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Airway Hydration and COPD

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Abstract

Chronic obstructive pulmonary disease (COPD) is one of the prevalent causes of worldwide mortality and encompasses two major clinical phenotypes, i.e., chronic bronchitis (CB) and emphysema. The most common cause of COPD is chronic tobacco inhalation. Research focused on the chronic bronchitic phenotype of COPD has identified several pathological processes that drive disease initiation and progression. For example, the lung's mucociliary clearance (MCC) system performs the critical task of clearing inhaled pathogens and toxic materials from the lung. MCC efficiency is dependent on: (i) the ability of apical plasma membrane ion channels such as the cystic fibrosis transmembrane conductance regulator (CFTR) and the epithelial Na^+ channel (ENaC) to maintain airway hydration; (ii) ciliary beating; and, (iii) appropriate rates of mucin secretion. Each of these components is impaired in CB and likely contributes to the mucus stasis/accumulation seen in CB patients. This review highlights the cellular components responsible for maintaining MCC and how this process is disrupted following tobacco exposure and with CB. We shall also discuss existing therapeutic strategies for the treatment of chronic bronchitis and how components of the MCC can be used as biomarkers for the evaluation of tobacco or tobacco-like-product exposure.

Keywords

Airway surface liquid; Cystic Fibrosis; CFTR; ENaC; Mucus; Tobacco smoke

Introduction

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death worldwide and is projected to become the third leading cause of death by 2030 [1]. COPD is characterized by a persistent airflow limitation that is progressive and associated with an augmented chronic inflammatory state [2]. COPD occurs after chronic environmental exposure to tobacco smoke and/or other noxious particles or gases [2, 3], but may also have a genetic component that predisposes certain populations to COPD [4].

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Conflicts of Interests

A Ghosh has no conflict to declare, R. Tarran is a founder of Spyryx Biosciences and R.C. Boucher is a founder of Parion Sciences.

Chronic bronchitis (CB) and emphysema are considered as the two major clinical and epidemiological phenotypes of COPD. Production of sputum and cough for at least 3 months over two consecutive years defines CB, whereas emphysema is defined by the "destruction of the gas-exchanging surfaces of the lung" [2]. Most COPD patients exhibit symptoms of both emphysema and CB [5]. However, CB is typically the more prevalent phenotype [6]. The clinical manifestations of CB (sputum production and a failure of mucus transport concomitant with chronic inflammation) are similar to the symptoms reported in early cystic fibrosis (CF) lung disease. CF is caused by mutations in the CFTR anion channel [7], which lead to reduced airway surface liquid (ASL) volume and subsequent mucus dehydration, mucus stasis and recurring airway infections [8–10]. Although the causal factors appear different, recent evidence suggests that the acquired dysfunction of CFTR-mediated ion transport following chronic tobacco exposure may also contribute to CB pathogenesis [11]. Indeed, the mucus hydration status of the airways has both direct and indirect effects on pulmonary health [12, 13]. Thus, it has been proposed that the pathophysiology of the two diseases, i.e. CF and COPD, share similar initiation and progression scenarios, with compromised mucus hydration being common [14, 15]. In this review, we shall discuss the pathophysiology of the chronic bronchitis form of COPD (see Figure 1), and how this knowledge may generate (i) novel treatment regimens and (ii) clinically relevant novel biomarkers of exposure.

Causes of COPD

Mortality in COPD in part reflects pulmonary failure, but may be dominated by comorbidities such as cardiovascular disease and lung cancer. Although the principal etiological factor for COPD is chronic tobacco smoke exposure, the involvement of genetic, epigenetic and host-factors is evident by the fact that only a fraction of smokers develop the disease. The CB incidence rate in smokers is typically suggested to be around 25% [16]. However, COPD may be underreported, and in the USA, for example, ~12 million adults have been diagnosed with the disease, but an equal number of people likely suffer from COPD without an official diagnosis. [17]. In addition to tobacco exposure, both indoor and outdoor pollution can also cause COPD in non-smokers [18–21]. As such, biomass smoke exposure (e.g. smoke from wood burning stoves) and occupational pollutant exposure are also considered significant risk factors for COPD [22, 23].

Apart from external etiological factors, the most crucial genetic risk factor for COPD is α1-anti trypsin deficiency [24]. The α1-anti trypsin protein is coded by the serpin peptidase inhibitor, clade A, member 1 (SERPINA1) gene [25]. α1-anti trypsin deficiency is an autosomal co-dominant state that leads to reduced levels of α1-anti trypsin in circulating blood and corresponding failure to inhibit neutrophil elastase [26]. Even without exposure to tobacco smoke, the subsequent uncontrolled neutrophil elastase activity can lead to lung damage and early onset COPD [27]. Significant pulmonary destruction is visible by the age of 30 in many patients [27], and α1-anti trypsin deficiency-induced lung disease worsens upon exposure to environmental toxicants such as tobacco smoke [4, 26, 28, 29].

The airway surface mediates normal mucus clearance and reduced ASL volume, i.e. hydration, produces the reduction in mucus clearance. Severe reductions in mucus clearance

are associated with increase mucus stasis/mucus adhesion, which lead to the increase in inflammation and bacterial infections characteristic of CF and CB [30]. Investigations into the pathophysiologic basis of chronic bacterial airways infection typically reveal that airways obstruction is the primary cause of this syndrome [31, 32]. Airways obstruction can be produced by intraluminal airway tumors and inhalation of foreign bodies. However, the most common form of airways infection is associated with the intraluminal obstruction produced by mucus adhesion, mucus plaques, and ultimately, mucus plugs [33–35]. For example, the pathologic studies of Hogg et al, indicated that this scenario is central to the pathogenesis and progression of the CB phenotype of COPD [36, 37].

The mucus clearance component of the lung's innate defense system

The lung is frequently exposed to inhaled pathogens and pollutants. However, by the time that inhaled air reaches the alveolar surfaces, it has been filtered and humidified [38]. These functions are largely performed by the innate defense system of the upper respiratory tract [38, 39]. Under normal conditions, pulmonary secretions contain soluble anti-microbial factors, macrophages, and mucins that each play a role in mediating the initial responses to toxic or infective inhalations [40–42]. The surface epithelium of the airways coordinates these responses via multiple specialized cell types, including columnar ciliated cells and mucous (goblet) cells, along with underlying basal cells. Mucins were secreted by the surface epithelial goblet cells in parallel with mucins derived from submucosal glands, which in the large airways open onto the airway surface through a series of ducts [43].

Most mucosal surfaces that interface with the outside world are "wet" mucosal surfaces [44]. As a general principle, "wetness" is a required characteristic for mechanical clearance, e.g., is required for the efficiency of blinking, swallowing, and indeed, mucus clearance [45]. Heretofore, it was generally assumed that the major determinants of the efficiency of mucus clearance were the actions of cilia and the rate of mucin secretion [46, 47]. Indeed, both are important contributors to the overall efficiency of mucus clearance, but evidence now suggests that the hydration status of the environment is the dominant variable for the efficiency of the mucus clearance process [48]. Under physiologic conditions, the mucus layer acts as a reservoir for water, i.e., it can donate or accept added liquid, to maintain apposition of the inner surface of the mucus layer with the tips of the cilia [48]. This regulation serves to preserve the hydration status of the pericilliary liquid layer (PCL), which is required for cell surface lubrication and for efficient ciliary beating. Mucus clearance can accelerate above basal rates when liquid is added to the airway lumen, consistent with the *in vitro* studies of human bronchial epithelial cultures (HBECs) with confocal and epifluorescence microscopy [48, 49]. When liquid is removed from the ASL in an inappropriate fashion, e.g., as in CF and CB [15], the supply of water "donated" from the mucus layer to the PCL can be exhausted, and the PCL will become dehydrated and collapse. The thickened (concentrated) mucus layer then comes into contact with cell surface microvilli, cilia and ultimately adheres (14). As a result of this interaction, mucus clearance ceases and the nidus for plaque/plug formation is generated.

