

Minireview

The Role of Proprotein Convertases in Upper Airway Remodeling

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Chronic rhinosinusitis (CRS) is a multifactorial, heterogeneous disease characterized by persistent inflammation of the sinonasal mucosa and tissue remodeling, which can include basal/progenitor cell hyperplasia, goblet cell hyperplasia, squamous cell metaplasia, loss or dysfunction of ciliated cells, and increased matrix deposition. Repeated injuries can stimulate airway epithelial cells to produce inflammatory mediators that activate epithelial cells, immune cells, or the epithelial–mesenchymal trophic unit. This persistent inflammation can consequently induce aberrant tissue remodeling. However, the molecular mechanisms driving disease within the different molecular CRS subtypes remain inadequately characterized. Numerous secreted and cell surface proteins relevant to airway inflammation and remodeling are initially synthesized as inactive precursor proteins, including growth/differentiation factors and their associated receptors, enzymes, adhesion molecules, neuropeptides, and peptide hormones. Therefore, these precursor proteins require post-translational cleavage by proprotein convertases (PCs) to become fully functional. In this review, we summarize the roles of PCs in CRS-associated tissue remodeling and discuss the therapeutic potential of targeting PCs for CRS treatment.

Keywords: airway remodeling, chronic rhinosinusitis, endoproteolytic cleavage, human nasal epithelial cells, nasal polyps, proprotein convertase

INTRODUCTION

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nose and paranasal sinuses that lasts longer than 12 weeks and is the most common upper respiratory tract disease associated with tissue remodeling. CRS has been divided into two major subtypes based on the presence (CRSwNP) or absence (CRSsNP) of nasal polyps (NPs) (Fokkens et al., 2012; Meltzer et al., 2004). CRSsNP comprises more than two-thirds of cases and is less likely to be managed by surgical intervention, whereas CRSwNP represents 20%–25% of cases. NPs are outgrowths of swollen inflammatory tissue that infiltrate the middle or superior meatus. They are the most severe form of pathological tissue remodeling in CRS and require surgical intervention. Tos et al. (2010) hypothesized that NP pathogenesis involves epithelial rupture and necrosis, leading to protrusions from the lamina propria and epithelial repair. Furthermore, Takabayashi et al. (2013a; 2013b) clarified that NP growth is due to the deposition of fibrin mesh within the tissue. However, why NPs only develop in some patients with CRS remains unclear.

The recent identification of appropriate CRS biomarkers has revealed new classification methods, such as the characterization of the CRS patient immune response, known as endotyping. Endotypes are classified according to distinct subsets of CD4⁺ T cells, namely T helper (Th)1, Th2, and Th17 cells, T cell products, infiltrating eosinophilic and noneosinophilic inflammatory cells, and remodeling markers

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(Schleimer, 2017; Staudacher et al., 2020). Age, as well as environmental and genetic factors, also influence CRS patient inflammatory endotypes (Mahdavinia et al., 2015; Stevens et al., 2019; Wang et al., 2016b; Zhang et al., 2017). However, some clusters cannot be classified using currently available endotype classification methods, highlighting the necessity for more specific biomarkers and indicating that CRS pathophysiology and pathogenesis remain to be fully understood. Comprehensive tissue remodeling processes, in particular, require further investigation, highlighting the necessity of elucidating regulatory mechanisms underlying tissue remodeling based on endotype classification.

Tissue remodeling is a secondary phenomenon, beginning in early-stage CRS development due to persistent inflammation (Bassiouni et al., 2013; Meng et al., 2013; Watelet et al., 2015). Tissue remodeling in CRS is the reorganization or renovation of nasal mucosa, which can be either physiological or pathological. Nasal mucosal inflammation induces remodeling processes within the mucosa characterized by changes in extracellular matrix (ECM) protein deposition, macrophage and lymphocyte infiltration, and histological structure. Structural alterations in the nasal epithelium include goblet cell hyperplasia, squamous metaplasia, epithelial–mesenchymal transition (EMT), epithelial barrier disruption, epithelial exfoliation, and basement membrane thickening. Structural changes in the lamina propria include stromal edema, bone thickening, fibrosis, angiogenesis, and submucosal gland hyperplasia. The various CRS subgroups can be differentiated by distinct remodeling features: for example, the eosinophilic forms of both CRSwNP and CRSsNP are characterized by increased edema, resulting in more severe disease presentation than the noneosinophilic forms of CRSwNP, which are characterized by increased glandular hyperplasia and dense

