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Airway Epithelial Barrier Dysfunction in Chronic Obstructive Pulmonary Disease: Role of Cigarette Smoke Exposure

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Abstract

The epithelial lining of the airway forms the first barrier against environmental insults, such as inhaled cigarette smoke, which is the primary risk factor for the development of chronic obstructive pulmonary disease (COPD). The barrier is formed by airway epithelial junctions, which are interconnected structures that restrict permeability to inhaled pathogens and environmental stressors. Destruction of the epithelial barrier not only exposes subepithelial layers to hazardous agents in the inspired air, but also alters the normal function of epithelial cells, which may eventually contribute to the development of COPD. Of note, disruption of epithelial junctions may lead to modulation of signaling pathways involved in

differentiation, repair, and proinflammatory responses. Epithelial barrier dysfunction may be particularly relevant in COPD, where repeated injury by cigarette smoke exposure, pathogens, inflammatory mediators, and impaired epithelial regeneration may compromise the barrier function. In the current review, we discuss recent advances in understanding the mechanisms of barrier dysfunction in COPD, as well as the molecular mechanisms that underlie the impaired repair response of the injured epithelium in COPD and its inability to redifferentiate into a functionally intact epithelium.

Keywords: COPD; cigarette smoke; epithelial barrier function; epithelial junctions

Chronic obstructive pulmonary disease (COPD) is a chronic lung disease with a high social and economic burden and mortality. COPD is characterized by an ongoing inflammatory process in the lungs that drives airway and lung tissue remodeling, including (small) airway fibrosis and emphysematous lung tissue destruction. The main risk factor for COPD is the inhalation of noxious gases and particles, including those present in cigarette smoke. The mucosal surface of the respiratory tract is in first contact with these hazardous agents, and is part of the innate immune defense against foreign substances. The mucosal defense mechanism encompasses the physical barrier activity of the airway epithelium, the mucociliary clearance system, production of antioxidants, protease inhibitors, and antimicrobial peptides, as well as mediators

that attract and activate cells of the immune response to prevent invasion of inhaled pathogens (1). Epithelial barrier function is maintained by tight junctions (TJs) and adherens junctions (AJs) that restrict epithelial permeability and movement of ions and solutes between cells, as well as migration of immune cells through the epithelial layer (2).

One of the leading causes of COPD is long-term direct or second-hand exposure to cigarette smoke (3). Cigarette smoke consists of gaseous and particulate phases that contain more than 7,000 chemicals, such as oxidative gases and heavy metals, and at least 70 carcinogenic substances (4). The detrimental effects of cigarette smoke exposure contribute to the pathogenesis of respiratory diseases, such as COPD and lung

cancer (5). Although cigarette smoking is considered as the main predisposing factor for COPD in large parts of the world, not all smokers develop COPD, indicating that other environmental factors and genetic susceptibility also contribute (6). Cigarette smoke is known to cause oxidative stress in the airway epithelium (7). This may eventually lead to sustained recruitment of immune cells, squamous metaplasia, mucus hypersecretion, and loss of ciliary beating on the airway epithelial surface (5, 8, 9), contributing to airflow limitation (10). In addition, oxidative stress induced by cigarette smoke disrupts the junctions between adjacent epithelial cells (11, 12), which may play a critical role in the pathogenesis of COPD, as outlined subsequently here.

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In this review, we discuss recent insights into the molecular mechanisms of cigarette smoke-induced loss of airway epithelial barrier function in COPD. Although various studies have also explored effects of cigarette smoke on alveolar epithelial cell barrier function, this is outside the scope of the present review.

Barrier Function in the Normal Respiratory Tract Epithelium

To better understand the role of cigarette smoke-induced barrier dysfunction in the pathogenesis of COPD, it is vital to discuss the normal architecture and function of airway epithelium. The epithelium of the (small) airways is lined with a cylindrical ciliated pseudostratified carpet, which is composed of four main types of cells (i.e., ciliated cells, secretory goblet cells, club cells, and basal cells [1]), of which the latter two have stem cell properties, acting as progenitor cells for ciliated cells and goblet cells.

Mucociliary clearance by the epithelial layer is provided by ciliated cells and goblet cells, which are mostly found in larger airways (13). Both goblet cells and submucosal glands produce mucus (14), which forms a gel layer on the epithelial surface of the respiratory tract, trapping pathogens and inhaled particles. Trapped pathogens and particles are removed by the concerted actions of cilia and by cough.

Barrier function of the epithelial layer is maintained by the formation of epithelial junctions. Epithelial junctions act to functionally segregate the basal from the apical compartment to allow epithelial polarization (15), and may thus be critical for differentiation of basal epithelial cells into mucociliary epithelium. In addition, apical junctional complexes between airway epithelial cells are an integral part of the mucosal immune system, regulating the protection against pathogens. Barrier function restricts transepithelial crossing of such inhaled pathogens, and barrier dysfunction may contribute to the increase in viral and bacterial infection in COPD. This may have important implications, as respiratory infections have been associated with the majority of COPD exacerbations

(16). The junctional complex consists of TJs and AJs. TJs are located in the apical part of the cell surface, limit permeability of the epithelium (17), and are composed of the transmembrane proteins, claudin (CLDN) (18), occludin (OCLN) (19), and junctional adhesion molecules (JAMs) (20). In addition, a number of other cytoplasmic molecules, such as zonula occludens (ZO)-1, ZO-2, ZO-3, cingulin, partitioning defective protein-3, Par-6, and afadin 6, have been implicated in the formation of TJs. Such molecules act as a scaffold by binding to the transmembrane proteins and linking them with actin microfilament and other cytoplasmic proteins that preserve the stability of TJs (21) (Figure 1).

AJs reside at the basolateral side of the more apically located TJs, connecting neighboring cells and initiating the formation of cell-cell contacts through homotypic, calcium-dependent adhesions by E-cadherin, a type I cadherin transmembrane glycoprotein. The cytoplasmic domain of E-cadherin is stabilized in the membrane when bound to the anchor proteins, p120 catenin, β -catenin, and α -catenin, linking the complex to the cytoskeleton (22).

It has been shown that α -catenin alone does not have the ability to join the E-cadherin/ β -catenin complex to the actin skeleton, and cooperates with other proteins, such as epithelial protein lost in the neoplasm (EPLIN) and vinculin (23). Binding of the p120-catenin to the transmembrane domain of E-cadherin has been shown to be critical for the stability of E-cadherin in AJs (22). E-cadherin is thought to provide the architecture required to form TJs, because the lack of proper E-cadherin expression in the epidermis results in delocalization of TJ proteins, ZO-1, OCLN, and CLDN (24). In addition, siRNA knockdown of E-cadherin resulted in decreased ZO-1 expression in association with reduced epithelial resistance in bronchial epithelial monolayers (25). Various researchers proposed that kinase families of epidermal growth factor receptor (EGFR), Src, and tyrosine phosphatases can be localized on the surface of AJs and cause interactions in the cytoplasmic domain of cadherin, β -catenin, and p120-catenin (26, 27).

Cigarette Smoke-induced Dysfunction of Cellular Junctions in COPD

Smoking has been reported to reduce known apical junction genes in the airway epithelium, of which the majority was further reduced in lung tissue of patients with COPD compared with smokers with a normal lung function (28). We have recently reported that TJ protein expression is disrupted in lung tissue of patients with end-stage COPD as well as in air-liquid interface differentiated epithelial cells from these patients with COPD compared with control subjects (17). This may have important consequences for the pathogenesis of COPD, as outlined subsequently here. Therefore, it is of interest to gain insight into the mechanisms responsible for airway epithelial barrier dysfunction and the impaired ability to redifferentiate into intact epithelium upon smoking in COPD.

Cigarette smoking induces changes in the airway epithelial layer, leading to goblet cell hyperplasia (12) and affecting cilia length as well as cilia recycling by a selective autophagy pathway, named ciliophagy (29). In addition, cigarette smoking impacts on epithelial barrier function (11). Already decades ago, *in vivo* models showed that cigarette smoke induces permeability of the airway mucosa (30). We and others have previously demonstrated that cigarette smoke also transiently impairs epithelial barrier function *in vitro*, disrupting OCLN and ZO-1 junctional expression (11, 12, 31–33). Moreover, Milara and colleagues (34) demonstrated that cigarette smoke extract reduces expression of E-cadherin and ZO-1 *in vitro* in primary epithelial cells from patients with COPD, but not control smokers, an effect that may be caused by reactive oxygen species (ROS)-dependent decrease in cAMP.

