rhinosinusitis (CRS) is a chronic inflammatory disease involving the nasal cavity and sinus mucosa, with an onset time of more than 12 weeks. Based on the presence or absence of polyps, CRS is divided into two types: CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP). The former accounted for the total one-third number of patients, and the latter accounted for two-thirds. CRSsNP is generally associated with type 1 inflammation, which is manifested by increased expression of IFN-γ and decreased expression of cytokines such as IL-5. Type 2 cytokines including IL-4,

Edited by: H.-U. Simon, Bern.

Karger@karger.com
www.karger.com/iaa

Correspondence to:
Hongtian Wang, wht301@263.net
Jinshu Yin, yinjinshu55@aliyun.com

Karger

Table 1. The difference between CRSsNP and CRSwNP

	CRSsNP	Eosinophilic CRSwNP	Noneosinophilic CRSwNP
Pathological characteristics	Epithelial mucosal fibrosis, basement membrane thickening, goblet cell proliferation, and subepithelial edema	Nasal polyps, basement membrane edema, albumin deposition, and pseudocyst formation	
Predominant infiltrating cell	Monocyte	Eosinophil	Neutrophil
Main symptoms	Facial pain or pressure, nasal discharge	Dysosmia	Nasal obstruction, nasal discharge

IL-5, and IL-13 produced by Th2 cells, ILC2s, and mast cells play important roles in CRSwNP.

The etiology and pathogenesis of CRS are complex. Relevant studies have shown that the pathogenesis of CRS is related to nasal anatomy, bacterial and fungal colonization, epithelial defense impairment, tissue remodeling, immune dysfunction, and other factors, which lead to functional defects of the nasal mucosa as a physical and immune barrier. Tissue remodeling in CRS results from long-term inflammatory stimulation. This transformation is associated with sustained damage and repair. During injury repair, some differences occur in tissue remodeling between the two subtypes of CRS.

CRSsNPs exhibit epithelial mucosal fibrosis, basement membrane thickening, goblet cell hyperplasia, subepithelial edema, collagen deposition, mucous gland hyperplasia, squamous metaplasia, and monocyte infiltration. CRSwNP is typically characterized by polypogenesis, severe basement membrane edema, albumin deposition, pseudocyst formation, and subepithelial and perivascular inflammatory cell infiltration. The type of inflammatory cell infiltration varies among species and regions. In Western countries, this manifested as Th2-mediated eosinophil inflammation, with marked eosinophil infiltration. In East Asia, approximately 50% of patients present with noneosinophilic inflammation, with neutrophil infiltration; edema is also milder than the former.

Different tissue remodeling patterns lead to different clinical features. CRSwNP is mainly characterized by nasal polyps, nasal obstruction, and dysosmia as the principle symptom, whereas CRSsNP manifests with edema and exudation, as well as facial pain or pressure and nasal discharge (shown in Table 1) [1]. The characteristics of different tissue remodeling have clear clinical guiding significance for the treatment of CRS. The specific mechanism of tissue remodeling is still not fully understood. At present, an increasing number of scholars have focused on epithelial-mesenchymal transition (EMT).

The Process of EMT

EMT is the transformation of polar epithelial cells into mesenchymal phenotypes under specific physiological and pathological conditions. The epithelium is a physical and immune barrier that maintains a balance between water and electrolytes. It also removes, degrades, or neutralizes environmental toxins and particles. Epithelial cells are essential for innate immune resistance to pathogens. In some cases, they activate pattern recognition receptors to produce protective enzymes, peptides, proteins, lipids, and ions, thus making the mucosal epithelium an effective barrier against microbial invasion [2]. Barrier dysfunction can be caused by tight junction protein defects, reduced protective antiproteases, dysregulation of the electrolyte and water transport systems, and other mechanisms. Several allergic diseases of the skin, lungs, and gastrointestinal tract are associated with disturbances in the epithelial barrier [3].

The nasal epithelium is mainly composed of ciliated epithelium, goblet cells, and basal airway cells. Ciliated epithelial cells are the main cell type in the airways. Goblet cells secrete mucin primarily on the inner surface of the airway to capture molecules in the environment. Muc5AC and Muc5B are the major mucin proteins in the airways [4]. In a healthy state, mucin production and clearance maintain dynamic balance. Hyper-differentiation of goblet cells driven by IL-4 and IL-13 disrupts the balance between Muc5AC and Muc5B; it is a phenomenon associated with asthma, AR, and CRS [5, 6]. Basal airway cells

are stem cell-like progenitors found in the upper and lower airways. Appropriate regulation of Notch signaling can produce ciliated mucus-secreting goblet cells or other specialized epithelial cells. Inhibition of Notch signaling promotes the fate of ciliated cells, whereas high levels of Notch promote differentiation into mucus-secreting goblet cells [7, 8]. Basal cells are closely attached to adjacent epithelial cells and anchored to the basement membrane by semidesmosomes [9]. In the steady state, the basal cells are relatively static. When the epithelium is damaged, basal cells are rapidly activated and temporarily leave the basement membrane to migrate to epithelial cells, thus forming a temporary barrier [10].

Epithelial cells are normally layered, tubular, or vesiculate, with the top exposed in the lumen as well as on the base of the basal membrane. Epithelial cells are closely bound to each other by special intercellular junctions (tight junctions, adherent junctions, desmosomes) to form apical-basal polarities [11].

Tight junction disintegration is an early event of EMT that results in the redistribution of occludin, claudin, and zonula occludens, along with the disruption of cell polarity and reorganization of the cytoskeleton [12]. EMT occurs when adhesion disintegrates, thereby resulting in the loss of its characteristic component, E-cadherin. The loss of E-cadherin expression is a basic event of EMT that triggers EMT [13]. With the breakdown of epithelial junctions, epithelial cells acquire mesenchymal characteristics. Consequently, increased expression levels of N-cadherin, vimentin, α-smooth muscle actin (α-SMA), collagen, fibronectin, and other extracellular matrix proteins were detected. During EMT, epithelial cells migrate in a directional manner due to the loss of intercellular junction structure and apical-to-basal polarity [12, 14]. During EMT, the expression and activity of extracellular proteases such as matrix metalloproteinase 9 (MMP-9) are increased, which leads to the degradation of extracellular matrix proteins. Thus, the behavior of cells is transformed from migration to invasion [15].

