Lung Disease in Cystic Fibrosis

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Cystic fibrosis (CF) is an autosomal recessive, life-threatening condition affecting many organs and tissues, the lung disease being the chief cause of morbidity and mortality. Mutations affecting the CF Transmembrane Conductance Regulator (CFTR) gene determine the expression of a dysfunctional protein that, in turn, triggers a pathophysiological cascade, leading to airway epithelium injury and remodeling.

Keywords: cystic fibrosis ; CFTR ; airway epithelium ; wound healing ; EGF/EGFR ; epithelial-mesenchymal transition ; curcumin ; CFTR modulators ; mesenchymal stem cells

1. Introduction

Cystic fibrosis (CF) is the most common autosomal recessive disorder in the Caucasian population, affecting more than 80,000 people around the world. CF affects many mucosal organs lined by an epithelium, including the lung and gastrointestinal tract, as well as many glands with secretory/adsorptive functions. The lung disease is, however, the major determinant of morbidity and mortality of CF individuals [1]. More than 2000 variants of the CFTR gene have been described, with a genotype-phenotype correlation for only 322 mutations (https://www.cftr2.org/, accessed on 4 March 2021) organized into six classes depending on the fate and function of the CFTR protein [2]. According to a newer classification [3], which includes a previous proposal of De Boeck and Amaral [4], CFTR mutations are still categorized into six classes, with the only exception that class I now comprises those of class 1A (no mRNA transcription) and class 1B (stop-codon mutations), both having the same outcome, i.e., absence of the CFTR protein (in case of class IB due to degradation of truncated mRNA by nonsense-mediated decay). Class II (to which the most common mutation—*F508del*—belongs) include mutants that do not overpass the endoplasmic reticulum quality control and are degraded in the proteasome. Class III and class IV mutations are those impairing gating or alter conductance of chloride and bicarbonate ions, respectively, of a CFTR channel correctly transported to the apical membrane. Class V mutations lead to a reduction in the CFTR protein levels due to alternative splicing. Class VI comprises those mutations that destabilize the CFTR protein at the apical membrane. Classes I, II, and III are associated with a more severe phenotype, whereas classes IV, V, and VI with milder phenotypes.

Since the discovery of the *CFTR* gene in 1989, many studies have elucidated the pathophysiological cascade occurring in the bronchi/bronchioli. The CFTR protein is a transmembrane glycoprotein which is comprised of two specular halves (each composed of six membrane-spanning domains and a nucleotide binding domain) connected by a regulatory domain, which makes it unique among the other members of the superfamily of ATP-binding cassette proteins. Functions classically ascribed to CFTR are linked to its activity as a channel of chloride and bicarbonate ions, involved in the proper hydration of airway surface liquid (ASL) [5]. Low or null CFTR protein expressed at the plasma membrane or its dysfunction [4] results in airway surface dehydration and ASL volume depletion, whose pathogenesis is attributable to abnormal ion and fluid homeostasis at the apical side of airway epithelial cells [6]. CFTR is involved also in sodium ions concentration in the ASL by a tonic inhibitory effect on the epithelial sodium channel (ENaC) [7][8], thereby in CF ENaC is hyper-activated and contributes to the liquid hypersorption from the airways [9]. Moreover, loss or dysfunctional CFTR is responsible for defective bicarbonate secretion, resulting in a reduced antimicrobial activity of ASL [10], as well as alterations in rheological properties of mucus which becomes dense with increased viscosity [11]. Altogether, these early events ultimately lead to reduced mucociliary clearance (MCC), mucus accumulation, airway plugging, bacterial colonisation, neutrophil inflammation, progressive tissue damage and decline in lung function in CF airways. A wealth of inflammatory and remodeling mediators has been found at altered levels in CF airways, i.e., in samples of sputum, bronchoalveolar lavage (BAL) fluid and exhaled breath condensate, as well as in the blood [12]. These mediators reflect the recruitment and activation of neutrophils (for example, HMGB-1, IL-1β, TNF-α, IL-8, GM-CSF, IL-17, elastase, myeloperoxidase), activation of adaptive immunity arms (IFN-γ, IL-17, IL-33), and events linked to angiogenesis and fibrosis (VEGF, TGF-β).

