Review

Crosstalk of Airway Smooth Muscle and Epithelial Cells in Chronic Lung Diseases

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Abstract: Chronic pulmonary diseases such as asthma, COPD, and Idiopathic pulmonary fibrosis are significant causes of mortality and morbidity worldwide. Currently, there is no radical treatment for many chronic pulmonary diseases, and the treatment options focus on relieving the symptoms and improving lung function. Therefore, efficient therapeutic agents are highly needed. Bronchial epithelial cells and airway smooth muscle cells and their crosstalk play a significant role in the pathogenesis of these diseases. Thus, targeting the interactions of these two cell types could open the door to a new generation of effective therapeutic options. However, the studies on how these two cell types interact and how their crosstalk adds up to respiratory diseases are not well established. With the rise of modern research tools and technology, such as lab-on-chip, organoids, co-culture techniques, and advanced immunofluorescence imaging, a substantial degree of evidence about these cell interactions emerged. Hence, this contribution aims to review the growing evidence of bronchial epithelial cells and airway smooth muscle cells crosstalk under normal and pathophysiological conditions. The review first deliberates the effects of both healthy and stressed epithelial cells on airway smooth muscle cells, taking into account three themes; contraction, migration, and proliferation. Then, it discusses the impact of airway smooth muscle cells on the epithelium in inflammatory settings. Later, it examines the role of airway smooth muscle cells in the early development of bronchial epithelial cells and their recovery after injury.

Keywords: epithelial; smooth muscle; interaction; pulmonary disease; airway; asthma; COPD; bronchial remodeling

1. Introduction

Inflammatory respiratory diseases are a prominent public health concern with high morbidity and mortality ratios (1). Asthma, with its various phenotypes, is a significant pulmonary disease affecting more than 300 million people globally, and its prevalence is increasing significantly in children and young adults (2). Meanwhile, Chronic Obstructive Pulmonary Disease (COPD) is a progressive airflow-restricting disease and is considered the third reason for death worldwide (3). In addition, other less common chronic pulmonary diseases, such as Idiopathic pulmonary fibrosis (IPF), have an enormous population burden and high mortality rate and treatment costs (4). Unfortunately, until now, there is no radical treatment for any of the previously mentioned diseases, and most of the current therapeutic strategies focus on suppressing inflammation and promoting bronchodilation. Therefore, understanding the physiology of the respiratory system and the pathophysiology of these diseases is highly important to develop future therapeutic strategies that treat or prevent these diseases.

The airway in the respiratory system consists of many layers formed in a certain way to create a tube with a lumen. The first layer from the lumen is a single sheet of cells called the epithelium. The epithelial layer involves many types of cells, such as ciliated cells, mucous-producing goblet cells, club cells, and basal cells tightly attached to a basement membrane and each other through tight junctions and adherens junctions. The basal membrane separates the epithelial layer from the next layer, lamina propria, which contains a

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variety of immune cells and fibroblasts inside a mixture of extracellular matrix (ECM) produced mainly by the later cells. Last but not least, bands of airway smooth muscle cells form the outer layer around the lamina propria (5). All the airway layers contain a complex of different cell types that maintain the hemostasis of the lung and defend the body against microbial and physical threads. Therefore, it is only natural that chronic lung diseases such as asthma, COPD, and pulmonary fibrosis progress due to the dysfunction of these cells and their interactions. Of these cells, bronchial epithelial cells (BECs) and airway smooth muscle cells (ASMCs) play a significant role in the pathogenesis of chronic lung diseases. For example, airway epithelial damage and abnormal repair accompanied by enlarged smooth muscle mass and increased cytokine production are some of the most well-established characteristics of asthma (5-7). In COPD, epithelial metaplasia and increased airway smooth muscle quantity correlate with airflow obstruction and, inversely, lung function (8). In addition, epithelial cells play a central role in initiating idiopathic pulmonary fibrosis and, alongside the myofibroblasts, contribute to the development of the disease (9, 10). Meanwhile, airway smooth muscle cells are associated with higher lung fibrosis and poor clinical conditions (11). Therefore, investigating the BEC and ASMC roles and interactions is vital for revealing new therapeutic opportunities for many chronic respiratory diseases.

Researchers have investigated the function of BECs and ASMCs and their roles in respiratory diseases for many years. However, the studies of how these two cell types interact and how their interactions add up to respiratory diseases have recently started to get more attention (7). Alongside the rise of modern innovative research tools and models, new information on the communication and the interaction between BECs and ASMCs in chronic respiratory disease development and progress has emerged. Nevertheless, the reviews that summarize the recent data on this subject are limited. Therefore, this review aims to recap the growing evidence of BECs and ASMCs interactions in the airways and the role of these interactions in chronic respiratory diseases. Hopefully, this contribution will facilitate future research planning and investigation. To better understand this broad complex topic, the review first discusses the effects of healthy and injured epithelium on airway smooth muscle cells. Next, it discusses the impact of airway smooth muscle cells on the epithelial cells in physiological and pathophysıological conditions.