According to the biological characteristics and biomarkers of EMT, EMT can be divided into three types: type 1 is related to embryogenesis and organ development; type 2 is associated with tissue damage and repair, organ fibrosis, and chronic inflammation; and type 3 is associated with the aggressive phenotype of malignant tumor cells. Mesenchymal-epithelial transition (MET) is a reversal of EMT that plays an important role in cellular development, induced pluripotent stem cell reprogramming, and tumor metastasis [16]. Cancer cells at metastatic sites are likely to regain their epithelial characteristics and develop MET. Epithelial plasticity enables cells to undergo multiple rounds of EMT and MET transition [17, 18] (shown in Fig. 1). EMT of nasal epithelial cells indicates aggravation of epithelial injury to some extent. If it can delay the process of EMT or MET, then promoting the repair of epithelial cells will be of great significance for EMT-related diseases.

EMT and CRS

Defects in the nasal epithelium of CRSwNP patients may be associated with elevated Th2 cytokine levels. In CRS, airway epithelial cells are stimulated by Th2 cytokines, such as IL-4 and IL-13. Additionally, Notch signaling is activated, thereby resulting in excessive goblet cell differentiation, which affects the balance between Muc5AC and Muc5B. The basal cells are then rapidly activated, thus migrating to the epithelial cells and forming a temporary barrier. Furthermore, IL-4 and IL-13 are key factors that control polyp formation [19]. Treatment with an anti-IL-4Rα antibody (dupilumab), which inhibits both IL-4 and IL-13 signaling, can reduce nasal polyp sizes as well as improve the quality of life and symptoms in patients with severe CRSwNP [20]. The in vitro stimulation of respiratory epithelial cells with cytokines, either IL-4 or IFN-γ, reportedly results in decreased epithelial barrier function [21]. In nasal polyp tissues, the proinflammatory factor, IL-6, which is a family of tumor suppressor M (OSM), is significantly elevated. OSM can disrupt the tight junctions of epithelial cells and increase their permeability, thus leading to epithelial barrier dysfunction [20]. The epithelial barrier plays an important role in CRSwNP pathogenesis.

Further proteomic analysis of nasal mucus from CRS patients revealed elevated levels of biological processes associated with EMT [22]. Compared with the normal control group, the expression of E-cadherin in the nasal mucosal epithelium of CRSwNP patients was decreased, while the expression of transforming growth factor-β1 (TGF-β1), α-SMA, fibrin, and vimentin was upregulated [23]. Additionally, α-SMA protein levels were negatively correlated with endoscopic scores and some postoperative symptoms [24]. In CRSwNP patients, the expression of occludin and occludens proteins in the nasal mucosal epithelium was decreased, which presents as irregular patches [21]. The study found that the EMT degree of early polyps was more active and significant than that of mature nasal polyps, thus indicating that early polyps have a more significant EMT dynamic process and continuous repair

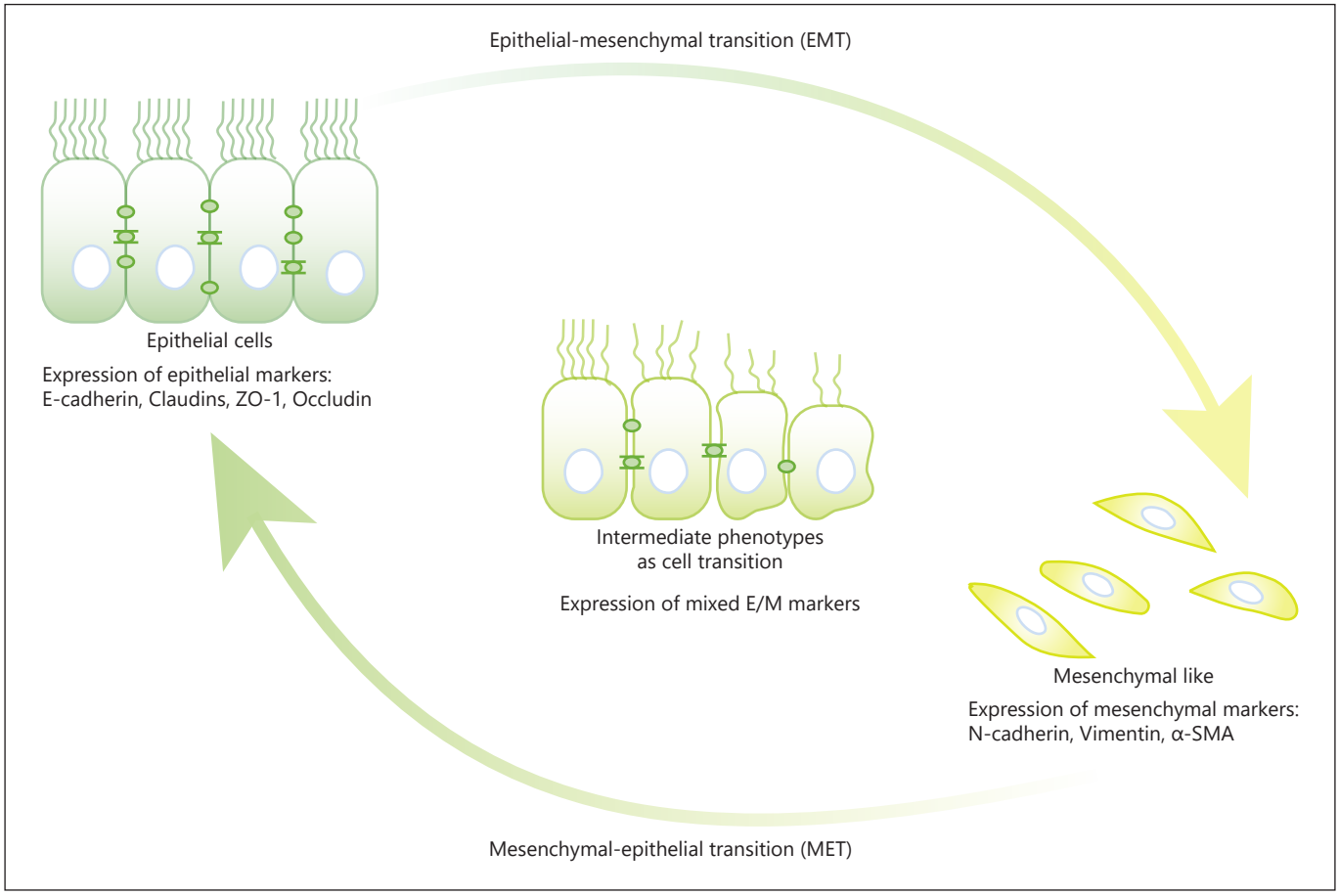

Fig. 1. The putative EMT and MET cycle. Under physiological or pathological conditions, epithelial cells can be transformed into cells with a mesenchymal phenotype. Epithelial cells lose epithelial markers like E-cadherin, claudins, occludin, and ZO-1 and gain mesenchymal markers like N-cadherin, vimentin, α-SMA. And cells undergo an intermediate transition state, express mixed epithelial and mesenchymal markers. Under certain conditions, these primary mesenchymal cells can be reinduced to form secondary epithelia by a MET.