Besides its role as a chloride/bicarbonate channel, CFTR is known to be involved in many cellular and tissue processes, such as fetal development [13], epithelial differentiation/polarization [14], regeneration [15], tight junction (TJ) formation [16] and epithelial–mesenchymal transition (EMT) [17]. The presence of mutated CFTR is associated with the dysregulation of differentiation and repair, eventually leading to cancer [18], that was described as “a wound that does not heal” [19]. Other alterations associated with CFTR loss/dysfunction are implicated in the maintenance of apical-basal polarity of the airway epithelium and cytoskeletal organization. The barrier function of the airway epithelium is guaranteed by integrity by the apical junction complexes (AJC), consisting of occluding TJs, anchoring adherens junctions (AJs), desmosomes, and gap junctions (GJs). It is well described that mislocalization of TJ proteins, disorganized actin cytoskeleton and lack of actin stress fibers are present in CF cells [20][21][22][23]. The link to these alterations was found to be the interaction of CFTR with a molecular complex, tethering CFTR to the apical side of airway epithelial cells. The scaffolding protein Na^+/H^+ exchanger regulatory factor isoform 1 (NHERF1) is essential to maintain CFTR at the apical location through its interaction with ezrin, an A-kinase anchoring protein, that tethers PKA in the proximity of CFTR, allowing cAMP-dependent control of chloride efflux [24][25][26][27][28][29]. On the other hand, a low transepithelial resistance (TEER), a measure of epithelial tightness, indicative of TJ disorganization, has been observed in CF bronchial epithelial cells as compared with a wt-CFTR expressing cells [14][30][31]. Castellani et al. [16] confirmed these previous results on TJ disorganization in CF cells by the lack of TJ proteins at cell-cell contacts (occludin, ZO-1, claudin 1, and JAM-1), and showed also that NHERF1 or CFTR overexpression in CFBE41o- cell (*F508del* homozygous) monolayers induced the reorganization of TJ proteins at the level of intercellular junctions and reduced the paracellular permeability to small solutes. Interestingly, they also found that in CFBE cells, ZO-1 and occludin were localized to the nuclei, whereas the plasma membrane signal was negligible [16], indicating that CFBE cells present a dislocation of TJ proteins at the nuclear level in basal condition. In keeping with these data, Ruan and colleagues [32] have shown that in a three-dimensional (3D) epithelial cell culture model, CFTR interacts with ZO-1 at TJ levels and keeps constrained the transcription factor ZO-1 nucleic acid binding protein (ZONAB) at the same location. Upon CFTR inhibition or knockdown, ZO-1 expression is reduced and the translocation of the transcription factor ZONAB from TJs to the nucleus is induced, and an increased proliferative activity occurs.

GJs are formed by connexins (Cxs), which assemble in the plasma membrane to form hemichannels or connexons, that dock to similar connexons on the neighboring cell allowing GJ intercellular communication (GJIC). By allowing the passage of ions and small solutes, GJs are involved in the regulation of proliferation/differentiation and the maintenance of tissue homeostasis [33][34] as well as in CFTR-related epithelial functions [35][36]. CFTR has been described to interact with Cxs to regulate their trafficking and regulation of GJIC by pro-inflammatory mediators [37]. To note, it was observed that Cx43 showed perinuclear localization in CuFi-5 cells (*F508del* homozygous), while Cx26 localization was unaffected, and both Cxs were at the correct position in non-CF NuLi cells [31], further demonstrating a defect in cell-to-cell contacts in CF airway epithelia.

All these observations link CFTR to epithelial tightness and thus to the modulation of epithelial differentiation and proliferation, as we shall see in the following Sections dedicated to epithelial wound repair and EMT.