Efficient mucus clearance from airway surfaces requires the coordinated activities of a "transported layer", i.e., the mucus layer, a cell surface layer, and cilia [50, 51]. The

biophysical requirements for a mucus layer to exhibit efficient clearance are daunting and only beginning to be understood. The mucus layer must have viscoelastic properties that enable the transfer of energy from ciliary beating to the vectorial movement of mucus towards the mouth [45]. The mucus layer must also have properties that will allow it to bind and entrap virtually all deposited particles so they may be removed from the pulmonary surface, yet not adhere to the cell surface [52]. Finally, the mucus layer must be able to adapt to local stresses to maintain its viscoelastic and other properties to provide optimum transport [44, 53, 54].

A new concept has emerged as to how the mucus layer is organized to provide these functions [55]. High molecular weight, extraordinarily long (0.5–20 μm) mucins (MUC5AC, MUC5B) are secreted onto the airway surface to form the mucus layer. However, the functional properties of this layer are also provided by interacting globular proteins. Indeed, this mucin interactome may provide both cross-links between mucin monomers and serve to build complex molecular compartments with their own innate defense activities [56, 57]. In parallel, our concept of the cell surface layer, i.e. the PCL, has undergone a dramatic evolution within the past several years. The emerging data on the identification of cell surface tethered mucins (MUC1, 4, 16) led to the hypothesis that the periciliary environment is comprised of a polyelectrolyte gel rather than a liquid layer [57]. This concept is important because it predicts that the distribution of water between the gels on the airway surface will in part reflect the relative concentration of mucus in each compartment. The “water drawing” power of each gel can be measured in terms of its osmotic modulus or pressure [58]. Importantly, mucin concentrations in diseased lungs cannot accurately be measured using Western blot-based approaches and instead must be measured by physic-chemical techniques. As a case in point, many of the epitopes in the mucins found in CF mucus that are recognized by antibodies are degraded in the highly proteolytic environment of the CF lung, yielding erroneous results by Western blot. However, both mass spectrometry analysis and refractometry measurements of the void volume indicated that CF mucin levels are increased relative to normal mucinlevels [58].

Goblet cells and mucins of the conducting airways

Chronic mucus hypersecretion has been identified as a potential risk factor for mortality in COPD because of the associated accelerated loss of lung function with this exposure [59]. One of the characteristic features of obstructive lung diseases is mucus hypersecretion that is driven in part by glandular hypertrophy but also by goblet cell hyperplasia and metaplasia, especially in the glandless small airways. As such, goblet cells contribute to the hypersecretion of mucins in CB airways [60]. The replacement of ciliated epithelial cells by goblet cells is complex and is in part driven by inhibition of apoptosis by EGFR, and/or IL-13-dependent activation of MEK/ERK [61–63]. The increase in mucin secretion in CB lungs may provide a protective coat of muc**us** that line the airways to limit toxicant exposure. Accordingly, while mucus obstruction is deleterious in the long term, in the short term, it may protect the airways against tobacco exposure by acting as a barrier to tobacco smoke diffusion. Mucin knock out mice exist ($MUC5b^{-/-}$) and exhibit increased susceptibility to infection [64], but the effects of tobacco exposure on these mice has not been established.

Often with a molecular mass over 500 kDa, mucins are 50–80% O-linked oligosaccharides by weight [65]. Mucins may be divided into two major groups, (i) membrane bound and (ii) secreted. Secreted mucins are classified as gel-forming and non-gel forming types. The secretion of mucins occurs through both the constitutive and regulated secretory pathways [66]. As part of the constitutive pathway, mucin-containing vesicles from the trans-Golgi network are released extracellularly, creating a “baseline activity” of mucin secretion that is Ca^{2+}-independent. [67]. Tethered mucins are typically trafficked to the cell surface by a constitutive pathway [68, 69]. In the regulated pathway, mucin-containing vesicles are transported from donor to storage compartments. For example, after synthesis in the endoplasmic reticulum, vesicles carrying mucins are transferred to the cis-Golgi for core glycosylation. They then undergo a maturation process in the Golgi and pass through the trans-Golgi to storage granules [66]. The mucin containing granules are trafficked towards the apical plasma membrane for secretion in a Rab GTPase-dependent fashion [66]. Studies with the SPOC1 rat goblet cell line demonstrated that increases in intracellular Ca^{2+} elicited by purinergic receptor stimulation triggered mucin secretion [70]. Further studies with the protein kinase C activator Phorbol 12-myristate 13-acetate and the Ca^{2+} ionophore ionomycin in goblet cell mucin secretion demonstrated the involvement of Ca^{2+} and protein kinase C in this process [71].

Upon secretion, the data of Verdugo et al. demonstrated that mucins “exploded” out of vesicles like a “jack in the box”. The mechanism for this phenomenon reflects the fact that a pore is formed between the secretory granule and the plasma membrane, extracellular Na^+ exchanges for Ca^{2+} in the granule, and mucins are osmotically expelled from the granule onto airway surfaces. A key aspect of this mechanism is that mucins contain negative charges that would normally repel other mucins when they are densely packed. However, the Ca^{2+} and H^+ in the granules shield mucin negative charges and allow the mucins to remain closely packed. This exchange of Ca^{2+} and H^+ for Na^+, causes mucins to expand ~600 times when they are secreted [72]. Consequently, mucins are secreted ‘dry’ with water being supplied by CFTR-mediated Cl^- secretion via the ciliated cells [73].

Ciliated cells of the conducting airways

The motive force for mucociliary clearance in the airways is provided by the ciliated cells of the respiratory tract. There are typically ~200 cilia per cell and their beating at ~10–15 Hz propels mucus and trapped pathogens/particles towards the oropharynx where it is expelled or swallowed in order to maintain pulmonary sterility [74]. Ciliary epithelia are polarized, with cilia being exclusively located at the apical plasma membrane. Ciliary orientation is determined by planar cell polarity proteins and their associated signaling pathways and is initiated before ciliogenesis[See [75] for a comprehensive review]. In airway epithelia, ciliogenesis is under the control of the forkhead box J1 (FoxJ1) transcription factor [76] and knockout of this gene in a mouse model prevents ciliogenesis [77]. More than 600 proteins have been identified in cilia [78]. Motile cilia typically exhibit the “9+2” arrangement where two singlet microtubules are surrounded by nine doublet microtubules along with several other components such as dynein arms, radial spokes and other proteins that are required for proper ciliary assembly and beating [79].

