Cystic fibrosis transmembrane conductance regulator in COPD: a role in respiratory epithelium and beyond

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Abstract

The cystic fibrosis transmembrane conductance regulator (CFTR) is a crucial ion channel for transport of chloride and bicarbonate anions. Functional roles of CFTR have been identified in a broad range of cell types including epithelial, endothelial, immune and structural cells. While CFTR has been investigated largely in the context of inborn dysfunction in cystic fibrosis, recent evidence shows that CFTR is also affected by acquired dysfunction in COPD. In patients with COPD and smokers, CFTR impairment has been demonstrated in the upper and lower airways, sweat glands and intestines, suggesting both pulmonary and systemic defects. Cigarette smoke, a key factor in COPD development, is the major cause of acquired CFTR dysfunction. Inflammation, bacterial byproducts and reactive oxygen species can further impair CFTR expression and function. CFTR dysfunction could contribute directly to disease manifestation and progression of COPD including disturbed airway surface liquid homeostasis, airway mucus obstruction, pathogen colonisation and inflammation. Mucus plugging and neutrophilic inflammation contribute to tissue destruction, development of dysfunction at the level of the small airways and COPD progression. Acquired CFTR dysfunction in extrapulmonary organs could add to common comorbidities and the disease burden. This review explores how CFTR dysfunction may be acquired and its potential effects on patients with COPD, particularly those with chronic bronchitis. The development of CFTR potentiators and the probable benefits of CFTR potentiation to improve tissue homeostasis, reduce inflammation, improve host defence and potentially reduce remodelling in the lungs will be discussed.

Introduction

The causative role of cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction in cystic fibrosis (CF) is well established, but its broad biological effects may also impact patients with COPD, a chronic disease that presents with features of chronic neutrophilic inflammation and small airways mucus obstruction similar to CF [1]. Evidence of CFTR dysfunction has been observed in patients with COPD [2–7]. Moreover, carriers of certain CFTR mutations have been shown to have increased risk of chronic bronchitis and bronchiectasis (1.3-fold and 1.9-fold, respectively) [8], challenging the idea that heterozygotes for CFTR mutations are asymptomatic [9].

The CFTR gene exhibits substantial natural variability, with >2000 variants reported, including >1000 pathogenic mutations [10], and at least six mutation classes defined by the nature of the molecular defect [11]. There is evidence from CF for a logarithmic relationship between CFTR function and clinical manifestation across the spectrum of disease [12]. Disease-causing variants often have more than one molecular defect [11] and ultimately lead to CFTR channel dysfunction [13, 14]. CFTR dysfunction can be acquired; for example, as discussed in detail later, as a consequence of cigarette smoking, the primary cause of COPD [6, 7, 15, 16], which can lead to reduced CFTR protein/mRNA expression, reduced CFTR cell surface expression and even direct modifications to the channel itself [17]. Furthermore, proteases such as neutrophil elastase, released as part of the inflammatory responses seen in chronic bronchitis, also degrade CFTR protein [18, 19]. In this review, we discuss CFTR, the effects of CFTR dysfunction, how such dysfunction could be acquired in patients with COPD and the potential impact it may have on patients based on clinical and pre-clinical data.

The role of CFTR in epithelial cells

The CFTR protein resides in the apical membrane of epithelial cells [2]. It belongs to the ATP-binding cassette transporter superfamily and functions as a crucial chloride and bicarbonate anion channel involved in salt and water transport across epithelial surfaces, requiring energy to fulfil its role [20–22]. Based on total expression levels, secretory and basal cells are responsible for the majority of CFTR expression in the superficial airway epithelium [23], whereas the rare pulmonary ionocyte cell type expresses the highest levels of CFTR per cell in the conducting airway epithelium [24, 25]. Mucus is produced by airway gland cells with CFTR-expressing serous cells producing most of the fluid component of gland secretions [26]. Mucus cells themselves do not express CFTR [27], but dysfunction in serous cell fluid secretion ultimately affects mucin secretion and maturation, leading to mucus plugging within the glands [26, 28]. Overall, CFTR regulates many mechanisms in epithelial physiology, including maintenance of epithelial surface hydration and luminal pH regulation [26], making it a key contributor to lung homeostasis [29]. Figure 1a illustrates a functional CFTR channel, contributing to epithelial surface fluid regulation and bicarbonate-dependent pH regulation. The function of CFTR in epithelial cells has been discussed in detail elsewhere [17]; the aim of this review is to look beyond epithelial cells to the other potential roles of CFTR in nonepithelial cells to broaden the understanding of the impacts of acquired CFTR dysfunction.

The role of CFTR in in nonepithelial cells

CFTR is expressed in many cell types of nonepithelial origin [30], including immune cells, fibroblasts, keratinocytes, airway smooth muscle cells, endothelial cells and bone cells. Figure 1b summarises the different cell types in which CFTR is functionally expressed and the consequences of CFTR impairment.

Immune cells

CFTR is expressed in macrophages [31–33]. Analysis of CFTR expression, apoptosis, polarisation, phagocytosis, bacterial killing and cytokine production in human CF and non-CF peripheral blood monocyte-derived macrophages (MDMs) found that CF MDMs display decreased CFTR expression, increased apoptosis and decreased phagocytosis compared with non-CF MDMs [34]. A study in CFTR-deficient mice of the effects of CFTR-proficient haematopoietic stem cell transfer on the outcome of severe pulmonary bacterial infection provided evidence for the role of CFTR in macrophage defence: CFTR-proficient CD68^{+} macrophage-like cells migrated into the lungs and replaced the recipients' CFTR-deficient macrophages, leading to a milder clinical course and higher survival following infection with *Pseudomonas aeruginosa* [35]. CFTR expression is associated with reduction of apoptosis; increases in CFTR expression correlate with decreased cellular apoptosis and CFTR knockout led to an increased propensity for macrophages to undergo apoptosis [36]. Interestingly, *ex vivo* treatment of macrophages from CF patients with CFTR modulators was able to augment phagocytosis of *P. aeruginosa* and reduce inflammatory status of the cells, demonstrating that macrophages are directly responsive to CFTR modulators [37]. In addition, a lack of CFTR has been found to be associated with hyperinflammatory responses [38], and treatment with CFTR modulators is associated with downregulation of inflammation [39, 40]. Macrophages are implicated in COPD pathogenesis directly through production of pro-inflammatory mediators and proteases and indirectly through defective phagocytosis of bacteria and reduced clearance of apoptotic cells [41, 42].