collagen deposition (Kountakis et al., 2004). Furthermore, fibrosis and collagen deposition, which are Th-1 biased inflammatory responses, are commonly observed in CRSsNP but not in CRSwNP. However, edema is a prominent feature of Th2-biased eosinophilic inflammation (Van Bruaene et al., 2009; 2012). Eosinophilic CRS shows heightened basement membrane thickening compared with noneosinophilic CRS (Lee et al., 2021). Previous studies have reported differences in tissue remodeling between polyps obtained from white and Asian patients (Shi et al., 2013; Van Bruaene and Bachert, 2011). Eosinophilic CRS is typically more common in the EU and USA, whereas noneosinophilic CRS is more common in Asia. However, the prevalence of eosinophilic CRS has increased in Asia due to an increasingly westernized lifestyle. Interestingly, a large histopathologic study of CRS in Wuhan, China, confirmed the link between eosinophilic infiltration and edema and the association of neutrophils with fibrosis (Cao et al., 2009).

Numerous mediators are implicated in airway tissue remodeling, including growth factors, enzymes, adhesion molecules, and ECM components (Ashley et al., 2017; Bassiouni et al., 2013; Maxfield et al., 2018; Samitas et al., 2018; Van Bruaene et al., 2012; Watelet et al., 2015) (Fig. 1). For example, insulin-like growth factor-1 (IGF-1) and its receptor are involved in epithelial cell hyperplasia, mucus overproduction, and ECM deposition (Chand et al., 2012; Krein et al., 2003). Notch signaling plays a critical role in the lineage selection of airway basal cells (BCs) during differentiation into either secretory or ciliated cells in many adults and embryonic tissues (Chiba, 2006; Koch and Radtke, 2010; Rock et al., 2011). However, the sustained activation of Notch signaling promotes the transition of airway BCs to a goblet cell fate (Gerovac et al., 2014; Gomi et al., 2015; Guseh et al., 2009;