Ciliary beating is typically under the control of second messengers similar to those that increase mucin secretion, and elevations in Ca^{2+}, cAMP and cGMP have been shown to increase beat frequency [80–84] [85–88]. This increase may be triggered by G_s-linked GPCRs such as P2Y2-R and adenosine receptors [89]. cAMP/PKA-dependent regulation of ciliary beating is best understood and PKA and A-kinase anchoring proteins (AKAPs) have been found in cilia [90]. In contrast, while Ca^{2+} is thought to directly increase ciliary beating, in a PKC-independent fashion, PKC activation is thought to slow ciliary beating [91].

Role of ion channels in airway mucus hydration

As noted above, in addition to ciliary beating, a major element that must be maintained for efficient mucociliarly clearance is the hydration status of the mucus [12]. The predominant experimental evidence has identified two airway epithelial major ion transport pathways which play crucial roles in determining hydration of airway surfaces, namely anion secretion and cation absorption. These opposing forces are balanced by the surface epithelium to osmotically regulate airway hydration [92]. Na^+ absorption across the apical plasma membrane is mediated by the epithelial Na^+ channel (ENaC) [93, 94]. Cl^- secretion can occur either by CFTR, or by the Ca^{2+} activated Cl^- channel (CaCC, imcluding Anoctamin 1/TMEM16A) [95, 96]. Ciliated cells are known to co-express Anoctamin 1, CFTR and ENaC [97], suggesting that they can both hydrate the ASL and move mucus to maintain lung sterility.

CFTR is an anion channel and adenine nucleotide binding cassette (ABC) protein, belonging to the ABC transporter subfamily [98]. CFTR is unique amongst ABC proteins in two ways. First, although CFTR has structural similarity to ABC proteins and has 12 transmembrane-spanning domains and two nucleotide-binding domains, it does not act as a transporter protein, but rather functions as an anion channel [99–101]. Second, CFTR has an extra intracellular segment known as the regulatory domain or R-domain, which is not a feature of other ABC proteins. The R-domain can be extensively phosphorylated which serves to gate the channel [100, 102]. Typically, ABC proteins bind ATP and use the energy to drive the transport of various molecules across cell membranes [103]. In CFTR, the interactions of ATP with nucleotide binding domains control opening and closing of the channel pore rather than driving solute transport [98]. Disease-causing CFTR mutations have been extensively studied [7]. For example, the F508 CFTR mutation, where phenylalanine at position 508 is deleted, induces CFTR retention in the endoplasmic reticulum, decreases CFTR open probability, and reduces residence time in the plasma membrane.

ENaC is a heterotrimer consisting of α, β and γ-subunits [104, 105]. While this is the subunit combination that is typically found in the lung, in other tissues, ENaC may be substituted by a δ-subunit [84–86]. The α- and γ-subunits must be proteolytically cleaved for ENaC to be activated and to conduct Na^+ [106, 107]. The function of the β-subunit is likely regulatory in nature [87]. α and γ ENaC subunits can also be ubiquitination on their N-termin by the E3 ubiquitin ligase Nedd4.2, which plays a significant role in determining the channel half-life [108]. Interestingy, knockdown of NEDD4.2 (NEDD4L) in mice leads to upregulation of ENaC and the development of significant lung pathology [109].

When CFTR is activated, Cl^- and HCO_3^- are secreted into the airway lumen, with Na^+ and H_2O following passively through the paracelluar pathway. This coordinated response results in an isotonic increase in ASL height/volume [12, 110–112]. Conversely, when ENaC is activated, Na^+ moves from the airway lumen into the blood and Cl^-/H_2O follow paracellularly in the same direction, decreasing ASL volume [113]. Cl^- is the most abundant anion in the ASL (~120 mM) and provides the driving force for the osmotically induced changes in ASL volume. In contrast, HCO_3^- is present at smaller concentrations (~30 mM) and likely has proportionately has effect on ASL volume [114]. In addition, HCO_3^- likely plays an important role in buffering ASL pH [72, 115]

Normal airway epithelia can sense ASL volume and adjust ion transport rates accordingly, using several feedback systems including: (i) soluble volume sensors encoded in the ASL, such as short palate lung and nasal epithelial clone 1 (SPLUNC1) ATP, and adenosine to regulate ENaC and CFTR [116, 117], and (ii) ciliary beating to directly sense ASL/mucus hydration and response with control of ATP release rates [118]. These processes have been extensively reviewed elsewhere [83, 89, 119, 120]. The optimal height of the periciliary layer has been determined to be ~7 μm for normal ASL. This height reflects a well hydrated PCL layer, which is optimal for mucus clearance since it provides a low frictional environment that allows cilia to beat and mucus to flow. A decreased PCL height due to CFTR deficiency in CF and COPD airways has been associated with reduced mucus clearance and impaired lung innate defense [15, 49, 121].

Whilst ENaC must be cleaved to be activated, it can then be inhibited via several different routes. ENaC's intracellular N-termini interact with PIP_2 to open the gate of the channel. Purine nucleotides, such as ATP, acting through G_q-linked $P2Y_2$ receptors, are able to deplete the intracellular face of the plasma membrane of PIP_2, which leads to a decrease in ENaC's open probability[122–124]. ENaC's open probability can also be affected by cAMP/PKA-induced phosphorylation, which in the airways can be mediated by 2 adrenergic receptors or A2BR purinergic receptors, both of which are G_s-linked and raise cAMP [95]. The number of ENaC subunits at the plasma membrane is also regulated by short palate lung and nasal epithelial clone 1 (SPLUNC1), a 25 kDa protein that is secreted into the ASL by the underlying epithelia. SPLUNC1 binds directly to β-ENaC [125], which causes ENaC to be internalized [126]. An 18 amino acid long region of the protein, termed the S18 region, has also been shown to inhibit ENaC and to slow ASL volume absorption [125]. SPLUNC1-dependent regulation of ASL volume is defective in CF airways [127]. That is, CF ASL is mildly acidic due to the lack of HCO_3^- secretion through CFTR (~pH 7.0 in normal ASL; ~pH 6.5 in CF ASL)[128] and SPLUNC1 is a pH-sensitive protein that fails to regulate ENaC at ≤ pH 6.5 [127, 129]. SPLUNC1 expression may be increased in COPD airways [130]. However, the possible effect of CFTR diminution on ASL pH and subsequent failure of SPLUNC1 to regulate ENaC remains to be tested.

The interactions between CFTR and ENaC are complicated and not fully understood [111, 131, 132]. However, electrophysiological studies have revealed that ENaC is inhibited by cAMP/PKA in the presence of CFTR and activated by cAMP/PKC in CFTR's absence [133, 134]. Further support of this regulatory interaction emerged from studies with Madin Darby Canine Kidney (MDCK) epithelial cells where recombinant ENaC activity was decreased by

coexpression with full-length CFTR [135]. Similar findings emanated from a study of freshly prepared lung slices from wild-type vs. $CFTR^{(-/-)}$ mice where basal open probability of ENaC increased fourfold in absence of CFTR [136]. Despite these studies, how CFTR actually regulates ENaC remains to be determined [111].

It is also not known whether decreased CFTR expression in COPD airways leads to ENaC hyperactivity and emerging *in vivo* data suggest that ENaC activity may be intrinsically normal in COPD [15, 137]. Crucially, despite reduced levels of CFTR in COPD airways, the remaining CFTR is wild-type and not a disease-causing mutant, and it may be that the residual wild type CFTR activity in COPD airways (~30% of normal activity) is sufficient to prevent ENaC hyperactivity. The situation may change with severe COPD, however, since ENaC is cleaved and activated by many proteases, including neutrophil elastase and cathepsins [138–142]. Indeed, chronic neutrophilia is seen in severe COPD, which may promote cleavage and activation of ENaC due to increased neutrophil elastase levels [143].