Events Involved in Epithelial Repair and EMT

In principle, epithelial repair follows different types of injury. In the case of CF, the damage to the airway epithelium can be caused by respiratory infections, in particular *Pseudomonas aeruginosa* (Figure 1) [38] and inflammatory mediators [39]. Also bacterial products secreted by *P. aeruginosa*, triggered by the quorum sensing (QS) network and involved in bacterial virulence, pathogenicity, and biofilm formation, have been shown to reduce airway epithelial repair rates [40][41][42]. On the other hand, heightened production of inflammatory mediators and reductions in anti-inflammatory molecule secretion by inflammatory and epithelial cells in CF lungs play a role in injury and remodeling of respiratory epithelia [43][44][45].

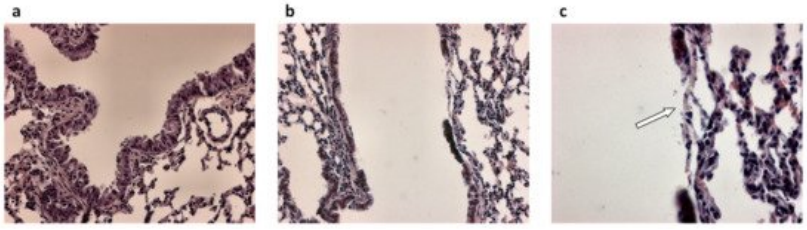

Figure 1. Airway epithelial damage in the airways following infection with *P. aeruginosa*. (**a**) Hematoxylin and eosin-stained lung section from control mice and (**b**,**c**) hematoxylin and eosin-stained lung sections from *P. aeruginosa* infected mice 48 h post-intratracheal instillation at a dose of 1×10^5 colony forming units. Panel **c** is an enlargement of panel **b**. Original magnification ×20 (**a**,**b**) and ×40 (**c**). White arrow in (**c**) indicates loss of the airway epithelium.

In the airways, epithelial repair follows general rules of reconstitution whose hallmarks are [46]: spreading and migration of neighboring epithelial cells using filopodial and lamellipodial extensions, a further step characterized by migration and proliferation of progenitor cells and, finally, differentiation. Recently, EMT induction, during which epithelial cells transform into mesenchymal-like cells, has been recognized as essential in physiologic and pathologic repair [47][48]. During EMT, epithelial cells lose their epithelial markers (i.e., E-cadherin) and present a migratory phenotype to re-epithelialize the wound. Fibroblast growth factor (FGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), and transforming growth factor (TGF)-β are growth factors orchestrating wound repair steps and are also involved in the initiation and regulation of the EMT [47].

The epithelium maintains its integrity through cell surface protein complexes, which contribute to form epithelial cell–cell junctions. The reorganization of the cytoskeletal architecture and polarity complexes, which result in cell shape changes, cell elongation, membrane protrusions and front–rear polarity, are essential in EMT and enable directional migration. Upon the initiation of EMT, junctions composed in the AJC are deconstructed and the junction proteins are relocalized and/or degraded [49]. A decreased claudin and occludin expression, and the diffusion of ZO-1 from cell–cell contacts has been described in early stages of EMT [50]. The findings of the nuclear localization of occludin and ZO-1 in CFBE cells [16] can be now viewed in the context of partial EMT state of CF airway epithelial cells (see below). E-cadherin—one of the main components of AJs—once cleaved and detached from the plasma membrane can be degraded [51]. Therefore, β-catenin is released from E-cadherin and act as transcription factor in the presence of WNT signaling which protects it from degradation [52]. The decrease in E-cadherin levels can even cause the accumulation of p120 catenin (also known as catenin δ1) in the nucleus where it works as transcription factor [53]. EMT initiation is also associated with disruption of desmosomes [50][51], and the decrease of Cx levels which in turn induce the loss of GJs [54]. During EMT progression, a decrease in the junction proteins expression at transcriptional level is observed, and this further stabilizes the loss of epithelial junctions [55][56]. Moreover, the apical–basal polarity, which is mediated by molecular complexes physically and functionally integrated with the cell junction architecture, is lost.