[25]. As with many chronic lung diseases, including asthma, chronic obstructive pulmonary disease, cystic fibrosis, idiopathic pulmonary fibrosis, and bronchiolitis, CRS is closely associated with type 2 EMT [26].

At present, most studies on the correlation between EMT and CRS are animal models or in vitro studies on cell culture and tissue biopsy. We found that current studies related to EMT are limited to CRSwNP. There is sufficient evidence that EMT plays an important role in nasal polyp tissue remodeling. However, the correlation between CRSsNP and EMT needs to be investigated. Ensuring the source of CRSsNP specimens and constructing an effective and reasonable CRSsNP in vitro model are the primary issues to be solved in this study.

Transcription Factors That Drive EMT in CRS

Altered gene expression is involved in the inhibition of epithelial phenotypes and the activation of mesenchymal phenotypes. The major transcription regulators include the SNAIL family (Snail1, Snail2/slug, and Snail3), TWIST family (Twist1 and Twist2), and zinc-finger E-box-binding (ZEB1 and ZEB2).

SNAIL Transcription Factor

The SNAIL family is a family of transcription factors that have been discovered to play an important role in tissue development, fibrosis, and tumorigenesis [27]. Snail and Slug levels in the nasal polyps of CRSwNP patients were significantly higher than those in patients with

healthy inferior turbinates. Nasal epithelial cells (NECs) and nasal polyp cells from the inferior turbinate of the same patient were treated with TGF-β. There were no significant differences in the mesenchymal EMT markers between the two tissues. The expression of Twist and fibronectin-1 increased rapidly after 12 h and decreased after 24 h in NECs of interior turbinates; in the nasal polyps, N-cadherin and Snail increased after 24 h. This suggests a different regulation of these markers in nasal polyps and inferior turbinates of CRS patients [28]. It was found that glucocorticoids inhibited TGF-β1-induced Snail and Slug expression; inhibiting Snail and Slug also improved TGF-β1-induced EMT process [29].

TWIST Transcription Factor

Similar to Snail, Twist overexpression downregulates epithelial gene expression and activates mesenchymal gene expression. The expression of Twist in the nasal polyps of CRSwNP patients was significantly higher than that of healthy inferior turbinates [28]. Elevated Twist expression has also been detected in a mouse model of nasal polyps [30]. The expression of E-cadherin decreased, but that of vimentin, fibronectin, α-SMA, Snail, and Slug increased after treatment with TGF-β1 [31]. The expression levels of vimentin and Twist are also elevated in hypoxia-induced EMT [32].

ZEB Transcription Factor

The ZEB family comprises two members: ZEB1 and ZEB2. Both trigger EMT through a combination of repression of epithelial cells and activation of mesenchymal proteins [33–36]. ZEB proteins act as powerful modulators of EMT by inhibiting multiple cell junction type proteins and cultivating mesenchymal properties. Snail and ZEB appeared together in the abnormal samples. Snail can activate but not completely affect ZEB [37]. ZEB usually binds to a specific structure in the promoter region of the epithelial cadherin gene to initiate EMT [38]. Diesel exhaust particle (DEP) and house dust mite co-exposure synergistically increased the number of nasal polyps, epithelial destruction, and ZEB2 expression. Inhibition of ZEB mitigated particulate induced EMT and reduced nasal polyps in mice [39].

EMT-Related Signaling Pathways in CRS

An increasing number of studies have been conducted on the pathways related to EMT in CRS. Researchers have found that TGF-β/Smad, Wnt/β-actin, hypoxia-inducible factor-1α (HIF-1α), and other signaling pathways are linked together and interact with each other to jointly regulate the EMT process (shown in Fig. 2).

TGF-β Signaling

The TGF-β family regulates a variety of biological processes, such as cell proliferation, differentiation, apoptosis, extracellular matrix synthesis, and the fate of stem and progenitor cells, thereby influencing embryonic development, wound healing, and immune responses [40]. TGF-β can be divided into TGF-β1, TGF-β2, and TGF-β3 subtypes, among which TGF-β1 accounts for the highest proportion and has the strongest biological activity, participates in tissue repair, and initiates tissue remodeling by activating EMT signals in the airway epithelium and nasal tissue [31]. Epithelial cells from nasal polyps and the inferior turbinate undergo EMT-like processes after in vitro exposure to TGF-β1 or EGF; however, only epithelial cells from nasal polyps show increased activity [24, 28]. At present, there are some differences in the expression levels of TGF-β1 in CRS patients. Some results revealed that the expression level of TGF-β1 in CRSwNP patients was higher than that in CRSsNP patients [23, 28, 41, 42]. However, other studies showed that compared with the normal control group, TGF-β1 expression in the mucosal epithelium of CRSsNP patients was upregulated, whereas the expression of TGF-β1 in the nasal mucosa epithelium of CRSwNP patients was downregulated [43, 44]. The reasons for the different levels of TGF-β1 expression in different studies on the two CRS subtypes are still unclear. Whether this is related to the specific site, timing, and progression of the specimen remains to be further studied. It may be a new research and treatment approach for EMT-related diseases to master the change rule of TGF-β in the progression of EMT as well as to influence the occurrence and development of EMT from TGF-β level regulation of EMT-related signaling pathways. TGF-β regulates cell function by activating a variety of signaling pathways through the activation of downstream mediators which regulate various transcription factors including Smad, Slug, Twist, and ZEB1/2, as well as typical (Smad-based) and atypical (non-Smad-based) signaling pathways.

TGF-β/Smad Signaling Pathway in EMT

The TGF-β/Smad signaling pathway is a classical pathway among various TGF-β signaling pathways. Thr key steps of the intracellular TGF-β signaling pathway are mediated by Smad proteins [45]. TGF-β induces EMT through a Smad2/3 dependent pathway. Compared to those in the control group, the levels of TGF-β1 and Smad3