Recent data indicate the regulation of ENaC may be abnormal in COPD. Recent data have indicated that extracellular ATP levels are reduced in COPD secretion, in part due to increased ecto-ATPase activities (Anderson, et al, Blue J. in press). Thus, ATP inhibition of ENaC may be reduced in COPD.

The effect of tobacco smoke on ciliary beating

Pyrolysed tobacco contains >5000 different chemicals including aldehydes, which are the product of combusted plant material (e.g. cellulose), heavy metals, CO and free radicals [144–146]. Since tobacco smoke is highly oxidized, it is very labile and its composition changes with time after its production [147]. Of the chemicals contained in tobacco smoke, acrolein, acetaldehyde, formaldehyde and Cd^{2+} are thought to have the biggest effect on the development of pulmonary disease [148]. Tobacco smoke components such as acrolein and acetaldehyde impair ciliary clearance [149]. Ciliary beat frequency has been shown to be significantly decreased in ciliated cells from nasal brushings of moderate and severe COPD patients compared to control and other at-risk subjects [150] and ciliary beat frequency was found to be lower in smoke-exposed subjects [151]. Volatile compounds in cigarette smoke extract are predicted to reduce ciliary beat frequency through a protein kinase C dependent mechanism [152]. Since these measurements were made under flooded conditions (i.e. the thin film ASL was washed away), this effect was likely due to changes in ultrastructure of the cilia rather than to changes in mucus viscosity.

Cigarette smoke (CS) exposure has been shown to impair ciliogenesis and to cause ciliary shortening [153]. Histone deacetylase 6, a ubiquitin ligase that also affects protein acetylation, has recently been implicated in ciliary shortening after CS exposure [154]. Specifically, CS is thought to upregulate histone deacetylase 6's ubiquitinase activity and promote removal of ciliary proteins, leading to ciliary shortening. Indeed, cells with reduced histone deacetylase 6 expression are resistant to CS-induced ciliary shortening.

Axonemal abnormalities, including changes in the microtubule's "9+2" arrangement, were also found to be about three fold greater in smokers and ex-smokers as compared to nonsmokers [155]. Abnormalities in radial spokes, dynein arms along with nexin links and

fused cilia were further observed in smokers [155–159]. Subsequent analyses revealed that hydrogen cyanide, formaldehyde, acrolein, acetaldehyde, ammonia, nitrogen dioxide and phenol all reduced ciliary beating in a similar fashion as cigarette smoke [160].

The effect of tobacco smoke on CFTR-mediated ion transport

Following both acute and chronic cigarette smoke exposure, CFTR mediated Cl^- secretion was found to be significantly reduced *in vitro* and *in vivo* [15, 137, 161]. This inhibition was both rapid in onset and sustained over extended periods of time. Several mechanisms have been proposed to account for the reduction in CFTR activity:

i. Effects on CFTR trafficking: After tobacco smoke exposure, CFTR has been shown to rapidly internalize [15, 162] (Figure 1). This internalization is Ca^{2+} dependent, with the Ca^{2+} emanating from lysosomes rather than from the endoplasmic reticulum or mitochondria [163]. Recent data has implicated activation of the MEK-Erk1/2-MAPK pathway in this response [162]. Tobacco exposure also caused CFTR aggregation and induced an alteration in solubility of CFTR, which we speculated caused CFTR to accumulate in a perinuclear aggresome-like compartment rather than traffic to the lysosomes or the proteasome [15]. Such changes in CFTR solubility have previously been observed with misfolded CFTR [164–166]. Because there is a peripheral quality control system for surface CFTR [167, 168], it is possible that tobacco exposure induces an acute mis-folding of surface CFTR, leading to rapid internalization.

ii. Direct effects of tobacco smoke metabolites on CFTR function: Acrolein is a highly reactive tobacco smoke metabolite that is known to form covalent adducts with DNA [169, 170] and with proteins [171]. In single channel patch clamp recordings, acrolein has been demonstrated to directly affect CFTR gating [172]. Cigarette smoke is acidic, and 50 standard cigarettes drawn through 50 mls of Frog Ringer solution acidified the solution by about 2 pH units [173]. This cigarette-exposed acidic media inhibited the activity of CFTR expressed in *Xenopus laevis* oocytes [125]. As the HCO_3^- secretion is also mediated by CFTR, tobacco smoke exposure also exhibits reduced secretion of HCO_3^- presumably resulting in further acidification of ASL [174, 175]. To date, however, there no evidence that tobacco exposure actually causes an acidification of the ASL *in vivo*.

iii. Effects of tobacco smoke and oxidative stress on CFTR expression: Tobacco smoke is highly oxidizing (5×10^{14} radicals per puff [176]). Oxidative stress is a well-known inhibitor of CFTR gene expression [177]. Indeed, Cd^{2+} and other oxidants present in tobacco smoke have been shown to decrease CFTR gene expression and to affect microRNAs, such as Mir-101 and Mir-144, that themselves influence CFTR expression [178]. Cd^{2+} was also shown to accumulate in the lungs of GOLD4 COPD patients and is negatively correlated with CFTR expression, suggesting that accumulated heavy metals in lung epithelia may act as toxins that continuously inhibit CFTR expression and drive mucus dehydration in severe COPD patients [178, 179].

Emerging data from multiple laboratories have indicated that tobacco exposure leads to airway dehydration in human bronchial epithelial cells cultured at an air-liquid interface (Figure 1) [15, 180]. A single bout of tobacco smoke exposure (i.e. the smoke from one cigarette) rapidly diminished ASL height (within 30 minutes of initiation of exposure), and it took 3–4 hours for ASL height to return to pre-exposure levels [15]. When cultures were exposed to smoke chronically, resting ASL height decreased permanently, indicating a more severe effect of tobacco exposure on ASL homeostasis [179]. The decrease in ASL height was due to changes in active ion transport, as opposed to a simple drying of the ASL by the smoke exposure, as evidenced by studies showing that [181, 182]. pre-inhibition of ENaC prevented the ASL depletion caused by tobacco smoke., Furthermore, the addition of ENaC antagonists after tobacco-smoke induced ASL dehydration resulted in a quicker restoration of ASL height to normal levels, indicating that continued ENaC activity in the absence of CFTR function contributed to the ASL depletion [181].

The effect of tobacco smoke on mucus secretion/mucus clearance

Nasal Mucociliary clearance (MCC) has been assessed in vivo with the saccharine transit test. Thus, this test involves placing saccharine in the inferior nasal turbinate and measuring the time taken for the saccharine to be tasted, which is directly proportional to the mean transit of saccharin through the nasal cavity. For example, the longer it takes to taste the saccharine, the slower the MCC rate [183]. Nasal MCC was significantly reduced in smokers compare to nonsmokers as measured using this method [184].