CFTR is expressed in neutrophils [43–45], with reduced expression observed in cells of patients with CF [43]. Neutrophils play a role in chlorination of phagocytosed bacteria, which is disrupted by CFTR channel dysfunction [44]. Additionally, suppression of CFTR function resulted in accumulation of cytosolic chloride in healthy neutrophils [43]. Activated neutrophils regulate CFTR distribution by mobilising it to the subcellular sites of activation, while neutrophils with the most common CFTR mutation, F508del, do not achieve this targeting and thus compromise host defence function [45]. In patients with CF with

a) Function of the CFTR channel
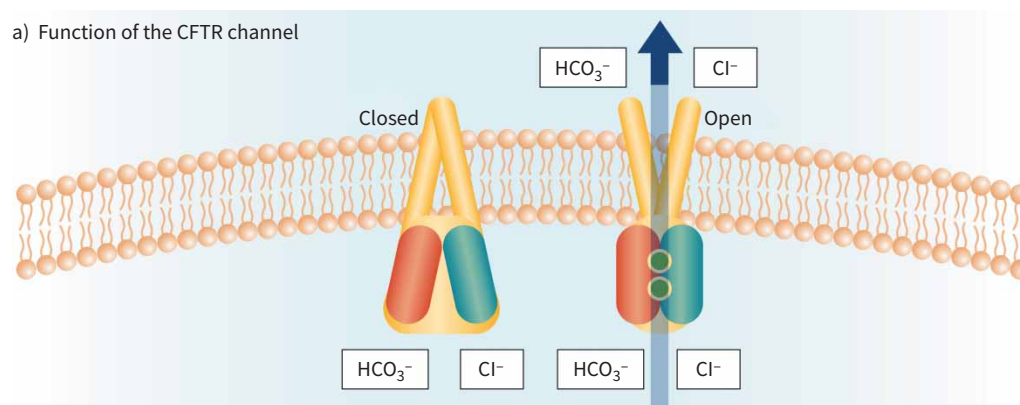
b) CFTR dysfunction and its impact in different cell types

Cell type expressing CFTR	Consequences of impaired CFTR function
Epithelial cells	Impaired cell surface hydration and luminal pH regulation Impacts on homeostasis Impaired regulation of other ion channels
Macrophages	Increased propensity to undergo apoptosis Hyperinflammatory responses Impaired phagocytosis
Neutrophils	Disruption to chlorination of phagocytosed bacteria Accumulation of cytosolic chloride Compromised host defences
Fibroblasts	Increased proliferation and myofibroblast differentiation Higher sensitivity to growth factors Increased responses to pro-inflammatory and fibrotic mediators
Airway smooth muscle	Potential altered function and contribution to airflow obstruction Intrinsic changes in proliferative and contractile properties
Endothelial cells	Potential impact on oxidative stress and homeostasis
Osteoclasts	Low bone mass Reduced ability to interact with other proteins and faulty chloride channel function, eventually impacting bone cell differentiation Altered porosity and chemical composition in the bones

FIGURE 1 Cystic fibrosis transmembrane conductance regulator (CFTR) function and expression in a variety of cell types in multiple organ systems. a) Function of the CFTR channel; b) CFTR dysfunction and its impact in different cell types. Cl^-: chloride; HCO_3^-: bicarbonate.

residual CFTR function, CFTR modulator therapy has been shown to bring about phenotypic changes in neutrophils, consistent with neutrophils becoming less activated [46]. Polymorphonuclear neutrophil (PMN)-dominated airway inflammation is a feature of CF. Compared with healthy controls, PMN counts are higher and PMN apoptosis is delayed in both those who are homozygous for CFTR mutations and heterozygotes, although it is unclear how this is modulated by CFTR [47]. Neutrophils have been implicated in many of the pathogenic effects of COPD features including emphysema and mucus hypersecretion [48], and airway neutrophilia is observed in COPD irrespective of clinical phenotype or disease severity [49].

CFTR is also expressed in B- and T-lymphocytes [50], which are a key part of lymphoid follicles frequently seen in CF [51, 52]. The role of lymphoid follicles in COPD is unclear, but association was found between the progression of COPD and the frequency of airways containing lymphoid follicles [53]; formation of lymphoid follicles is part of the molecular signature of emphysema [54].

Fibroblasts and keratinocytes

Fibroblasts are involved in the maintenance of extracellular matrix in the lungs; inappropriate recruitment and activation of fibroblasts is implicated in airway fibrosis in multiple chronic lung diseases [55].

Fibroblasts from CF mice display an altered phenotype compared with wild-type littermates, having increased proliferation and myofibroblast differentiation, higher sensitivity to growth factors and increased responses to pro-inflammatory and fibrotic mediators [56]. A CF stromal lung model based on fibroblasts from patients with CF found that fibroblasts maintain an activated pro-fibrotic state *in vitro* and a high proliferation rate. Furthermore, this model showed enhanced pro-fibrotic markers and upregulation of genes involved in epithelial function and inflammatory response [57]. In patients with COPD, fibroblasts demonstrate decreased capacity for sustaining tissue repair [58]. Keratinocytes impact wound healing [59–61] and are a source of matrix metalloproteinase (MMP)-9, which plays a role in matrix remodelling [62]. CFTR knockdown in mice led to delayed cutaneous wound healing with inflammation, increased keratinocyte proliferation and aberrant differentiation [60], while knockdown of CFTR in cultured human keratinocytes promoted cell migration, but inhibited differentiation. Suppressed migration with enhanced differentiation was associated with overexpression of CFTR, suggesting that CFTR helps regulate keratinocyte behaviour [59].