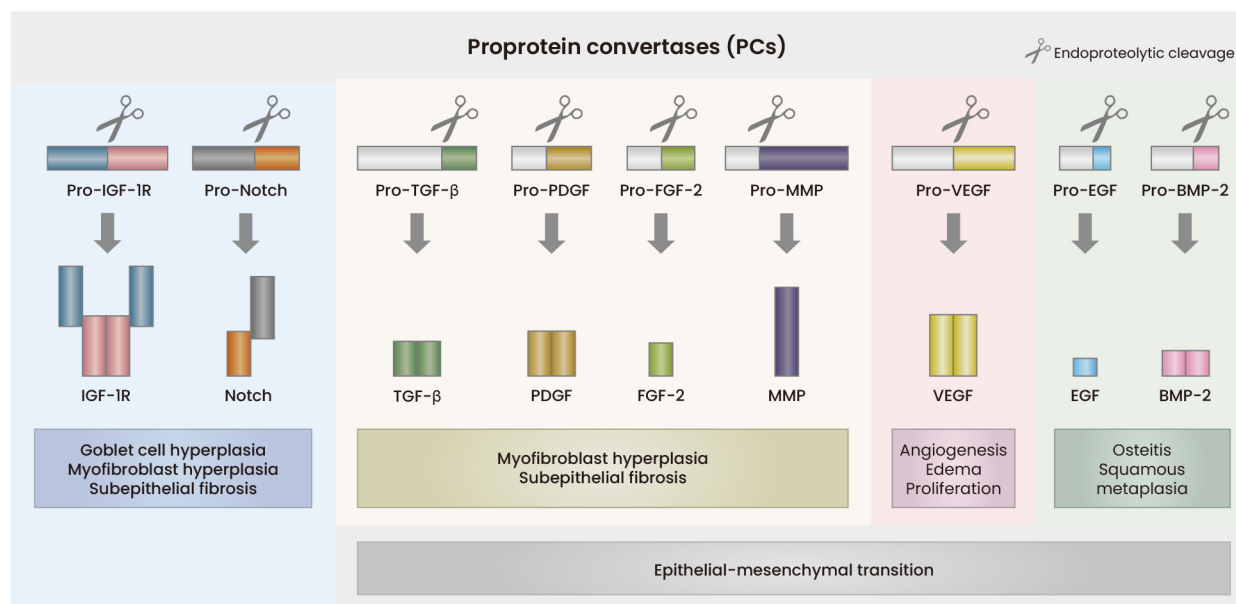


Fig. 1. Schematic representation of PC processing resulting in upper airway remodeling. Depicted are proforms of the numerous PC substrates that are associated with tissue remodeling, their mature forms, and their effects on upper airway remodeling.

Table 1. Substrates activated by PCs

Typical substrates	
PC1/3	Growth hormone-releasing hormone, insulin, glucagon, corticotropin, β -lipotropin, ACTH
PC2	Insulin, glucagon, α -MSH, met-enkephalin, somatostatin
PC4	Pituitary adenylate cyclase-activating polypeptide, IGF-2
Furin	Albumin, factor IX, VWF, neurotrophins, adhesins, α - and β -secretases, TNF- α , TGF- β , IGF-1, IGF-1R, integrins, Notchs, PDGF, VEGFs, MMPs, BMPs, bacterial toxins (anthrax toxin, diphtheria toxin, pseudomonas exotoxin A, aerolysin toxin, Shiga toxins, Clostridium specum α -toxin), viral glycoproteins (HIV gp160, Evola gp, influenza HA, measles, cytomegalovirus, respiratory syncytial virus, coronavirus)
PC5/6	GDF11, PTPRM, L1CAM, α 4 integrin, BMPs <i>In vitro</i> and <i>ex vivo</i> redundancy with furin and PACE4
PACE4	Nodal, Lefty, L1CAM, MMPs, BMPs <i>In vitro</i> and <i>ex vivo</i> redundancy with furin and PC5/6
PC7	Transferrin receptor 1 Partial redundancy with furin, PC5/6, and PACE4

ACTH, adrenocorticotropin hormone; α -MSH, α -melanocyte-stimulating hormones; IGF, insulin-like growth factor; VWF, Von Willebrand factor; TNF- α , tumor necrosis factor α ; TGF- β , transforming growth factor β ; IGF-1R, insulin-like growth factor 1 receptor; VEGF, vascular endothelial growth factor; HIV gp 160, human immunodeficiency virus envelope glycoprotein 160; HA, hemagglutinin; GDF11, growth differentiation factor 11; PTPRM, protein tyrosine phosphatase receptor type M; L1CAM, neural cell adhesion molecule L1.

Rock et al., 2011). Transforming growth factor β (TGF- β), platelet-derived growth factor (PDGF), fibroblast growth factor 2 (FGF-2), vascular endothelial growth factor (VEGF), and matrix metalloproteinase (MMP) all induce the pathological conversion of epithelial cells into fibroblasts, resulting in tissue fibrosis (Cámara and Jarai, 2010; Davies, 2009; Strutz et al., 2002). Furthermore, VEGF promotes edema, angiogenesis, and epithelial cell growth in NPs (Fruth et al., 2012). Upregulation of epidermal growth factor (EGF) skews the airway BC fate toward the squamous and EMT-like phenotypes with decreased epithelial junctional barrier integrity (Shaykhiev et al., 2013). Additionally, bone morphogenetic protein 2 (BMP-2) is associated with both osteitis (Kim et al., 2021) and squamous metaplasia (Lee et al., 2015) in patients with refractory CRSwNP. Importantly, numerous secreted and cell surface proteins, including the proteins mentioned above, are initially synthesized as inactive precursor proteins, requiring endoproteolytic cleavage by proprotein convertases (PCs) for activation (Fig. 1, Table 1) (Artenstein and Opal, 2011; Seidah and Chrétien, 1999). Earlier work by our lab indicated that the expression of four PCs (furin, PC1/3, PC5/6, and PACE4) is significantly upregulated in CRS patient NP mucosa (Fig. 2). These results indicate that these enzymes may play important roles in NP pathogenesis. Furthermore, PCs show promise as diagnostic markers for CRS and may ultimately be targeted by molecular therapy. We summarize the general properties and biological relevance of PCs, as well as current discoveries