Mucus adhesion contributes to the pathophysiology of chronic infectious airways diseases in different ways. The notion that mucus adhesion imparts airflow obstruction emerged from micro-CT studies. Specifically, Hogg et al, found that the survival rate of COPD patients was dominated by events internal to the airway basement membrane, i.e., epithelial hyperplasia and mucus obstruction [6, 36]. It has also been reported that mucus plugs from COPD lungs are ~7% solids [15, 185]. Our experimental data and theoretical analyses indicate that when mucus dehydration (as indexed by % solids) exceeds 6% solids, mucus clearance slows [48, 55, 186]. Indeed, the biophysical properties of 6.5% mucus became markedly abnormal (2 log differences) as compared to normal 2% mucus [52, 186]. These data predict that more concentrated mucus will be transported by cilia less effectively. In theory, when mucus concentrations exceed 6%, the osmotic pressure exerted by the mucins in the mucus layer will exceed that of the PCL, leading to a collapse of the PCL and adhesion of mucus to airway surfaces [55]. Mucus from CF subjects exhibited similar properties with elevated % solids content and subsequent mucus stasis [35, 187, 188], suggesting that mucus dehydration plays a role in the pathogenesis of both diseases. The ASL dehydration induced by tobacco-smoke mediated down-regulation of CFTR is further exacerbated by the concomitant increase in mucin secretion [189–191]. For example, acrolein increased MUC5AC expression, which contributes to an imbalance between mucin production and salt/water secretion [192, 193].

Animal models of COPD

The *in vivo* and *in vitro* human data are complemented by data from animal models of COPD. Historically, mice, rats and ferrets have been chronically exposed to tobacco smoke [194–197]. For example, chronic tobacco exposure in mice causes emphysema, which has been attributed to macrophage elastase (MME) production. Six months of cigarette smoke exposure caused macrophage accumulation in murine lungs along with an enlargement of alveolar airspaces [198]. MME $^{(-/-)}$ mice were protected against this response, indicating that protease production is required for alveolar damage [198]. Interestingly, while mice develop the emphysema phenotype, they do not typically exhibit mucus obstruction/CB. One reason for this observation may be that reduced CFTR activity is critical for mucus dehydration in humans, CFTR is little expressed in murine lungs. Consistent with this notion, CFTR knockout mice do not exhibit a significant pulmonary phenotype [199, 200]. The mucociliary clearance rates observed in rodents is also slower than reported in humans, which may further contribute to the species differences in response to cigarette smoke [201, 202]. Currently, and to the best of our knowledge, there are no good rodent spontaneous or environmentally produced models of CB dehydration.

Genetically, mucus obstruction has been induced in mice by transgenically overexpressing the β-subunit of ENaC, which produced a phenotype of airway surface liquid (ASL) volume depletion that was associated with mucus adhesion/plaque formation and the inability to clear inhaled bacteria that phenocopies CF and CB [203]. Importantly, these animals exhibited a spontaneous mortality of ~60% after 30 days that reflected mucus obstruction [203]. This model has helped establish the relative importance of hydration on airway health. In contrast, a series of mouse models with either no cilia or dysfunctional cilia surprisingly exhibited little or no pulmonary disease phenotype [202]. Diseases that affect other aspects of the innate defense mechanisms of the lung, e.g., alveolar macrophage dysfunction, neutrophil dysfunction, dysfunction of immunoglobulins (both secretory, IgA, and IgG) tend to produce disease of the alveoli rather than airways. Thus, we believe that it is a reasonable conclusion that mechanical (mucus) clearance is the dominant form of innate defense of the airways, and failure to provide this activity produces obstruction and the propensity for chronic infection.

Treatment for CFTR diminution and mucus dehydration in COPD

The increased awareness that CB is at least, in part, a disease of CFTR dysfunction and mucus dehydration, suggested that ASL/mucus rehydration would be beneficial in clearing airway mucus obstruction in COPD subjects. This goal may be achieved either by direct rehydration of the mucus with osmotic agents such as hypertonic saline or mannitol, by restoring CFTR function, or by inhibiting ENaC (Figure 2). The use of hypertonic saline for the treatment of dehydrated airways in CF patients has shown promise by improving mucus clearance and lung function, as well as decreasing the number of acute exacerbations (Figure 2a) [204, 205]. Hypertonic saline has also been shown to accelerate MCC in COPD patients [15, 206]. In addition to increasing airway hydration, inhaled hypertonic saline treatment also decreased the number of neutrophils in the lung, suggesting that it may be a viable therapy for COPD patients [207].

Interestingly, the duration of action of hypertonic saline is relatively short in normal and COPD airways as compared to CF airways [15, 204]. This short response likely reflects the fact that in normal airways, the high salt concentration achieved on airway surfaces following aerosol delivery of hypertonic saline generates chemical gradients for Cl^- to be absorbed through CFTR and the paracellular pathway, with Na^+ being absorbed through ENaC and also the paracellular pathway, thus limiting the sustainablilty of osmotic effects of hypertonic saline on airway surfaces. In contrast, the absence of CFTR function in CF airways eliminates the transcellular path for Cl^- absorption, resulting in the maintenance of high concentrations of NaCl on airway surfaces. Because the high ASL Cl^- concentration caused cannot be dissipated as rapidly, a greater, osmotically-induced hydration is generated [135].

The open probability of wild-type CFTR typically ranges from 0.35 to 0.40 [208, 209], and P_o is reduced following acrolein exposure to <0.1 [172]. The G551D disease-causing CFTR mutation exhibits an open probability that is ~0 [210]. However, potentiator drugs such as VX770 (Ivacaftor/Kalydeco) have been developed which increase the open probability of both wild-type and G551D CFTR [211]. It has been hypothesized that such potentiators may be a suitable treatment to increase open probability, and hence restore Cl^- secretion, in CFTR-deficient COPD airways (Figure 2b). Indeed, acute VX770 addition increases CFTR-mediated Cl^- secretion after cigarette smoke extract exposure in HBECs [180]. In contrast, while the F508 CFTR mutant is retained in the endoplasmic reticulum, it may be induced in vitro to traffic to the plasma membrane with a reduced open probability of ~0.1 [212]. CFTR correctors have also been developed that help traffic CFTR to the plasma membrane, although their effects on CS-exposed HBECs have not been determined [213]. As a caveat, it has recently been shown that chronic VX770 exposure may inhibit wild type CFTR by reducing CFTR's dwell time in the plasma membrane, suggesting that VX770 may not be the optimal potentiator to restore CFTR function in COPD patients [214]. Thus, potentiator/corrector based approaches to restore CFTR function are relatively new and the continued development of compounds designed to improve trafficking of CFTR in tobacco-exposed/CB airways may yield novel therapeutics for the treatment of CFTR dysfunction in CB airways.

There are more conventional approaches to activating CFTR in COPD subjects. Since CFTR is a cAMP-activated channel, phosphodiesterases such as roflumilast may also serve to increase CFTR activation levels (Figure 2c) [182, 215]. This effect may also be present in COPD airways, given that ~30% of CFTR function remains [15, 137, 161]. Similarly, inhaled 2 adrenergic receptor agonists are a mainstay of COPD treatment, and they are thought to relieve the airway obstruction by inducing smooth muscle relaxation, as seen in asthmatic patients [216]. However, these agonists also activate CFTR [217]. To date, no COPD study has been completed in order to determine the most effective 2 agonist for activating CFTR. Such optimization of existing/FDA approved 2 agonists may provide a useful tool for reducing mucus dehydration in CB airways.