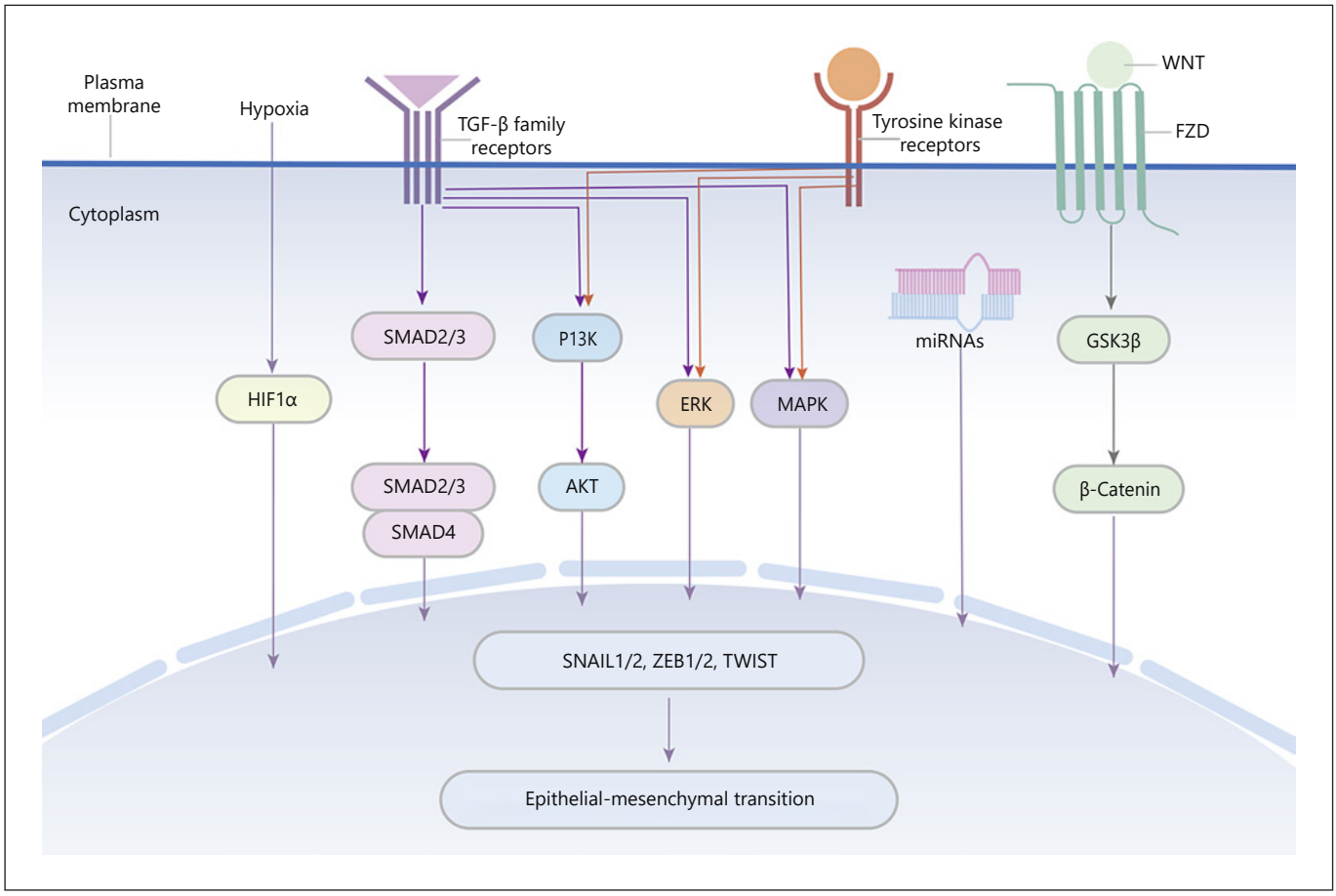

Fig. 2. Signaling pathways promoting EMT in CRSwNP. The process of EMT is regulated by multiple signaling pathways. TGF-β signaling complies with SMAD2 and SMAD3 to lead to EMT. TGF-β can also activate p13K/Akt, ERK and MAPK signaling pathways. The WNT signaling pathway promotes EMT by stabilizing β catenin by inhibiting GSK3β. The cellular microenvironment can regulate EMT. Hypoxia promotes EMT through HIF-1α. Receptor tyrosine kinases activate PI3K-ATK, ERK, and MAPK pathway via activation of growth factors, such as EGF, VEGF, and FGF. Several microRNAs can also be involved in EMT regulation. These signaling pathways induce EMT by activation of EMT transcription factors, including SNAI1/2, ZEB1/2, TWIST. EMT can be increased by interaction and cooperation between different pathways. GSK3β, glycogen synthase kinase 3β.

in CRSsNP patients were significantly increased [46]. Although the upstream signaling pathway of TGF-β in CRSsNP patients is enhanced, Smad7 overexpression may inhibit the antiproliferative effects of downstream signaling pathway components such as pSmad3, and TGF-β on nasal epithelial cells [47]. TβRI and TβRII were also highly expressed in the nasal mucosa of CRSsNP patients [46]. Inhibition of TBX1 can reduce EMT and inflammatory processes to some extent. Through TGF-β-Smad2/3 signaling pathway, it can increase the expression of Th1 cytokines and E-cadherin, as well as reduce the expression of Th2 cytokines, vimentin, and α-SMA [48].

TGF-β/Non-Smad Signaling Pathways in EMT

In epithelial cells undergoing EMT, TGF-β activates AKT through PI3K, which in turn promotes the EMT process [49]. Additionally, miR-21 mediates TGF-β1-induced EMT in human nasal epithelial cells (hNECs) through the PTEN/Akt pathway [23]. Reduced levels of PTEN and increased levels of phosphorylated Akt were detected in TGF-β-induced hNECs; miR-21 downregulation eliminated these effects. In hNECs, miR-21 overexpression can downregulate PTEN and upregulate Akt phosphorylation, whereas a specific inhibitor of Akt activation can significantly eliminate miR-21-regulated EMT.

TGF-β also activates the extracellular signal-regulated kinase (ERK), p38, and JUN N-terminal kinase MAPK pathways. In vitro, glucocorticoids prevented tissue remodeling in CRSwNP by blocking EMT initiation by the MAPK and Snail/Slug signaling pathways induced by TGF-β1 in hNECs [29].

Wnt/β-Catenin Signaling

Wnt signaling is controlled by several Wnt ligands that bind to a variety of frizzled receptors to regulate intracellular signaling. Typical Wnt signaling pathways rely on β-catenin as a signal mediator for transport to the nucleus, thereby promoting gene expression and cell function through Wnt ligand binding. However, the role of atypical Wnt ligands remains unclear. Long-term excessive Wnt signaling can activate and drive EMT. Due to the decrease in ciliary cells, cell polarity may change and ciliary activity may be uncoordinated, which is not conducive to mucilage clearance [50–52].

The presence of Wnt, absence of E-cadherin, and increase of β-catenin are typical features of EMT [51]. Activation of the Wnt signaling pathway contributes to the maintenance of the inflammatory environment and tissue remodeling in CRSwNP [50]. Upregulation of Wnt signaling has been found in CRSwNP; activation of Wnt signaling in hNECs can trigger cytokine release and lead to a series of changes. These changes are consistent with those during tissue remodeling in nasal polyps, including the loss of cell differentiation and abnormal epithelial morphology. In a mouse model of nasal polyps, activation of the Wnt signaling pathway induced more frequent polypoid lesions. Wnt-related molecules, such as β-catenin, WNT3A, and cyclin D1, and EMT-related molecules, such as E-cadherin and α-SMA, were upregulated. The same trend was observed in Wnt signaling and EMT in CRSwNP patients [30]. The morphology of epithelial cells changed as well as intercellular adhesion decreased in hNECs treated with recombinant human WNT3A [50].