Airway smooth muscle

Airway smooth muscle (ASM) displays a hyperproliferative phenotype in COPD [63], and airway wall remodelling in patients with COPD features increased thickness of the ASM. Patients with CF often have symptoms typical of asthma and bronchial hyperresponsiveness, suggesting that CFTR may play a role in ASM function. Loss of CFTR alters porcine ASM function and may contribute to the airflow obstruction phenotype observed in human CF [64]. A study of ASM cells isolated from healthy donor and CF transplant lungs found that impaired CFTR function in ASM cells causes intrinsic changes in proliferative and contractile properties [65]. The CFTR modulator ivacaftor may relax smooth muscle in patients with CF, suggesting that CFTR dysfunction could cause increased smooth muscle tone in people with CF [66]. CFTR expression is greatly decreased in pulmonary artery smooth muscle in human and animal models of pulmonary hypertension [67], and radiographic evidence of pulmonary hypertension is associated with abnormal sweat chloride [68]. CFTR plays a key role in hypoxic pulmonary vasoconstriction, modulating the response to sphingolipids, which are important signal mediators of this physiological response [69].

Endothelial cells

Endothelial cell apoptosis and senescence are features of COPD [70]. Significant loss of markers of healthy endothelium has been observed in lung tissue isolated from patients with COPD. Furthermore, loss of several endothelial markers was found to correlate with reduced pulmonary function and increased emphysema [71]. CFTR is expressed by endothelial cells. Functional endothelial CFTR limits oxidative stress and contributes to the normal anti-inflammatory state of human umbilical vein endothelial cells (HUVEC) [72]. Studies on HUVEC suggest a role for CFTR in maintenance of endothelial cell homeostasis [73], while other studies suggest CFTR upregulation protects against palmitate-induced endothelial dysfunction by improving autophagic flux [74]. Although CFTR impairment downregulates endothelial cell proliferation, migration and autophagy, defective CFTR function leads to endothelial cell activation and a persisting pro-inflammatory endothelium state with increased leukocyte adhesion. CFTR modulators partially reduce the increase in endothelial cell activation markers and leukocyte adhesion shown in CF patient-derived endothelial cells [75]. Endothelial cells in human and animal models of pulmonary hypertension decrease CFTR expression [67].

Bone cells

Osteoporosis is a frequent comorbidity in patients with COPD [76]. CFTR is expressed in osteoblasts and osteoclasts at lower levels than in epithelial cells [77]. Genetic inactivation of CFTR in osteoblasts has been found to contribute to low bone mass [78]. The main effects of CFTR mutations are diminished ability to interact with other proteins and faulty chloride channel function, which ultimately influence bone cell differentiation [79]. CFTR deficiency alters porosity and chemical composition in the bones of newborn pigs [80], as well as reducing bone length, growth plate thickness, bone content and insulin-like growth factor-I in CFTR-knockout rats [81]. In patients with CF, CFTR mutation may impact NF-κB and Wnt signalling, resulting in disruption to osteoblast differentiation as well as decreased bone formation and increased bone resorption [79, 82]. Cartilage may also be affected, as ultrasounds of human tracheas showed that those in patients with CF had a greater width and were less circular in shape compared with those without CF [83].

Mechanisms of acquired CFTR dysfunction

Multiple internal and external factors may lead to acquired CFTR dysfunction (figure 2) through multiple direct and indirect effects [17]. In persons without CF, there are several means by which CFTR dysfunction may develop outside of inherited genetic mutations, with cigarette smoking being the most documented [3, 5, 6, 15, 16, 84, 85]. Thus, the predominant cause of COPD is also the most common
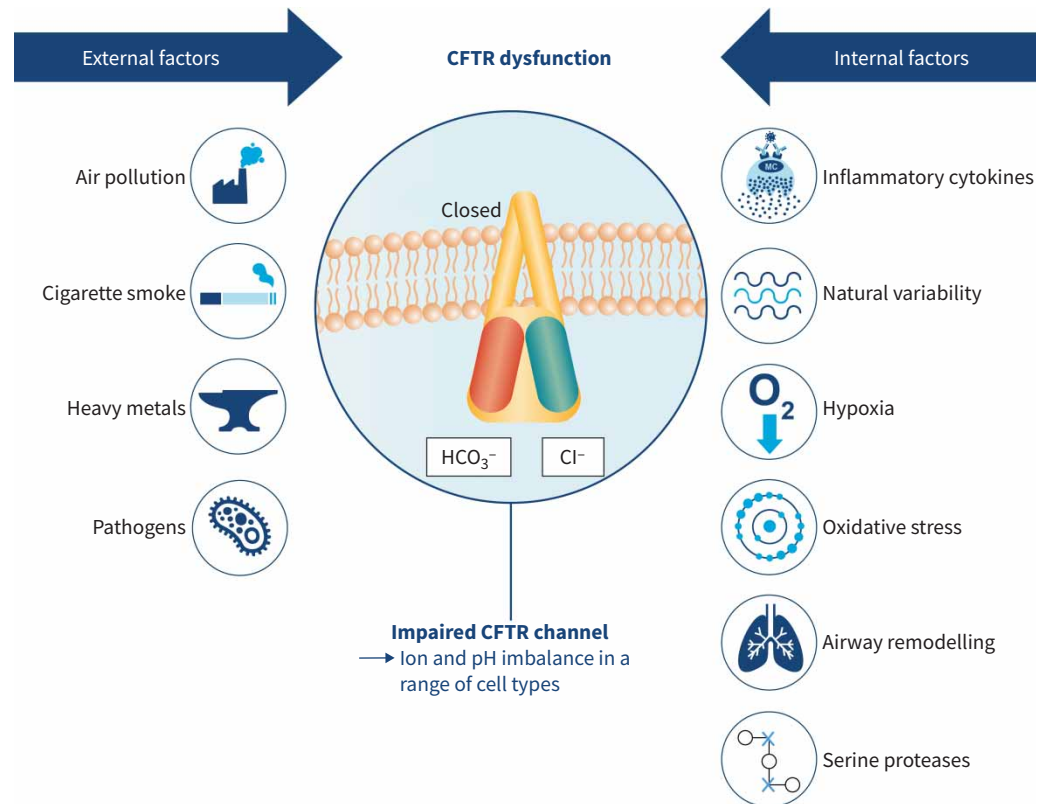
FIGURE 2 Multiple internal and external factors can lead to cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction. O_2: oxygen; Cl^-: chloride; HCO_3^-: bicarbonate.

cause of acquired CFTR dysfunction. Cigarette smoke decreases the expression of the CFTR gene, thus influencing mRNA levels, protein and function *in vitro*. Compared with nonsmokers, acquired CFTR deficiency was observed in the nasal respiratory epithelium of cigarette smokers [15]. Cigarette smoke can directly modify specific amino acid residues in CFTR, as can acrolein, an aldehyde contained in tobacco smoke [86]. CFTR dysfunction occurs in smokers who use e-cigarettes (which produce acrolein when heated) [87]. Cigarette smoking reduces CFTR function at extrapulmonary sites, removed from direct exposure, including the intestinal epithelia (mice), sweat glands and distal rectum (humans) suggesting that acquired CFTR dysfunction is a systemic phenomenon [7]. CFTR dysfunction can be transmitted to the fetus of smoke-exposed animals [88]. Acquired CFTR dysfunction in patients with COPD persists in the lungs following smoking cessation [3]. The level of CFTR activity in patients with COPD who smoke is roughly halfway between levels of activity in control individuals who have neither COPD nor CF and patients with CF [7] (figure 3). The relationship between protein levels and function is nonlinear. Even when individuals have "half-normal" CFTR function alongside acquired dysfunction, they still have substantially worse CFTR activity than individuals who are genetic heterozygotes [89, 90]. The extent to which individuals who are heterozygotes for CFTR mutations are impacted by cigarette smoke is debated. A Danish study showed reduced fecundity in F508del heterozygote smokers [91] and it has been suggested that this could be explained by cigarette smoke inducing a further decrease in CFTR function in reproductive tissues of F508del heterozygotes [15]. In contrast, *in vitro* and *in vivo* studies have shown that CFTR mutation heterozygosity does not affect the magnitude of reduction in CFTR activity induced by cigarette smoke [92].

In addition to cigarette smoke, other air pollutants and occupational exposure to certain vapours, dusts, gases and fumes have detrimental effects in patients with COPD [93–95]. Such environmental factors can also impact CFTR. Exposure to cadmium, a component of cigarette smoke and byproduct of smelters and therefore a prevalent air pollutant, inhibits CFTR protein expression in the lungs of COPD patients [96]. Pollutants such as cigarette smoke and cadmium can upregulate certain microRNAs such as miR-101 [97], which suppress CFTR expression [98]. Exposure to environmental arsenic can induce CFTR dysfunction and bronchitis symptoms [99]. Ozone downregulates the expression and function of CFTR [100, 101].
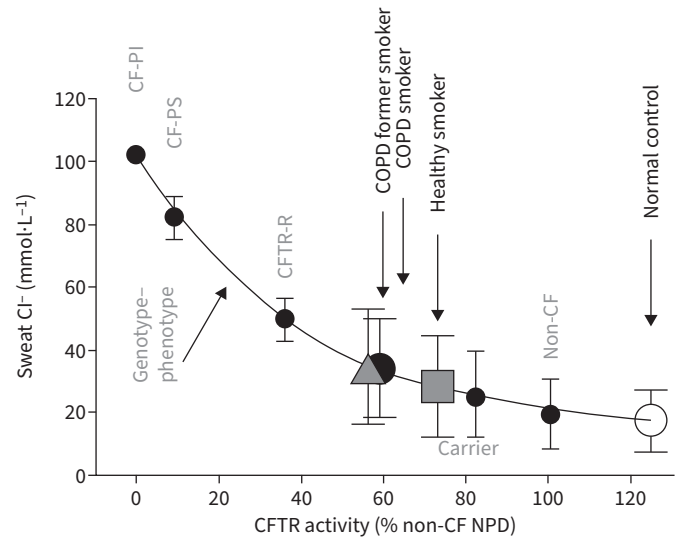
FIGURE 3 Level of cystic fibrosis transmembrane conductance regulator (CFTR) function in cigarette smokers with and without COPD compared with that of patients with cystic fibrosis (CF) with various severities of CFTR mutations grouped by clinical phenotype. PI: pancreatic-insufficient; PS: pancreatic-sufficient; CFTR-R: CFTR-related disorders; Cl⁻: chloride; NPD: nasal potential difference. Reproduced from [7] with permission.

There is conflicting evidence for the effects of treatments such as corticosteroids on CFTR function [102]: glucocorticoids in particular may reduce CFTR expression and channel activity, as shown in rats [103, 104], while cell culture studies showed that dexamethasone can rescue mutated CFTR expression through inhibition of a ubiquitin ligase [105]. It has been shown in human cell culture that dexamethasone regulates wild-type CFTR protein post-transcriptionally, enhancing maturation and expression [106], and induces SGK1, which selectively increases wild-type CFTR in the plasma membrane by inhibiting its endocytic retrieval from the membrane [107]. Additionally, dexamethasone stimulates CFTR activity through nongenomic signalling in rats [108].