A fourth therapeutic approach for COPD focuses on inhibiting ENaC to restore airway hydration (Figure 2d). To date, no ENaC inhibitors have been used to successfully treat airway dehydration by limiting Na^+ absorption through the airways and previous trials have

failed due to both safety issues and lack of efficacy [218] (amilioride trials- safe- no effect). Amiloride and its analogues are a series of ENaC antagonists that were initially designed to inhibit ENaC in the kidneys with a goal of treating hypertension [219]. Whilst efficacious at increasing MCC, amilioride failed therapeutically to treat CF because it was rapidly absorbed across the airways via organic cation transporters into the systemic circulation where they can gain exposure to ENaC in the kidneys [220]. Inhibition of ENaC in the distal convoluted tubules of the kidneys directly blocks Na^+ absorption and indirectly blocks K^+ secretion, leading to a diuresis and natriuresis that is “potassium sparing” [219]. Consequently, if ENaC inhibitors delivered to the lung via aerosol are absorbed systemically and cleared renally, hyperkalemia can result. Two inhaled ENaC antagonists (amiloride and GS9411) have elicited a renal response [218, 221]. Recently, novel ENaC antagonists have been administered by inhalation and shown to increase rehydration and ciliary beating without eliciting this response, suggesting that ENaC antagonism may be a viable therapy for ASL rehydration in COPD airways [222, 223]. Furthermore, ENaC antagonists have been shown to reverse ASL dehydration seen in tobacco-exposed airway epithelia, and to increase ciliary beat frequency *in vitro*, suggesting that if safety issues can be overcome, then ENaC antagonists may be a useful treatment for CB.

Using components of the MCC pathway as biomarkers of exposure for the study of new and emerging tobacco products

In addition to the existing brands of tobacco that have been well characterized (e.g. Marlboro, Camel cigarettes etc.), a large number “new and emerging” tobacco products and e-cigarettes are reaching the market that are currently unregulated by the US Food and Drug Administration. These products include little cigars, E-cigarettes and hookah [224]. For these new and emerging tobacco products, their effects on the lung are only beginning to be studied. It has been proposed that E-cigarettes, which produce a vapor with an electronic coil, may lessen the risk of developing COPD and/or lung cancer because they do not combust tobacco nor require pyrolysis to produce the vapor [225, 226]. Whilst the principle ingredients of E-cigarettes are typically propylene glycol, nicotine and flavorings, the chronic effect of these devices on the lungs is only beginning to be understood, and their propensity for triggering COPD is not known. For example, it has recently been reported that E-cigarette liquids promoted IL-6 production and inhibited SPLUNC1 expression [227]. IL-6 is known to increase mucin gene expression and secretion [228], while SPLUNC1 not only regulates ENaC (see above, but also plays multiple other roles in innate defense) and its absence could lead to altered epithelial function. Furthermore, formaldehyde has recently been detected in e-cigarette vapor, and this agent may be a breakdown product from the propylene glycol propellant [229]. As these tobacco products are newly emerging, appropriate human studies will need to be performed before these experiments are fully validated and their long term effects understood.

Chemical flavors are now widely being used in new and emerging tobacco products [230–232]. However, while these flavors are generally safe to eat, by and large, their effects on the lung are unknown. Recent data have indicated that flavors such as menthol can stimulate transient potential receptor (TRP) channels in the airways. TRP channels are involved in

nociception in the airways and their stimulation reduces irritation caused by tobacco exposure, making menthol cigarettes easier to smoke than regular cigarettes [233]. Indeed, genetic variants in TRPA1 amongst the general population have been linked to an increased preference for menthol cigarettes and for heavier tobacco intake in a subset of the population [234]. Another flavor that has received attention is diacetyl, which is used in the food industry to provide a buttery flavor and is added to many food products, including popcorn and beer [235]. Diacetyl is thought to be safe when ingested orally and is approved for human usage [235]. However, diacetyl inhalation is known to cause bronchiolitis obliterans or "popcorn workers lung" [236]. Additionally, the authors reported a twofold greater risk of diacetyl causing COPD, although these cases may have been bronchiolitis obliterans that were misdiagnosed. Thus, it may wise that every flavor used in new and emerging tobacco products and e-cigarettes be retested for lung toxicity in order to prevent the development of chronic airway disease.

Considering the present shift in tobacco use from conventional cigarettes to new and emerging tobacco products like little cigars, smokeless tobacco and e-cigarettes, the effects that these new products will exert on airway hydration and associated mucociliary clearance are as yet untested [237–239]. Importantly, components of the mucociliary transport system will serve as useful biomarkers of exposure both in vivo and in vitro. In vivo, CFTR activity/expression, mucus clearance rates, induced sputum properties (mucus hydration, protease activity) are all useful and relatively accessible biomarkers that can be used to measure effects of exposure. Similarly, CFTR function, ciliogenesis, mucin expression and mucus clearance rates can all be assayed in well-differentiated HBECs in vitro following tobacco/e-cig exposure, suggesting that these measures will be important biomarkers that can be utilized to assess the relative toxicity of exposure to new and emerging tobacco products.

The differences between chronic bronchitis and cystic fibrosis

Although we speculate that CF and CB are characterized by relative dehydration of mucus and mucus adhesion, the pathophysiologic sequences that produce this state are very different in the two diseases. We hypothesize that the pathogenesis of CF lung disease directly reflects abnormal regulation of electrolyte transport to produce ASL volume depletion. Specifically, we speculate that the failure of CFTR function in CF airways produces an intrinsic defect in regulation of Na^+ absorption and a failure to secrete anions via the tonic basal anion secretory pathway, i.e., CFTR itself. Concurrently, reduced HCO_3^- secretion due to the lack of CFTR contributes to a mildly acidic pH [127]. Thus, the primary defect is one of ASL volume depletion, and only later, when chronic infection sets in, does mucin hypersecretion commence, which worsens the relative dehydration state of mucus.

In contrast, mucus dehydration in CB is likely multifactorial. Mucin hypersecretion has been thought to be the primary defect in COPD that leads to relative airway surface dehydration [74]. Indeed, it is highly likely that high rates of secretion of mucins into the airway lumen, coupled with upregulated ecto-ATPase mediated reductions in extracellular ATP concentrations, contribute to increased mucus percent solids content. However, new data suggests that CFTR dysfunction contributes equally to this phenotype [11] [Anderson, Blue

J 2015). Compared to CF, COPD is a relatively slowly evolving disease when left untreated. Data from CF subjects suggest that severe CF mutations lead to a relatively complete inhibition of CFTR function and relatively rapid mucus plugging and heterogeneous loss of mucus clearance. In contrast, since tobacco exposure results in a progressive decline in CFTR function (50% in smokers, >70% in COPD patients), mucus dehydration is not so severe (~10% solids in COPD; up to 20% solids in CF) so the mucus obstruction is not so profound, consistent with a slower onset of disease.

Both diseases exhibit chronic inflammation. Inflammation in CF is characterized by high intraluminal IL 8 levels and high concentrations of polymorphonuclear cells [240]. The inflammatory response of the airway in COPD patients is likely more complex [241]. Perhaps this complexity reflects the chronic exposure to a tobacco smoke mixture that contains more than 5,000 chemical entities. Often, early in the disease, the inflammatory response is characterized by increased intraluminal and airway wall mononuclear cells, particularly CD4 and CD8 cells [241]. Only later in the disease, once severe obstruction of the airways obstruction and chronic infection sets in, does persistent neutrophilic accumulation in mucus become characteristic of this disease process [143]. Despite these differences, our knowledge of CF has helped drive a better understanding of the CFTR and mucus defect in COPD.