2. The Effects of Epithelium on Airway Smooth Muscle Cells:

The airway epithelial cells, with their tight junctions, are the first line of contact against various pollutants and pathogens in the lungs. They also contribute to the airway clearance system consisting of secreted mucus, beating cilia, and antimicrobial peptides. Consequently, for many years it has been thought that the fundamental purpose of the epithelial cells is to provide a mechanical first line of defense against inhaled foreign objects and particles (12). However, the epithelium's role in normal and pathophysiological conditions is relatively more complicated to be restricted to its physical structure. Epithelial cells can recognize a variety of foreign particles thanks to their set of pattern recognition receptors (PRRs) such as the toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), C-type lectin receptors (CLRs), and protease-activated receptors (PARs) (12). In addition, bronchial epithelial cells can produce a wide range of chemokines and cytokines to recruit and activate numerous kinds and phenotypes of cells. These include but are not limited to interleukin-1 beta (IL-1B), interleukin 6 (IL-6), interleukin-25 (IL-25), interleukin-33 (IL-33), C-X-C motif chemokine ligand 8 (CXCL8), C-C motif chemokine ligands (CCL5, CCL17, and CCL20), granulocyte-macrophage colony-stimulating factor (GM-CSF), and thymic stromal lymphopoietin (TSLP) (12-14).

The lamina propria layer separates the epithelium sheet from the smooth muscle layer in a healthy airway. Thus, there is no direct contact between the epithelial cells and the airway smooth muscle cells. Therefore, soluble mediators must diffuse through the lamina propria layer for the epithelium to affect ASMCs (15). For example, the epithelium

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could release such mediators when it encounters air pollutants and environmental pathogens, leading to airway narrowing, obstruction, and exacerbation in asthmatic and COPD patients (6). Therefore, targeting these paracrine signals from the airway epithelium to the underlying smooth muscle layer is promising for developing a new treatment strategy for asthma, COPD, and other chronic pulmonary diseases (16). In addition, recent studies suggest that the epithelium layer can also influence ASMCs under normal conditions. Hereafter, perhaps these new strategies could prevent the development of the disease and the structural remodeling accompanying them. The epithelial cells in the airway tend to have different and complex effects on ASMCs. Depending on the outcome of these effects, the review will categorize them into three main themes: Effects that impact ASMC contractility, actions that affect ASMC migration, and interactions that alter ASMC proliferation.

The Effects of Epithelium on Airway Smooth Muscle Cell Contraction

Since the mid-eighties of the last century, researchers have attempted to understand the effect of the airway epithelium on the airway smooth muscle tone. The first experiment using canine and dog bronchi demonstrated *in vitro* that physical deletion of the epithelium increases the responsiveness of airway smooth muscle to a range of constricting agonists (17). In the next few years, many researchers confirmed this phenomenon. In addition, they reported that removal of the epithelium decreases airway smooth muscle responsiveness to some relaxing agents using *in vitro* cattle, guinea pigs, and rabbit trachea (18). However, now it is well established that prostaglandins and nitric oxide released from the epithelium are responsible for this effect, and these studies were previously reviewed (17-19). Therefore, to serve its purpose, this review will focus on emerging new data about the epithelium effects on ASMCs outside prostaglandins and leukotrienes pathways, outlining new possible paths and the outcome of studies that use innovative research models.

The epithelium plays a complex role in affecting ASMCs in normal and pathophysiological conditions. The new data demonstrate that the prostaglandins pathway is not the only means the epithelium could influence the ASMCs under normal conditions. Although these pathways vary in their mechanism of action, many studies suggest that the epithelium most likely backs the relaxation of ASMCs in normal conditions but contributes to ASMCs' hyperresponsiveness under stressful environments (Fig. 1 demonstrates the adaption of the airway wall layers to stressful settings). *Gallos et al.* (20) have reported that the airway epithelium is the primary source of endogenous γ-Aminobutyric acid (GABA) in the airways of Guniea pigs and humans. GABA released from the epithelium spontaneously contributed to ASMCs relaxation both *in vitro* and *in vivo*. In addition, GABA receptor antagonist Gabazine increased airway smooth muscle response to acetylcholine *in vitro* both in healthy human airway tissue and Guniea pig trachea. In this aspect, Brain natriuretic peptide (BNP) is an endogenous hormone secreted primarily by the ventricular myocardium under normal conditions and elevated in heart failure. *Calzetta et al.* reported that BNP increased the levels of acetylcholine and nitric oxide in the supernatant of BECs when added to the cell culture. Remarkably, the acetylcholine-high supernatant of BECs contributed to ASMCs relaxation in cell culture. To explain the pro-relaxant effect of the previously mentioned supernatant in the study, the authors investigated the role of the muscarinic M2 receptor. As the selective blocking of this receptor demolished this effect, the authors suggested that the substance discharged from BECs enhances the second messenger cyclic guanosine monophosphate (cGMP) concentration inside ASMCs, acting specifically on the muscarinic M2 receptor. Consequently, the study demonstrated the possible role of BNP in protecting against airway hyperresponsiveness (AHR) in asthma by employing BEC and ASMC interactions (21). In another study, the supernatant of healthy unstressed human BECs and commercial epithelial cell line BEAS-2B contributed to the relaxation of ASMCs when added to ASMCs culture. Further, the supernatant of unstimulated BECs altered the contractile phenotype of ASMCs. This alteration was

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characterized by downregulating a-smooth muscle actin (a-SMA) in ASMCs. Furthermore, the new phenotype expressed higher cyclic adenosine monophosphate (cAMP) in response to constricting agonists such as histamine (22).

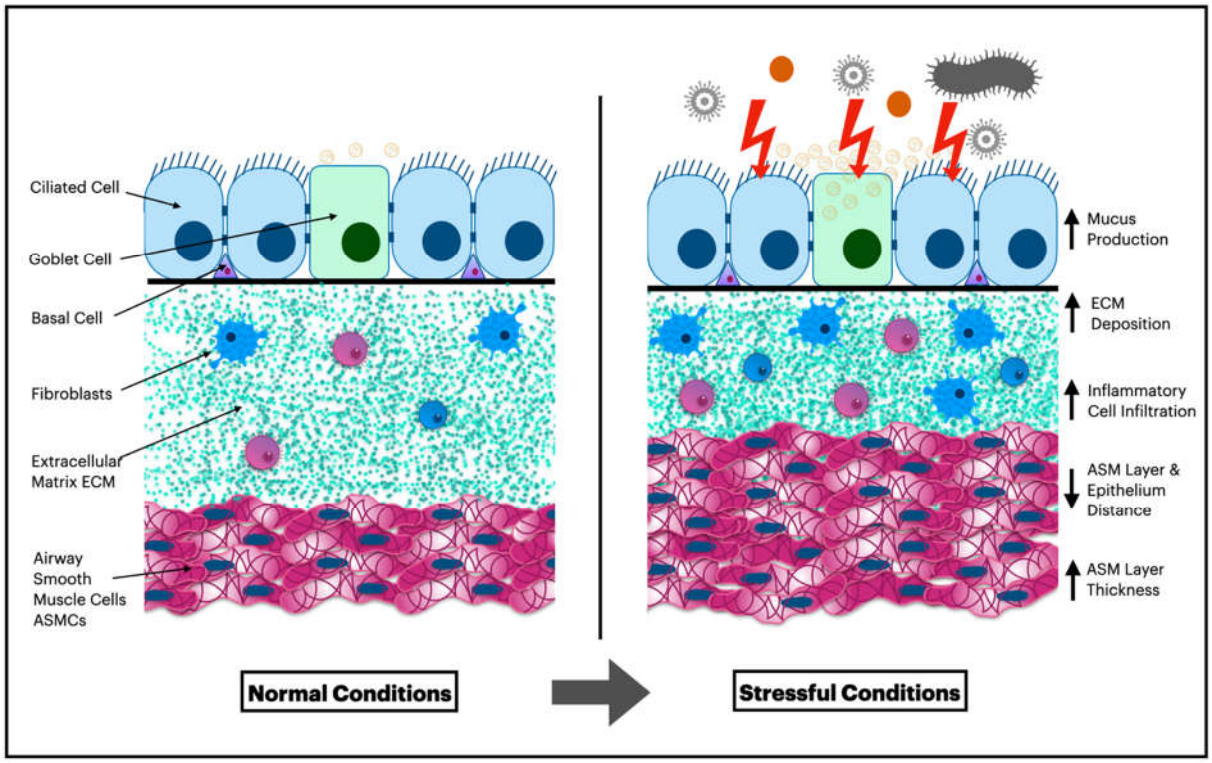

Figure 1. Alteration of the airway wall components as a response to stressful conditions.

On the contract, BECs tend to exaggerate the contractile response of ASMCs under stressful and tense conditions. These conditions could result from endogenous or exogenous stimuli or internal or external stressors. For instance, The supernatant of mechanically compressed human BECs amplified the contraction of ASMCs to histamine *in vitro* (23). Considering that BECs experience a tremendous amount of mechanical compression during acute bronchoconstriction, *Lan et al.* results emphasize the importance of targeting the impact of BECs on ASMCs as a possible therapeutic strategy for bronchodilators-resistant airway diseases. The authors also reported that this effect was endothelin-1 depending, as blockage of endothelin receptors in ASMCs eradicated this observation. *Zhou et al.* (24) also stated that physical BECs injury was responsible for the contraction of ASMCs using an *ex-vivo* lung slices model in rats. Moreover, even a single epithelial cell rupture induced rapid and global airway constriction. This action took place after the single ruptured cell triggered a distinct instantaneous calcium wave in the epithelium and multiple ones in ASMCs. Therefore, the authors suggested a release of soluble mediators from the epithelium into the smooth muscle layer under the physical stress of BECs.

Inflammatory stressors such as infection may also contribute to the constrictor outcome of BECs on ASMCs. Rhinovirus (RV) is one of the most encountered infections in the respiratory system of children and adults. In addition, RV infection in children is associated with a higher risk of asthma and is responsible for exacerbations in asthmatic adults. Therefore, the use of the RV in asthma research and disease models is widespread (25). In a study, *Parikh et al.* demonstrated that epithelium infection by rhinovirus induces AHR using an ex vivo human model of asthma. The AHR is associated with increased responses of ASMCs to carbachol and an increased influx of intercellular calcium (26). The infected epithelium cells expressed higher levels of IFN-g–induced protein 10 (IP-10) and macrophage inflammatory protein-1b (MIP-1b). It is merit to mention that RV infection did not increase the release of cytokines known to cause AHR, such as IL-13 and IL-33,

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from the epithelial cells. Actually, the cytokine IL-13 induced AHR even without its direct effects on ASMCs. Using genetically modified mice that only express functional IL-13 receptors in the bronchial epithelial cells but not in ASMCs, *Kuperman et al.* reported that the cytokine IL-13 trigged BECs to amplify the response of ASMCs towards agonists (27).

β2-adrenoreceptor agonists are the most widely used effective bronchodilators in asthma. Still, there is growing evidence that activation of the β2 adrenoreceptor–β arrestin pathway has been implicated in contradictory actions in murine asthmatic models. Using akin methodology to the latest mentioned study, *P. Nguyen et al.* (28) developed genetically modified mice expressing β2-adrenoreceptors only in the epithelial cells. Using IL-13 as a stressor, the authors reported all the classical effects of IL-13 on the airways, such as AHR, eosinophilic inflammation, and mucus production. Furthermore, the study demonstrated that β2-adrenoreceptors blockage in the epithelial cells eradicated all previously mentioned effects of IL-13, and the stimulation of β2-adrenoreceptors aggravated them. Therefore, the authors could show that this outcome depends on the activation of β arrestin pathway in BECs only independently from the direct pathway actions in ASMCs. However, the authors did not identify the mechanism of how BECs influence ASMCs contraction, but they suggested the role of cytokines released from BECs towards ASMCs, especially that production of CCL2, CCL24, CXCL1 from BECs significantly increased after the exposure to IL-13.

