Role of Airway Epithelial Cells in Development of Chronic Obstructive Pulmonary Disease

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Abstract: Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation of peripheral airways that is not fully reversible. COPD is associated with airway remodeling, which thickens the airway walls and narrows the airway as a result. Exposure to cigarette smoke is the major risk factor for this condition. Pathogens including viruses and bacteria may induce COPD exacerbations. Now there is more evidence to suggest a significant relationship between airway epithelial cells and the pathogenesis of COPD. Airway epithelial cells make up an efficient barrier against pathogens and aggressive molecules. However, after exposure to cigarette smoke and pathogens, epithelial cells are stimulated to release a variety of pro-inflammatory mediators, including chemokines, cytokines, and growth factors through signaling pathways. These mediators recruit or stimulate other types of cells to induce an increased inflammatory response, production of mucus, and finally result in airway remodeling. After epithelium injury, epithelial cells participate in the process of repair and regeneration to maintain the epithelial integrity. A better understanding of the role of airway epithelial cells in COPD may be valuable to provide a basis for new therapeutic strategies.

Keywords: Airway epithelial cell, chronic obstructive pulmonary disease, inflammation, remodeling, wound repair.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a condition characterized by airflow limitation of peripheral airways that is not fully reversible and usually becomes progressively worse over time. The key determinants to this limit in airflow are an increase in the resistance of small conducting airways less than 2 mm in diameter and emphysematous destruction of the lung elastic recoil force available to drive expiratory flow [1]. COPD is typically associated with chronic bronchitis and emphysema. Inhalation of noxious particles or gases, particularly cigarette smoking (CS), is the major risk factor. Pathogens, including viruses and bacteria, may induce COPD exacerbation. COPD currently affects between approximately 10 and 24 million adults in the United States alone [2]. COPD is the fourth leading cause of death worldwide, and further increases in its prevalence and mortality rate are predicted [3].

ANATOMY AND PHYSIOLOGICAL FUNCTION OF AIRWAY EPITHELIAL CELLS

The surface of airway epithelium consists of ciliated cells, goblet cells, Clara cells and basal cells. Ciliated cells are found from the trachea to the last respiratory bronchiole, but their height decreases from pseudostratified columnar cells to simple columnar cells, and eventually cuboidal cells, with the reduction of the airway diameter. The frequency of goblet cells also decreases toward the periphery; until in the bronchioles, they are replaced by Clara cells. These airway epithelial cells make up an efficient barrier through intercellular epithelial junctions against pathogens and aggressive molecules. Moreover, they exert important effects in airway defense mechanisms through mechanical clearance of the mucus, homeostasis of ion and water transport, biochemical antibacterial, antioxidant, antiprotease functions, and so on [4].

ROLE OF AIRWAY EPITHELIAL CELLS IN CIGARETTE SMOKE EXPOSURE

CS exposure is a key risk factor in the development of COPD and is identified as the primary cause of 80–90% of all COPD cases in the United States [5]. A series of studies have previously found that expression of Interleukin-8 (IL-8) and mucin was significantly increased and the barrier function (trans-epithelial electric resistance, TEER) was reduced when airway epithelial cells were exposed to CS [6-9]. The reduction of TEER reflects a breakdown of the epithelial integrity, which can affect the defense mechanisms of the airway. Further studies have confirmed that the epidermal growth factor receptor (EGFR) mediated the production of IL-8 and mucin induced by CS in airway epithelial cells [8,9]. Besides EGFR, niflumic acid-sensitive chloride channels (probably calcium-activated chloride channel) also affect the mucin production as a part of a single complex signaling pathway [10]. Then by activating the mitogen-activated protein kinase (MAPK) pathway in airway epithelial cells, nicotine contributes to the development and progression of the inflammatory reactions in COPD patients [11].

Baginski *et al.* have reported that CS induced early growth response gene 1 (Egr-1) to up-regulate in epithelial cells. Through the Egr-1-mediated mechanisms, pro-inflammatory cytokines, such as IL-1β and TNF-α, were significantly up-regulated in pulmonary epithelial cells exposed to CS [12]. And then CS had the potential to amplify induction of respiratory mucins by pro-inflammatory stimuli.