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References

1. WHO. Projections of mortality and causes of death, 2015 and 2030. 2013.
2. GOLD, Global Initiative for Chronic Obstructive Lung Disease (GOLD). 2014 Global Strategy for the Diagnosis, Management and Prevention of COPD. 2014.
3. Barnes PJ. Cellular and molecular mechanisms of chronic obstructive pulmonary disease. Clin Chest Med. 2014; 35(1):71–86. [PubMed: 24507838]
4. DeMeo DL, Silverman EK. Alpha1-antitrypsin deficiency. 2: genetic aspects of alpha(1)-antitrypsin deficiency: phenotypes and genetic modifiers of emphysema risk. Thorax. 2004; 59(3):259–64. [PubMed: 14985567]
5. Kim V, Criner GJ. Chronic bronchitis and chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2012; 187(3):228–37. [PubMed: 23204254]
6. Hogg JC, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med. 2004; 350(26):2645–53. [PubMed: 15215480]
7. Tsui LC. The spectrum of cystic fibrosis mutations. Trends Genet. 1992; 8(11):392–8. [PubMed: 1279852]
8. Harris A, Argent BE. The cystic fibrosis gene and its product CFTR. Semin Cell Biol. 1993; 4(1): 37–44. [PubMed: 7680915]
9. Chmiel JF, Davis PB. State of the art: why do the lungs of patients with cystic fibrosis become infected and why can’t they clear the infection? Respir Res. 2003; 4:8. [PubMed: 14511398]

10. Collawn JF, Matalon S. CFTR and lung homeostasis. Am J Physiol Lung Cell Mol Physiol. 2014; 307(12):L917–23. [PubMed: 25381027]
11. Rab A, et al. Cigarette smoke and CFTR: implications in the pathogenesis of COPD. Am J Physiol Lung Cell Mol Physiol. 2013; 305(8):L530–41. [PubMed: 23934925]
12. Chambers LA, Rollins BM, Tarran R. Liquid movement across the surface epithelium of large airways. Respir Physiol Neurobiol. 2007; 159(3):256–70. [PubMed: 17692578]
13. Ehre C, Ridley C, Thornton DJ. Cystic fibrosis: an inherited disease affecting mucin-producing organs. Int J Biochem Cell Biol. 2014; 52:136–45. [PubMed: 24685676]
14. Boucher RC. Relationship of airway epithelial ion transport to chronic bronchitis. Proc Am Thorac Soc. 2004; 1(1):66–70. [PubMed: 16113415]
15. Clunes LA, et al. Cigarette smoke exposure induces CFTR internalization and insolubility, leading to airway surface liquid dehydration. FASEB J. 2012; 26(2):533–45. [PubMed: 21990373]
16. Lundback B, et al. Not 15 but 50% of smokers develop COPD?--Report from the Obstructive Lung Disease in Northern Sweden Studies. Respir Med. 2003; 97(2):115–22. [PubMed: 12587960]
17. Mannino DM, et al. Chronic obstructive pulmonary disease surveillance--United States, 1971–2000. Respir Care. 2002; 47(10):1184–99. [PubMed: 12354338]
18. Peacock JL, et al. Outdoor air pollution and respiratory health in patients with COPD. Thorax. 2011; 66(7):591–6. [PubMed: 21459856]
19. Sunyer J. Urban air pollution and chronic obstructive pulmonary disease: a review. Eur Respir J. 2001; 17(5):1024–33. [PubMed: 11488305]
20. Kurmi OP, et al. COPD and chronic bronchitis risk of indoor air pollution from solid fuel: a systematic review and meta-analysis. Thorax. 2010; 65(3):221–8. [PubMed: 20335290]
21. Schikowski T, et al. Ambient air pollution: a cause of COPD? Eur Respir J. 2013; 43(1):250–63. [PubMed: 23471349]
22. Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. Lancet. 2007; 370(9589):765–73. [PubMed: 17765526]
23. Salvi SS, Barnes PJ. Chronic obstructive pulmonary disease in non-smokers. Lancet. 2009; 374(9691):733–43. [PubMed: 19716966]
24. Laurell CB, Eriksson S. The electrophoretic alpha1-globulin pattern of serum in alpha1-antitrypsin deficiency. 1963. COPD. 2013; 10(Suppl 1):3–8. [PubMed: 23527532]
25. Zorzetto M, et al. SERPINA1 gene variants in individuals from the general population with reduced alpha1-antitrypsin concentrations. Clin Chem. 2008; 54(8):1331–8. [PubMed: 18515255]
26. Stoller JK, Aboussouan LS. Alpha1-antitrypsin deficiency. Lancet. 2005; 365(9478):2225–36. [PubMed: 15978931]
27. Cox DW, Levison H. Emphysema of early onset associated with a complete deficiency of alpha-1-antitrypsin (null homozygotes). Am Rev Respir Dis. 1988; 137(2):371–5. [PubMed: 3257661]
28. Brantly ML, et al. Clinical features and history of the destructive lung disease associated with alpha-1-antitrypsin deficiency of adults with pulmonary symptoms. Am Rev Respir Dis. 1988; 138(2):327–36. [PubMed: 3264124]
29. Dowson LJ, Guest PJ, Stockley RA. Longitudinal changes in physiological, radiological, and health status measurements in alpha(1)-antitrypsin deficiency and factors associated with decline. Am J Respir Crit Care Med. 2001; 164(10 Pt 1):1805–9. [PubMed: 11734427]
30. Boucher RC. Airway surface dehydration in cystic fibrosis: pathogenesis and therapy. Annu Rev Med. 2007; 58:157–70. [PubMed: 17217330]
31. Mall MA, Hartl D. CFTR: cystic fibrosis and beyond. Eur Respir J. 2014; 44(4):1042–54. [PubMed: 24925916]
32. Smith A. Pathogenesis of bacterial bronchitis in cystic fibrosis. Pediatr Infect Dis J. 1997; 16(1): 91–5. discussion 95–6, 123–6. [PubMed: 9002117]
33. Maestrelli P, et al. Remodeling in response to infection and injury. Airway inflammation and hypersecretion of mucus in smoking subjects with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2001; 164(10 Pt 2):S76–80. [PubMed: 11734472]