HIF-1α Signaling Pathway

In hypoxia-related EMT, HIF-1α signaling is activated and promotes the expression of EMT-related genes, including Twist, TGF-β, and Lox [53]. HIF-1α is responsible for hypoxia-induced EMT [32]. Inhibition of HIF-1α expression restored E-cadherin levels under hypoxia conditions. E-cadherin expression is decreased by HIF-1α under normal oxygen conditions. HIF-1α expression is associated with the loss of E-cadherin and α-SMA in CRS; HIF-1α inhibitors inhibit the formation of nasal polyps in a mouse model.

Sirtuin1 (SIRT1) deacetylates HIF-1α and HIF-2α and inhibits their transcriptional activity [54]. In the nasal polyp model of SIRT1 transgenic mice, the number of polypoid lesions in the mice was reduced compared to that in wild-type mice [55]. SIRT1 levels were downregulated in the sinus mucosa of CRS patients compared to those in patients without nasal polyps. Resveratrol (SIRT1 activator) and sirtinol (SIRT1 inhibitor) can inhibit and promote nasal polyp formation, respectively. Resveratrol inhibits hypoxia-induced EMT and restores morphologic changes in hNECs.

Advanced Glycation End Products/ERK Signaling Pathway

Advanced glycation end products (AGEs) are products of nonenzymatic glycation and oxidation of proteins and lipids. Their interactions with AGE receptors (RAGE) and other receptors activate several pathways involved in diseases characterized by collagen metabolism disorder [56, 57]. Compared to those in the control group, the levels of AGE, RAGE, and MMP3 in polyps were higher, and RAGE was widely overexpressed in the deep lamina propria, submucosa, and around blood vessels. There was no significant difference in ERK levels between the two groups, but the expression level of p-ERK in polyps was increased, indicating activation of ERK signaling in CRSwNP patients. This evidence confirms that AGE/RAGE cascades are involved in EMT processes in polyp disease [58]. The interaction between AGE and RAGE seems to induce connective tissue remodeling through changes in MMP-1, TIMP, and P38 mitogen-activated protein (MAPK), and NF-κB. ERK signaling is influenced by the AGE/RAGE pathway and induces EMT.

High-Mobility Group Box 1 and RAGE Signaling Pathway

High mobility group box1 (HMGB1), a RAGE associated with injury, was upregulated in refractory CRSwNP and correlated with the severity of the disease [59]. Compared with the control group, HMGB1 showed a high level of expression in eosinophilic CRS with nasal polyps (ECRSwNP) and accumulated significantly in the cytoplasm [54, 55]. TGF-β1 and HMGB1 expression is also significantly upregulated in nasal epithelial cells under hypoxia [32, 56]. Compared with the control group, HMGB1 and interstitial markers in polyps were increased, whereas epithelial markers were decreased. HMGB1 upregulated N-cadherin and vimentin but downregulated ZO-1 and E-cadherin in epithelial cells isolated from ECRSwNP in a dose-dependent manner [60]. HMGB-1

induced the expression of α-SMA, fibronectin, and collagen, inhibited RAGE and its downstream molecules P38, JUN N-terminal kinase, and AP-1, and blocked the induction of HMGB-1 [61]. By studying the association between Snail and HMGB1, it was found that the downregulation of Snail1 reduced the expression of HMGB1, thus leading to the reduction of proinflammatory cytokines; in contrast, inhibition of HMGB1 reduced the expression of Snail1, thus inhibiting the EMT process [62]. Hypoxia-induced HMGB1 can induce EMT through RAGE signal transduction [60].

Interferon-γ/P38/ERK Signaling Pathway

IFN-γ plays an important role not only in innate immunity against potential pathogens but also in adaptive immunity, which coordinates leukocyte attraction, maturation, and differentiation of multiple cell types [63]. IFN-γ is a marker of Th1 type inflammation and is present in large quantities in many inflammatory diseases, including CRS [64]. In patients with neutrophil CRSwNP, IFN-γ levels in epithelial cells were upregulated and correlated with EMT-related markers [65]. Under normoxic conditions, IFN-γ induces HIF-1α upregulation, which results in E-cadherin loss. IFN-γ induces EMT through the p38 and ERK pathways; IFN-γ induced EMT in airway epithelial cells is unaffected by HIF-1α and TGF-β signaling. The use of p38 and ERK inhibitors reversed IFN-γ-induced changes in EMT marker levels and prevented nasal polyp formation and chemotactic cytokine secretion by neutrophils. Combined inhibition of p38 and ERK was more significant than that of ERK alone.

The Role of microRNA in CRS

MicroRNAs (miRNAs) are single-stranded noncoding RNAs, 19–24 nucleotides in length, and highly conserved during evolution. They regulate the expression of posttranscriptional genes by inducing translation inhibition and mRNA degradation [66]. Moreover, miRNAs can target hundreds of messenger RNAs (mRNAs) and affect the expression of many genes [67]. As regulators of gene expression, miRNAs are active in a variety of cellular biological processes, such as proliferation, apoptosis, differentiation, and survival [68–71]. Dysregulation of miRNAs has been found in diseases associated with excessive inflammation and immune dysfunction such as psoriasis, rheumatoid arthritis, pulmonary fibrosis, allergic asthma, allergic rhinitis, atopic dermatitis, and eosinophilic esophagitis [72–75]. Studies have shown that miRNAs are closely related to the occurrence and development of CRS [76].

Some miRNAs are associated with CRS inflammation; changes in their expression levels regulate inflammatory responses via different signaling pathways. Upregulated miR-125b levels may lead to increased mucosal eosinophils in eosinophilic CRSwNP [76]. Conversely, the upregulation of miR-335-5p expression has an anti-inflammatory effect on CRS. In contrast, overexpression of miR-335-5p inhibits the activation of the Akt signaling pathway by targeting TPX2, as well as the release of inflammatory cytokines, thus reducing CRS inflammation [77]. Knockdown of miR-761 stimulates inflammatory responses and exacerbates CRS symptoms in a mouse model [78].