Another key role of EMT programme is to provide cells with the ability to migrate by invading through ECM. This process requires the reorganization of cytoskeleton so as to permit the dynamic elongation and directional motility [51][57][58]. Cells produce several types of membrane protrusion, which are rich of actin and facilitate cell movement and act as sensory extensions of the cytoskeleton. These projections include lamellipodia, which are sheet-like membrane protrusions, and spike-like extensions called filopodia at the edge of them [59]. Invadopodia degrade ECM by proteolytic activities, thus facilitating cell invasion [59][60]. The family of RHO GTPases is involved in these processes with RHOA promoting actin stress fibre formation and RAC1 and CDC42 promoting the formation of filopodia and lamellipodia. The conversion from apical-basal polarity (epithelial cells) to front-rear polarity (mesenchymal cells), which is one of the main aspects of EMT, involves RHO GTPases [61][62].

The repression of genes which are fundamental for the epithelial structure (e.g., E-cadherin) is counterbalanced by the activation of genes encoding proteins involved in mesenchymal adhesion such as N-cadherin (mesenchymal neural cadherin). Depending on the cell type and the extent to which cells advance through an EMT programme, cells undergoing an EMT may begin to express vimentin, to suppress cytokeratin, to shift expression of key integrins and so forth. These changes in the intermediate filament composition enable cell motility as vimentin can interact with motor proteins [63].

Remodelling of the ECM and changes to cell interactions with the ECM are essential in the initiation and progression of EMT. While acquiring a mesenchymal phenotype, epithelial cells lose their interaction with basal membrane and communicate with an inflammatory ECM.

As integrin complexes enable cells to receive ECM signals and integrate those elicited by growth factors, some epithelial integrins are down-regulated while others are activated in EMT. Some of these newly expressed integrins have key roles in EMT progression [51]. Thus, $\alpha_6\beta_4$, that mediates contacts with the basement membrane, is epigenetically down-regulated [64], whereas β_1 integrins increase during EMT [65][66][67][68]. During wound repair, β1-integrin subunit was found to be expressed by repairing cells on their basolateral side as well as on the apical side [69]. Kim and colleagues [65] demonstrated the crucial role of the laminin receptor $\alpha_3\beta_1$ integrin in E-cadherin turnover and the remarkable crosstalk and interdependence of TGF-β signalling and β-catenin–SMAD signalling systems, in the control of EMT. Also the fibronectin receptor $\alpha_5\beta_1$ integrin expression is augmented during EMT increasing cell adhesion to fibronectin, the expression of which is also activated during EMT, and promoting cell migration and protection from apoptosis. The increased expression of the β_1 integrins and their ligation of collagen type I initiates a signaling cascade, leading to disassembly of the E-cadherin adhesion complex and the nuclear translocation of β-catenin, ultimately determining cell proliferation [68]. A strict connection of changes to the integrin repertoire and ligation as well as intracellular signals and

increased expression of metalloproteinases (MMP2 and MMP9) has been demonstrated [49][70][71]. MMPs will enhance ECM protein degradation where integrin adhesion receptors focally interact with inflammatory ECM proteins, thus enabling invasion [51][59][72]. Other effects of MMPs related to the EMT activation are related to the trimmering of the extracellular domain of E-cadherin, thus contributing to the loss of AJs [70], and to increased SNAIL1 expression operated by increased levels of reactive oxygen species [73]. Finally, it has to be recognized that some growth factors are stored in the ECM and that localized ECM degradation may release them, such as is the case for TGF-β that is present in a latent form and is activated by MMPs and $\alpha_v\beta_6$ [74][75]. TGF-β, in turn, stimulates the expression of collagens and fibronectin, which are involved in the matrix remodeling.

EMT is induced by an interplay of soluble growth factors such as HGF, members of the TGF (e.g., TGF-β) and FGF families, insulin-like growth factor (IGFs, e.g., IGF-1), EGF as well as extracellular matrix such as collagen or hypoxic conditions. These factors activate signaling pathways leading to either expression or post-transcriptional and post-translational modification of EMT-associated transcription factors (EMT-TFs) [76][77]. Three main families of EMT-TFs have been described with the SNAI (SNAI1/Snail and SNAI2/Slug), ZEB (ZEB1 and ZEB2), and TWIST (TWIST1 and TWIST2) nuclear proteins, playing pivotal roles in the orchestration of EMT [78]. These EMT-TFs have been shown to interact with a variety of proteins involved in transcriptional regulation including proteins that function in epigenetic modification, forming together regulatory complexes.