Mechanisms of Cigarette Smoke-induced Disruption of Cell-Cell Contacts

Several mechanisms have been implicated in the cigarette smoke-induced barrier dysfunction, which are summarized in Figure 2. We found that cigarette smoke exposure induces disruption of E-cadherin-mediated barrier function in airway epithelial cells *in vitro* by downregulation of A-kinase anchoring

Figure 1. Schematic illustration representing the structure of barriers in normal airway epithelium. In normal airway epithelium, tight junctions (TJs) reside in the apical side and consist of the anchoring proteins, occludin (OCLN), claudins, and junctional adhesion molecules (JAMs). Zonula occludens (ZO)-1, ZO-2, and ZO-3 act as a connector between JAMs and cytoskeleton. Adherens junctions (AJs) are located more basally than TJs and function to connect the actin cytoskeleton to neighboring cells through binding of the Ca^{2+} -dependent E-cadherin-p120 complex to the cytoskeleton through cytoplasmic β -catenin and α -catenin. At the basal side, desmosomes constitute the most basolateral contact. AF6 = afadin 6; β -cat = β -catenin; BM = basement membrane; Cdc42 = cell division cycle 42; ERK = extracellular signal-regulated kinase; PAR = partitioning defective protein.

protein (AKAP)-9 expression (35). AKAP-9 regulates sublocalization of protein kinase (PK) A, which was shown to be involved in localization of E-cadherin to the basolateral membrane (35). As PKA is a downstream effector of cAMP, these findings may help to explain why decreased cAMP levels lead to disrupted expression of E-cadherin (34). Of note, a decrease in E-cadherin protein expression was observed in lung tissue of patients with COPD compared with control subjects matched for smoking history (35). Activation of EGFR and downstream extracellular signal-regulated kinase (ERK) upon the generation of ROS has been observed upon cigarette smoke exposure in airway epithelial cells (31, 36). Cigarette smoke exposure and subsequent ROS production have also been shown to induce EGFR phosphorylation at Tyr-845, leading to Src kinase phosphorylation and inhibiting EGFR degradation (37). In addition, cigarette smoke has been shown to induce EGFR activation through Ras-related C3 botulinum toxin substrate (Rac) 1 and cell division cycle (Cdc) 42 and p120-catenin-dependent mechanism (38, 39). The cigarette smoke extract-induced decrease in transepithelial resistance and cleavage junctional delocalization of scaffolding proteins ZO-1 and OCLN in airway epithelial cells *in vitro* was shown to be EGFR dependent (11). Cigarette smoke extract-induced downregulation of junctional-related genes and reduction of transepithelial resistance in basal airway epithelial cells has also been shown to be mediated by EGFR activation (40, 41). In a recent *in vitro* study, Mishra and colleagues (42) uncovered another mechanism for cigarette smoke-induced airway epithelial barrier dysfunction, in which human epidermal growth factor 2 (HER2)-dependent EGFR activation followed by mitogen-activated protein kinase-mediated IL-6 release decreases transepithelial resistance through an unknown IL-6-dependent mechanism. Cigarette smoke has been demonstrated to activate Rho kinase and phosphorylate ZO-1-binding tyrosine residue in OCLN in airway epithelial cells, thereby dissociating these two proteins and consequently disrupting epithelial integrity (43). Finally, it has been shown that ROS present in cigarette smoke induces fragmentation of hyaluronan in airway epithelial cells *in vitro*, impairing barrier integrity by binding to its epithelial surface receptor

Figure 2. Disruption of airway epithelial junctions in response to cigarette smoke exposure. After cigarette smoke exposure, the airway epithelium and, in particular, intercellular contacts undergo significant changes. Cigarette smoke can induce disruption of TJs upon phosphorylation of the ZO-1 binding tyrosine residue (ZBR) in OCLN. In addition, it can decrease gene expression of ZO-1 and OCLN. Cigarette smoke also increases the production of mitochondrial reactive oxygen species (ROS), which, in turn, activate epidermal growth factor receptor (EGFR) through Src-mediated phosphorylation of ERK signaling. Activated ERK can induce TJ dissociation. In addition, cigarette smoke can induce disruption in cell-cell contacts through EGFR-dependent mechanisms upon its activation by EGF and amphiregulin (AREG) ligands, as well as by EGFR-independent mechanisms. Furthermore, ROS present in cigarette smoke can induce hyaluronan fragmentation, which leads to Rho kinase (ROCK) phosphorylation via its surface receptor layilin. ROCK activation can disrupt AJs through decrease in E-cadherin gene and protein expression. AKAP = A-kinase anchoring protein; CDH1 = E-cadherin; CS = cigarette smoke; fHA = fragmented hyaluronic acid; HA = hyaluronic acid; Ub-Cbl = E3 ubiquitin ligase.

layilin and mediating RhoA/Rho kinase-dependent decrease in E-cadherin expression, both at the gene and protein level (44). Importantly, it has previously been shown that superoxide dismutase (SOD) 3, a susceptibility gene for COPD (45), abrogates hyaluronan fragmentation (46). Increased fragmentation of hyaluronan as a result of lower SOD3 expression in COPD may thus induce disruption of epithelial junctions and increase permeability of the airway epithelium in smokers with COPD. In line, higher levels of low-molecular hyaluronan have been observed in lung tissue of patients with severe COPD (47). Furthermore, in smokers with COPD, a polymorphism in the antioxidant genes, SOD3 as well as glutathione S-transferase isoenzyme, was associated with reduced lung function compared with asymptomatic smokers (48, 49).

Epithelial to Mesenchymal Transition

The loss of epithelial barrier function with downregulation of E-cadherin is an important aspect of a process called epithelial to mesenchymal transition (EMT). EMT is a process involved in cell migration, repair, and tissue remodeling, with loss of epithelial markers and junctional proteins and gain of mesenchymal markers (50). During EMT, E-cadherin-mediated disruption of cell-cell contacts leads to liberation of β -catenin, and its degradation can be prevented by GSK-3 β inactivation upon activation of transforming growth factor (TGF)- β or wntless/integrase-1 (WNT) signaling. Subsequently, β -catenin translocates to the nucleus, where it activates transcription of various genes, including such E-cadherin repressors as Snail1, Slug, zinc finger E-box binding homeobox (ZEB) 1, 2, and mesenchymal markers, such as vimentin, fibronectin, and remodeling metalloproteinase (MMP)-2 and -9 (51). The possible molecular mechanisms responsible for EMT are discussed subsequently here, but there are indications that cigarette smoke-induced oxidative stress can result in epithelial phenotype shift and EMT (34, 52). Reduced antioxidant responses may render susceptible smokers more prone to undergo ROS-induced epithelial barrier disruption and/or have abnormal repair responses to damaging insults, leading to EMT (48, 53–58). These epithelial changes may subsequently contribute to (small) airway

wall remodeling in COPD, inducing abnormal proliferation and differentiation of epithelial cells (59) and aberrant expression of growth factors, MMPs, and extracellular matrix, leading to subepithelial fibrosis, an important hallmark of COPD (34, 52, 60). EMT may lead to increased production of collagen I and MMP-9 (34, 60), promoting thickening of the subepithelial (small) airway wall and airway remodeling. Indeed, the observed fragmentation of the basement membrane (52, 60) may support epithelial migration into the subepithelial layer and EMT *in vivo*, whereas features of EMT in the airway epithelium positively correlated with airway obstruction. Similarly, cigarette smoke has been shown to induce EMT in alveolar epithelial cells (61–63), which may impair alveolar re-epithelization upon damage (64, 65), thus also having implications for the development of emphysema. Cigarette smoke affects WNT/ β -catenin signaling and EMT in alveolar epithelial cells (66, 67), although these studies show an inhibition of β -catenin signaling, which is not in line with previous studies showing the induction of EMT (68, 69).

Of interest, the phenotype switch of airway epithelial cells to a more mesenchymal-like profile by EMT has also been implicated in the pathogenesis of lung cancer (70). The molecular mechanisms underlying EMT process include multiple interconnected cascades with a multitude of drivers that have been implicated in both COPD and lung cancer (70–77), including WNT/ β -catenin, TGF- β , Hedgehog (Hh), integrin-linked kinase, urokinase plasminogen activator receptor (uPAR), and Notch signaling pathways (78). TGF- β , which is recognized as a key regulator of tissue remodeling in COPD (79), is a well-known inducer of the EMT process (e.g., through phosphorylation of Smad2/3/4 complex and non-Smad-associated kinases, such as mitogen-activated protein kinase and Akt) (11, 51, 80–82). Translocation of phosphorylated Smad to the nucleus triggers upregulation of EMT-inducing transcription genes, β 1-integrin and WNT. Our group and others (71, 75, 83) observed aberrant regulation of WNT ligands, WNT-4 and WNT-5B, in cigarette smoke extract-exposed airway epithelial cells from patients with COPD. Cigarette smoke-induced WNT-5B augmented the expression of mesenchymal markers

through TGF- β /Smad3 signaling (71). Hh signaling overlaps with WNT and TGF- β cascades to induce EMT through E-cadherin suppression (84). Of interest, a polymorphism in Hh-interacting protein (HHIP) has been associated with both COPD and lung cancer, and Hh signaling has been implicated in cigarette smoke-induced EMT (85). Altered expression of several Hh and WNT ligands has also been observed in lung cancer tissues and cells, which was associated with tumor invasion (86, 87). In addition, *in vitro* and *in vivo* studies have shown that hypoxia-inducible factor 1- α has been implicated in cigarette smoke-mediated EMT process in both COPD and lung cancer (61). Studies by Wang and colleagues (74, 88, 89) observed a significant correlation between activation of uPAR signaling and airway remodeling in patients with COPD. Accordingly, cigarette smoke extract-induced activation of uPAR induced EMT through phosphatidylinositol (PI) 3-Akt-dependent inhibition of GSK-3 β *in vitro* in airway epithelial cells from patients with COPD. In addition, increased expression of uPA has been observed *in vitro* in airway epithelium of patients with COPD, which may contribute to the emergence of mesenchymal hallmarks (88, 89). More recently, Chen and colleagues found that high-mobility group box (HMGB)-1, which has been found to be increased in COPD (90), induces PI3 kinase/Akt-dependent accumulation of nuclear β -catenin in human airway epithelial cell *in vitro*, resulting in apical junction impairment and EMT phenotype (91, 92). In line, cigarette smoke extract was shown to induce EMT through uPAR-dependent PI3-Akt activation *in vitro* in lung cancer epithelial cells (77) (Figure 3). Hence, airway epithelial barrier dysfunction may be the consequence of abnormal activity of various pathways that have been implicated in the pathogenesis of COPD, leading to abnormal repair and EMT.