Internal factors that influence CFTR function include inflammatory cytokines such as interferon-γ, which negatively affects the expression and activity of CFTR [109]. *In vitro* and *in vivo*, the protease neutrophil elastase induces CFTR degradation through the activation of intracellular calpains [18]. Localised acquired CFTR deficiency and subsequent abnormalities of fluid and electrolytes within the sinuses may be created by an oxygen-restricted environment in patients with chronic rhinosinusitis [84].

There is substantial variability of endogenous CFTR function. Variations in natural processes such as post-translational modifications (PTMs) can influence CFTR expression. PTMs and functional crosstalk between different PTMs regulate protein activity in addition to influencing protein turnover, conformation and localisation, as well as interactions with other proteins [110]. The folding state of CFTR determines how the channel is trafficked intracellularly: misfolding of CFTR prevents intracellular recycling and facilitates its degradation [111]. CFTR can also be post-transcriptionally regulated by microRNAs [112]. Some microRNAs are induced by inflammation and remodelling, perpetuating dysfunction [113, 114]. CFTR expression and biogenesis is downregulated by transforming growth factor-β1 exposure [115], potentially mediated through miR-145 [116]. There are variable degrees of CFTR function in healthy individuals without variants/mutations in the CFTR gene. This is evidenced by the large variability in CFTR function detected by *in vivo* measurements of nasal potential difference in healthy individuals (which partially overlaps with CF patients) [117] and studies of CFTR function in mice from different genetic backgrounds suggesting that genetic modifiers may play an important role in influencing wild-type CFTR activity [118]. This suggests potential for an interaction between host and gene-modifier environment, whereby cigarette smoke and environmental factors may have detrimental effects on non-CF individuals with low endogenous CFTR activity.

Certain aspects of COPD pathogenesis itself may contribute to CFTR dysfunction. Hypoxia has been shown to alter CFTR mRNA, protein and function in human cells, and the expression of CFTR in airways, gastrointestinal tissues and the liver was repressed in mice subjected to hypoxia *in vivo* [119]. Chronic

infection is indelibly linked to chronic bronchitis and plays an important role in the initiation and elaboration of CFTR dysfunction. Hypoxia-inducible factor-1α is a transcriptional regulator of cellular responses to hypoxia that can upregulate the platelet-activating factor receptor (PAFR) on the airway epithelial surface, thereby facilitating infection by PAFR-dependent bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *P. aeruginosa* [120]. Infection of the respiratory tract may lead to CFTR dysfunction; the *P. aeruginosa* virulence factor Cif negatively affects CFTR expression [121]. *P. aeruginosa* infection or exposure to *P. aeruginosa* exoproducts impair CFTR proteins in non-CF airway epithelial cells [122, 123]. Treatment of *P. aeruginosa* cultures with a quorum-sensing inhibitor prevents the negative effect of *P. aeruginosa* exoproducts on wild-type CFTR [124]. Oxidative stress by pyocyanin, a redox-active virulence factor produced by *P. aeruginosa*, impairs CFTR chloride ion transport [125]. Viral infection influences CFTR activity, *e.g.* influenza matrix protein 2 decreases CFTR activity by increasing secretory organelle pH [126]. Similarly, influenza infection reduces CFTR function in murine respiratory and alveolar epithelia, which persists beyond the infection period [127]. CFTR expression is repressed by prolonged exposure to oxidative stress in human bronchial cells [128]. Dysfunctional CFTR leads to reduced transport of the natural antioxidants glutathione and thiocyanate, and can therefore trigger an imbalance between antioxidants and reactive oxygen species [129]. Oxidative stress may be a common mechanism through which many of these factors (tobacco smoke, particulate pollutants, cytokines, hypoxia) mediate loss of cell-surface CFTR. This can then become a circular feed-forward loop: the initial CFTR dysfunction leads to mucus stasis and infection with inflammation, which worsen the dysfunction, especially at the local level. Interrupting this cycle could have important implications for patients with acquired CFTR dysfunction. CFTR dysfunction may also affect lipid metabolism: abnormal essential fatty acid profiles have been observed in CF [130]. A pro-inflammatory fatty acid imbalance could further exacerbate CFTR dysfunction [129].

Role of acquired CFTR dysfunction in COPD airways

COPD is characterised by persistent airflow limitation, frequently taking the form of chronic bronchitis, where small airway obstruction and tissue damage accelerates loss of lung function and mortality [131–133]. Small airway mucus plugging is an issue for patients with the emphysema phenotype of COPD [53, 134] and contributes to the development of emphysema in patients with chronic bronchitis. The chronic bronchitis phenotype of COPD has many phenotypical features in common with CF including chronic neutrophilic airway inflammation, goblet cell metaplasia and mucin hypersecretion, mucus obstruction of the small airways and chronic bacterial infection [3, 4, 135]. The clinical consequences for the chronic bronchitis phenotype of COPD encompasses a predisposition to lower respiratory tract infection, higher exacerbation frequency, additional comorbidities and worse overall mortality [136]. Patients with COPD and chronic bronchitis have a greater exacerbation frequency, are more likely to have gastro-oesophageal reflux, allergic ocular and nasal symptoms and poor lung function than those without chronic bronchitis [137, 138]. Current smoking and increased airway wall thickness are associated with chronic bronchitis [138], a finding recapitulated in a ferret model of acquired CFTR dysfunction [139].

CFTR dysfunction leads to defective chloride and bicarbonate ion transport into the airway lumen, which results in dehydration and acidification of airway surface liquid (ASL), and production of highly viscoelastic and acidic secretions [26, 27]. Bicarbonate ion secretion helps regulate local pH [26, 140, 141] and allows mucins to unfold [142], and supports innate immunity [2]. These processes are disrupted by the lack of chloride and bicarbonate ion secretion when CFTR is dysfunctional. Moreover, as the epithelial sodium channel (ENaC) is inhibited by CFTR, and its activity is increased by exposure to cigarette smoke [143], CFTR dysfunction (and the key factor leading to its acquisition) results in increased sodium absorption *via* ENaC, aggravating ASL dehydration [144].