In addition to their effects on inflammation, some miRNAs can regulate the EMT process in airway epithelial cells [79]. When the expression levels of the miRNAs of miR-34 and miR-449 families were changed, the epithelial cilium function of CRSwNP patients was significantly impaired [80]. Furthermore, miR-761 overexpression upregulated N-cadherin and downregulated E-cadherin expression by inactivating the LCN2/Twist1 signaling pathway. It also inhibited nasal mucosal remodeling and EMT in a mouse model of CRS [78]. Under stressful conditions, rat alveolar macrophages produce high levels of miR-21-5p in exosomes and transport miR-21-5p to tracheal epithelial cells, thereby promoting EMT of rat tracheal epithelial cells through the TGFβ1/Smad7 signaling pathway [23]. Downregulation of miR-155-5p inhibits EMT in nasal mucosal epithelial cells by targeting SIRT1 [81]. Overexpression of miR-30a-5p alleviates TGF-β1-induced EMT in the nasal mucosa by inhibiting the expression of cyclin-dependent kinase 6 [82]. The expression level of miR-1287-5p was also decreased in CRS and LPS-induced hNECs upregulation of miR-1287-5p inhibited EMT and inflammation in LPS-induced hNECs through the Snail1/HMGB1 pathway [62].

Therapeutic Approaches of CRS

Traditional treatment for CRS is to be treated with nasal saline rinses and intranasal corticosteroids (INCSs). In more severe cases, oral corticosteroids, possibly in combination with antibiotics, for acute exacerbations and surgery may be warranted. Drug therapy focuses on inflammation. Recent studies have found that glucocorticoids may have some interventional effects on EMT. In addition to their anti-inflammatory effects, glucocorticoids

inhibited TGF-β-induced EMT by blocking the MAPK and Snail/Slug signaling pathways [29]. These medications are effective, but may be limited by side effects, complications, and lack of long-term follow-up. In recent years, monoclonal antibody-related drugs targeting the cytokines IL-4, IL-5, IL-13, and IgE have provided ideas for targeted EMT therapy. It may be a new direction for effective long-term disease control by intervening in EMT while controlling inflammation and adding EMT-targeted drugs before surgery.

Conclusion

CRS involves a variety of biological mechanisms; inhibition of inflammation alone cannot prevent its development. The occurrence of EMT is an important mechanism of CRS; inhibiting the occurrence and development of EMT or even reversing EMT provides new ideas and methods for the prevention and treatment of CRS. The specific regulatory mechanism of EMT in CRS has not been fully understood; its occurrence and development are jointly regulated by multiple factors and signaling pathways. An in-depth study of the mechanism of EMT in CRS is of great significance for the treatment of CRS and the development of therapeutic drugs.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was supported by the Beijing Municipal Commission of Education and Natural Science Foundation of Beijing Municipality (Grant No. KZ202110025030), the Central Laboratory of Capital Medical University Affiliated Beijing Shijitan Hospital (Grant No. 2020-KF27), the National Natural Science Foundation of China (Grant No. 81670901, 81371074).

Author Contributions

Hongtian Wang and Jinshu Yin developed the idea for the review, and Yifan Xia wrote the manuscript.

References

1 Huvenne W, van Bruaene N, Zhang N, van Zele T, Patou J, Gevaert P, et al. Chronic rhinosinusitis with and without nasal polyps: what is the difference? Curr Allergy Asthma Rep. 2009;9:213–20.
2 Schleimer RP, Kato A, Kern R, Kuperman D, Avila PC, et al. Epithelium: at the interface of innate and adaptive immune responses. J Allergy Clin Immunol. 2007;120:1279–84.
3 Schleimer RP, Berdnikovs S. Etiology of epithelial barrier dysfunction in patients with type 2 inflammatory diseases. J Allergy Clin Immunol. 2017;139:1752–61.
4 Bonser LR, Erle DJ. Airway mucus and asthma: the role of MUC5AC and MUC5B. J Clin Med. 2017;6.
5 Kiyokawa H, Morimoto M. Notch signaling in the mammalian respiratory system, specifically the trachea and lungs, in development, homeostasis, regeneration, and disease. Dev Growth Differ. 2020;62:67–79.
6 Whitsett JA. Airway epithelial differentiation and mucociliary clearance. Ann Am Thorac Soc. 2018;15:S143–148.
7 Rock JR, Gao X, Xue Y, Randell SH, Kong YY, Hogan BL, et al. Notch-dependent differentiation of adult airway basal stem cells. Cell stem cell. 2011;8:639–48.
8 Morimoto M, Nishinakamura R, Saga Y, Kopan R. Different assemblies of Notch receptors coordinate the distribution of the major bronchial Clara, ciliated and neuroendocrine cells. Development. 2012;139:4365–73.
9 Evans MJ, Cox RA, Shami SG, Wilson B, Plopper CG. The role of basal cells in attachment of columnar cells to the basal lamina of the trachea. Am J Respir Cell Mol Biol. 1989;1:463–9.
10 Kim SH, Matthay MA, Mostov K, Hunt CA. Simulation of lung alveolar epithelial wound healing in vitro. J R Soc Interface. 2010;7:1157–70.
11 Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol. 2001;2:285–93.
12 Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. Cell Res. 2009;19:156–72.
13 Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009;139:871–90.
14 Gruenert S, Jechlinger M, Beug H. Diverse cellular and molecular mechanisms contribute to epithelial plasticity and metastasis. Nat Rev Mol Cell Biol. 2003;4:657–65.
15 Moustakas A, Heldin CH. Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression. Cancer Sci. 2007;98:1512–20.
16 Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer. 2009;9:265–73.
17 Chaffer CL, Thompson EW, Williams ED. Mesenchymal to epithelial transition in development and disease. Cells Tissues Organs. 2007;185:7–19. 6
18 Lim J, Thiery JP. Epithelial-mesenchymal transitions: insights from development. Development. 2012;139:3471–86.
19 Kato A. Immunopathology of chronic rhinosinusitis. Allergol Int. 2015;64:121–30.
20 Bachert C, Han JK, Desrosiers M, Hellings PW, Amin N, Lee SE, et al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. Lancet. 2019;394:1638–50.
21 Soyka MB, Wawrzyniak P, Eiwegger T, Holzmann D, Treis A, Wanke K, et al. Defective epithelial barrier in chronic rhinosinusitis: the regulation of tight junctions by IFN-γ and IL-4. J Allergy Clin Immunol. 2012;130:1087–96.
22 Kao SS, Bassiouni A, Ramezanpour M, Finnie J, Chegeni N, Colella AD, et al. Proteomic analysis of nasal mucus samples of healthy patients and patients with chronic rhinosinusitis. J Allergy Clin Immunol. 2021;147:168–78.