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Reynolds *et al*. have reported that CS synergistically increased gene expression and protein production of MUC5AC mucin, which was not only induced by lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α), and tumor growth factor-α (TGF-α), but also by amphiregulin in airway epithelial cells [13].

ROLE OF AIRWAY EPITHELIAL CELLS IN THE INFECTIONS OF PATHOGENS

Pathogens, including viruses and bacteria, can cause injury to the epithelial cells lining the airway, and then exacerbate COPD and accelerate deterioration of lung function. Acute exacerbations of COPD are major causes of hospitalizations and death, and account for 70% of health care costs for the disease [14].

Viruses such as rhinovirus (RV), influenza, parainfluenza, respiratory syncytial virus (RSV), adenovirus (AV), and coronavirus contribute to the exacerbation of COPD. Of these viruses, RV serves as the dominant viral pathogen [15-18]. Respiratory viral infections are associated with COPD exacerbations that are more frequent, severe, and have longer recovery times [16].

The airway epithelial cell is the principle site of respiratory viral infection and plays a central role in viral modulation of airway inflammation through the release of cytokines, chemokines, and growth factors. Viruses enter into the epithelial cells based on interaction with host-cell surface receptors. RV attaches to epithelial cells through intercellular adhesion molecule-1 (ICAM-1), then enters into the cells. However, blockage of ICAM-1 may prevent RV infection [19-21]. Following viral infection, many inflammatory mediators are produced from airway epithelial cells. For instance, RV infection has been shown to induce production of a variety of pro-inflammatory chemokines, including Epithelial neutrophil-activating protein-78 (ENA-78), IL-8, INF-γ-inducible protein 10 (IP-10), regulated on activation normal T-expressed and secreted proteins (RANTES), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), as well as cytokines, such as IL-1, IL-6, IL-11, IL-16, TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF) from epithelial cells [15,20]. These inflammatory mediators contribute to recruitment and activation of inflammatory cells including T cells, NK cells, macrophages and neutrophils, as shown in Fig. (**1**). Though such responses facilitate clearance of virus, these cells may also serve to amplify pre-existing inflammation, then induce a pulmonary and systemic inflammatory response, mucus hypersecretion and airway remodeling, all of which contribute to the exacerbation of COPD.

Further studies have confirmed that Toll-like receptor (TLRs)-3 and epidermal growth factor receptor (EGFR) were induced to increase double –stranded RNA responsiveness and inflammatory reaction in the airway epithelial cells after RV infection [22-25]. And then a series of signal transduction pathways, which take part in the control of transcriptional and post-transcriptional regulation of epithelial cytokine and chemokine production, are induced by viruses [24,26-30]. These signal transduction pathways includes Phosphatidylinositol 3-Kinase (PI3K), MAPK, STATs, Nuclear Factor-κb (NF-κB) and so an. However, our under-