The resultant abnormal airway milieu, with impaired innate host defences, favours chronic airway infection and inflammation, leading to increased lung injury [2]. Chronic bacterial colonisation and persistent neutrophilic inflammation are features of COPD. These processes may be amplified by CFTR impairment in macrophages and neutrophils, leading to reduced bacterial killing and increased inflammatory responses [145]. In addition, debris from pathogens and extracellular DNA from apoptotic cells can entangle with mucins, causing further mucus obstruction and potentially plugging [146].

Dysfunction in CFTR contributes to disruption of the microbiome. Healthy ASL is a primary innate defence mechanism of the lungs, and the disruption of ASL homeostasis provides a suitable environment for bacterial colonisation and dysbiosis [147–149]. A heterogeneous environment is created by the changing oxygen and pH gradients; these environmental changes favour coexistence and proliferation of a wide range of microbial species [148, 150]. Proteobacteria and Actinobacteria are over-represented in the CF pulmonary microbiome [151]. There is some overlap with the sputum microbiota of patients with

COPD, where Proteobacteria dominance is associated with disease severity [152]. Indeed, *P. aeruginosa* infections are associated with exacerbations and high mortality in patients with COPD [153]. Dysbiosis has been observed in infants and children with CF compared with those without CF, characterised by increased relative abundances of Proteobacteria, especially *Escherichia coli*, and decreased Clostridiales [154, 155]. Overall, the airway microbiome is less diverse in patients with CF compared with healthy individuals and the reduction in diversity is associated with increasing age, antibiotic use, lung function decline and disease progression, often leading to dominance of typical CF pathogens such as *P. aeruginosa* or *Staphylococcus aureus* [156–159]. It has been demonstrated in germ-free mice that mutated CFTR alone is sufficient to alter the microbiome [160] and there is variety in microbial representation in faecal microbiota depending on CFTR variants [161]. It has been shown that pharmacological restoration of CFTR activity led to rapid decreases in the sputum abundance of bacteria, in particular *P. aeruginosa* [162]. A possible link between CFTR mutations and allergic bronchopulmonary aspergillosis (ABPA), a pulmonary disorder characterised by a hypersensitivity response to the allergens of the fungus *Aspergillus fumigatus*, has been proposed [163], suggesting that acquired CFTR dysfunction in patients with COPD could make them more susceptible to this condition. Augmented CFTR activity in CF reduces the prevalence of *Aspergillus* infection [164, 165]. ABPA is increasingly recognised in COPD [166]. *Aspergillus* sensitisation in COPD is associated with frequent exacerbations, poor lung function and bronchiectasis [167–169].

Increased inflammation is associated with CFTR dysfunction in a probable circular fashion, with CFTR dysfunction leading to inflammation that results in worsening of the CFTR dysfunction. In patients with CF, airway inflammation is dominated by neutrophils, which produce reactive oxygen species, neutrophil extracellular traps, proteases such as neutrophil elastase, and pro-inflammatory mediators, leading to progressive tissue damage, immune cell recruitment and inflammation [145, 170]. Increased mucus viscosity affects neutrophil motility, impairing phagocytosis and consequently supporting chronic bacterial infection [132, 171]. Patients with COPD, particularly those with chronic bronchitis, already exhibit impaired airway clearance, chronic bacterial colonisation and persistent neutrophilic inflammation like patients with CF, which may be exacerbated by acquired CFTR dysfunction [3, 19, 137, 172]. Small airway disease (SAD), a key feature of COPD, is a major cause of airflow obstruction and is characterised by mucus plugging, airway remodelling and neutrophilic inflammation [173–175]. In turn, these changes may result in increased COPD exacerbations and bacterial colonisation, which may further contribute to COPD progression through increased SAD and emphysema development [175]. Patients with COPD and SAD have poorer health status, worse spirometry results and more severe lung hyperinflation than those without SAD [174, 176]. In the small airways of patients with COPD who experience frequent exacerbations, measures of SAD were associated strongly with neutrophilic inflammation [177]. Disease progression in COPD is heterogeneous and can take the form of small airway dysfunction and emphysema preceding large airway wall abnormalities or large airway wall abnormalities may precede emphysema and small airway dysfunction [178]. Acquired CFTR dysfunction may contribute to COPD pathogenesis *via* chronic airway inflammation, mucus hypersecretion and recurrent infections and SAD. ASM plays a pivotal role in airway remodelling in both CF [65] and COPD [179]. Airway epithelial remodelling in COPD may induce an abnormal localisation and expression of ion channel proteins such as CFTR [180]. As COPD progresses, large areas of the conducting airway are replaced by squamous epithelium with little to no CFTR expression. Cellular injury and inflammation in COPD is diverse and complex; recent single-cell RNA sequencing profiles of explanted lung tissue from patients with advanced COPD showed distinct changes in epithelial cell populations and associated CFTR expression [181]. Abnormal expression and distribution of CFTR protein has been observed in non-CF inflamed and/or remodelled airway tissues [182].

CFTR dysfunction may have important consequences for disease initiation and progression in COPD, including destruction of airway and lung parenchyma epithelial cells causing tissue remodelling, reduction of gas exchange area, impairment of lung function [183], damage to the pulmonary matrix through neutrophil elastase and matrix metalloproteins [184], and effects on contractility of ASM [185]. Additionally, dehydrated and acidified ASL and airway inflammation contribute to mucociliary dysfunction and ultimately support increased bacterial colonisation and locally amplified neutrophilic inflammation. Many of these pathways have the potential to further perpetuate CFTR dysfunction.