Link between Inflammatory Mediators and Permeable Mucosal Barrier

Structural and subsequent functional disruption of apical junctions is a common hallmark of chronic inflammation, particularly in the respiratory and gastrointestinal epithelium (93). Many

mediators of innate and adaptive immunity that may be increased upon chronic cigarette smoke exposure are known to regulate the physical barrier function of the airway epithelium, including cytokines, chemokines, and lipophilic factors (Figure 4). Among cytokines, especially T helper (Th) 2 and 17 cytokines have been proposed as key disruptive factors for epithelial integrity (94, 95). The direct exposure of airway epithelial cells to IL-4 and IL-13 *in vitro* was shown to induce enhanced permeability of the epithelium through the activation of Janus-associated kinase (JAK) (95). Gene expression analysis of the airway epithelium in COPD tissue also suggested an impact of Th2 cytokines on these cells in COPD (96). Results from another gene expression analysis have shown an elevation in IL-13 expression in lung tissue of patients with severe COPD compared with control subjects without COPD (97), and Th2-like eosinophilic inflammation has especially been associated with virus-induced COPD exacerbations (98). Furthermore, higher levels of the Th2 cytokines, IL-4 and IL-13, have been observed in the airway epithelium of smokers with chronic bronchitis versus healthy smokers (99). Thus, especially in a subset of patients, Th2 cytokines may contribute to epithelial barrier dysfunction. Nevertheless, to the best of our knowledge, there is no evidence of association between Th2 cytokine levels and increased permeability in smoke-exposed airway epithelium. Though higher IL-4 levels have been reported in the bronchoalveolar fluids of patients with COPD, reduced IL-4 expression has been observed in lung tissue of patients with COPD compared with control subjects without COPD, and this was shown to be associated with the severity of disease (100, 101). Th17 cells express different isoforms of IL-17 (IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F), and airway epithelial cells express various of these isoforms, including IL-17A and F (102, 103). The number of Th17 cells has been reported to be elevated in blood samples and airway tissue of patients with COPD compared with control subjects without COPD (104, 105). Furthermore, increased expression of IL-17A and IL-17F has been observed in the airway epithelium of stable patients as well as patients with severe COPD, which was accompanied by a decline in lung function (106, 107). An *in vivo* study has revealed that cigarette

Figure 3. Molecular mechanisms involved in cigarette smoke-induced epithelial to mesenchymal transition (EMT) in airway epithelium. Cigarette smoke-induced ROS can activate several signaling pathways in airway epithelium, including urokinase plasminogen activator receptor (uPAR), Hedgehog (Hh), WNT, and transforming growth factor (TGF)- β , leading to dissociation of cellular contacts by suppression of CDH1 expression, and subsequent gaining of mesenchymal characteristics. Activation of uPAR and high-mobility group box (HMGB)-1 by smoke exposure can prevent β -catenin degradation upon its release from E-cadherin-mediated contacts through phosphatidylinositol (PI) 3-Akt-dependent inactivation of GSK-3 β , resulting in its translocation to the nucleus, where it induces mesenchymal genes, including vimentin, fibronectin, metalloproteinase (MMP)-2 and MMP-9, and E-cadherin repressors, Snail, Slug, and zinc finger E-box-binding homeobox (ZEB). In addition, TGF- β can induce EMT through Smad-dependent pathways. Nuclear translocation of the phosphorylated Smad2/3 complex can lead to activation of EMT-inducing WNT and β 1-integrin transcription genes. WNT-5B can also induce EMT through activation of the TGF- β /Smad3 pathway. Non-Smad TGF- β pathway acts through mitogen-activated protein kinase (MAPK)/Akt-dependent inactivation of GSK-3 β and further translocation of liberated β -catenin, which overlaps with uPAR and WNT signaling pathways.

smoke increases secretion of IL-17 from the airway epithelium (102). Recently, Ramezanpour and colleagues (94) found that Th17 cytokines are the predominant

inducers of AJs disruption in a rhinosinusitis animal model, whereas no significant change was observed with either Th1 or Th2 cytokines. In contrast, earlier

findings indicate that IL-17 is not able to induce epithelial barrier dysfunction in primary cultures of human sinonasal epithelial cells from patients with rhinosinusitis, whereas IL-4 and IFN- γ induced epithelial barrier disruption (108). Higher levels of the Th1 cytokine, IFN- γ , have been observed in the lung tissue, BAL fluid, and sputum samples of patients with COPD (109–112). Pretreatment with both IFN- γ and proinflammatory mediator, TNF- α , has been described to induce EGFR-mediated airway junctional disintegration in epithelial cells *in vitro* (113, 114). In addition, recent evidence shows that TNF- α can induce loss of E-cadherin expression in a Src-dependent fashion in airway epithelial cells *in vitro* (115, 116). Although earlier studies showed increased levels of TNF- α both in sputum and BAL fluid of patients with COPD (110, 117), lower levels of TNF- α have been observed in sputum samples of patients with COPD compared with the control (112). Another proinflammatory cytokine that may participate in airway junctional dysfunction in COPD is IL-1 β . Decades ago, Rusznak and coworkers (118) observed a marked elevation in IL-1 β levels from mainstream cigarette smoke-exposed airway epithelial cells of smokers with COPD and asymptomatic smokers compared with nonsmokers. Recent investigations have reported that, upon *in vitro* exposure of airway epithelial cells to exogenous IL-1 β , HER2 is activated through a disintegrin and metalloproteinase (ADAM) 17-dependent release of neuregulin (NRG)-1 ligand, which resulted in dissociated intercellular β -catenin–E-cadherin adhesion complex and a reduction in barrier function (119). As described subsequently here, IL-6, as a cytokine extensively described for its implication in pathogenesis of COPD (120), has also been demonstrated to disrupt airway epithelial integrity upon HER2 activation (42). Together, several of the proinflammatory mediators in COPD have been shown capable of inducing airway epithelial barrier function. Of these, TNF- α , IFN- γ , and IL-1 β are most likely to contribute to barrier dysfunction in COPD, as overviewed in Figure 4.

In turn, loss of barrier function may lead to alterations in the production of immune modulators by the airway epithelium. Findings from our group indicate that airway epithelial disruption

Figure 4. Proinflammatory mediators regulating epithelial barrier function. The inflammatory responses mediated by various cytokines, including T-helper cell type 1 (Th1), Th2, and Th17, and chemokines (IL-1 β and TNF- α) can alter different intercellular signaling pathways, leading to barrier dysfunction. Increased release of IL-6 upon human epidermal growth factor (HER) 2 activation leads to decline in barrier function. Moreover, HER2 activation mediated by IL-1 β can break β -catenin–E-cadherin complex. TNF- α can activate Src kinase, which leads to AJ disruption by downregulation of E-cadherin. Activation of Janus-associated kinase (JAK) upon Th2 cytokines, IL-4 and IL-13, leads to enhanced permeability of airway epithelium. On the other hand, IFN- γ in combination with TNF- α can affect epithelial barrier function through EGFR-mediated TJ disruption. Increased IL-17A can also induce airway epithelial barrier disruption through an unknown pathway.

induced by siRNA knockdown of E-cadherin promotes the release of proinflammatory cytokines by activation of EGFR and downstream signaling pathways (121). In line, Hackett and colleagues (122) reported an increased proinflammatory cytokine response in air–liquid interface–differentiated airway epithelial cultures upon epithelial damage. These observations reinforce the importance of airway barrier function in the regulation of immune mediators.

There are also barrier-protective mediators released by airway epithelial cells that may alter in response to cigarette smoke–induced barrier dysfunction (123). Club cell secretory protein-10 (CC10) acts as an essential barrier protective factor for airway epithelium (124). Downregulation

of CC10 has been observed in lung tissue of patients with COPD and cigarette smoke–exposed animals, and may indirectly contribute to the leaky manifestation of airway epithelium (124–127). Moreover, we reported an association between the elevated expression of RNase7, an epithelial antimicrobial peptide, and EGFR-dependent airway epithelial barrier disruption induced by cigarette smoke, implying a protective role for RNase7 upon disruption of the epithelial barrier (32). Herr and colleagues (128) reported that cigarette smoke reduces bacteria-induced expression of the antimicrobial peptide, hBD-2/DEFB4, and we have recently extended these findings to show that whole smoke derived from a single cigarette not only caused a transient

decrease in epithelial barrier function, but also impaired production of inducible antimicrobial peptides, such as hBD-2/DEFB4, S100A7, and lipocalin/LCN2 (129). Furthermore, we showed that antibacterial activity and expression of selected antimicrobial peptides were decreased in differentiated cultures of patients with moderate COPD compared with smoking control subjects, whereas no difference in epithelial barrier activity was noted. This indicates that both the chemical barrier function of the airway epithelium provided by antimicrobial peptides and physical barrier are impaired by smoke exposure and affected in COPD.

Cigarette smoking may also impact directly on the microbiome. For instance, it

has been reported that *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae* are overrepresented in smokers compared with nonsmokers (123). Furthermore, it has been shown that upper airways from smokers display higher microbial diversity than nonsmokers (130), with an overrepresentation of *Eubacterium* spp., *Abiotrophia* spp., *Anaerovorax*, *Eggerthella*, *Dorea*, and *Erysipelotrichaceae* I.S. in the nasopharynx of smokers compared with nonsmokers. In COPD, an overrepresentation of the *Proteobacteria* phylum (131, 132) and the *Firmicutes* phylum (133–135) has been observed. As far as we know, there are no studies available assessing the effect of *Firmicutes* on human airway epithelial barrier function, whereas various studies have shown adverse effects of respiratory pathogens on cultured airway epithelial cells. For instance, products of *Proteobacterium Pseudomonas aeruginosa* are cytotoxic to airway epithelial cells, and will thus impact on airway epithelial barrier integrity (136). Furthermore, in a previous study, we have shown that *S. pneumoniae* reduces transepithelial resistance in human airway epithelial cells (137). Therefore, smoking may affect epithelial barrier function through direct and indirect effects on the lung microbiome.

In addition to the effects on the airway epithelium, cigarette smoke may affect the respiratory host defense to microbes by effects on additional innate, as well as adaptive, immune cells (4). A number of studies have shown that cigarette smoke diminishes the phagocytic ability of alveolar macrophages to engulf apoptotic cells, a process known as efferocytosis (138–140), which is also impaired in COPD (141). This impaired efferocytosis may lead to necrotic processes, which increase danger signals and proinflammatory mediators (142), thus promoting barrier disruption. In addition, it has been shown that cigarette smoke induces a decrease in alveolar macrophage responses to double-stranded RNA by downregulation of Toll-like receptor 3, thereby making patients with COPD more susceptible to undergoing viral exacerbation (143). Cigarette smoke increases Th17 responses by overexpression of IL-17A in lung CD4⁺ and $\gamma\delta$ T lymphocytes *in vivo*, which may affect barrier function, as described previously here (144).

Recent Developments in Therapeutic Approaches Based on the Restoration of Airway Epithelial Barrier Activity in COPD

Remodeling of the airway wall is a hallmark of COPD that mainly arises from long-term detrimental smoking, leading to persistent inflammation and tissue damage. The repair response of airway epithelial cells in COPD is thought to be abnormal, with an inability to restore epithelial integrity and normal function of the intact, fully differentiated layer. Therapeutic interventions specifically targeting the restoration of epithelial barrier function may be beneficial in COPD, but are currently lacking. The current therapies for COPD are aimed at suppression of inflammation and bronchodilation, including inhaled corticosteroids and long-acting bronchodilators. These drugs do not halt or reverse disease progression, although they may slow it down and provide temporary relief of symptoms during exacerbation (145). The GLUCOLD study showed an improvement in lung function of patients with COPD upon treatment with corticosteroids (146). Pathway analysis with gene set enrichment analysis on genome-wide gene expression has shown that this improvement in lung function is associated with upregulation of genes that are enriched for epithelial barrier function (147). This indicates that corticosteroids may affect epithelial barrier function, and further supports the notion that loss of barrier function is related to lung function decline in COPD. In line with these findings, we showed that the inhaled corticosteroid budesonide protects against cigarette smoke-induced airway epithelial barrier disruption *in vitro*, which likely involved modulation of EGFR-dependent pathways (137). However, pretreatment of airway epithelial cells with dexamethasone was not sufficient to reverse TGF- β -induced EMT (148). In addition to corticosteroids, Milara and colleagues (34, 149, 150) showed that treatment with cAMP-elevating compounds successfully restores airway epithelial barrier dysfunction induced by either cigarette smoke extract or TGF- β *in vitro*. Therefore, it will be of interest to study effects of PDE4 inhibitors on epithelial barrier dysfunction in COPD.

Schamberger and colleagues (12) showed that treatment with exogenous TGF- β 1 restores the cigarette smoke extract-induced damage to the airway epithelial barrier by upregulation of junctional proteins (ZO-1 and ZO-2) *in vitro*. This is in contrast with the previously defined role for TGF- β 1 in tissue remodeling in COPD (72). Pretreatment of airway epithelial cells with EGF has also been shown to protect epithelial TJs against cigarette smoke extract-induced junctional damage *in vitro* (33) and to promote airway epithelial repair *in vitro* (151), which is again in contrast to the role of EGFR in smoke-induced barrier dysfunction. Regardless of this, due to their pleiotropic effects, TGF and EGF may not be suitable therapeutic strategies to improve epithelial barrier function.

Several studies have noted effectiveness of pharmacological inhibition in restoration of TJ activity. Rezaee and colleagues (152) have provided new understanding regarding the mechanism of airway barrier disruption induced by respiratory syncytial virus and showed that PKD inhibition attenuates respiratory syncytial virus-induced disruption of junctional assembly *in vitro*. In line with this, inhibition of PKD3 at baseline has been shown to enhance electrical resistance of airway epithelial cells *in vitro*, possibly via upregulation of CLDN1 (153). Moreover, as described previously here, the use of AKAP inhibitor St-Ht31 peptides has been demonstrated to counteract the cigarette smoke extract-induced impairment of E-cadherin mediated cell–cell contacts in 16HBE cells (35), and may thus have therapeutic benefits.

Recently published *in vitro* studies raise attention to the capability of chemotherapy with various compounds to block pathways involved in the disruption of the airway epithelial barrier upon smoke exposure (154, 155). Furthermore, a recent study showed that treatment with vitamin D may rescue cigarette smoke extract-induced disruption in airway epithelial E-cadherin *in vitro* through downregulation of ERK pathway (156). Finally, it has been shown that corilagin, a polyphenolic compound, can restore the integrity of lung epithelial cellular junctions in cigarette smoke-induced disrupted TJ-related protein connexin 40, possibly through its antioxidant properties (157). Among these compounds, PKD inhibitors

Table 1. Potential Therapeutic Candidates Regulating Airway Epithelial Barrier Function

Therapeutic compounds	Molecular Targets	Effects on Airway Epithelial Barrier Function	Type of Study	Reference
Budesonide	EGFR	Protection against cigarette smoke–induced barrier disruption by increase in TEER and ZO-1 expression	<i>In vitro</i> /16HBE cells	137
EGF			<i>In vitro</i> /differentiated HBECs	33
Exogenous TGF- β	TGF- β receptor	Upregulation of ZO-1 and ZO-2 and inhibition of TEER decrease in cigarette smoke–induced barrier disruption	<i>In vitro</i> /16HBE and HBECs	12
PDE4 inhibitor	cAMP	Inhibition of cigarette smoke–induced E-cadherin and ZO-1 downregulation	<i>In vitro</i> /differentiated HBECs	34, 149, 150
PKD inhibitor	PKD3	Increase in TEER and CLDN1 expression upon calcium depletion	<i>In vitro</i> /16HBE cells	153
AKAP inhibitor	AKAP-cAMP	Reversion of cigarette smoke–induced impairment of cell membrane E-cadherin	<i>In vitro</i> /16HBE cells	35
Vitamin D	ERK	Rescue of E-cadherin and β -catenin protein loss and maintenance of TEER upon cigarette smoke extract exposure	<i>In vitro</i> /16HBE cells	156
Corilagin	NF- κ B	Prevention of cigarette smoke–induced decrease in TJ-related connexin 40 gene expression and protein levels	<i>In vitro</i> /Calu-3	157

Definition of abbreviations: AKAP = A-kinase anchoring protein; cAMP = cyclic adenosine monophosphate; CLDN = claudin; EGF = epithelial growth factor; EGFR = EGF receptor; ERK = extracellular signal-regulated kinase; HBECs = human bronchial epithelial cells; PDE4 = phosphodiesterase 4; PKD = protein kinase D; TEER = transepithelial electrical resistance; TGF- β = transforming growth factor- β ; TJ = tight junction; ZO-1/ZO-2 = zonula occludens-1/2.

have exerted the most promising effect on restoration of airway epithelial barrier function by means of recovering both AJs and TJs.

Concluding Remarks and Future Directions

Together, evidence for loss of epithelial junctions and dysregulated airway epithelial barrier function in patients with COPD is emerging. Both oxidative stress and proinflammatory responses induced by cigarette smoke may disrupt airway epithelial barrier function. Subsequently, EMT may contribute to abnormal repair of the airway epithelium in COPD. Polymorphisms in specific genes associated with COPD may contribute to increased

susceptibility to cigarette smoke–induced damage, as well as the abnormal repair response. Insight in to the mechanisms of loss of epithelial integrity in COPD has been provided by *in vitro* and *in vivo* studies, including ROS-induced EGFR activation, but these cannot completely mimic the chronic nature of the disease. Thus, more insight may be provided by junctional gene knockdown in animal models. Table 1 summarizes the results of *in vitro* studies on airway epithelial barrier function. We suggest that restoration of airway barrier function in COPD with drug interventions that restore epithelial barrier function, regulate EMT and epithelial repair, and especially those that target EGFR and its downstream signaling, may be beneficial. Such strategies should be

addressed in future studies. In addition, it will be of interest to assess the effects of cigarette smoke on alveolar barrier function as well as the impact of the altered microbiome in COPD on lung epithelial barrier function. There is also an increasing interest in emerging smoke products, such as flavored electronic cigarettes, and various studies have evaluated their effects on epithelial cell function. These studies show that the toxicity of such products is less compared with conventional tobacco cigarettes, but do highlight a range of possible adverse effects of electronic cigarettes on epithelial function (158). The importance of such findings for lung health requires further investigation. ■

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References

- Hiemstra PS, McCray PB Jr, Bals R. The innate immune function of airway epithelial cells in inflammatory lung disease. *Eur Respir J* 2015; 45:1150–1162.
- Nawijn MC, Hackett TL, Postma DS, van Oosterhout AJ, Heijink IH. E-cadherin: gatekeeper of airway mucosa and allergic sensitization. *Trends Immunol* 2011;32:248–255.
- Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2013;187: 347–365.
- Centers for Disease Control and Prevention (US), National Center for Chronic Disease Prevention and Health Promotion (US) and Office on Smoking and Health (US). The health consequences of smoking: 50 years of progress: a report of the Surgeon General. Atlanta: Centers for Disease Control and Prevention; 2014.
- Hogg JC, Timens W. The pathology of chronic obstructive pulmonary disease. *Annu Rev Pathol* 2009;4:435–459.
- Wood AM, Stockley RA. The genetics of chronic obstructive pulmonary disease. *Respir Res* 2006;7:130.
- van der Toorn M, Rezayat D, Kauffman HF, Bakker SJ, Gans RO, Koëter GH, et al. Lipid-soluble components in cigarette smoke induce mitochondrial production of reactive oxygen species in lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L109–L114.

8. Rigden HM, Alias A, Havelock T, O'Donnell R, Djukanovic R, Davies DE, *et al.* Squamous metaplasia is increased in the bronchial epithelium of smokers with chronic obstructive pulmonary disease. *PLoS One* 2016; 11:e0156009.
9. Yaghi A, Zaman A, Cox G, Dolovich MB. Ciliary beating is depressed in nasal cilia from chronic obstructive pulmonary disease subjects. *Respir Med* 2012;106:1139–1147.
10. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, *et al.* The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004;350:2645–2653.
11. Heijink IH, Brandenburg SM, Postma DS, van Oosterhout AJ. Cigarette smoke impairs airway epithelial barrier function and cell-cell contact recovery. *Eur Respir J* 2012;39:419–428.
12. Schamberger AC, Mise N, Jia J, Genoyer E, Yildirim AO, Meiners S, *et al.* Cigarette smoke-induced disruption of bronchial epithelial tight junctions is prevented by transforming growth factor- β . *Am J Respir Cell Mol Biol* 2014;50:1040–1052.
13. Ganesan S, Comstock AT, Sajjan US. Barrier function of airway tract epithelium. *Tissue Barriers* 2013;1:e24997.
14. Bals R, Hiemstra PS. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *Eur Respir J* 2004;23:327–333.
15. Cereijido M, Valdés J, Shoshani L, Contreras RG. Role of tight junctions in establishing and maintaining cell polarity. *Annu Rev Physiol* 1998; 60:161–177.
16. Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. *N Engl J Med* 2008;359:2355–2365.
17. Heijink IH, Noordhoek JA, Timens W, van Oosterhout AJ, Postma DS. Abnormalities in airway epithelial junction formation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2014; 189:1439–1442.
18. Krause G, Winkler L, Mueller SL, Haseloff RF, Piontek J, Blasig IE. Structure and function of claudins. *Biochim Biophys Acta* 2008;1778:631–645.
19. McCarthy KM, Skare IB, Stankewich MC, Furuse M, Tsukita S, Rogers RA, *et al.* Occludin is a functional component of the tight junction. *J Cell Sci* 1996;109:2287–2298.
20. Ebnet K, Suzuki A, Ohno S, Vestweber D. Junctional adhesion molecules (JAMs): more molecules with dual functions? *J Cell Sci* 2004;117:19–29.
21. Anderson JM. Molecular structure of tight junctions and their role in epithelial transport. *News Physiol Sci* 2001;16:126–130.
22. Hartsock A, Nelson WJ. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta* 2008;1778:660–669.
23. Dufour S, Mège RM, Thiery JP. α -catenin, vinculin, and F-actin in strengthening E-cadherin cell-cell adhesions and mechanosensing. *Cell Adhes Migr* 2013;7:345–350.
24. Tunggal JA, Helfrich I, Schmitz A, Schwarz H, Günzel D, Fromm M, *et al.* E-cadherin is essential for *in vivo* epidermal barrier function by regulating tight junctions. *EMBO J* 2005;24:1146–1156.
25. Heijink IH, Brandenburg SM, Noordhoek JA, Postma DS, Slebos DJ, van Oosterhout AJ. Characterisation of cell adhesion in airway epithelial cell types using electric cell-substrate impedance sensing. *Eur Respir J* 2010;35:894–903.
26. Niessen CM, Gottardi CJ. Molecular components of the adherens junction. *Biochim Biophys Acta* 2008;1778:562–571.
27. Hoschuetzky H, Aberle H, Kemler R. β -catenin mediates the interaction of the cadherin-catenin complex with epidermal growth factor receptor. *J Cell Biol* 1994;127:1375–1380.
28. Shaykhiev R, Otaki F, Bonsu P, Dang DT, Teater M, Strulovici-Barel Y, *et al.* Cigarette smoking reprograms apical junctional complex molecular architecture in the human airway epithelium *in vivo*. *Cell Mol Life Sci* 2011;68:877–892.
29. Cloonan SM, Lam HC, Ryter SW, Choi AM. “Ciliophagy”: the consumption of cilia components by autophagy. *Autophagy* 2014;10:532–534.
30. Hogg JC. Bronchial mucosal permeability and its relationship to airways hyperreactivity. *Eur J Respir Dis Suppl* 1982;122:17–22.
31. Petecchia L, Sabatini F, Varesio L, Camoirano A, Usai C, Pezzolo A, *et al.* Bronchial airway epithelial cell damage following exposure to cigarette smoke includes disassembly of tight junction components mediated by the extracellular signal-regulated kinase 1/2 pathway. *Chest* 2009;135:1502–1512.
32. Amatngalim GD, van Wijck Y, de Mooij-Eijk Y, Verhoosel RM, Harder J, Lekkerkerker AN, *et al.* Basal cells contribute to innate immunity of the airway epithelium through production of the antimicrobial protein RNase 7. *J Immunol* 2015;194:3340–3350.
33. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, *et al.* Defective epithelial barrier function in asthma. *J Allergy Clin Immunol* 2011;128:549–556.e541–512.
34. Milara J, Peiró T, Serrano A, Cortijo J. Epithelial to mesenchymal transition is increased in patients with COPD and induced by cigarette smoke. *Thorax* 2013;68:410–420.
35. Oldenburger A, Poppinga WJ, Kos F, de Bruin HG, Rijks WF, Heijink IH, *et al.* A-kinase anchoring proteins contribute to loss of E-cadherin and bronchial epithelial barrier by cigarette smoke. *Am J Physiol Cell Physiol* 2014;306: C585–C597.
36. Heijink IH, van Oosterhout A, Kapus A. Epidermal growth factor receptor signalling contributes to house dust mite-induced epithelial barrier dysfunction. *Eur Respir J* 2010;36:1016–1026.
37. Khan EM, Lanir R, Danielson AR, Goldkorn T. Epidermal growth factor receptor exposed to cigarette smoke is aberrantly activated and undergoes perinuclear trafficking. *FASEB J* 2008;22:910–917.
38. Zhang L, Gallup M, Zlock L, Finkbeiner W, McNamara NA. p120-catenin modulates airway epithelial cell migration induced by cigarette smoke. *Biochem Biophys Res Commun* 2012;417:49–55.
39. Zhang L, Gallup M, Zlock L, Finkbeiner WE, McNamara NA. Rac1 and Cdc42 differentially modulate cigarette smoke-induced airway cell migration through p120-catenin-dependent and -independent pathways. *Am J Pathol* 2013;182:1986–1995.
40. Shaykhiev R, Zuo WL, Chao I, Fukui T, Witover B, Brekman A, *et al.* EGF shifts human airway basal cell fate toward a smoking-associated airway epithelial phenotype. *Proc Natl Acad Sci USA* 2013;110:12102–12107.
41. Zuo WL, Yang J, Gomi K, Chao I, Crystal RG, Shaykhiev R. EGF-amphiregulin interplay in airway stem/progenitor cells links the pathogenesis of smoking-induced lesions in the human airway epithelium. *Stem Cells* 2017;35:824–837.
42. Mishra R, Foster D, Vasu VT, Thaikootathil JV, Kosmider B, Chu HW, *et al.* Cigarette smoke induces human epidermal receptor 2-dependent changes in epithelial permeability. *Am J Respir Cell Mol Biol* 2016;54:853–864.
43. Olivera D, Knall C, Boggs S, Seagrave J. Cytoskeletal modulation and tyrosine phosphorylation of tight junction proteins are associated with mainstream cigarette smoke-induced permeability of airway epithelium. *Exp Toxicol Pathol* 2010;62:133–143.
44. Forteza RM, Casalino-Matsuda SM, Falcon NS, Valencia Gattas M, Monzon ME. Hyaluronan and layilin mediate loss of airway epithelial barrier function induced by cigarette smoke by decreasing E-cadherin. *J Biol Chem* 2012;287:42288–42298.
45. Siedlinski M, van Diemen CC, Postma DS, Vonk JM, Boezen HM. Superoxide dismutases, lung function and bronchial responsiveness in a general population. *Eur Respir J* 2009;33:986–992.
46. Gao F, Koenitzer JR, Tobolewski JM, Jiang D, Liang J, Noble PW, *et al.* Extracellular superoxide dismutase inhibits inflammation by preventing oxidative fragmentation of hyaluronan. *J Biol Chem* 2008; 283:6058–6066.
47. Dentener MA, Vernooy JH, Hendriks S, Wouters EF. Enhanced levels of hyaluronan in lungs of patients with COPD: relationship with lung function and local inflammation. *Thorax* 2005; 60:114–119.
48. Cheng SL, Yu CJ, Chen CJ, Yang PC. Genetic polymorphism of epoxide hydrolase and glutathione S-transferase in COPD. *Eur Respir J* 2004;23:818–824.
49. Dahl M, Bowler RP, Juul K, Crapo JD, Levy S, Nordestgaard BG. Superoxide dismutase 3 polymorphism associated with reduced lung function in two large populations. *Am J Respir Crit Care Med* 2008;178:906–912.
50. Hackett TL. Epithelial-mesenchymal transition in the pathophysiology of airway remodelling in asthma. *Curr Opin Allergy Clin Immunol* 2012;12:53–59.
51. Bartis D, Mise N, Mahida RY, Eickelberg O, Thickett DR. Epithelial-mesenchymal transition in lung development and disease: does it exist and is it important? *Thorax* 2014;69:760–765.

52. Gohy ST, Hupin C, Fregimillicka C, Detry BR, Bouzin C, Gaide Chevroney H, *et al.* Imprinting of the COPD airway epithelium for dedifferentiation and mesenchymal transition. *Eur Respir J* 2015;45: 1258–1272.
53. Hackett NR, Heguy A, Harvey BG, O'Connor TP, Luetlich K, Flieder DB, *et al.* Variability of antioxidant-related gene expression in the airway epithelium of cigarette smokers. *Am J Respir Cell Mol Biol* 2003;29: 331–343.
54. Ammous Z, Hackett NR, Butler MW, Raman T, Dolgalev I, O'Connor TP, *et al.* Variability in small airway epithelial gene expression among normal smokers. *Chest* 2008;133:1344–1353.
55. Pierrou S, Broberg P, O'Donnell RA, Pawlowski K, Virtala R, Lindqvist E, *et al.* Expression of genes involved in oxidative stress responses in airway epithelial cells of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2007;175:577–586.
56. Tilley AE, O'Connor TP, Hackett NR, Strulovici-Barel Y, Salit J, Amoroso N, *et al.* Biologic phenotyping of the human small airway epithelial response to cigarette smoking. *PLoS One* 2011;6:e22798.
57. Juul K, Tybjaerg-Hansen A, Marklund S, Lange P, Nordestgaard BG. Genetically increased antioxidative protection and decreased chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2006;173:858–864.
58. Young RP, Hopkins R, Black PN, Eddy C, Wu L, Gamble GD, *et al.* Functional variants of antioxidant genes in smokers with COPD and in those with normal lung function. *Thorax* 2006;61:394–399.
59. Ganesan S, Sajjan US. Repair and remodeling of airway epithelium after injury in chronic obstructive pulmonary disease. *Curr Respir Care Rep* 2013;2:145–154.
60. Sohal SS, Walters EH. Epithelial mesenchymal transition (EMT) in small airways of COPD patients. *Thorax* 2013;68:783–784.
61. Eurlings IM, Reynaert NL, van den Beucken T, Gosker HR, de Theije CC, Verhamme FM, *et al.* Cigarette smoke extract induces a phenotypic shift in epithelial cells; involvement of HIF1 α in mesenchymal transition. *PLoS One* 2014;9:e107757.
62. Shen HJ, Sun YH, Zhang SJ, Jiang XJ, Dong XW, Jia YL, *et al.* Cigarette smoke-induced alveolar epithelial-mesenchymal transition is mediated by Rac1 activation. *Biochim Biophys Acta* 2014;1840: 1838–1849.
63. Checa M, Hagood JS, Velazquez-Cruz R, Ruiz V, García-De-Alba C, Rangel-Escareño C, *et al.* Cigarette smoke enhances the expression of profibrotic molecules in alveolar epithelial cells. *PLoS One* 2016; 11:e0150383.
64. Chilosi M, Carloni A, Rossi A, Poletti V. Premature lung aging and cellular senescence in the pathogenesis of idiopathic pulmonary fibrosis and COPD/emphysema. *Transl Res* 2013;162:156–173.
65. Zhong Q, Zhou B, Ann DK, Minoo P, Liu Y, Banfalvi A, *et al.* Role of endoplasmic reticulum stress in epithelial-mesenchymal transition of alveolar epithelial cells: effects of misfolded surfactant protein. *Am J Respir Cell Mol Biol* 2011;45:498–509.
66. Baarsma HA, Skronska-Wasek W, Mutze K, Ciolek F, Wagner DE, John-Schuster G, *et al.* Noncanonical WNT-5A signaling impairs endogenous lung repair in COPD. *J Exp Med* 2017;214:143–163.
67. Skronska-Wasek W, Mutze K, Baarsma HA, Bracke KR, Alsafadi HN, Lehmann M, *et al.* Reduced frizzled receptor 4 expression prevents WNT/ β -catenin-driven alveolar lung repair in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2017;196:172–185.
68. van der Velden JIJ, Guala AS, Leggett SE, Sluimer J, Badura EC, Janssen-Heininger YMW. Induction of a mesenchymal expression program in lung epithelial cells by wingless protein (Wnt)/ β -catenin requires the presence of c-Jun N-terminal kinase-1 (JNK1). *Am J Respir Cell Mol Biol* 2012;47:306–314.
69. Zou W, Zou Y, Zhao Z, Li B, Ran P. Nicotine-induced epithelial-mesenchymal transition via Wnt/ β -catenin signaling in human airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2013;304: L199–L209.
70. Vu T, Jin L, Datta PK. Effect of cigarette smoking on epithelial to mesenchymal transition (EMT) in lung cancer. *J Clin Med* 2016;5: E44.
71. Heijink IH, de Bruin HG, Dennebos R, Jonker MR, Noordhoek JA, Brandsma CA, *et al.* Cigarette smoke-induced epithelial expression of WNT-5B: implications for COPD. *Eur Respir J* 2016;48:504–515.
72. Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, *et al.* Increased expression of transforming growth factor-beta1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med* 2001;163:1476–1483.
73. Tilley AE, Harvey BG, Heguy A, Hackett NR, Wang R, O'Connor TP, *et al.* Down-regulation of the notch pathway in human airway epithelium in association with smoking and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2009;179:457–466.
74. Zhang Y, Xiao W, Jiang Y, Wang H, Xu X, Ma D, *et al.* Levels of components of the urokinase-type plasminogen activator system are related to chronic obstructive pulmonary disease parenchymal destruction and airway remodelling. *J Int Med Res* 2012;40:976–985.
75. Heijink IH, de Bruin HG, van den Berge M, Bennink LJ, Brandenburg SM, Gosens R, *et al.* Role of aberrant WNT signalling in the airway epithelial response to cigarette smoke in chronic obstructive pulmonary disease. *Thorax* 2013;68:709–716.
76. Zhou X, Baron RM, Hardin M, Cho MH, Zielinski J, Hawrykiewicz I, *et al.* Identification of a chronic obstructive pulmonary disease genetic determinant that regulates HHIP. *Hum Mol Genet* 2012;21: 1325–1335.
77. Wang Q, Wang H, Zhang Y, Zhang Y, Xiao W. Activation of uPAR is required for cigarette smoke extract-induced epithelial-mesenchymal transition in lung epithelial cells. *Oncol Res* 2013; 21:295–305.
78. Nowrin K, Sohal SS, Peterson G, Patel R, Walters EH. Epithelial-mesenchymal transition as a fundamental underlying pathogenic process in COPD airways: fibrosis, remodeling and cancer. *Expert Rev Respir Med* 2014;8:547–559.
79. Aschner Y, Downey GP. Transforming growth factor- β : master regulator of the respiratory system in health and disease. *Am J Respir Cell Mol Biol* 2016;54:647–655.
80. Zhang L, Gallup M, Zlock L, Basbaum C, Finkbeiner WE, McNamara NA. Cigarette smoke disrupts the integrity of airway adherens junctions through the aberrant interaction of p120-catenin with the cytoplasmic tail of MUC1. *J Pathol* 2013;229:74–86.
81. Gohy ST, Hupin C, Pilette C, Ladjemi MZ. Chronic inflammatory airway diseases: the central role of the epithelium revisited. *Clin Exp Allergy* 2016;46:529–542.
82. Câmara J, Jarai G. Epithelial-mesenchymal transition in primary human bronchial epithelial cells is Smad-dependent and enhanced by fibronectin and TNF- α . *Fibrogenesis Tissue Repair* 2010;3:2.
83. Durham AL, McLaren A, Hayes BP, Caramori G, Clayton CL, Barnes PJ, *et al.* Regulation of Wnt4 in chronic obstructive pulmonary disease. *FASEB J* 2013;27:2367–2381.
84. Katoh Y, Katoh M. Hedgehog signaling, epithelial-to-mesenchymal transition and miRNA [review]. *Int J Mol Med* 2008;22:271–275.
85. Young RP, Whittington CF, Hopkins RJ, Hay BA, Epton MJ, Black PN, *et al.* Chromosome 4q31 locus in COPD is also associated with lung cancer. *Eur Respir J* 2010;36:1375–1382.
86. Hong Z, Bi A, Chen D, Gao L, Yin Z, Luo L. Activation of hedgehog signaling pathway in human non-small cell lung cancers. *Pathol Oncol Res* 2014;20:917–922.
87. Harada T, Yamamoto H, Kishida S, Kishida M, Awada C, Takao T, *et al.* Wnt5b-associated exosomes promote cancer cell migration and proliferation. *Cancer Sci* 2017;108:42–52.
88. Wang Q, Wang Y, Zhang Y, Zhang Y, Xiao W. The role of uPAR in epithelial-mesenchymal transition in small airway epithelium of patients with chronic obstructive pulmonary disease. *Respir Res* 2013;14:67.
89. Wang Q, Wang Y, Zhang Y, Zhang Y, Xiao W. Involvement of urokinase in cigarette smoke extract-induced epithelial-mesenchymal transition in human small airway epithelial cells. *Lab Invest* 2015;95: 469–479.
90. Ferhani N, Letuve S, Kozhich A, Thibaudou O, Grandsaigne M, Maret M, *et al.* Expression of high-mobility group box 1 and of receptor for advanced glycation end products in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010;181:917–927.
91. Chen YC, Statt S, Wu R, Chang HT, Liao JW, Wang CN, *et al.* High mobility group box 1-induced epithelial mesenchymal transition in human airway epithelial cells. *Sci Rep* 2016;6:18815.

92. Xia Q, Xu J, Chen H, Gao Y, Gong F, Hu L, *et al.* Association between an elevated level of HMGB1 and non-small-cell lung cancer: a meta-analysis and literature review. *Onco Targets Ther* 2016;9:3917–3923.
93. Rezaee F, Georas SN. Breaking barriers: new insights into airway epithelial barrier function in health and disease. *Am J Respir Cell Mol Biol* 2014;50:857–869.
94. Ramezani M, Moraitis S, Smith JL, Wormald PJ, Vreugde S. Th17 cytokines disrupt the airway mucosal barrier in chronic rhinosinusitis. *Mediators Inflamm* 2016;2016:9798206.
95. Saatian B, Rezaee F, Desando S, Emo J, Chapman T, Knowlden S, *et al.* Interleukin-4 and interleukin-13 cause barrier dysfunction in human airway epithelial cells. *Tissue Barriers* 2013;1:e24333.
96. Christenson SA, Steiling K, van den Berge M, Hijazi K, Hiemstra PS, Postma DS, *et al.* Asthma-COPD overlap: clinical relevance of genomic signatures of type 2 inflammation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2015;191:758–766.
97. Alevy YG, Patel AC, Romero AG, Patel DA, Tucker J, Roswit WT, *et al.* IL-13-induced airway mucus production is attenuated by MAPK13 inhibition. *J Clin Invest* 2012;122:4555–4568.
98. Papi A, Bellettato CM, Braccioni F, Romagnoli M, Casolari P, Caramori G, *et al.* Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. *Am J Respir Crit Care Med* 2006;173:1114–1121.
99. Miotto D, Ruggieri MP, Boschetto P, Cavallero G, Papi A, Bononi I, *et al.* Interleukin-13 and -4 expression in the central airways of smokers with chronic bronchitis. *Eur Respir J* 2003;22:602–608.
100. Perotin JM, Adam D, Vella-Boucaud J, Delepine G, Sandu S, Jonvel AC, *et al.* Delay of airway epithelial wound repair in COPD is associated with airflow obstruction severity. *Respir Res* 2014;15:151.
101. Barceló B, Pons J, Fuster A, Sauleda J, Noguera A, Ferrer JM, *et al.* Intracellular cytokine profile of T lymphocytes in patients with chronic obstructive pulmonary disease. *Clin Exp Immunol* 2006;145:474–479.
102. Shen N, Wang J, Zhao M, Pei F, He B. Anti-interleukin-17 antibodies attenuate airway inflammation in tobacco-smoke-exposed mice. *Inhal Toxicol* 2011;23:212–218.
103. Di Stefano A, Caramori G, Gnemmi I, Contoli M, Vicari C, Capelli A, *et al.* T helper type 17-related cytokine expression is increased in the bronchial mucosa of stable chronic obstructive pulmonary disease patients. *Clin Exp Immunol* 2009;157:316–324.
104. Vargas-Rojas MI, Ramírez-Venegas A, Limón-Camacho L, Ochoa L, Hernández-Zenteno R, Sansores RH. Increase of Th17 cells in peripheral blood of patients with chronic obstructive pulmonary disease. *Respir Med* 2011;105:1648–1654.
105. Zhang J, Chu S, Zhong X, Lao Q, He Z, Liang Y. Increased expression of CD4⁺IL-17⁺ cells in the lung tissue of patients with stable chronic obstructive pulmonary disease (COPD) and smokers. *Int Immunopharmacol* 2013;15:58–66.
106. Doe C, Bafadhel M, Siddiqui S, Desai D, Mistry V, Rugman P, *et al.* Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. *Chest* 2010;138:1140–1147.
107. Roos AB, Sandén C, Mori M, Björner L, Stampfli MR, Erjefält JS. IL-17A is elevated in end-stage chronic obstructive pulmonary disease and contributes to cigarette smoke-induced lymphoid neogenesis. *Am J Respir Crit Care Med* 2015;191:1232–1241.
108. 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;130:1087–1096.e10.
109. Di Stefano A, Caramori G, Capelli A, Gnemmi I, Ricciardolo FL, Oates T, *et al.* STAT4 activation in smokers and patients with chronic obstructive pulmonary disease. *Eur Respir J* 2004;24:78–85.
110. Hodge G, Nairn J, Holmes M, Reynolds PN, Hodge S. Increased intracellular T helper 1 proinflammatory cytokine production in peripheral blood, bronchoalveolar lavage and intraepithelial T cells of COPD subjects. *Clin Exp Immunol* 2007;150:22–29.
111. Brozyna S, Ahern J, Hodge G, Nairn J, Holmes M, Reynolds PN, *et al.* Chemotactic mediators of Th1 T-cell trafficking in smokers and COPD patients. *COPD* 2009;6:4–16.
112. Moermans C, Heinen V, Nguyen M, Henket M, Sele J, Manise M, *et al.* Local and systemic cellular inflammation and cytokine release in chronic obstructive pulmonary disease. *Cytokine* 2011;56:298–304.
113. Petecchia L, Sabatini F, Usai C, Caci E, Varesio L, Rossi GA. Cytokines induce tight junction disassembly in airway cells via an EGFR-dependent MAPK/ERK1/2-pathway. *Lab Invest* 2012;92:1140–1148.
114. Coyne CB, Vanhook MK, Gambling TM, Carson JL, Boucher RC, Johnson LG. Regulation of airway tight junctions by proinflammatory cytokines. *Mol Biol Cell* 2002;13:3218–3234.
115. Georas SN, Rezaee F. Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation. *J Allergy Clin Immunol* 2014;134:509–520.
116. Hardyman MA, Wilkinson E, Martin E, Jayasekera NP, Blume C, Swindle EJ, *et al.* TNF- α -mediated bronchial barrier disruption and regulation by src-family kinase activation. *J Allergy Clin Immunol* 2013;132:665–675.e8.
117. Aaron SD, Angel JB, Lunau M, Wright K, Fex C, Le Saux N, *et al.* Granulocyte inflammatory markers and airway infection during acute exacerbation of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;163:349–355.
118. Rusznak C, Mills PR, Devalia JL, Sapsford RJ, Davies RJ, Lozewicz S. Effect of cigarette smoke on the permeability and IL-1 β and sICAM-1 release from cultured human bronchial epithelial cells of never-smokers, smokers, and patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2000;23:530–536.
119. Finigan JH, Faress JA, Wilkinson E, Mishra RS, Nethery DE, Wyler D, *et al.* Neuregulin-1-human epidermal receptor-2 signaling is a central regulator of pulmonary epithelial permeability and acute lung injury. *J Biol Chem* 2011;286:10660–10670.
120. Rincon M, Irvin CG. Role of IL-6 in asthma and other inflammatory pulmonary diseases. *Int J Biol Sci* 2012;8:1281–1290.
121. Heijink IH, Kies PM, Kauffman HF, Postma DS, van Oosterhout AJ, Vellenga E. Down-regulation of E-cadherin in human bronchial epithelial cells leads to epidermal growth factor receptor-dependent Th2 cell-promoting activity. *J Immunol* 2007;178:7678–7685.
122. Hackett TL, Singhera GK, Shaheen F, Hayden P, Jackson GR, Hegele RG, *et al.* Intrinsic phenotypic differences of asthmatic epithelium and its inflammatory responses to respiratory syncytial virus and air pollution. *Am J Respir Cell Mol Biol* 2011;45:1090–1100.
123. Garmendia J, Morey P, Bengoechea JA. Impact of cigarette smoke exposure on host-bacterial pathogen interactions. *Eur Respir J* 2012;39:467–477.
124. Hiemstra PS, Bourdin A. Club cells, CC10 and self-control at the epithelial surface. *Eur Respir J* 2014;44:831–832.
125. Laicho-Contreras ME, Polverino F, Gupta K, Taylor KL, Kelly E, Pinto-Plata V, *et al.* Protective role for club cell secretory protein-16 (CC16) in the development of COPD. *Eur Respir J* 2015;45:1544–1556.
126. Park HY, Chung A, Wright JL, Li Y, Tam S, Man SF, *et al.* Club cell protein 16 and disease progression in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2013;188:1413–1419.
127. Zhu L, Di PY, Wu R, Pinkerton KE, Chen Y. Repression of CC16 by cigarette smoke (CS) exposure. *PLoS One* 2015;10:e0116159.
128. Herr C, Beisswenger C, Hess C, Kandler K, Suttrop N, Welte T, *et al.*; R Bals for the CAPNETZ Study Group. Suppression of pulmonary innate host defence in smokers. *Thorax* 2009;64:144–149.
129. Amatngalim GD, Schrupf JA, Henic A, Dronkers E, Verhoosel RM, Ordóñez SR, *et al.* Antibacterial defense of human airway epithelial cells from chronic obstructive pulmonary disease patients induced by acute exposure to nontypeable *Haemophilus influenzae*: modulation by cigarette smoke. *J Innate Immun* 2017;9:359–374.
130. Charlson ES, Chen J, Custers-Allen R, Bittinger K, Li H, Sinha R, *et al.* Disordered microbial communities in the upper respiratory tract of cigarette smokers. *PLoS One* 2010;5:e15216.
131. Erb-Downward JR, Thompson DL, Han MK, Freeman CM, McCloskey L, Schmidt LA, *et al.* Analysis of the lung microbiome in the “healthy” smoker and in COPD. *PLoS One* 2011;6:e16384.

132. Monsó E. Microbiome in chronic obstructive pulmonary disease. *Ann Transl Med* 2017;5:251.
133. Huang YJ, Kim E, Cox MJ, Brodie EL, Brown R, Wiener-Kronish JP, et al. A persistent and diverse airway microbiota present during chronic obstructive pulmonary disease exacerbations. *OMICS* 2010;14:9–59.
134. Sze MA, Dimitriu PA, Hayashi S, Elliott WM, McDonough JE, Gosselink JV, et al. The lung tissue microbiome in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2012; 185:1073–1080.
135. Kim HJ, Kim YS, Kim KH, Choi JP, Kim YK, Yun S, et al. The microbiome of the lung and its extracellular vesicles in nonsmokers, healthy smokers and COPD patients. *Exp Mol Med* 2017;49:e316.
136. van 't Wout EF, van Schadewijk A, van Boxtel R, Dalton LE, Clarke HJ, Tommassen J, et al. Virulence factors of *Pseudomonas aeruginosa* induce both the unfolded protein and integrated stress responses in airway epithelial cells. *PLoS Pathog* 2015;11:e1004946.
137. Heijink IH, Jonker MR, de Vries M, van Oosterhout AJ, Telenga E, Ten Hacken NH, et al. Budesonide and fluticasone propionate differentially affect the airway epithelial barrier. *Respir Res* 2016;17:2.
138. Noda N, Matsumoto K, Fukuyama S, Asai Y, Kitajima H, Seki N, et al. Cigarette smoke impairs phagocytosis of apoptotic neutrophils by alveolar macrophages via inhibition of the histone deacetylase/Rac/CD9 pathways. *Int Immunol* 2013;25:643–650.
139. Richens TR, Linderman DJ, Horstmann SA, Lambert C, Xiao YQ, Keith RL, et al. Cigarette smoke impairs clearance of apoptotic cells through oxidant-dependent activation of RhoA. *Am J Respir Crit Care Med* 2009;179:1011–1021.
140. Wang Y, Luo G, Chen J, Jiang R, Zhu J, Hu N, et al. Cigarette smoke attenuates phagocytic ability of macrophages through down-regulating milk fat globule-EGF factor 8 (MFG-E8) expressions. *Sci Rep* 2017;7:42642.
141. McCubrey AL, Curtis JL. Efferocytosis and lung disease. *Chest* 2013;143:1750–1757.
142. Ravichandran KS. Find-me and eat-me signals in apoptotic cell clearance: progress and conundrums. *J Exp Med* 2010;207: 1807–1817.
143. Todt JC, Freeman CM, Brown JP, Sonstein J, Ames TM, McCubrey AL, et al. Smoking decreases the response of human lung macrophages to double-stranded RNA by reducing TLR3 expression. *Respir Res* 2013;14:33.
144. Shan M, Yuan X, Song LZ, Roberts L, Zarinkamar N, Seryshev A, et al. Cigarette smoke induction of osteopontin (SPP1) mediates T(H)17 inflammation in human and experimental emphysema. *Sci Transl Med* 2012;4:117ra9.
145. Baker KE, Bonvini SJ, Donovan C, Foong RE, Han B, Jha A, et al. Novel drug targets for asthma and COPD: lessons learned from *in vitro* and *in vivo* models. *Pulm Pharmacol Ther* 2014;29:181–198.
146. Lapperre TS, Snoeck-Stroband JB, Gosman MM, Jansen DF, van Schadewijk A, Thiadens HA, et al.; Groningen Leiden Universities Corticosteroids in Obstructive Lung Disease Study Group. Effect of fluticasone with and without salmeterol on pulmonary outcomes in chronic obstructive pulmonary disease: a randomized trial. *Ann Intern Med* 2009;151:517–527.
147. van den Berge M, Steiling K, Timens W, Hiemstra PS, Sterk PJ, Heijink IH, et al. Airway gene expression in COPD is dynamic with inhaled corticosteroid treatment and reflects biological pathways associated with disease activity. *Thorax* 2014;69:14–23.
148. Doerner AM, Zuraw BL. TGF- β 1 induced epithelial to mesenchymal transition (EMT) in human bronchial epithelial cells is enhanced by IL-1 β but not abrogated by corticosteroids. *Respir Res* 2009;10: 100.
149. Milara J, Peiró T, Serrano A, Guíjarro R, Zaragoza C, Tenor H, et al. Roflumilast N-oxide inhibits bronchial epithelial to mesenchymal transition induced by cigarette smoke in smokers with COPD. *Pulm Pharmacol Ther* 2014;28:138–148.
150. Milara J, Peiró T, Serrano A, Artigues E, Aparicio J, Tenor H, et al. Simvastatin increases the ability of roflumilast n-oxide to inhibit cigarette smoke-induced epithelial to mesenchymal transition in well-differentiated human bronchial epithelial cells *in vitro*. *COPD* 2015;12:320–331.
151. Burgel PR, Nadel JA. Roles of epidermal growth factor receptor activation in epithelial cell repair and mucin production in airway epithelium. *Thorax* 2004;59:992–996.
152. Rezaee F, DeSando SA, Ivanov AI, Chapman TJ, Knowlden SA, Beck LA, et al. Sustained protein kinase D activation mediates respiratory syncytial virus-induced airway barrier disruption. *J Virol* 2013;87: 11088–11095.
153. Gan H, Wang G, Hao Q, Wang QJ, Tang H. Protein kinase D promotes airway epithelial barrier dysfunction and permeability through down-regulation of claudin-1. *J Biol Chem* 2013;288:37343–37354.
154. Steelant B, Seys SF, Boeckxstaens G, Akdis CA, Ceuppens JL, Hellings PW. Restoring airway epithelial barrier dysfunction: a new therapeutic challenge in allergic airway disease. *Rhinology* 2016;54: 195–205.
155. Heijink IH, Nawijn MC, Hackett TL. Airway epithelial barrier function regulates the pathogenesis of allergic asthma. *Clin Exp Allergy* 2014;44:620–630.
156. Zhang R, Zhao H, Dong H, Zou F, Cai S. 1 α ,25-dihydroxyvitamin D₃ counteracts the effects of cigarette smoke in airway epithelial cells. *Cell Immunol* 2015;295:137–143.
157. Muresan XM, Cervellati F, Sticozzi C, Belmonte G, Chui CH, Lampronti I, et al. The loss of cellular junctions in epithelial lung cells induced by cigarette smoke is attenuated by corilagin. *Oxid Med Cell Longev* 2015;2015:631758.
158. Hiemstra PS, Bals R. Basic science of electronic cigarettes: assessment in cell culture and *in vivo* models. *Respir Res* 2016;17:127.