23 Li X, Li C, Zhu G, Yuan W, Xiao ZA. TGF-β1 induces epithelial-mesenchymal transition of chronic sinusitis with nasal polyps through microRNA-21. Int Arch Allergy Immunol. 2019;179:304–19.
24 Li H, Liu Q, Wang H, Sun XC, Yu HP, Hu L, et al. Epithelial-mesenchymal transition in chronic rhinosinusitis (CRS) and the prognostic value of α-SMA in postoperative outcomes of patients with CRS. Mol Med Rep. 2019;20:2441–9.
25 Meng J, Zhou P, Liu Y, Liu F, Yi X, Liu S, et al. The development of nasal polyp disease involves early nasal mucosal inflammation and remodelling. PLoS One. 2013;8:e82373.
26 Knight DA, Grainge CL, Stick SM, Kicic A, Schuliga M. Epithelial mesenchymal transition in respiratory disease: fact or fiction. Chest. 2020;157:1591–6.
27 Barrallo-Gimeno A, Nieto MA. The Snail genes as inducers of cell movement and survival: implications in development and cancer. Development. 2005;132:3151–61.
28 Könnecke M, Burmeister M, Pries R, Böscke R, Bruchhage KL, Ungefroren H, et al. Epithelial-mesenchymal transition in chronic rhinosinusitis: differences revealed between epithelial cells from nasal polyps and inferior turbinates. Arch Immunol Ther Exp. 2017;65:157–73.
29 Yang HW, Lee SA, Shin JM, Park IH, Lee HM. Glucocorticoids ameliorate TGF-β1-mediated epithelial-to-mesenchymal transition of airway epithelium through MAPK and Snail/Slug signaling pathways. Sci Rep. 2017;7:3486.
30 Bae JS, Ryu G, Kim JH, Kim EH, Rhee YH, Chung YJ, et al. Effects of Wnt signaling on epithelial to mesenchymal transition in chronic rhinosinusitis with nasal polyp. Thorax. 2020;75:982–93.
31 Park IH, Kang JH, Shin JM, Lee HM. Trichostatin a inhibits epithelial mesenchymal transition induced by TGF-β1 in airway epithelium. PLoS One. 2016;11:e0162058.
32 Shin HW, Cho K, Kim DW, Han DH, Khalmuratova R, Kim SW, et al. Hypoxia-inducible factor 1 mediates nasal polypogenesis by inducing epithelial-to-mesenchymal transition. Am J Respir Crit Care Med. 2012;185:944–54.
33 Spaderna S, Schmalhofer O, Wahlbuhl M, et al. The transcriptional repressor ZEB1 promotes metastasis and loss of cell polarity in cancer. Cancer Res. 2008;68:537–44.
34 Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat Rev Cancer. 2007;7:415–28.
35 Vandewalle C, Van Roy F, Berx G. The role of the ZEB family of transcription factors in development and disease. Cell Mol Life Sci. 2009;66:773–87.
36 Sánchez-Tilló E, Liu Y, de Barrios O, Siles L, Fanlo L, Cuatrecasas M, et al. EMT-activating transcription factors in cancer: beyond EMT and tumor invasiveness. Cell Mol Life Sci. 2012;69:3429–56.
37 Peña C, García JM, García V, Silva J, Domínguez G, Rodríguez R, et al. The expression levels of the transcriptional regulators p300 and CtBP modulate the correlations between SNAIL, ZEB1, E-cadherin and vitamin D receptor in human colon carcinomas. Int J Cancer. 2006;119:2098–104.
38 Abba ML, Patil N, Leupold JH, Allgayer H. MicroRNA regulation of epithelial to mesenchymal transition. J Clin Med. 2016;5.
39 Lee M, Lim S, Kim YS, Khalmuratova R, Shin SH, Kim I, et al. DEP-induced ZEB2 promotes nasal polyp formation via epithelial-to-mesenchymal transition. J Allergy Clin Immunol. 2021;149:340–57.
40 Saito A, Horie M, Nagase T. TGF-β signaling in lung health and disease. Int J Mol Sci. 2018;19:2460.
41 Balsalobre L, Pezato R, Perez-Novo C, Alves MT, Santos RP, Bachert C, et al. Epithelium and stroma from nasal polyp mucosa exhibits inverse expression of TGF-β1 as compared with healthy nasal mucosa. J Otolaryngol Head Neck Surg. 2013;42:29.
42 Yamin M, Holbrook EH, Gray ST, Busaba NY, Lovett B, Hamilos DL. Profibrotic transforming growth factor beta 1 and activin A are increased in nasal polyp tissue and induced in nasal polyp epithelium by cigarette smoke and Toll-like receptor 3 ligation. Int Forum Allergy Rhinol. 2015;5:573–82.
43 Li J, Liu X, Sha M, Li Y. The balance between HGF and TGF-β1 acts as a switch in the tissue remodeling of chronic rhinosinusitis. Int J Clin Exp Pathol. 2019;12:933–40.
44 Kou W, Hu GH, Yao HB, Wang XQ, Shen Y, Kang HY, et al. Regulation of transforming growth factor-β1 activation and expression in the tissue remodeling involved in chronic rhinosinusitis. ORL J Otorhinolaryngol Relat Spec. 2012;74:172–8.
45 Massagué J. TGF-beta signal transduction. Annual review of biochemistry. 1998;67:753–91.
46 Li YC, An YS, Wang T, Zang HR. Analysis of transforming growth factor β signaling in chronic rhinosinusitis. Chin Med J. 2013;126:3340–3.
47 Wang T, Zang HR, Li YC, An Y, Wang H, Li L, et al. The role of Smad signaling pathway in the repair of mucosal epithelium in chronic sinusitis with nasal polyps and without nasal polyps. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 2019;33:9–13.
48 Zhong Y, Li Y, Zhang H. Silencing TBX1 exerts suppressive effects on epithelial-mesenchymal transition and inflammation of chronic rhinosinusitis through inhibition of the TGFβ-Smad2/3 signaling pathway in mice. Am J Rhinol Allergy. 2020;34:16–25.
49 Lamouille S, Derynck R. Cell size and invasion in TGF-beta-induced epithelial to mesenchymal transition is regulated by activation of the mTOR pathway. J Cell Biol. 2007;178:437–51.
50 Böscke R, Vladar EK, Könnecke M, Hüsing B, Linke R, Pries R, et al. Wnt signaling in chronic rhinosinusitis with nasal polyps. Am J Respir Cell Mol Biol. 2017;56:575–84.
51 Ghahhari NM, Babashah S. Interplay between microRNAs and WNT/β-catenin signalling pathway regulates epithelial-mesenchymal transition in cancer. Eur J Cancer. 2015;51:1638–49.
52 Tilley AE, Walters MS, Shaykhiev R, Crystal RG. Cilia dysfunction in lung disease. Annu Rev Physiol. 2015;77:379–406.
53 Lu X, Kang Y. Hypoxia and hypoxia-inducible factors: master regulators of metastasis. Clin Cancer Res. 2010;16:5928–35.
54 Lim JH, Lee YM, Chun YS, Chen J, Kim JE, Park JW. Sirtuin 1 modulates cellular responses to hypoxia by deacetylating hypoxia-inducible factor 1alpha. Mol Cell. 2010;38:864–78.
55 Lee M, Kim DW, Yoon H, So D, Khalmuratova R, Rhee CS, et al. Sirtuin 1 attenuates nasal polypogenesis by suppressing epithelial-to-mesenchymal transition. J Allergy Clin Immunol. 2016;137:87–98.e7.
56 Ramasamy R, Yan SF, Schmidt AM. Advanced glycation endproducts: from precursors to RAGE: round and round we go. Amino Acids. 2012;42:1151–61.
57 Hu H, Jiang H, Ren H, Hu X, Wang X, Han C. AGEs and chronic subclinical inflammation in diabetes: disorders of immune system. Diabetes Metab Res Rev. 2015;31:127–37.
58 Vetuschi A, Pompili S, Di Marco GP, Calvaruso F, Iacomino E, Angelosante L, et al. Can the AGE/RAGE/ERK signalling pathway and the epithelial-to-mesenchymal transition interact in the pathogenesis of chronic rhinosinusitis with nasal polyps? Eur J Histochem. 2020;64:3079.
59 Dzaman K, Szczepanski MJ, Molinska-Glura M, Krzeski A, Zagor M. Expression of the receptor for advanced glycation end products, a target for high mobility group box 1 protein, and its role in chronic recalcitrant rhinosinusitis with nasal polyps. Arch Immunol Ther Exp. 2015;63:223–30.
60 Min HJ, Choe JW, Kim KS, Yoon JH, Kim CH. High-mobility group box 1 protein induces epithelialmesenchymal transition in upper airway epithelial cells. Rhinology. 2020;58:495–505.
61 Lee SH, Cho JH, Park JH, Cho JS, Lee HM. High mobility group box chromosomal protein-1 induces myofibroblast differentiation and extracellular matrix production via RAGE, p38, JNK and AP-1 signaling pathways in nasal fibroblasts. Am J Rhinol Allergy. 2021;35:774–80.