Figure 4 illustrates different aspects of CFTR dysfunction in the lungs. The ASL is acidified through reduced CFTR-mediated bicarbonate ion transport, which leads to altered mucus properties and attracts pathogen colonisation. In addition, dehydration of the ASL impairs mucociliary clearance. Furthermore, CFTR impairment in immune cells involved in pathogen defence leads to reduced bacterial killing, increased apoptosis and inflammatory responses [145]. Together, these effects lead to increased pathogen colonisation and subsequent immunological effects (increased inflammasome activity). The pathogen
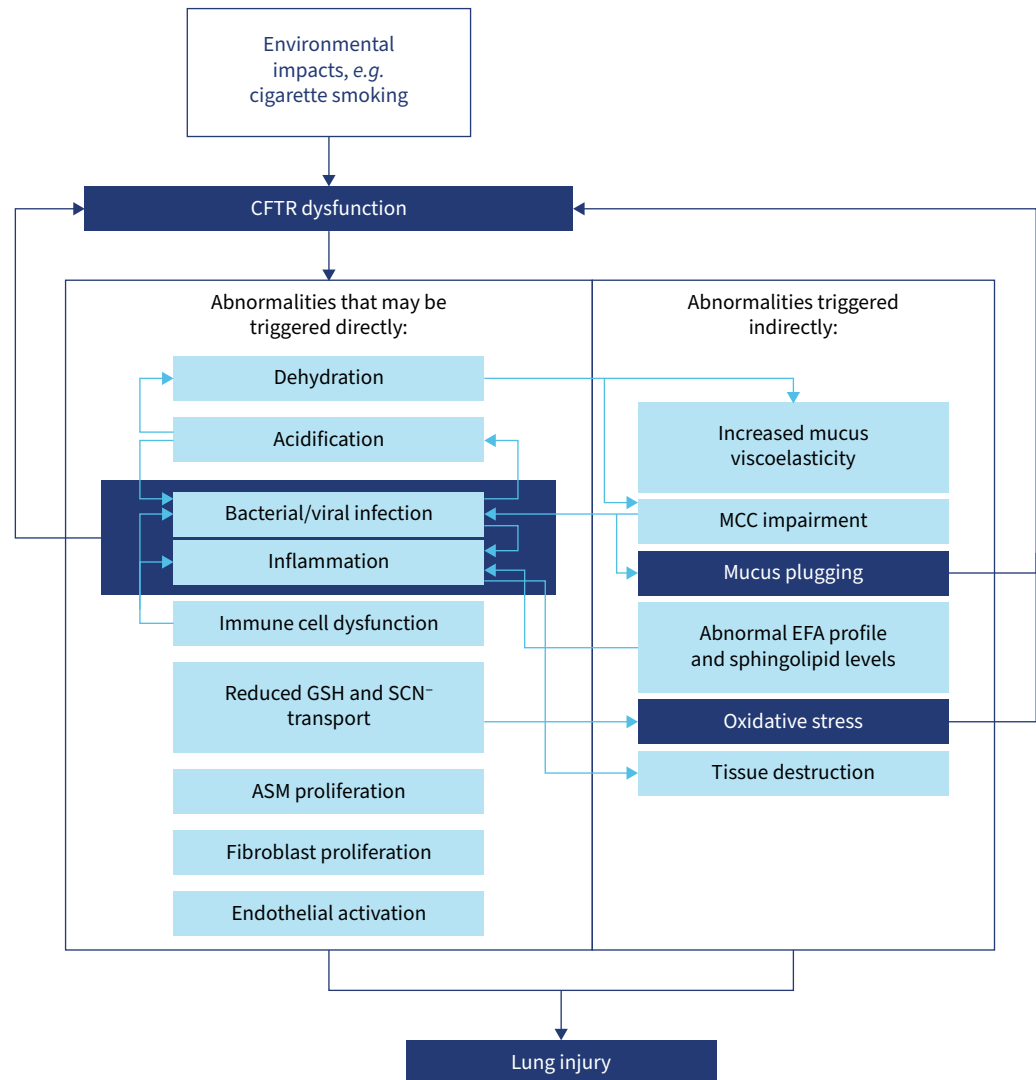
FIGURE 4 Interplay of multiple impacts of cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction in the lungs. GSH: glutathione; SCN$^-$: thiocyanate; ASM: airway smooth muscle; MCC: mucociliary clearance; EFA: essential fatty acid.

metabolism can further acidify the ASL, which feeds back into reduced bacterial clearance. Table 1 summarises the multiple consequences of CFTR impairment in the body.

Development of CFTR potentiators for COPD with chronic bronchitis

Development of CFTR modulator therapies to improve CFTR function systemically represents a significant step in the treatment of patients with CF [10, 144, 186–188]. These new therapies include CFTR potentiators, which increase the flow of ions through activated CFTR channels on the cell surface [189]. Potentiators augment CFTR activity in the presence of endogenous stimuli, in contrast to CFTR activators which stimulate CFTR function irrespective of the surrounding physiologic situation [189]. Potentiators act on multiple mutant forms of CFTR with channel gating defects [190] and can also increase wild-type CFTR activity [16, 189]. The benefits of CFTR potentiation include augmented airway surface hydration, improved mucus rheology, accelerated mucus clearance, reduced inflammatory burden and improved bacterial clearance; CFTR potentiators have good safety profiles and are well tolerated [86, 169, 191, 192]. Furthermore, there is evidence that the systemic impacts of CF are ameliorated by CFTR potentiators in the gastrointestinal tract [193], pancreas [194, 195], sweat ducts [196] and bones [197, 198]. CFTR activity is augmented by increasing cAMP-mediated activation using the phosphodiesterase-4 inhibitor roflumilast. Roflumilast improves ion transport in cell culture studies and smoke-exposed mice, and consequently

TABLE 1 Multifaceted roles of cystic fibrosis transmembrane conductance regulator (CFTR) impairment in the body	
Epithelial homeostasis	CFTR dysfunction acidifies ASL by limiting bicarbonate secretion [26]
	CFTR dysfunction dehydrates ASL by reduced anion secretion and increased sodium absorption *via* ENaC [144]
	ASL acidification and dehydration contributes to impaired MCC and consequential mucus overload and plugging [17]
	Suboptimal pH for antimicrobial proteins leads to weakened host defence [140]
Bacterial infection	CFTR dysfunction in phagocytes impairs bacterial defence [34]
	pH alteration fuels bacterial colonisation [140]
	Impaired MCC facilitates accumulation of bacteria and biofilm formation [148]
	Imbalance of microbiome [151, 160]
Inflammatory status	CFTR dysfunction in macrophages leads to increased release of inflammatory cytokines (*e.g.* IL-6 and IL-8) [42]
	Impaired CFTR affects neutrophil function, and can lead to overexuberant pro-inflammatory responses to acute insults such as LPS [145]
	Bacterial exoproducts trigger inflammasome activation and release of IL-8 [145]
Lung remodelling	Direct effects of CFTR dysfunction on specific cells and downstream effects of airway dehydration and mucus accumulation [65]
	Remodelling itself may impact CFTR expression [180]
Extrapulmonary comorbidities	CFTR dysfunction is systemic in smokers [7]
	CFTR dysfunction has been associated with multisystem morbidities, which worsen COPD outcomes, such as GORD, sinusitis, diabetes and bone fractures [208]

ASL: airway surface liquid; ENaC: epithelial sodium channel; MCC: mucociliary clearance; IL: interleukin; LPS: lipopolysaccharide; GORD: gastro-oesophageal reflux disease.

increases ASL depth in epithelial cells [192, 199]; this may also explain why roflumilast has been shown to be helpful in COPD patients who specifically exhibit chronic bronchitis [200]. Taken together, CFTR potentiators or other CFTR activators may therefore represent a viable therapeutic option for patients with COPD of both chronic bronchitis and emphysema phenotypes. Improving CFTR function in patients with COPD has the potential to improve lung health, facilitate mucus clearance and reduce bacterial colonisation in patients with COPD. Patients with COPD with predominant chronic bronchitis (*i.e.* cough and sputum overproduction) who continue to experience exacerbations and symptoms despite inhaled therapies are expected to benefit the most with CFTR potentiation. CFTR potentiation has been shown to be effective and well tolerated in CF patients, even with chronic use [196]. Given the potential role of CFTR dysfunction in other airway diseases such as asthma, bronchiectasis and ABPA, CFTR modulators could also prove to be a therapy option beyond CF and COPD [201].

Ivacaftor is approved for treatment of patients with CF with specific CFTR mutations [202]. It is a potentiator of CFTR chloride channel function, facilitating increased chloride transport by potentiating channel-open probability (or gating) of the surface-localised CFTR protein [189]. Ivacaftor potentiates mutant CFTR forms with defects in channel gating caused by CFTR gating (class III) mutations in addition to normal CFTR and F508del-CFTR in *in vitro* studies [203]. It increases channel opening time by stabilising the open state of the CFTR [204]. Ivacaftor restores CFTR-dependent chloride transport in cigarette smoke exposed epithelial cells and improves mucociliary transport, ASL depth and ciliary beating *in vitro*, demonstrating the potential of CFTR modulators to address pathological airway physiology evident in acquired CFTR dysfunction [16, 86]. In a pilot study, ivacaftor augmented measures of CFTR function and clinical symptoms in patients with COPD and chronic bronchitis [202]. Ivacaftor improves extrapulmonary disease manifestations in patients with CF with gating mutations, suggesting potential systemic benefits to CFTR potentiation [165]. Furthermore, in a ferret model of COPD with chronic bronchitis, the CFTR potentiator GLPG2196 reversed CFTR dysfunction and pathological chronic bronchitis features [205].

Icenticaftor is an oral, small-molecule CFTR potentiator currently in development. In patients with CF, icenticaftor demonstrated clinically meaningful changes in lung function and sweat chloride level across a broad array of mutations [206]. In a phase II safety, tolerability and efficacy study (www.clinicaltrials.gov identifier NCT02449018) conducted in 92 patients with moderate-to-severe COPD, icenticaftor significantly increased forced expiratory volume in 1 s ($\geqslant$50 mL) at 28 days. Moreover, icenticaftor reduced systemic inflammation, with a trend towards reduced sputum bacterial colonisation in patients with COPD; however, it was not observed to improve lung clearance index [207]. An ongoing phase II study (www.clinicaltrials.gov identifier NCT0426882) aims to assess the mode of action of icenticaftor in an estimated 100 patients with COPD. Dose selection for icenticaftor is being assessed in a phase II dose-range finding study in 974 patients (www.clinicaltrials.gov identifier NCT04072887).

Conclusions

Smoking is the most prevalent cause of both COPD and acquired CFTR dysfunction, which affects both epithelial and nonepithelial cells in pulmonary and extrapulmonary systems. Certain aspects of COPD pathogenesis can amplify CFTR dysfunction, and CFTR dysfunction can persist even after smoking cessation. Acquired CFTR dysfunction may contribute to COPD pathogenesis through CFTR impairment in a range of different cell types, including epithelial, immune and structural cells. The consequence of CFTR dysfunction could be amplifying effects resulting in chronic airway inflammation, recurrent infections and sputum overload. These pathological manifestations have been linked to chronic bronchitis, small airway disease, structural alterations and development of emphysema. In addition, the demonstrated systemic impairment of CFTR in smokers and its functional roles in organs beyond the lungs suggests that CFTR impairment may contribute to the systemic burden of COPD. Therefore, systemic CFTR potentiation for these patients may be beneficial, leading to an overall improvement in lung health through supporting epithelial homeostasis, controlling infection and inflammation and reducing lung remodelling.

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