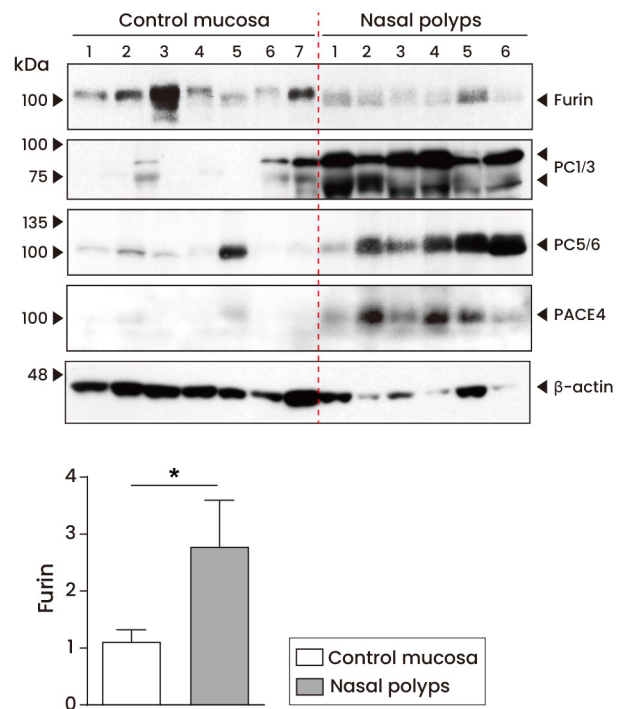


Fig. 2. PC expression in control nasal mucosa and nasal polyps. Western blot analysis reveals that furin, PC1/3, PC5/6, and PACE4 are expressed in both control nasal mucosa and nasal polyps, and the expression of four PCs is significantly upregulated in nasal polyps compared to the control mucosa. Right panel shows densitometric analysis of furin, normalized to β -actin and relative to control mucosa. Data represent the mean \pm SEM. * P < 0.05.

regarding the pathophysiological roles of furin, PC1/3, PC5/6, and PACE4 in CRS.

PROPROTEIN CONVERTASES

PCs are a family of calcium-dependent serine endoproteases. Examples of these enzymes include furin, PC1/3, PC2, PC4, PC5/6, PACE4, and PC7 (Artenstein and Opal, 2011). These enzymes activate precursor proteins through cleavage at doublets of the basic amino acids arginine (R) or lysine (K) or at paired basic motifs [R/K-(X) n -R/K-R], where the arrow indicates the cleavage site, X represents any amino acid except cysteine and n = 0, 2, 4, or 6 (Seidah and Chrétien, 1999; Steiner et al., 1967). In various organs, PCs are essential for key physiological functions, such as embryonic development and tissue homeostasis, due to their involvement in the proteolytic activation of many secretory proteins, including growth/differentiation factors and their receptors, adhesion molecules, enzymes, neuropeptides, and peptide hormones (Table 1) (Artenstein and Opal, 2011; Thomas, 2002; Turpeinen et al., 2013). Although PC inactivation in mice and humans has revealed specific phenotypes caused by unique, tissue-specific processing events (Seidah et al., 2013), additional investigation into the specific physiologic substrates for PCs is required. Furthermore, some PCs are associated with

various pathophysiological states, including endocrinopathies, cancer, viral/bacterial/parasitic infection, atherosclerosis, and neurodegenerative (Table 2) (Artenstein and Opal, 2011; Chrétien et al., 2008; Seidah and Prat, 2012; Thomas, 2002; Turpeinen et al., 2013). These PCs therefore, represent potential therapeutic targets for the treatment of various human diseases. We summarize the reported PC inhibitors that are expected to affect human pathologies (Table 2). It should be noted that none of these PC inhibitors is highly specific to only one PC.

INHIBITION OF FURIN-MEDIATED NOTCH1 PROCESSING IN AIRWAY BASAL CELLS PROMOTES CILIATED CELL DIFFERENTIATION

Airway BCs are a long-lived, multipotent stem cell population responsible for normal epithelium homeostasis and regeneration after injury, which is accomplished through their capacities for self-renewal and differentiation into multiple cell types, including secretory and ciliated cells (Rock et al., 2010). However, chronic repetitive injuries disrupt the balance be-

tween BC proliferation and differentiation and ultimately lead to pathological tissue remodeling, such as BC hyperplasia, goblet cell hyperplasia, squamous cell metaplasia, loss or dysfunction of ciliated cells, and increased matrix deposition (Araya et al., 2007; Rock et al., 2010; Samitas et al., 2018). These dramatic structural and functional changes contribute to disease susceptibility, initiation, and progression in the airway. Therefore, clarifying the fundamental mechanisms underlying BC lineage choice and differentiation during airway inflammation and remodeling is clinically relevant.

The Notch signaling pathway plays an essential role in regulating the differentiation of airway BCs into secretory and ciliated cells in both the developmental and adult phases (Chiba, 2006; Koch and Radtke, 2010; Rock et al., 2011). Four mammalian Notch receptors (Notch1-4) have been identified (Chiba, 2006). Steady-state Notch signaling is present in relatively few BCs due to low epithelial turnover. However, Notch signaling is greatly increased during repair after epithelial injury (Rock et al., 2011). *In vivo* studies have shown that the sustained activation of Notch1 signaling promotes luminal differentiation of airway BCs, primarily toward goblet cell lineages

Table 2. Therapeutic potential of PCs

	Diseases	Proposed therapies	References
PC1/3	Neuroendocrine tumors (pheochromocytoma, pituitary adenoma, carcinoids, pancreatic cancer, small-cell lung carcinoma)	Small-molecule inhibitors (2,5-dideoxystreptamine derivatives, peptidomimetic analogs, temozolomide), PC1/3 propeptide	Becker et al., 2012; Boudreault et al., 1998; Chrétien et al., 2008; Rose et al., 2020; Vivoli et al., 2012
PC2	Neuroendocrine tumors, liver colorectal metastases	Small-molecule inhibitors (bicyclic guanidines, pyrrolidine bis-piperazines, 2,5-dideoxystreptamine derivatives), PC2 propeptide	Chrétien et al., 2008; Kowalska et al., 2009; Muller et al., 2000; Tzimas et al., 2005; Vivoli et al., 2012
PC4	Male contraceptive	Small-molecule inhibitors (flavonoid derivatives)	Becker et al., 2012; Majumdar et al., 2010
Furin	Cancer and metastasis, viral, bacterial and parasitic infections	Bi: shRNA-Furin GMCSF, locked nucleic acid (LNA), neutralizing antibodies, small-molecule inhibitors (2,5-dideoxystreptamine, dicoumarol derivatives, B3, phenylacetyl-Arg-Val-Arg-4-amidinobenzylamide, decarboxylated P1 arginine peptide mimetics, guanidylated streptamine derivatives, peptidomimetic analogs, temozolomide, dicoumarol derivatives), alpha-1-antitrypsin derivatives, nanobodies, furin propeptide	Becker et al., 2010; 2012; Coppola et al., 2008; Couture et al., 2012; Dahms et al., 2021; Jiao et al., 2006; Klein-Szanto and Bassi, 2017; Komiyama et al., 2009; Rose et al., 2020; 2021; Senzer et al., 2012
PC5/6	Atherosclerosis, cancer, viral infections, reproduction, dyslipidemia	Small-molecule inhibitors (guanidylated streptamine derivatives, peptidomimetic analogs, dicoumarol derivatives), alpha-1-antitrypsin derivatives), PC5/6 propeptide	Becker et al., 2012; Dahms et al., 2021; Klein-Szanto and Bassi, 2017; Rose et al., 2021
PACE4	Cancer and metastasis, arthritis, viral and pathogenic infections	shRNA, Small-molecule inhibitors (Multi-Leu peptide, peptidomimetic analogs, temozolomide, guanidylated streptamine derivatives, dicoumarol derivatives), alpha-1-antitrypsin derivatives	Becker et al., 2012; Byun et al., 2010; Couture et al., 2012; Klein-Szanto and Bassi, 2017; Levesque et al., 2012; Rose et al., 2020; 2021
PC7	Anxiety	Small-molecule inhibitors (guanidylated streptamine derivatives, dicoumarol derivatives), PC7 propeptide	Dahms et al., 2021; Klein-Szanto and Bassi, 2017; Rose et al., 2021