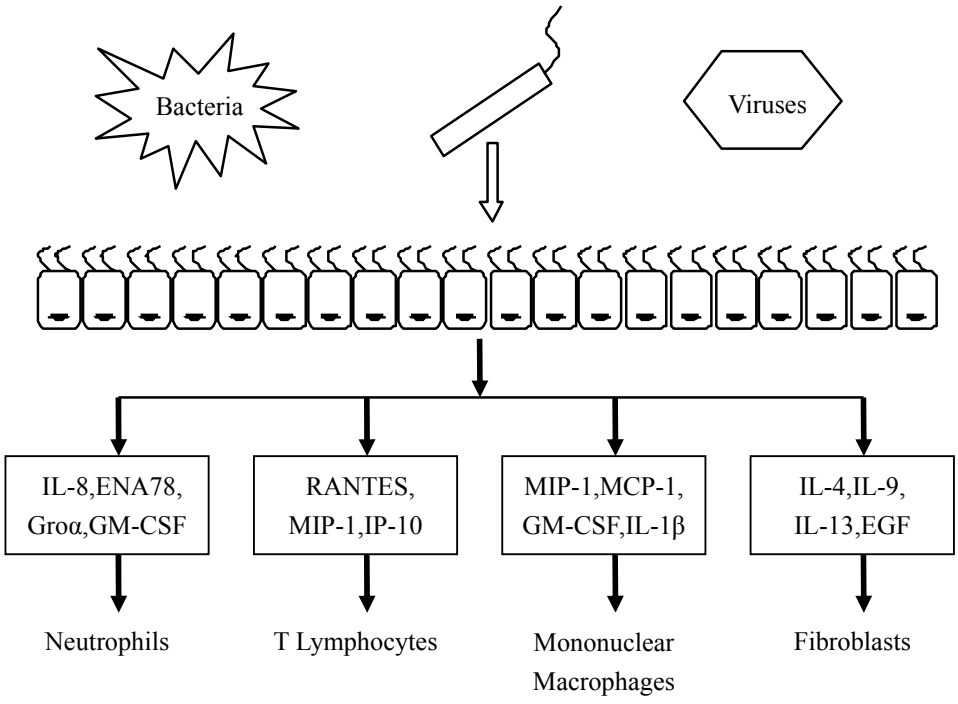

Fig. (1). Cigarette smoking and pathogens act on airway epithelium, then stimulate cells to release pro-inflammatory mediators. These mediators recruit or stimulate neutrophils, T lymphocytes, mononuclear macrophages, and fibroblasts, which induce a pulmonary and systemic inflammatory response, mucus hypersecretion, and airway remodeling.

standing of the mechanisms by which viruses induce cytokine and chemokine production still remains limited.

Beside viruses, bacteria can also trigger exacerbations in patients with COPD by colonizing and infecting the lower respiratory tract [31,32]. Bacteria are capable of efficiently adhering and invading the airway epithelium, especially binding to damaged cells or cells still undergoing repair. Then such as *M. catarrhalis* and *H. influenzae* induce inflammatory response in airway epithelial cells through the MAPK and NF-κB activation pathways, which is characterized by the release of IL-8, GM-CSF, IL-1β, and TNF-α [33-35]. Furthermore, Slevogt *et al.* have shown that the inflammatory response reduced by *M. catarrhalis* was of a strong time and dose dependent [35]. However, a prospective, longitudinal cohort study of patients with COPD has shown change in bacterial load was unlikely to be an important mechanism for exacerbations [36]. Therefor better understanding of the host–pathogen interaction, rather than enumerating bacteria in respiratory samples, is potentially required to provide new insights into bacterial infection in COPD.

As early as 1986, Plotkowski *et al*. have demonstrated that pneumococcal adherence to respiratory epithelium is maximal in the repairing migratory cells after viral infection [37]. Similarly, Sajjan U *et al*. have found that RV facilitated binding, translocation, and persistence of bacteria by disrupting airway epithelial barrier function [38]. Moreover, another study has shown H. influenzae infection increased the expression of the airway epithelial cell ICAM-1 and TLR3, leading to enhanced binding of RV and a potential for RV-induced chemokine release [39]. These data suggest bacteria and viruses may synergistically induce exacerbation of COPD.

ROLE OF AIRWAY EPITHELIAL CELLS IN OXIDATIVE STRESS

Oxidative stress plays an important role in the pathogenesis of COPD, which contributes to oxidative inactivation of antiproteases and surfactants, mucus hypersecretion, membrane lipid peroxidation, alveolar epithelial injury, remodeling of extracellular matrix, and apoptosis [40]. As the physical barrier between submucosal tissues and the external environment, airway epithelial cells are the initial targets of reactive oxygen species (ROS), which are injured by oxidants inhaled as atmospheric pollutants or produced during inflammatory responses. Many previous studies have demonstrated that CS and pathogens could induce oxidative stress in airway epithelial cells, though airway epithelial cells could synthesize and secrete antioxidants such as glutathione peroxidase [32,41-44]. For example, Pierrou *et al*. have observed significant changes in oxidant response genes *in vivo* and vitro exposed to CS, some of which were further amplified in a nonlinear fashion, in COPD [41]. Another study has confirmed that RV infection induced depletion of reduced glutathione in epithelial cells by activating xanthine oxidase [44].