In the airways, the final step of wound repair is the terminal differentiation which means the reconstitution of a pseudostratified epithelium with mucus-secreting cells (goblet), secretory (Club cells), ciliated cells, PNEC (pulmonary neuroendocrine cells), and basal cells. Recent studies have identified another cell type which has been called “ionocyte” and expresses higher levels of CFTR than other airway cells do [33][34]. Ionocytes derive directly from basal cells (BCs), some of which appear to function as classic multipotent stem cells, while other BCs are thought to be progenitors already committed to a ciliated or secretory fate [79][80]. As genetic (single-cell RNA sequencing) and more detailed histological and functional studies advance, not only ionocytes but also other rare cell types derived from BCs (PNEC, tuft cells, mucous ciliated cells and deuterosomal cells) are being studied during airway epithelium regeneration and repair [81][82].

The processes of epithelial repair are modulated by several growth factors, including EGF and related cytokines (TGF-α, amphiregulin, heparin-binding EGF), which through EGF receptors (EGFR), produce motility, proliferative responses, differentiation and survival [43][83][84]. MMPs and a disintegrin and metalloproteases (ADAMs) release from the cell surface EGFR ligands, including TGF-α, heparin-binding EGF (HB-EGF) and amphiregulin (AREG), that in turn can bind and activate EGFR in an autocrine or paracrine manner, while the transmembrane (pro) form may activate EGFR in adjacent cells (juxtacrine) [85]. During migration, cells attach to a provisional matrix of fibronectin and other extracellular matrix (ECM) components such as fibrin and fibrinogen. Platelet-derived growth factor (PDGF) and TGF-β act as the main modulators of ECM deposition by attracting and activating fibroblasts, in this way regulating airway repair [86][87]. Migrating epithelial cells overexpress MMPs to degrade the provisional matrix which provides novel attachment sites for lamellipodia. MMP-9 (gelatinase B), MMP-3 and MMP-11 (stromelysin 1 and 3, respectively), and MMP-7 (matrilysin), have been implicated in the matrix remodeling and acquisition of a typical epithelial-mesenchymal phenotype [88]. It has been suggested that the high expression and activation levels of MMPs can be regulated by an increase in IL-8, a pro-inflammatory cytokine [89]. TGF-β plays also a role in this remodelling process by up-regulating MMP-2 [90]. Furthermore, epithelial repair is stimulated by other growth factors, namely insulin, IGFs, HGF, keratinocyte growth factor (KGF), calcitonin gene-related peptides, and the cathelicidin LL-37 peptide. HGF and KGF acts as chemotactic and growth-stimulating factors. They also stimulate the synthesis of ECM and facilitate interactions with MMPs through specific cell receptors [91][92]. The main features of regeneration and wound repair in a non-CF airway epithelium are represented in Figure 2.