(Gerovac et al., 2014; Gomi et al., 2015; Guseh et al., 2009; Rock et al., 2011). A similar result was obtained from *in vitro* experiments using Notch signaling agonists and antagonists in air-liquid interface (ALI)-human bronchial epithelial cell cultures initiated with BCs (Guseh et al., 2009). Importantly, the Notch receptor is activated after cleavage by a furin-like convertase (Logeat et al., 1998; Rand et al., 2000). Using an *in vitro* ALI-human nasal epithelial (HNE) cell culture model of airway injury (Puchelle et al., 2006; Whitcutt et al., 1988), we found that inhibiting PC activity during BC differentiation using decanoyl-RVKR-chloromethylketone (CMK) treatment (Hallenberger et al., 1992) skews differentiation toward the ciliated cell phenotype. This skewed differentiation was evidenced by increased numbers of ciliated cells and the upregulation of various genes associated with ciliated cell differentiation (Lee et al., 2017). Furthermore, furin knockdown resulted in suppressed Notch1 processing and increased ciliated cell numbers in ALI-HNE cell culture, indicating that furin is the enzyme responsible for Notch1 activation in HNE cells. These observations and previous studies collectively suggest that furin may play a critical role in BC lineage choice toward goblet cell lineages, as well as the pathogenesis of goblet cell hyperplasia during chronic injury. Therefore, targeting furin has potential as an attractive therapeutic approach for airway epithelial repair and regeneration after injury.

OVEREXPRESSED PC1/3 CONTRIBUTES TO NASAL POLYPOGENESIS THROUGH EMT INDUCTION

EMT is a process for an epithelial cell to undergo profound biochemical changes to acquire a mesenchymal phenotype, which includes the loss of epithelial cell–cell junctions, the generation of apical-basal polarity, interactions with the basement membrane, and the upregulation of mesenchymal markers, such as α -smooth muscle actin (α -SMA), vimentin, MMPs, collagen I, and epithelial transcriptional suppressors (Snail and Twist) (Câmara and Jarai, 2010; Davies, 2009; Kalluri and Neilson, 2003; Willis and Borok, 2007). Disrupting cell–cell adhesions during EMT allows contact between ligand/receptor pairs that do not typically interact due to segregation into either the apical or basolateral membrane domains. Additionally, this disruption initiates signal transduction cascades that affect epithelial cell activation and differentiation, resulting in tissue remodeling (Georas and Rezaee, 2014; Gibson and Perrimon, 2003; Vermeer et al., 2003). Furthermore, airway epithelial injury and abnormal epithelial repair responses induce persistent epithelial cell activation by undergoing EMT, leading to a pathological process associated with fibrogenesis (Hackett, 2012; Hackett et al., 2009; Shin et al., 2012; Willis and Borok, 2007). Thus, understanding the precise molecular interactions underlying EMT could lead to the identification of novel therapeutic targets to treat tissue fibrosis in chronic inflammatory airway diseases. Hackett et al. (2009) demonstrated that TP63⁺ KRT5⁺ BCs in a multilayered, differentiated ALI-airway epithelial cell culture derived from asthmatic subjects undergo EMT after exposure to TGF- β 1, which is a known major inducer of EMT. EMT was evidenced by the loss of epithelial markers, E-cadherin and zonular occludin-1, and the upregulation of

mesenchymal markers, EDA-fibronectin, vimentin, α -SMA, and collagen-1 (Hackett et al., 2009). Another study reported that hypoxic conditions present in inflamed sinus tissue drive EMT through a Smad3-dependent mechanism (Shin et al., 2012). Importantly, results from our lab revealed that both NP epithelium and ALI-HNE cell cultures undergoing TNF- α /IL-1 β -induced EMT highly express PC1/3, together with the mesenchymal marker proteins N-cadherin, collagen I, and MMP-2 (Lee et al., 2013). Specifically, PC1/3 expression was mostly confined to the basal and suprabasal layers in healthy control nasal epithelium but was upregulated in the entire NP epithelial layer (Lee et al., 2013). Because EMT is intimately linked with the acquisition of epithelial stem cell properties with greater phenotypic plasticity (Mani et al., 2008), differentiated epithelial cells may dedifferentiate into regressed basal/progenitor cells in disease states (Tata et al., 2013). Moreover, overexpressing PC1/3 in stably transfected human lung mucoepidermoid carcinoma HCl-H292 cells resulted in decreased E-cadherin expression and increased mesenchymal marker expression (N-cadherin, vimentin, collagen I, α 5 integrin, fibronectin, MMP2, Snail, and Twist), concurrent with the transition to a fibroblast-like morphology driven by actin cytoskeleton remodeling (Lee et al., 2013). Taken together, these observations suggest that PC1/3 contributes to tissue remodeling and CRSwNP pathogenesis and play crucial roles in EMT and fibrosis. PC1/3 likely contributes to CRSwNP pathogenesis due to altered processing of integrins, collagen I, fibronectin, neuropeptides, and MMPs (Artenstein and Opal, 2011; Cheng et al., 2001). Further research is required to fully define the precise molecular mechanisms underlying PC1/3-mediated EMT. Greater clarification is also needed to identify the physiological PC1/3 substrates that could provide new therapeutic targets for CRSwNP treatment.

PC5/6A PROMOTES THE SQUAMOUS DIFFERENTIATION OF HUMAN NASAL EPITHELIAL CELLS BY ACTIVATING BMP-2

Squamous metaplasia of the airway is a pathologic process by which normal, pseudostratified epithelium transdifferentiates into stratified epithelium consisting of flattened, squamous cells (Auerbach et al., 1961; Puchelle et al., 2006; Rock et al., 2010). Thus, squamous differentiation in airway epithelial cells and epidermal differentiation share many morphological and biochemical characteristics (Jetten, 1989). Chronic repetitive injuries to the airway epithelium induce tissue remodeling, such as epithelial cell hyperproliferation and squamous metaplasia, resulting in impaired mucociliary clearance (Puchelle et al., 2006). Interestingly, results from our lab revealed the significant upregulation of PC5/6A and BMP-2 in both the metaplastic squamous epithelium of NPs and a retinoic acid (RA) deficiency-induced squamous metaplasia model of ALI-HNE cells (Lee et al., 2015). RA deficiency is well-known to induce conversion from normal pseudostratified epithelium into stratified squamous airway epithelium (McDowell et al., 1984; Wolbach and Howe, 1925; Yoon et al., 2000). In a study by Pearton et al. (2001), four PCs, including furin, PACE4, PC5/6, and PC7, had significant roles in terminal keratinocyte differentiation in the epidermis. Additionally, BMP

signaling is implicated in pathophysiological processes including wound-healing, fibrosis, and allergic inflammation in the skin and lungs (Botchkarev, 2003; Rosendahl et al., 2002; Sountoulidis et al., 2012; Yan et al., 2010). BMP signaling is also known to be involved in the regulation of embryonic development and adult homeostasis (Botchkarev, 2003; Hogan, 1999; Sountoulidis et al., 2012). Yan et al. (2010) demonstrated that TNF- α -induced EMT is mediated by the BMP-2 signaling pathway in wound-healing and fibrosis of human skin. A study from the Zou lab revealed the induction of a smoking-related abnormal phenotype in human airway BCs mediated by exaggerated BMP-4/BMPR1A/Smad signaling, generating squamous metaplasia (Zuo et al., 2019). Importantly, BMPs are known to be physiological substrates for PACE4 and PC5/6A (Table 1) (Constam et al., 1996; Tsuji et al., 2003). Our lab demonstrated that PC5/6A knockdown and pharmacological inhibition of PC activity in RA-deficient ALI cultures resulted in significant reductions in BMP-2 protein expression and processing, accompanied by the downregulation of squamous cell marker genes (*cornifin/SPRR1* and *involucrin*) and the upregulation of secretory (*MUC5AC*, *TFE3*, and *MUC5B*) and ciliated cell marker genes (*Tektin* and *DNAI1*) (Lee et al., 2015). Conversely, PC5/6A overexpression using adenoviral-mediated transduction and exogenous BMP-2 resulted in the upregulation of squamous cell marker genes and the broad downregulation of ciliated and secretory cell differentiation genes (Lee et al., 2015) under RA-sufficient culture conditions for the mucociliary differentiation of HNE cells (Yoon et al., 2000). These observations suggest that PC5/6A-mediated BMP-2 maturation contributes to squamous metaplasia on the NP mucosal surface. Furthermore, Kim et al. (2021) reported that in CRSwNP, BMP-2 is upregulated in NP tissues, associated with osteitis severity, advanced disease extent, and disease refractoriness after surgery. Taken together, these results indicate that PC5/6A can serve as a new CRSwNP biomarker reflecting the pathophysiology of nasal mucosa with squamous metaplasia. Targeting PC5/6A may, therefore, be a viable therapeutic strategy for treating refractory CRSwNP.