ROS to the airway epithelial cells increases the gene expression of cytokines, such as TNF-a, which in turn activate the cells to induce pro-inflammatory genes, such as TNF-a, IL-8, IL-1, iNOS, cyclooxygenase-2, ICAM-1, IL-6, MIP-1a, GM-CSF, and stress response genes, such as heat shock protein (HSP)-27, 70, 90, hemeoxygenase-1 (HO-1), and antioxidant enzymes, such as glutamate cysteine ligase, MnSOD, and thioredoxin [45]. Rahman *et al*. have found that the levels of 4-hydroxy-2-nonenal-modified (4-HNE) proteins were increased in airway epithelial cells in subjects with COPD [46]. 4-HNE is a diffusible and highly reactive lipid peroxidation end-product, the modification of which results in abnormal regulation of cellular functions, such as cell proliferation and inhibition, T-cell apoptosis, and activation of various signaling pathways. Further studies have indicated that ROS was implicated in initiating inflammatory responses in the lungs through the activation of signal transduction pathways, such as the MAPK and PI-3K pathways, and transcription factors, such as NF-κB and activator protein-1 (AP-1), leading to enhanced gene expression of pro-inflammatory mediators [45].

Because oxidative stress is implicated in the pathogenesis of COPD, therapeutic administration of antioxidants may be effective in the treatment of COPD. A study by Di Stefano *et al.* has demonstrated the expression of the p65 protein of NF-κB was increased in airway epithelium in COPD patients, which was correlated with the degree of airflow limitation [47]. A related study about the potential therapeutic effect of an small interfering RNA against p65 in airway epithelial cells has demonstrated the cells treated with TNF-α showed a reduced p65 expression and concomitant IL-6 and IL-8 expression [40,45].

ROLE OF AIRWAY EPITHELIAL CELLS IN MUCUS HYPERSECRETION

With the progression of COPD, an association with mucus accumulation develops, though mucus acts as a physical barrier. In addition, polluted air, CS, pathogens can increase the production of mucus [9,10,13, 48-51]. For instance, RV can increase the mucin production in epithelial cells by the MAPK or TLR3-EGFR-dependent pathway [48,50]. The surface epithelial goblet cells are the main source of secretory gelforming mucins in the airways, and the sub-mucosal glands serves as a secondary source. Since COPD mainly implicates in peripheral small airways, which do not contain sub-mucosal glands, it appears that goblet cells maybe play an important role in the initiation and development of COPD. Furthermore, the number of goblet cells also increase in COPD. Moreover, some cytokines influence the formation of goblet cells. For example, IL-1β increases MUC5AC expression and secretion in bronchial epithelial cells [51]. Some previous studies have demonstrated that the activation of EGFR increased goblet cells metaplasia and mucus production through MAPK and PI3K signaling pathway [52-54].

Besides the oversecretion of mucus, the reduction of mucociliary clearance also results in mucus accumulation. Mucociliary clearance relies on appropriate interactions between the ciliated epithelium, the height of the periciliary fluid and mucus, which may be disrupted by viral and bacterial infections or by inhalation toxins and other causes [55]. It has been shown that ciliated cells represent approximately 25-30% of surface epithelial cells in never-smokers, 10% in smokers with normal pulmonary function, and almost none in COPD patients [56].

ROLE OF AIRWAY EPITHELIAL CELLS IN APOPTOSIS

Apoptosis, programmed cell death, is a physiologic mechanism for elimination of unnecessary, damaged, or infected cells. Many previous studies of patients with COPD have indicated a greater presence of apoptotic cells in the lungs than those in the control group [57-62]. These apoptotic cells include bronchial and alveolar epithelial cells, as well as endothelial cells in the parenchyma.