62 Hao W, Zhu Y, Guo Y, Wang H. miR-1287-5p upregulation inhibits the EMT and pro-inflammatory cytokines in LPS-induced human nasal epithelial cells (HNECs). Transpl Immunol. 2021;68:101429.
63 Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol. 2005;5:375–86.
64 Van Bruaene N, Pérez-Novo CA, Basinski TM, Van Zele T, Holtappels G, De Ruyck N, et al. T-cell regulation in chronic paranasal sinus disease. J Allergy Clin Immunol. 2008;121:1435–41.
65 Lee M, Kim DW, Khalmuratova R, Shin SH, Kim YM, Han DH, et al. The IFN-γ-p38, ERK kinase axis exacerbates neutrophilic chronic rhinosinusitis by inducing the epithelial-to-mesenchymal transition. Mucosal Immunol. 2019;12:601–11.
66 Tubita V, Callejas-Díaz B, Roca-Ferrer J, Marin C, Liu Z, Wang Y, et al. Role of microRNAs in inflammatory upper airway diseases. Allergy. 2021;76:1967–80.
67 Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol. 2018;141(4):1202–7.
68 Liu M, Zhang Y, Zhang J, Cai H, Zhang C, Yang Z, et al. MicroRNA-1253 suppresses cell proliferation and invasion of non-small-cell lung carcinoma by targeting WNT5A. Cell Death Dis. 2018;9:189.
69 Slattery ML, Mullany LE, Sakoda LC, Wolff RK, Samowitz WS, Herrick JS. Dysregulated genes and miRNAs in the apoptosis pathway in colorectal cancer patients. Apoptosis. 2018;23:237–50.
70 Li L, Miu KK, Gu S, Cheung HH, Chan WY. Comparison of multi-lineage differentiation of hiPSCs reveals novel miRNAs that regulate lineage specification. Sci Rep. 2018;8:9630.
71 Martello A, Mellis D, Meloni M, Howarth A, Ebner D, Caporali A, et al. Phenotypic miRNA screen identifies miR-26b to promote the growth and survival of endothelial cells. Mol Ther Nucleic Acids. 2018;13:29–43.
72 Dissanayake E, Inoue Y. MicroRNAs in allergic disease. Curr Allergy Asthma Rep. 2016;16:67.
73 Pandit KV, Corcoran D, Yousef H, Yarlagadda M, Tzouvelekis A, Gibson KF, et al. Inhibition and role of let-7d in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2010;182:220–9.
74 Sonkoly E, Janson P, Majuri ML, Savinko T, Fyhrquist N, Eidsmo L, et al. MiR-155 is overexpressed in patients with atopic dermatitis and modulates T-cell proliferative responses by targeting cytotoxic T lymphocyte-associated antigen 4. J Allergy Clin Immunol. 2010;126:581–9.e1–20.
75 Sonkoly E, Wei T, Janson PC, Sääf A, Lundeberg L, Tengvall-Linder M, et al. MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? PLoS One. 2007;2:e610.
76 Zhang XH, Zhang YN, Li HB, Hu CY, Wang N, Cao PP, et al. Overexpression of miR-125b, a novel regulator of innate immunity, in eosinophilic chronic rhinosinusitis with nasal polyps. Am J Respir Crit Care Med. 2012;185:140–51.
77 Gu X, Yao X, Liu D. Up-regulation of microRNA-335-5p reduces inflammation via negative regulation of the TPX2-mediated AKT/GSK3β signaling pathway in a chronic rhinosinusitis mouse model. Cell Signal. 2020;70:109596.
78 Cheng J, Chen J, Zhao Y, Yang J, Xue K, Wang Z. MicroRNA-761 suppresses remodeling of nasal mucosa and epithelial-mesenchymal transition in mice with chronic rhinosinusitis through LCN2. Stem Cell Res Ther. 2020;11:151.
79 Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol. 2014;15:178–96.
80 Callejas-Díaz B, Fernandez G, Fuentes M, Martínez-Antón A, Alobid I, Roca-Ferrer J, et al. Integrated mRNA and microRNA transcriptome profiling during differentiation of human nasal polyp epithelium reveals an altered ciliogenesis. Allergy. 2020;75:2548–61.
81 Yang N, Cheng H, Mo Q, Zhou X, Xie M. miR-155-5p downregulation inhibits epithelial-to-mesenchymal transition by targeting SIRT1 in human nasal epithelial cells. Mol Med Rep. 2020;22:3695–704.
82 Zhang T, Zhou Y, You B, You Y, Yan Y, Zhang J, et al. miR-30a-5p inhibits epithelial-to-mesenchymal transition by targeting CDK6 in nasal polyps. Am J Rhinol Allergy. 2021;35:152–63.