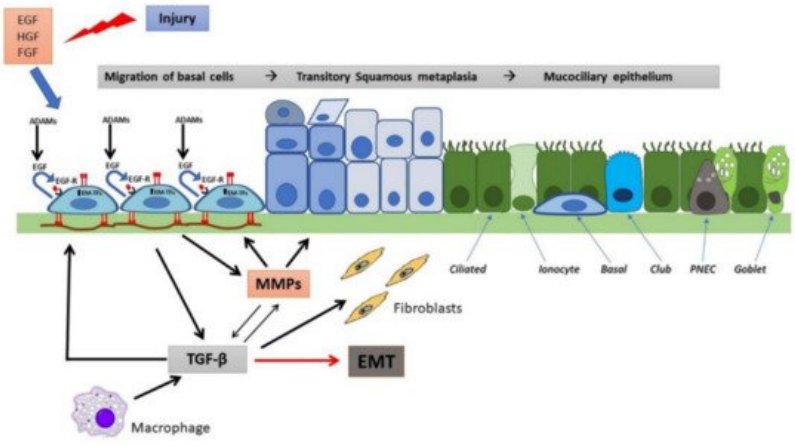

Figure 2. Wound repair and regeneration in a non-CF airway epithelium. Following injury, many cytokines and growth factors (e.g., EGF, HGF, FGF) are secreted in the wound repair microenvironment, which incite in basal cells a migratory and proliferative phenotype. Shedding of EGFR ligands (e.g., EGF) by ADAMs and binding to EGFR in an autocrine or iuxtacrine manner are key events involved in stimulation of cell migration and proliferation. Afterwards, MMP secretion by regenerating epithelial cells and subsequent TGF-β activation lead to genetic expression changes, related to EMT stimulation and activation of EMT-transcription factors (EMT-TFs). Epithelial cells and macrophages release TGF-β that induce ECM component deposition by epithelial cells and stimulate fibroblast activation, resulting in further matrix deposition. These events provoke alterations in junctional complexes and reorganization of actin cytoskeleton (not shown), modification of various integrin expression with β_1-integrins increase at basal side and ectopic expression on the apical side, deposition of inflammatory ECM glycoproteins (e.g., fibronectin is shown) and its remodeling exerted by MMPs. TGF-β and MMPs enhance each other in a positive way. This process proceeds with the formation of a squamous stratified epithelium and subsequent pseudostratification and mucociliary differentiation.

2. Pathological Processes in the CF Airways

Dorothy Andersen was the first to describe the condition named “cystic fibrosis of the pancreas” and observe in 49 pediatric cases at the levels of the lungs some pathological features characteristics of CF: mild tubular dilatation of small bronchi and bronchioles, the presence of mucopurulent material in the larger bronchi and trachea with “some congestion of the underlying mucosa”, and multiple small abscesses in the smaller bronchi [93]. Her report also described the infection of airways and she observed that most of the patients in her study died of pneumonia before the age of 6 months. She and others also described squamous metaplasia of the respiratory epithelium, to be related to vitamin A deficiency and a factor in perpetuating the respiratory infections due to the absence of the protective role of mucus [94][95].

Pathological changes occurring in the airways of toddlers and older children with CF are characterized by mucopurulent plugging of small and medium size bronchioles and development of bronchiectasis, secondary to proteolysis and chondrolysis of airway support tissues [96]. The dilated airways contribute to reduced mucociliary and cough clearance and the persistence of mucus inspissation and endobronchial inflammation.

Bacterial infections and the chronic hyper-inflammatory response generated as a consequence, lead to ensuing repairing mechanisms in response to the epithelial damage [97][98][99]. Neutrophils represent the main inflammatory cell type found into the lumen of CF airways and they have been shown to contribute to the pathophysiology of CF lung disease. Indeed, these immune cells, once entered into the CF airways, produce damaging mediators (proteases, reactive oxygen species, elastase) [100] that induce epithelial apoptosis [101] and/or premature senescence [102], and herald the remodeling of the airway epithelium by upregulating mucin expression and inducing goblet cell metaplasia [103][104]. Besides the hyperactivation of the CF airway epithelium, which triggers neutrophil attraction and activation in the CF airways by producing cytokines and chemokines [105], other immune cells and mediators have been found to be involved. Elevated levels of IL-17 and IL-23 in the sputum of CF patients, and particularly in those chronically infected with *P. aeruginosa*, implicate a role for Th17 cells in the persistent neutrophil infiltration in CF lung disease and chronic infection with *P. aeruginosa* [106]. Pretreatment of immortalized CF bronchial cells (NuLi) with IL-17 determined much greater IL-8 secretion in response to an agonist of NOD1, a cytosolic innate immune receptor, and *P. aeruginosa* diffusible material, identifying an amplification mechanism by which CF epithelial cells may trigger the inflammatory response to bacterial ligands [107]. Furthermore, the treatment of primary CF bronchial epithelial cultures with IL-17 increased production of IL-8, IL-6 and granulocyte macrophage colony-stimulating factor, confirming a positive feedback element in CF airway inflammation involving adaptive immunity.