PACE4 UPREGULATION IS ASSOCIATED WITH AIRWAY GOBLET CELL HYPERPLASIA

Goblet cell hyperplasia is a common feature of chronic airway diseases, which include asthma, allergic rhinitis, and CRSwNP (Jackson, 2001; Jiao et al., 2020; Tomazic et al., 2020). Recent unpublished results from our lab revealed that in the human nasal epithelium within a Th2 milieu, PACE4 upregulation is associated with goblet cell hyperplasia and mucus overproduction. In both NP epithelium and ALI-HNE cells treated with the Th2 cytokine IL-4, which induces goblet cell hyperplasia (Park et al., 2007), PACE4 expression was mostly confined to the basal and suprabasal layers. Furthermore, PACE4 knockdown in ALI-cultured BCs inhibited IL-4-induced goblet cell differentiation, which implies this enzyme is an attractive therapeutic target for CRSwNP treatment. Supporting this finding, a microarray-based study of the transcriptomes of eosinophilic CRSwNP (ECRSwNP) and noneosinophilic CRSwNP (non-ECRSwNP) showed that mRNA levels of Th2

cytokines and *PCSK6*, which is the gene *PACE4*, are significantly increased in ECRSwNP (Wang et al., 2016a). These results suggest PACE4 involvement in Th2 inflammation in ECRSwNP. ECRSwNP exhibits a poorer outcome compared to non-ECRSwNP. Indeed, ECRSwNP exhibits greater objective disease severity and a high recurrence rate after surgery (Nakayama et al., 2011; Szucs et al., 2002). Therefore, PACE4 may increase the risk of ECRSwNP, making it a potential diagnostic and prognostic biomarker and treatment target. Further investigation into the mechanism of how PACE4 is involved in ECRSwNP and the potential therapeutic benefits of targeting PACE4 in ECRSwNP is required.

CONCLUSION

There is still much more to learn about pathological endotyping or subphenotyping of tissue remodeling features in CRS patients, which has the potential to identify patients at a higher risk of recurrent or persistent disease. Here, we assert that PCs have crucial impacts on various types of CRS pathological tissue remodeling, including goblet cell hyperplasia, fibrosis, and squamous metaplasia. Therefore, PCs could be considered promising diagnostic and prognostic biomarkers in CRS patients. Targeting PCs has great potential to treat CRS. However, PC substrate specificity remains unknown in both the physiological and pathophysiological context. This lack of knowledge is largely due to substantial redundancies in the substrates and functions among PCs and the co-expression of some PCs in cells. Therefore, further studies to elucidate the precise mechanisms of PC activity and PC substrate specificity in tissue remodeling and CRS pathogenesis will enable the development of specific biomarkers for disease progression and more individualized treatment strategies. The challenge of identifying potent and safe PC inhibitors has great potential to yield an alternative CRS therapeutic option that could ultimately improve human health.

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AUTHOR CONTRIBUTIONS

S.-N.L. and J.-H.Y. conceived the study, wrote the manuscript, and secured funding.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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