CS plays an important role in the process of apoptosis of airway epithelial cells. Even for the ex-smokers with COPD, there is no significant difference in apoptosis of airway epithelial cells between current and ex-smokers with COPD, which implies excess apoptosis persists after the cessation of smoking [63]. On the one hand, CS activates effector caspases ,stimulates proteases and chemokines, and induces epithelial apoptosis *via* IL-18Rα-dependent pathways [64]. Besides, CS decreases histone deacetylase activity, resulting in increased expression of autophagic proteins, which induces autophagy and apoptosis [65]. On the other hand, CS impairs the clearance of apoptotic cells by suppressing phagocytic ability of alveolar macrophages [66-68]. An inability to effectively remove apoptotic cells may not only be the result of post-apoptotic cytolysis (secondary necrosis), but also interfere with normal cell replacement [60]. CS inhibits efferocytosis through oxidant-dependent activation of the RhoA-Rho kinase pathway [67]. The process is a dose-dependent, reversible, and cell type-independent manner, whereas more intense CS exposure had an irreversible effect. HO-1 provides cytoprotection against oxidative stress. Recent studies have demonstrated that HO-1 protected against CS-induced cell death by downregulating apoptosis and autophagy-related signaling [68,69]. Moreover, CS causes the loss of cellular ATP and rapid depolarization of mitochondrial membrane potential, and then blockade of mitochondrial respiratory chain, which switches epithelial cells apoptosis into necrosis [70]. However, HO-1 has the potential role in defense against mitochondria mediated apoptosis during CS exposure [69].

ROLE OF AIRWAY EPITHELIAL CELLS IN AIRWAY REMODELING

COPD is always associated with the remodeling of the airway epithelium, such as squamous metaplasia, mucous secretory cell hyperplasia, and mucus accumulation. This contributes to the decline of lung function, irreversible airflow obstruction, and predisposition of the patient to infection and hospitalization. The remodeling is the result of disruption in normal cell and tissue dynamics, which are caused by inhaled noxious particles or gases and pathogens, such as bacteria or viruses.

After injury, airway epithelial cells need to repair and regenerate to restore epithelial integrity and function in a rapid and dynamic manner. The current consensus is that many cells can contribute to repair of an injury, but that basal cells likely represent a stem cell compartment in the adult pseudostratified epithelium [71]. Besides basal cells, Clara cells may also be stem cells to restore the airway epithelium after injury. On the basis of studies in experimental animals and limited studies in humans, the process of repair and regeneration includes the spreading and migration of the basal cells neighboring the wound, proliferation and active mitosis, and squamous metaplasia, which is followed by progressive redifferentiation with the emergence of preciliated cells. The final step of the repair and regeneration process is ciliogenesis and complete regeneration of a pseudostratified mucociliary epithelium, as shown in Fig. (**2**) [71,72]. Lately, Park *et al*. have reported that in order to maintain the integrity of the epithelium of mice, ciliated epithelial cells transdifferentiated into squamous cells and spreaded beneath injured Clara cells within 6-12 hours of an injury, then transdifferentiated from squamous to cuboidal to columnar cell types as differentiation-specific cell markers typical of the mature airway were restored, as shown in Fig. (**2**) [73]. Though the study bases on animal model, it suggests human ciliated epithelial cells or other non-stem cells maybe have the same potential to repair the airway epithelium. Moreover, recently there are two studies identifying genes which mediate regeneration of human airway epithelial cells *in vivo* and *in vitro* respectively [74,75]. *In vivo*, Heguy *et al*. denuded the airway epithelium of healthy individuals, sequentially sampled the same region 7 and 14 days later. At 7 days compared with resting epithelium, there were substantial differences in gene expression pattern. The repair transcriptome was dominated by cell cycle, signal transduction, metabolism and transport, and transcription genes. However, at 14 days postinjury, the expression profile was similar to that of resting airway epithelium [74]. *In vitro*, Ross *et al*. cultured human bronchial epithelial cells over a 28-day period and identified over 2,000 genes that displayed statistically significant 2-fold or greater changes in expression during the time course. Many of these genes are involved in processes associated with airway epithelial biology, such as cell adhesion, immunity, transport, and cilia formation [75].