Away from tense and stressful conditions, genetic reasons may contribute to the effects of BECs on ASMCs. *Li et al.* (16) have reported that specific phenotypes of murine epithelium that lack the transcription factors forkhead box P1 and P4 (Foxp1 and Foxp4) could evoke AHR and ASMCs contractility. Notably, the authors mentioned that the loss of Foxp1 and Foxp4 expression induces the release of neuropeptide Y from the epithelial cells. This potent vasoconstrictor peptide increased Rho kinase activity and, therefore, the phosphorylation of the myosin light chains in ASMCs without changing the inflammatory profile and cytokine levels.

The epithelial cells in the airways are considered the first line of defense against micro bacteria and other outsider stressors and harmful conditions. Thus, to limit their access, it is only logical to have the ability to influence the airway smooth muscle cells to act inversely in standard and alarming settings (Fig. 2 recaps the influence of BECs on ASMC contractility). However, the dysfunction of this tight airway control may open the door to some pathophysiological conditions. Therefore, a better understanding of the effects of BECs on ASMCs' contractability could contribute to developing novel therapeutic strategies for various respiratory diseases. These new options could be vital for some patients, especially those with abnormal resistance to typical bronchodilators.

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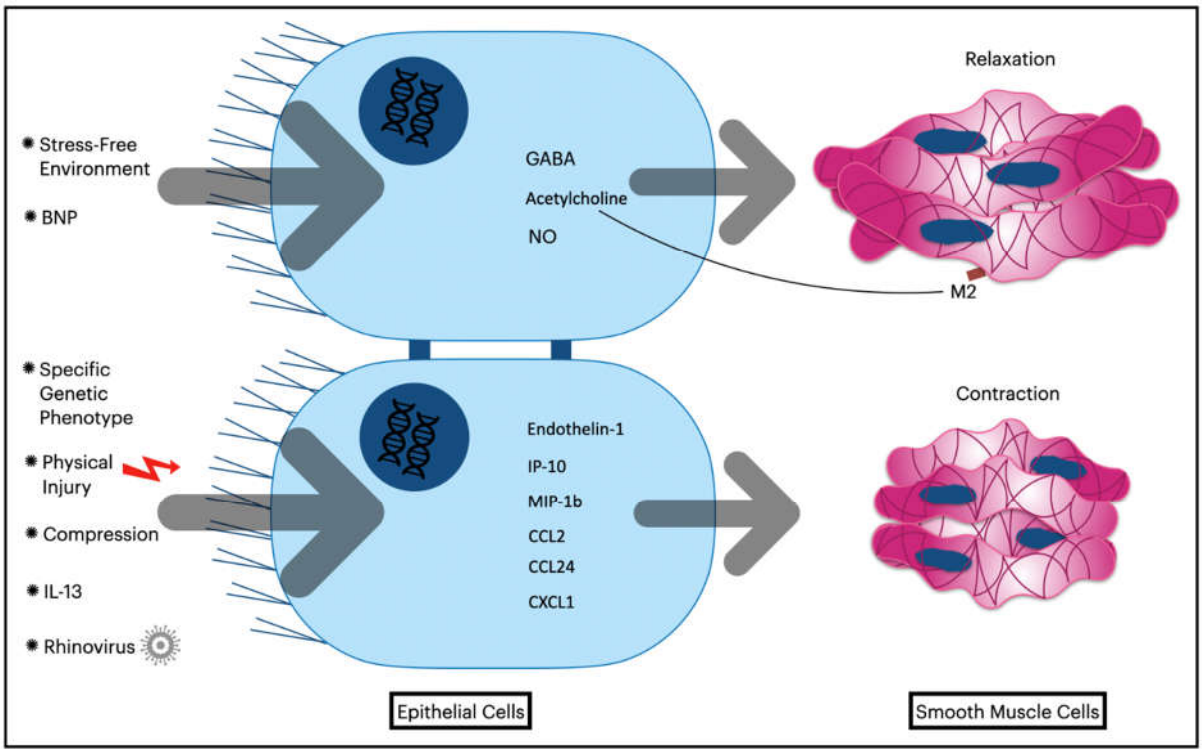

Figure 2. The impact of bronchial epithelial cells on airway smooth muscle cell contractility.

The Effects of Epithelium on Airway Smooth Muscle Cell Migration

Airway remodeling is a cluster of chronic changes in the structure of the airways detected in some respiratory diseases. This cluster includes observations such as epithelial injury, unpaired recovery, increased airway wall thickness, and airway smooth muscle cell mass (29). For many years researchers believed that airways smooth muscle cells hypertrophy and hyperplasia are the main reason for the increase in wall thickness and ASMC mass. Nevertheless, recent studies suggest that ASMC migration may contribute to the rise in ASMC mass, especially in asthmatic patients (30).