The structural alterations accumulating with time in CF airways include hyperplasia of goblet and basal cells [39][97][108][109], squamous metaplasia [109][110], increase in epithelial height [39][108][111], cell shedding [97][98][108][109][111], and increased thickness of reticular basement membrane (RBM) [97][112]. Airway ECM remodeling and thus structural changes follow enhanced degradation of ECM proteins such as elastin, collagen, and glycosaminoglycans. These alterations are associated with a marked and early protease/antiprotease imbalance and the release of unopposed amounts of neutrophil elastase (NE), MMPs and other proteases that are involved in tissue damage and remodeling [113][114][115][116]. In vivo data show considerable evidence of an imbalance of MMPs and their inhibitors (tissue inhibitors of MMP, TIMPs) in the CF airways with prevalence and activation of MMPs [114][117][118][119]. The action of MMPs, as well as of serine (NE) and cysteine (cathepsins) proteases, secreted by epithelial cells, macrophages and the recruited neutrophils, in the pathogenesis of CF airway disease is complex and multi-faceted, including the enhancement of mucin/mucus production and secretion, the activation of PARs (protease-activated receptors) leading to proinflammatory signaling, the trans-activation of other proteases by cleaving pro-domains and degrading cognate antiproteases, the aggravation of basic CF ion transport defects by the proteolytic degradation of CFTR and activation of ENaC, and the cleavage of various host

protein substrates precipitating either activation (in the case of some proinflammatory cytokines) or inactivation (in the case of some antimicrobial peptides and surfactant proteins) [120].

Some other molecules involved directly in the remodeling of CF airways have been identified and studied. TGF-β, which has been found at elevated levels in blood (plasma) and BAL levels in CF patients and have been associated with pulmonary exacerbations [121][122], plays multiple roles in the pathogenesis of CF lung disease [123]. Besides downregulation of chloride transport in airway epithelial cells by acting negatively on CFTR and on Calcium-activated Chloride Conductance (operated by TMEM16A) [124][125][126], TGF-β signaling may drive goblet cell hyperplasia and increased mucin secretion, as indicated by studies in mouse models [127][128]. Immune and nonimmune cells, including airway epithelial cells [129][130], can secrete the latent form of TGF-β, which, upon activation orchestrates the lung immunity [131][132]. The increased RBM thickness in children with CF was found to be significantly related to BAL concentrations of TGF-β1 but unrelated to the raised levels of inflammatory cells and other cytokines [113]. Importantly, lung samples obtained from CF patients showed a significant peribronchiolar remodeling associated with prominent myofibroblast differentiation and fibrosis, i.e., TGF-β-dependent processes [129][133].

The EGFR is involved in mucin expression and secretion in the CF airways [134]. Indirect activation of MMPs and ADAMs by TNF-α stimulation can induce the EGFR pathway by evoking EGFR ligand shedding and inducing the EGFR pathway [135][136][137] in epithelial cells [138]. Both EGFR [139][140] and IL-13 receptor [141] have been associated with mucous cell metaplasia and mucin synthesis. More recently, the EGFR has been functionally coupled to ADAM17 at the level of the airway epithelium, and the EGFR/ADAM17 axis and its signaling pathway has been linked to TGF-α and AREG release and mucin expression [142]. AREG autocrine signaling affects mucus expression [143] and cytokine secretion [144], whereas its paracrine signaling has been linked to TGF-β-induced fibrosis [145]. Moreover, CF bronchial epithelial CFBE41o- cells displayed an enhanced ADAM17-mediated shedding of AREG compared with genetically identical cells with induced wt-CFTR expression and this correlated with enhanced apical presentation and phosphorylation of EGFR [146]. Further studies are necessary to clearly determine the role of EGFR/ADAM17 axis in CF, wound repair and other associated pathological hallmarks of lung disease. Nevertheless, all these changes indicate that epithelial differentiation and likely migration occurring to repair damage are somehow altered in CF airways. In the following Sections, we shall review those studies trying to elucidate the underlying mechanisms of these alterations, with particular reference to regeneration and wound repair.

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