In the process of repair and regeneration, there are many cellular and molecular factors involved. In spreading and migration of the epithelial cells, the polymerization and accumulation of actin in the lamellipodia of the dedifferentiated and flattened basal cells results in forming adhesive contacts with the extracellular matrix (ECM). And then, by degrading components of the ECM, metalloproteases (MMPs) remodel the provisional matrix to allow migrating cells to form new contacts which may be used by cells to exert traction through actin filament bundles to move further along. MMPs such as MMP-7, MMP-9 play a key role in the migration to promote airway epithelial wound [76-81]. A further study has confirmed that it existed an exclusive expression of MMPs at the apical part of the well-differentiated regenerated airway epithelium, and incubation of the regenerating epithelial cells with MMP inhibitors leaded to an abnormal epithelial differentiation [81]. However, in this process, most of the bacteria identified in COPD bind especially to damaged cells or cells still undergoing repair. These bacteria also release virulence factors that impede or delay the migration of the epithelial cells and induce a disordered repair which undermines the epithelial barrier function. For instance, such as *H. influenzae, S. pneumoniae,* and *P. aeruginosa* can bind to ECM in areas of incomplete repair and virulence factors from *P. aeruginosa* and *S. aureus* induce actin skeleton disorganization and overactivation of MMP-2, which are responsible for delayed epithelial wound closure [4].

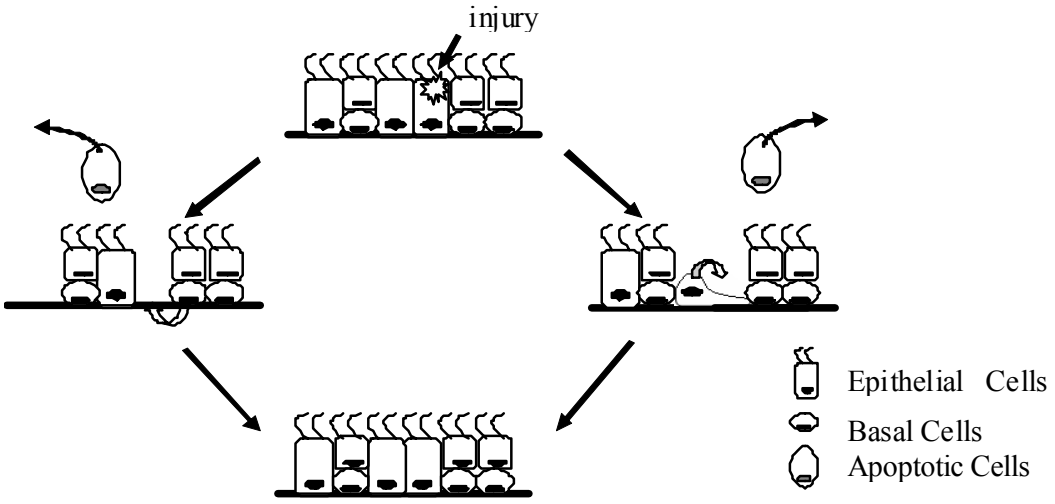

Fig. (2). Injury induces airway epithelial cells to undergo apoptosis and removal. Basal cells neighboring the wound participate in the process of repair and regeneration, including spread, migration, proliferation, squamous metaplasia, finally transdifferentiate mature epithelial cells. Ciliated epithelial cells also have the ability to spread and transdifferentiate into distinct epithelial cell types to repair the airway epithelium.

During injury and repair, epithelial cells also are able to secrete cytokines and growth factors. Coraux *et al*. have reported that during the cell migration and proliferation steps airway epithelial cells expressed IL-8 at a relatively high level, whereas airway epithelial pseudostratification and surface airway epithelium differentiation were associated with an increased expression of MMPs and a progressive decrease in the level of IL-8 [81]. TGF-β1 modulates the composition of the provisional matrix over which the epithelial cells migrate and has been shown to increase in airway wound repair through MMP-2 up-regulation [82].

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