In recent years, the *in vitro* studies of two different types of co-culture for BECs and ASMCs started to become more common. In direct co-culture, cells share the culture in direct contact; meanwhile, in indirect co-culture, the cells are physically separated but share the culture's medium. Alongside with availability of new migration assay tools and techniques in the cell culture models, the potential effects of BECs on ASMCs migration started to emerge. Until today, studies demonstrating any significant influence of the epithelium on ASMC migration under normal conditions are absent. Therefore, we could say that the co-culture of BECs and ASMCs has no impact on ASMC migration in a peaceful environment. However, scholars have reported that many endogenous and exogenous stressors are essential in triggering BECs to increase the migration of ASMCs in cell culture. For instance, YKL-40 is a glycoprotein endogenously expressed and secreted by numerous cell types. Its serum level is associated with many respiratory diseases, such as asthma and COPD (31). Many studies indirectly associated YKL-40 with increased migration of human ASMCs through the outcome of its effects on the bronchial epithelial cells. *Tang et al*. (31) have reported that YKL-40 increased the expression and the production of IL-8 by human BECs and BEAS-2B epithelial cell lines in a dose-dependent manner. This action depended on MAPK and NF-κB Pathways as blockage of either one altered the amplified chemokine levels. When the researchers treated ASMCs with the supernatant of the previously mentioned epithelial cells, the heightened levels of IL-8 were the leading player in the increased migration of human ASMCs *in vitro*, as the latter effect vanished after the depletion of IL-8 from the cell culture medium. It is merit to mention that this glycoprotein could increase the migration of ASMCs directly, even if deprived of

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the contribution of BECs. In this aspect, Bara et al. (32) reported that YKL-40 increased ASMC migration *in vitro* without affecting the cytokine production profile of ASMCs. However, its expression in the epithelial cells was positively correlated with higher ASMCs mass in asthmatic patients. Tumor necrosis factor alpha (TNFα) is another endogenous mediator indirectly associated with amplified ASMC migration. The supernatants of both BEAS-2B epithelial cell line and human BECs expressed higher levels of IL-8 and RANTES (CCL5) but lower levels of TGF-B after being exposed to TNFα in cell culture for 24 hours in a concentration-dependent manner. These increased two chemokines directly increased the migration of human ASMCs in Boyden chamber analysis, as blocking one of them decreased the migration of ASMCs (30). *Su et al.* (33) have reported similar results by stimulating BECs with TNFα in cell culture and testing the effect of their supernatants on ASMC migration. BECs significantly increased the migration of ASMCs in vitro. This action was associated with increased levels of IL-8 and CCL5 as well. More interestingly, the authors also reported that BECs taken from asthmatic patients increased the migration of ASMCs *in vitro*, even without external stimulation.

The respiratory system, specifically the epithelium, is in direct contact with foreign organisms and materials. Many of these exogenous factors trigger or aggravate chronic respiratory diseases. For instance, cigarette smoking is a crucial risk factor for COPD, while second-hand cigarette smoke exposure contributes to developing the same condition (34). In addition, rhinovirus is highly associated with asthma development and COPD exacerbations (8, 25). Notably, various of these exogenous factors seem responsible for increasing the migration of ASMCs by acting on BECs. Lu et al. (35) demonstrated that cigarette smoke extract increased the production of IL-8 and TGF-B by human epithelial cell line A549 in cell culture. Both of these cytokines were responsible for the increased migration of human ASMCs when they existed in the cell culture. Blocking either one demolished the migration of ASMCs toward cigarette smoke-exposed epithelial cells. Furthermore, the impact of epithelial cells on ASMC migration in the study further intensified in the presence of muscarinic agonist carbachol. Carbachol acted on the epithelial cell's M3 receptor as this effect was overturned by selectively blocking this receptor. Therefore, the study of Lu et al. indicates that the M3 receptor could be a possible target to prevent the influence of BECs on ASMC migration.

Nonylphenol, another environmental pollutant with estrogen-like activities, augmented the production of IL-6 and IL-8, but not CCL5, from HBE135-E6E7 and BEAS-2B human epithelial cell lines in cell culture. Although nonylphenol increased the apoptosis of these cell lines, the conditional medium created from the supernatant of these cells significantly increased the migration of ASMCs in vitro. This effect depended on both IL-6 and IL-8, as depleting either from the conditioned medium altered the migration amplification (36). Regarding exogenous factors, the supernatant of human BECs increased the migration of human ASMCs in indirect co-culture after infection with rhinovirus. This increase was associated with higher production of CXCL10, CXCL8, and CCL5 from BECs. However, the depletion of only CCL5 from the supernatant of BECs prevented the migration of ASMCs, suggesting this chemokine's vital role in ASMC migration. Fascinatingly, the authors also reported that even if ASMCs highly express the CXCR3 receptor, its natural ligand, CXCL10 has less to do with the increased migration of ASMCs. However, ASMCs also presented, with less degree, CCR1, CCR3, and CCR5 receptors. The effect of CCL5 on ASMC migration could be through its action on one of these receptors. However, as the authors chose to use an anti-CCL5 antibody, not receptor blockage, to investigate the impact of CCL5, it is still tricky to make a stronger assumption (37). In parallel with the previous study, human BECs also increased the migration of human asthmatic ASMCs, but not those of healthy subjects, after infection with rhinovirus in indirect co-culture. The authors also reported that this outcome is associated with higher production of CXCL10 and CXCL9. In addition, CXCR3 antibodies blocked this effect; hence they suggested that CXCL10 drives this specific migration through the activation of CXCR3. In addition, *Celle et al.* remarkably reported that the distance between the basal membrane

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of epithelial cells and the smooth muscle layer in the airways of the asthmatic patient was shorter compared to healthy subjects (38).