34. Thompson AB, et al. Intraluminal airway inflammation in chronic bronchitis. Characterization and correlation with clinical parameters. Am Rev Respir Dis. 1989; 140(6):1527–37. [PubMed: 2604284]
35. Worlitzsch D, et al. Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients. J Clin Invest. 2002; 109(3):317–25. [PubMed: 11827991]
36. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. Lancet. 2004; 364(9435):709–21. [PubMed: 15325838]
37. Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic obstructive lung disease. N Engl J Med. 1968; 278(25):1355–60. [PubMed: 5650164]
38. Knowles MR, Boucher RC. Mucus clearance as a primary innate defense mechanism for mammalian airways. J Clin Invest. 2002; 109(5):571–7. [PubMed: 11877463]
39. Antunes MB, Cohen NA. Mucociliary clearance--a critical upper airway host defense mechanism and methods of assessment. Curr Opin Allergy Clin Immunol. 2007; 7(1):5–10. [PubMed: 17218804]
40. Ali M, et al. Analysis of the proteome of human airway epithelial secretions. Proteome Sci. 2011; 9:4. [PubMed: 21251289]
41. Pennington JE. Respiratory tract infections: intrinsic risk factors. Am J Med. 1984; 76(5A):34–41. [PubMed: 6372477]
42. Rogan MP, et al. Antimicrobial proteins and polypeptides in pulmonary innate defence. Respir Res. 2006; 7:29. [PubMed: 16503962]
43. Puchelle E, et al. Airway epithelial repair, regeneration, and remodeling after injury in chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2006; 3(8):726–33. [PubMed: 17065381]
44. Thornton DJ, Rousseau K, McGuckin MA. Structure and function of the polymeric mucins in airways mucus. Annu Rev Physiol. 2008; 70:459–86. [PubMed: 17850213]
45. Randell SH, Boucher RC. Effective mucus clearance is essential for respiratory health. Am J Respir Cell Mol Biol. 2006; 35(1):20–8. [PubMed: 16528010]
46. Fahy JV, Dickey BF. Airway mucus function and dysfunction. N Engl J Med. 2010; 363(23): 2233–47. [PubMed: 21121836]
47. Nadel JA, Davis B, Phipps RJ. Control of mucus secretion and ion transport in airways. Annu Rev Physiol. 1979; 41:369–81. [PubMed: 373597]
48. Tarran R, et al. The relative roles of passive surface forces and active ion transport in the modulation of airway surface liquid volume and composition. J Gen Physiol. 2001; 118(2):223–36. [PubMed: 11479349]
49. Tarran R, et al. The CF salt controversy: in vivo observations and therapeutic approaches. Mol Cell. 2001; 8(1):149–58. [PubMed: 11511368]
50. Kilburn KH. Cilia and mucus transport as determinants of the response of lung to air pollutants. Arch Environ Health. 1967; 14(1):77–91. [PubMed: 6017099]
51. Schlesinger RB. The interaction of inhaled toxicants with respiratory tract clearance mechanisms. Crit Rev Toxicol. 1990; 20(4):257–86. [PubMed: 2178627]
52. Cone RA. Barrier properties of mucus. Adv Drug Deliv Rev. 2009; 61(2):75–85. [PubMed: 19135107]
53. Grotberg JB. Respiratory fluid mechanics and transport processes. Annu Rev Biomed Eng. 2001; 3:421–57. [PubMed: 11447070]
54. Tarran R, Button B, Boucher RC. Regulation of normal and cystic fibrosis airway surface liquid volume by phasic shear stress. Annu Rev Physiol. 2006; 68:543–61. [PubMed: 16460283]
55. Button B, et al. A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia. Science. 2012; 337(6097):937–41. [PubMed: 22923574]
56. Cao R, et al. Mapping the protein domain structures of the respiratory mucins: a mucin proteome coverage study. J Proteome Res. 2012; 11(8):4013–23. [PubMed: 22663354]
57. Kesimer M, et al. Molecular organization of the mucins and glycocalyx underlying mucus transport over mucosal surfaces of the airways. Mucosal Immunol. 2013; 6(2):379–92. [PubMed: 22929560]

58. Henderson AG, et al. Cystic fibrosis airway secretions exhibit mucin hyperconcentration and increased osmotic pressure. J Clin Invest. 2014; 124(7):3047–60. [PubMed: 24892808]
59. Prescott E, Lange P, Vestbo J. Chronic mucus hypersecretion in COPD and death from pulmonary infection. Eur Respir J. 1995; 8(8):1333–8. [PubMed: 7489800]
60. Rogers DF. Airway goblet cells: responsive and adaptable front-line defenders. Eur Respir J. 1994; 7(9):1690–706. [PubMed: 7995400]
61. Cohn L. Mucus in chronic airway diseases: sorting out the sticky details. J Clin Invest. 2006; 116(2):306–8. [PubMed: 16453018]
62. Tyner JW, et al. Blocking airway mucous cell metaplasia by inhibiting EGFR antiapoptosis and IL-13 transdifferentiation signals. J Clin Invest. 2006; 116(2):309–21. [PubMed: 16453019]
63. Atherton H, et al. Preliminary pharmacological characterisation of an interleukin-13-enhanced calcium-activated chloride conductance in the human airway epithelium. Naunyn Schmiedebergs Arch Pharmacol. 2003; 367(2):214–7. [PubMed: 12595965]
64. Roy MG, et al. Muc5b is required for airway defence. Nature. 2014; 505(7483):412–6. [PubMed: 24317696]
65. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. Nat Rev Cancer. 2004; 4(1):45–60. [PubMed: 14681689]
66. Davis CW, Dickey BF. Regulated airway goblet cell mucin secretion. Annu Rev Physiol. 2008; 70:487–512. [PubMed: 17988208]
67. Forstner G. Signal transduction, packaging and secretion of mucins. Annu Rev Physiol. 1995; 57:585–605. [PubMed: 7778879]
68. Hattrup CL, Gendler SJ. Structure and function of the cell surface (tethered) mucins. Annu Rev Physiol. 2008; 70:431–57. [PubMed: 17850209]
69. Rose MC, Voynow JA. Respiratory tract mucin genes and mucin glycoproteins in health and disease. Physiol Rev. 2006; 86(1):245–78. [PubMed: 16371599]
70. Abdullah LH, et al. P2u purinoceptor regulation of mucin secretion in SPOC1 cells, a goblet cell line from the airways. Biochem J. 1996; 316 (Pt 3):943–51. [PubMed: 8670174]
71. Abdullah LH, et al. Protein kinase C and Ca2+ activation of mucin secretion in airway goblet cells. Am J Physiol. 1997; 273(1 Pt 1):L201–10. [PubMed: 9252557]
72. Quinton PM. Role of epithelial HCO3(−) transport in mucin secretion: lessons from cystic fibrosis. Am J Physiol Cell Physiol. 2010; 299(6):C1222–33. [PubMed: 20926781]
73. Verdugo P. Goblet cells secretion and mucogenesis. Annu Rev Physiol. 1990; 52:157–76. [PubMed: 2184755]
74. Livraghi A, Randell SH. Cystic fibrosis and other respiratory diseases of impaired mucus clearance. Toxicol Pathol. 2007; 35(1):116–29. [PubMed: 17325980]
75. Vladar EK, Antic D, Axelrod JD. Planar cell polarity signaling: the developing cell's compass. Cold Spring Harb Perspect Biol. 2009; 1(3):a002964. [PubMed: 20066108]
76. You Y, et al. Role of f-box factor foxj1 in differentiation of ciliated airway epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2004; 286(4):L650–7. [PubMed: 12818891]
77. Gomperts BN, Gong-Cooper X, Hackett BP. Foxj1 regulates basal body anchoring to the cytoskeleton of ciliated pulmonary epithelial cells. J Cell Sci. 2004; 117(Pt 8):1329–37. [PubMed: 14996907]
78. Ostrowski LE, et al. A proteomic analysis of human cilia: identification of novel components. Mol Cell Proteomics. 2002; 1(6):451–65. [PubMed: 12169685]
79. Satir P, Christensen ST. Overview of structure and function of mammalian cilia. Annu Rev Physiol. 2007; 69:377–400. [PubMed: 17009929]
80. Davis CW, Lazarowski E. