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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

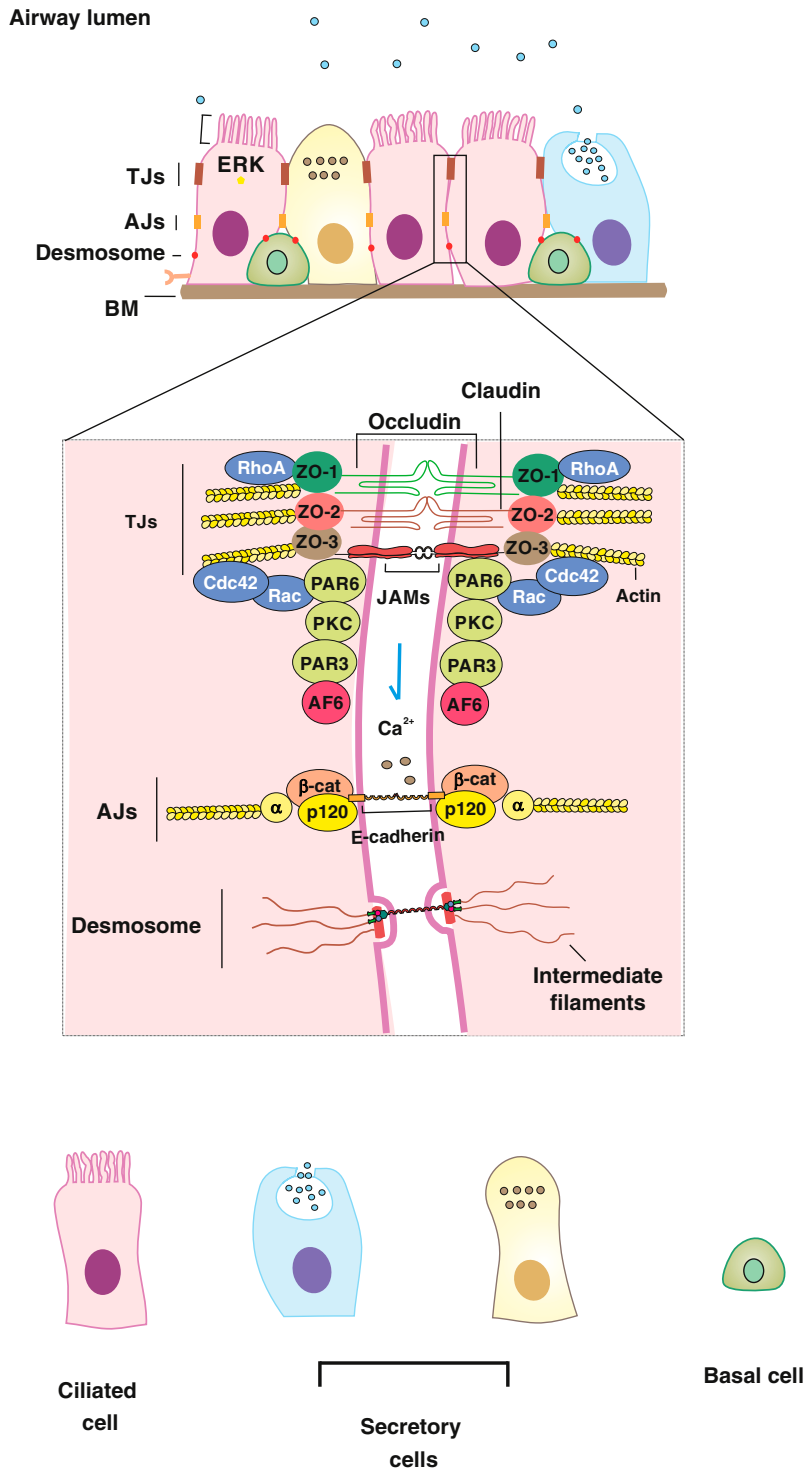

Figure 1. Schematic illustration representing the structure of barriers in normal airway epithelium. In normal airway epithelium, tight junctions (TJs) reside in the apical side and consist of the anchoring proteins, occludin (OCLN), claudins, and junctional adhesion molecules (JAMs). Zonula occludens (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 EFGR 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

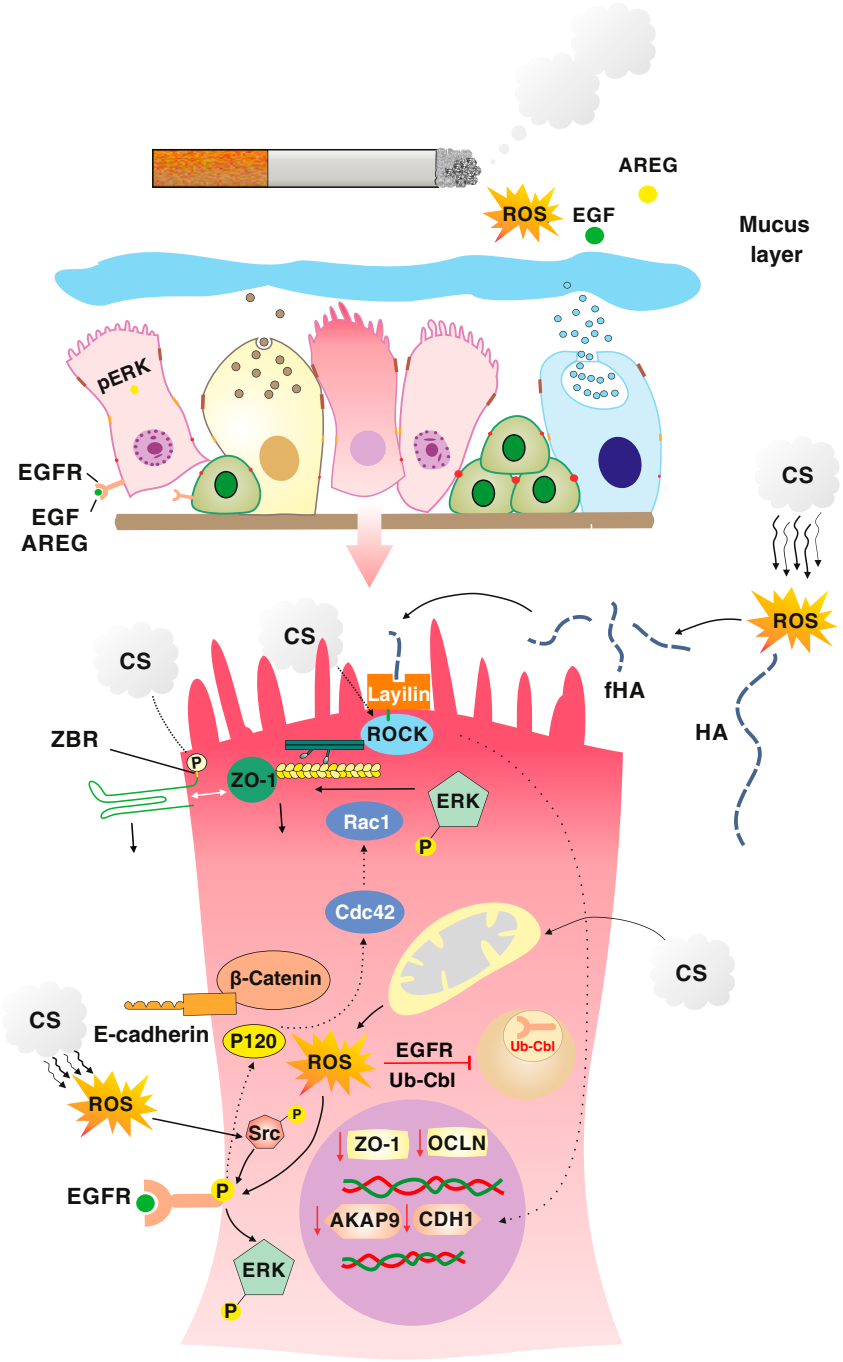

Figure 2. Disruption of airway epithelial junctions in response to cigarette smoke exposure. After cigarette smoke exposure, the airway epithelium and, in particular, intercellular contacts undergo significant changes. Cigarette smoke can induce disruption of TJs upon phosphorylation of the ZO-1 binding 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 hyalorunan fragmentation (46). Increased fragmentation of hyalorunan 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-tranferase 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-3β inactivation upon activation of transforming growth factor (TGF)-β or wingless/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, β1-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-3β *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