Many studies have reported the positive effect of IL-8 (CXCL8), CXCL10, and RANTES (CCL5) in smooth muscle cell migration in the airways. This matter gains great importance, knowing that BECs can produce all of these cytokines in response to a stressful environment, especially in asthmatic patients. Perhaps these cytokines could be the leading player in decreasing the distance between the epithelium and smooth muscle layer observed in airway remodeling. To draw a more definite statement about the role of these cytokines, we still need further studies and investigations. However, CXCL8, CXCL10, and CCL5 with their receptors (CXCR1 and CXCR2), CXCR3, and CCR5, respectively (39, 40) seem to be a potential therapeutic target to prevent ASMC migration in bronchial remodeling and therefore aggravation of the disease in many chronic lung disease patients.

The Effects of Epithelium on Airway Smooth Muscle Cell Proliferation

Increased smooth muscle layer thickness in the airway wall is one of the most critical features of bronchial remodeling. In addition, many studies associated asthma severity in adults and children with increased smooth muscle mass (41). Hence, innovative asthma therapies such as bronchial thermoplasty target the airway's smooth muscle cells. Further, even though most of the classical treatments for asthma and COPD are focused on bronchodilation and inhibiting inflammatory cell activation and infiltration into the airways (42), these medications are considered potent inhibitors of airway smooth muscle cell proliferation as well.

The epithelium tends to have more complex effects on ASMC proliferation than their migration and contraction. The outcome of many studies indicates that relaxed and stressed BECs increased the proliferation of ASMCs both *in vitro* and *in vivo*. The indirect co-culture of healthy human BECs and BEAS-2B cell lines with human ASMCs shifted the phenotype of the latter cells into a proliferative phenotype. As a result, ASMCs downregulated a-smooth muscle actin (a-SMA) and myocardin and expressed less intracellular calcium and higher cAMP levels in response to histamine (22). In this aspect, Malavie et al. have reported that the direct co-culture of unstimulated and healthy human BECs with human ASMCs has increased the proliferation index of ASMCs in vitro. The co-culture supernatant expressed higher levels of IL-6, IL-8, monocyte chemotactic protein (MCP-1), and matrix metalloproteinase (MMP-9) than only ASMCs culture. All of the previously mentioned mediators correlated with the degree of ASMC proliferation. In addition, the increased proliferation index of human ASMCs in co-culture decreased by inhibiting IL-6 and/or IL-8 and abolished by inhibiting MCP-1 and/or MMP-9 pathways. Nevertheless, physically injured human BECs have increased the proliferation index of human ASMCs in vitro even more than healthy BECs, and the co-culture expressed even higher concentrations of IL-6, IL-8, MCP-1, and MMP-9. However, when comparing the injured and healthy BECs cultures, only the production of MMP-9 tends to increase between the wounded and uninjured epithelial cells. These findings suggest the importance of injury-induced MMP-9 release by epithelial cells in the proliferation of ASMCs (15). Since mechanical compression also increased BECs-induced ASMC proliferation, the BECs-ASMCs interactions are fundamental in how bronchoconstriction adds to bronchial remodeling (23). In vivo studies also provide similar results, in which mechanical injury of the epithelium layer of rabbit trachea stimulated proliferation in the airway smooth muscle layer (15). It is merit to mention that physical injury or compression is not the only factor that could arouse the proliferation of ASMCs by BECs. Many exogenous and endogenous stressors could produce such an effect. Tang et al. demonstrated that both human BECs and BEAS-2B cells increased the proliferation of human ASMCs *in vitro* in response to YKL-40 exposure. This exposure was responsible for a surge in the cytokine IL-8 (CXCL8) production from BECs (31). In addition, YKL-40 increased the proliferation of ASMCs in cell culture directly, even without the presence of BECs in the culture, by activating the

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PAR-2-receptor signal (32). In the endogenous stimulators aspect, amphiregulin is a member of the epidermal growth factor (EGF) family and a potent growth stimulator. Amphiregulin encouraged BECs to increase the growth factor expression in ASMCs mainly by acting on the COX-2 enzyme of BECs (7). Furthermore, the leukotriene D4 also induced BECs to increase the production of TGF-B1 in cell culture. The TGFB1-rich supernatant of BECs significantly increased the proliferation of ASMCs in vitro (43). As LTD4 can increase TGFB1 production from the BECs acting on cys-LT receptor 1 (CysLT1), the authors hypothesized a paracrine loop of TGFB1 secretion from BECs and ASMCs could be involved in ASMC proliferation in vivo as well.

Respiratory inflammatory diseases such as asthma and COPD are well known for periodic degrades of the situation called exacerbations. These exacerbations are provoked mainly by outside or exogenous factors or stimuli such as bacteria, cigarette smoke, house dust mites (HDM), and other allergens (34, 44-46). *Lu et al.* reported that human alveolar epithelial cells exposed to cigarette smoke extract (CSE) increased the ability of human ASMCs to repair injury (35). In this aspect, epithelial cells stimulation by house dust mite extract increases the proliferation of asthmatic ASMCs but not that of subjects without asthma. The mechanism of this effect involves a protease-activated receptor (PAR)-2–dependent epithelial production of leukotrienes C4. In addition to overexpression of leukotrienes receptor, CysLT1 is asthmatic ASMCs (46). In addition, the environmental pollutant Nonylphenol increased human BECs and BEAS-2B cells' production of IL-6 and IL-8 in cell culture. Hence, the conditioned culture medium of human BECs and BEAS-2B cells increased ASMC proliferation in indirect co-culture experiments (36).

In conclusion, many researchers have associated IL-8 and IL-6 with increased ASMC proliferation in epithelial cell injury models. Since the cytokine IL-8 is also strongly related to increased ASMC migration in these settings, the pharmacological targeting of this cytokine or its receptors could be the center of a future possible therapeutic strategy that aims to prevent irreversible bronchial remodeling in many patients. However, we are still in need of more valuable research to understand the mechanisms of how external and internal stimuli alter the airway epithelial cells to initiate their impact on bronchial smooth muscle cells. Such research could improve our treatment for chronic lung diseases even more with agents that act on the beginning of the disease cascades and loops. Table 1 summarizes the research that aims to understand the impact of BECs on ASMCs. Meanwhile, the graphical abstract represents a visual summary of the outcomes of this impact.

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Table 1. Summary of the impact of BECs on ASMCs in a relaxed and stressful microenvironment.

Outcome	*Model*	*Animals*	*cell type*	*Stressor*	*Means of interaction*	*Mechanism*	*Reference*
Relaxation	*In vitro*	Human Guinea Pig	BEC*	None	GABA	*Unknown*	*[20]*
	In vitro	Human	BEAS-2B ASMC**	None	Acetylcholine Nitric oxide	Unknown	[21]
	In vitro	Human	ASMC BEAS-2B BEC	None	ASMCs COX-1 Prostaglandin E receptors 2 and 4	Unknown	[22]
Contraction	*In vitro*	Human	ASMC BEC	Mechanical pressure	Endothelin-1	Unknown	[23]
	Ex vivo / In vitro	Rat Human	BEC	Physical injury	Unknown soluble mediators	*Unknown*	*[24]*
	Ex vivo / In vitro	Human	ASMC	Rhinovirus	IP-10 MIP-1b	Unknown	[26]
	In vivo	Mouse	NA	IL-13	Unknown	Unknown	[27]
	In vivo / In vitro	Mouse Human	BEC	IL-13	CCL2 / CCL24 CXCL1	B-arrestin pathway	*[28]*
	In vivo / Ex vivo	Mouse Human	NA	Genetic	Neuropeptide Y	*Unknown*	*[16]*
Migration	*In vitro*	Human	BEAS-2B BEC ASMC	YKL-40	IL-8	Activating MAPK and NF-κB Pathways	*[31]*
	In vitro	Human	ASMC BEC BEAS-2B	TNFα	IL-8 RANTES	Unknown	[30]
	In vitro	Human	ASMC BEC	TNFα	IL-8 CCL5	Unknown	[33]
	In vitro	Human	ASMC A549	CSE	IL-8 TGF-β1	M3 mAChR	[35]
	In vitro	Human	ASMC BEAS-2B HBE135	Nonylphenol	IL-6 / IL-8	Unknown	[36]
	In vitro	Human	ASMC BEC	Rhinovirus	CXCL8 CCL5	Unknown	[37]
	In vitro	Human	ASMC BEC	Rhinovirus	CXCL10	Unknown	[38]
Proliferation	*In vitro*	Human	ASMC BEC BEAS-2B	None	ASMCs COX-1 Prostaglandin E receptors 2 and 4	*Unknown*	*[22]*
	In vitro / In vivo	Human Rabbit	ASMC BEC	Physical injury	IL-6 / IL-8 MCP-1 MMP-9	*Unknown*	[15]
	In vitro	Human	ASMC BEC	Mechanical pressure	Endothelin-1	*Unknown*	[23]
	In vitro	Human	BEAS-2B BEC ASMC	YKL-40	IL-8	Activating MAPK and NF-κB Pathways	*[31]*
	In vitro	Human	ASMC BEC	Amphiregulin	Unknown	COX-2 activity in BEC	*[7]*
	In vitro	Human	ASMC 293LT1 A549 BEC	Leukotriene D4 LTD4	TGF-B1	CysLT1 receptor	*[43]*
	In vitro / Ex vivo	Human	ASMC BEC	HDM	Leukotriene C4	PAR-2 receptor	*[46]*
	In vitro	Human	ASMC	Nonylphenol	IL-6 / IL-8	Unknown	[36]

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	BEAS-2B
	HBE135

** BEC: Primary bronchial epithelial cell, ** ASMC: Primary airway smooth muscle cell*

3. The Effects of Airway Smooth Muscle Cells on Epithelial Cells

The presence purpose of the airway smooth muscle layer in adult airways is still widely debated. Some researchers describe it as the airways appendix, and others restrict its physiological role to contraction only; hereafter, its actual function is still not entirely understood in healthy adults (47). However, their contribution to lung pathophysiological conditions and inflammatory diseases is well established. In addition to their solid contracting ability that narrows the airway lumen, ASMCs can produce many proinflammatory growth factors, such as transforming growth factor-β (TGF-beta), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF). In addition to a wide range of cytokines, such as IL-1β, IL-5, IL-6, IL-8, and IL-17 (48). Furthermore, ASMCs can also switch their phenotype from contractile to proliferative and increase their migration (49). *Faiz et al.* reported that IL-1B increased the secretion of CCL20 from the ASMCs taken from both healthy and asthmatic patients. However, this augmentation was more noticeable in cells taken from asthmatic patients. CCL20 released from ASMCs induced mucus production in BECs, mainly by acting on CCR6 on mucus-producing goblet cells in the epithelium (50). Under specific conditions, airway smooth muscle cells can also influence the cytokine production of BECs. *Deacon et al.* reported that human ASMCs increased amphiregulin secretion in response to bradykinin exposure. This augmented level of amphiregulin directly increased the production of CXCL8, Vascular endothelial growth factor (VEGF), and COX-2 in airway epithelial cells (7). A recent study showed that CCL20 released from human ASMCs has significantly increased rhinovirus replication within the epithelium *in vitro*. This action which involves the antiviral protein kinase RNA-activated (PKR) pathway, reveals another dimension of ASMCs impact on respiratory diseases where frequent exacerbations due to respiratory viruses are often observed, such as Asthma and COPD (51).

Compared to adult life, the role of the airway smooth muscle cells in lung development is better elaborated. Many studies suggest that ASMCs are essential for the development of the epithelia during organ development. As the development of the lung begins as a simple epithelial tube surrounded by mesenchyme cells that later develop into several different cell types, smooth muscle cells develop from fibroblast growth factor 10-expressing mesenchymal cells (52, 53). During lung development, the epithelium layer experiences branching morphogenesis to generate the conducting airways, such as bronchi and bronchioles (54). However, epithelial proliferation is insufficient to create branches, and smooth muscle wrapping is required to shape the epithelium into a tube-like shape (55). This task of ASMCs is particularly true in the cartilage-lacking bronchioles case, where smooth muscle cells are indispensable for providing the required elasticity to keep the airway open (56). The smooth muscle cells could physically apply mechanical forces to sculpt the epithelia, thanks to their contractile phenotype. However, recent computational models suggested that epithelial folding can be driven by the stiffness of the smooth muscle layer surrounding the epithelial cells rather than by contraction forces (57). *Goodwine et al.* also confirm this hypothesis in a recent study. The authors published a preprint showing that only the existence of smooth muscle cells was sufficient for airway branching morphogenesis in mice. Even if these smooth muscle cells genetically lack the contractile phenotype (58). In addition, *Palmer et al.* demonstrated that this stiffness helps as a mechanical wall where fluid pressure pushes the epithelial cells against during epithelial branching in the lizard lung. For a better understanding of this effect, the authors draw an example of a stress ball; the fluid pressure inside the developing tube pushes the epithelial cells against the holes of a meshwork of smooth muscle layer (59). We can also find similar outcomes if we look at the innovative method of 3D cell culture studies. *Güney et al.* (60) seeded airway epithelial cells inside an ECM-mimicking structure of agarose and hydrogel (agrogel) and observed their development and proliferation for about three

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weeks. The authors of this study have reported that when seeded alone, epithelial cells formed spheres inside the 3D structure of agrogel. However, adding stromal cells such as ASMCs has increased the survival of epithelial cells, but most importantly, it has transferred the sphere-shaped 3D structure into the tubule. The tubule formation was epithelial cell-driven and surrounded by stromal cells. Therefore, the crosstalk between BECs and ASMCs was vital for spheres branching into a tube-like structure the authors called bronchotubules.

In mice, α-smooth muscle actin (SMA α)-expressing smooth muscle cells border the airway epithelium before the epithelial branching in the embryonic mouse lung. This localized presence of smooth muscle cells is necessary for the epithelium branching, as surgically removing smooth muscle abolished the branching entirely (52). In this aspect, *Goodwin et al.* also revealed that epithelial proliferation during mouse lung development is insufficient to generate domain branches. In addition, the authors stated that smooth muscle wrapping is essential to shape the epithelium into a branch (55). After the epithelium branching, the fibroblast growth factor 10-expressing cells translocate proximally along the airway and express smooth muscle-specific genes, such as α-actin-2 (61). These cells also release fibroblast growth factor 10 to act on the epithelial progenitors to prevent their differentiation and promote their proliferation (62). ASMC defects during mice's lung development are associated with a different epithelial phenotype characterized by decreased basal cell number, precocious club cell differentiation, and increased secretoglobin expression (56). Even after lung development, the study of *Volckaert et al.* reported that the embryonic fibroblast growth factor 10 signaling pathway is also reactivated in mature ASMCs after BECs injury in adult mouse lungs. This action allows ASMCs to form an airway epithelial stem cell niche that services BECs repair (62). In this aspect, *Moiseenko et al.* reported this BECs-repair supporting ASMC population is distinct from pre-existing airway smooth muscle cells and is critical for renewing BECs of the adult lung (63). Nevertheless, in a recently published study, *young et al.* reported that differentiation of airway smooth muscle during lung development is unnecessary for lung branching morphogenesis in mice. Still, it is indispensable for establishing airway size and tracheal cartilage segmentation (64) (The graphical abstract outlines the impact of ASMCs on bronchial epithelial cells).

Understanding the interaction between airway smooth muscle cells and bronchial epithelial cells during organ development could seem less important to develop new treatment strategies for lung diseases at first. However, these interactions are vital in organ engineering and developing novel 3D ex vivo models such as organoids and lung-on-chip that help to test and develop new possible medications. These modern models have great importance since they provide a 3D structure that mimics the lung microenvironment and the organ complexity in which cells interact with the surrounding extracellular matrix and the external environment without losing close interaction with each other (54, 65).

4. Conclusion:

Bronchial epithelial and airway smooth muscle cell interactions are promising targets for developing novel therapeutic strategies for chronic lung diseases. The epithelium strongly influences ASMC contractility, migration, and proliferation; therefore, it is a robust initiator of airway remodeling. Furthermore, all stress, compression, and injury aggravated this influence, making the epithelium one of the suspects in inducing a loop of continuous remodeling in the airway wall. Understanding the impact of airway smooth muscle cells on the epithelium is also essential because manipulating these effects is the base of developing advanced experimental 3D models. The 3D models are up-and-coming to increase the credibility of *in vitro* studies taking advantage of their ability to use human cells in a 3D structure that mimics the tissue microenvironment.

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