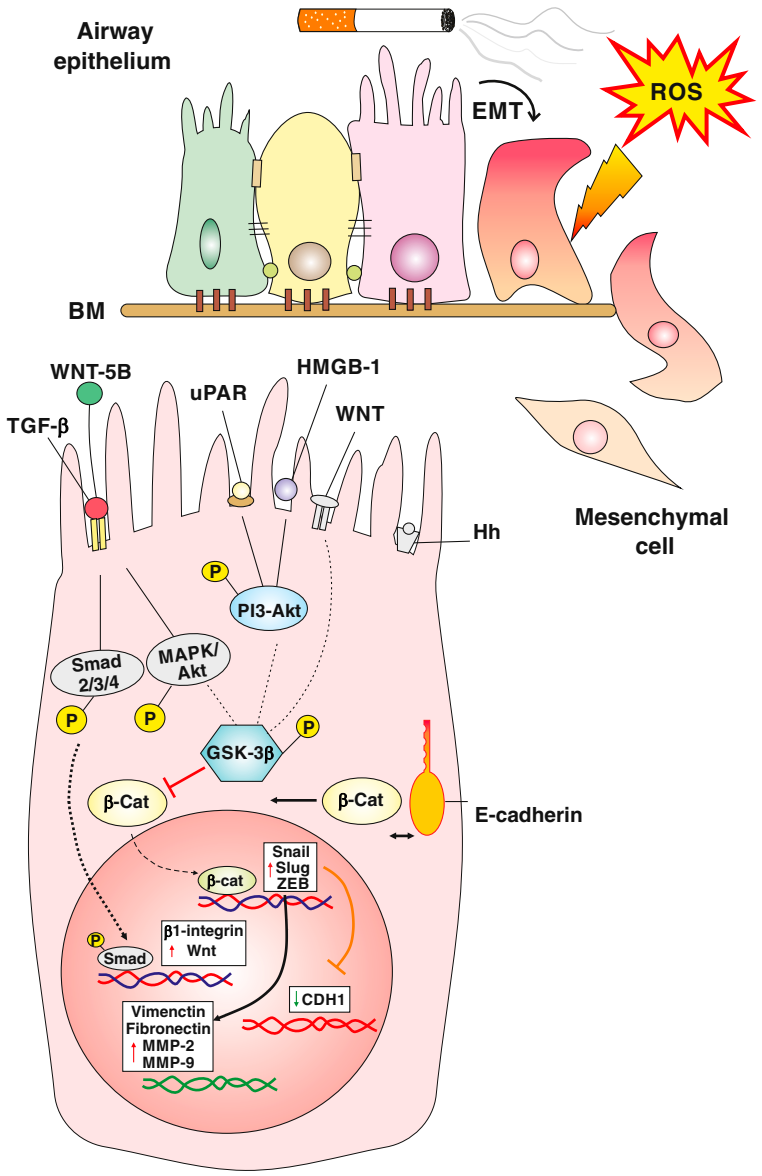

Figure 3. Molecular mechanisms involved in cigarette smoke–induced epithelial to mesenchymal transition (EMT) in airway epithelium. Cigarette smoke–induced ROS can activate several signaling pathways in airway epithelium, including urokinase plasminogen activator receptor (uPAR), Hedghog (Hh), WNT, and 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-3β, 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 β1-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-3β 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-1β. Decades ago, Rusznak and coworkers (118) observed a marked elevation in IL-1β 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-1β, 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-1β 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

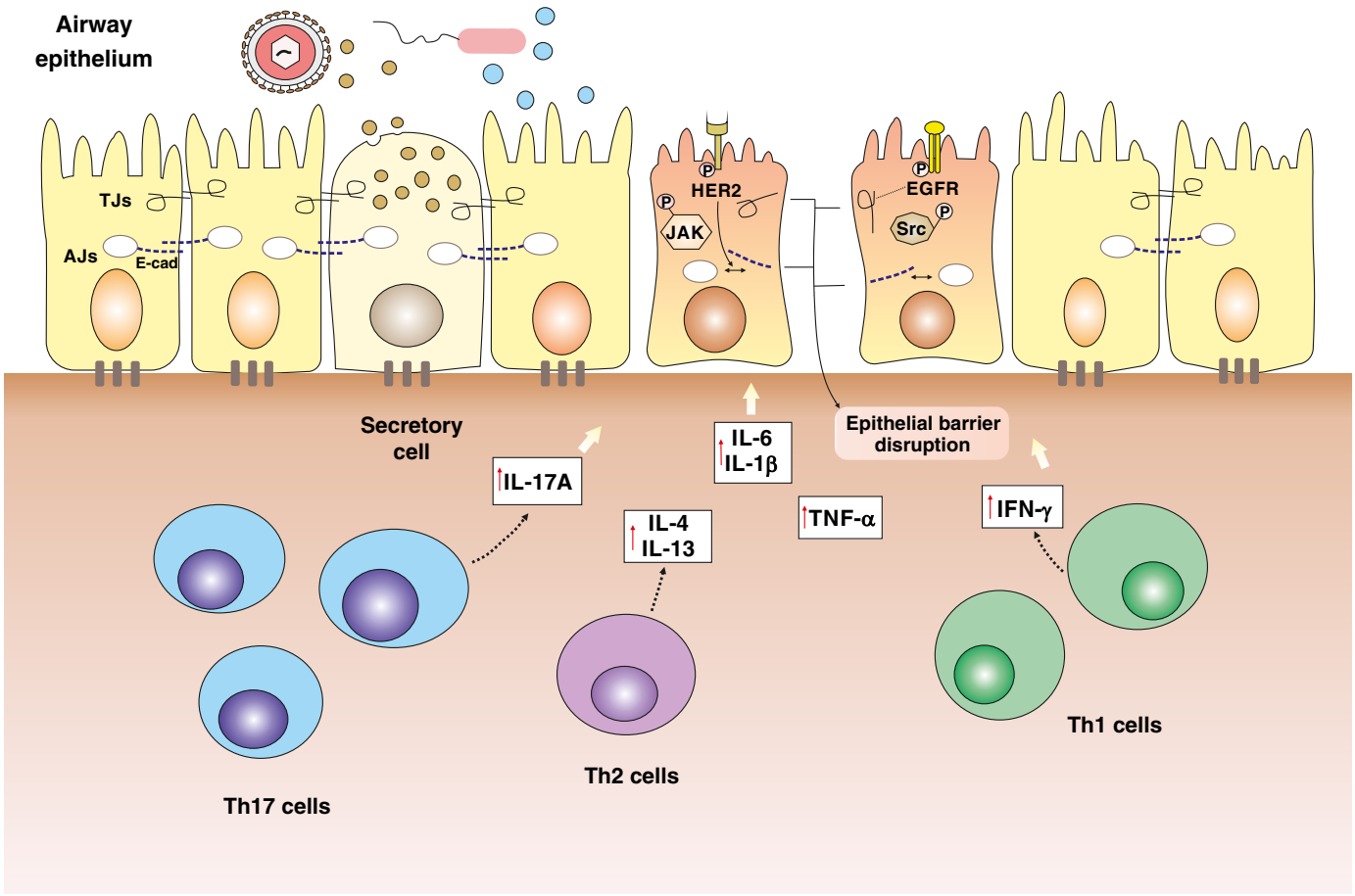

Figure 4. Proinflammatory mediators regulating epithelial barrier function. The inflammatory responses mediated by various cytokines, including T-helper cell type 1 (Th1), Th2, and Th17, and chemokines (IL-1β and TNF-α) can alter different intercellular signaling pathways, leading to barrier dysfunction. 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-1β 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 γδ 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-β1 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-β1 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	*In vitro*/16HBE cells	137
EGF			*In vitro*/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	*In vitro*/16HBE and HBECs	12
PDE4 inhibitor	cAMP	Inhibition of cigarette smoke–induced E-cadherin and ZO-1 downregulation	*In vitro*/differentiated HBECs	34, 149, 150
PKD inhibitor	PKD3	Increase in TEER and CLDN1 expression upon calcium depletion	*In vitro*/16HBE cells	153
AKAP inhibitor	AKAP-cAMP	Reversion of cigarette smoke–induced impairment of cell membrane E-cadherin	*In vitro*/16HBE cells	35
Vitamin D	ERK	Rescue of E-cadherin and β-catenin protein loss and maintenance of TEER upon cigarette smoke extract exposure	*In vitro*/16HBE cells	156
Corilagin	NF-κB	Prevention of cigarette smoke–induced decrease in TJ-related connexin 40 gene expression and protein levels	*In vitro*/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 = phosphdiesterase 4; PKD = protein kinase D; TEER = transepithelial electrical resistance; TGF-β = transforming growth factor-β; TJ = tight junction; ZO-1/ZO-2 = zonula ocludens-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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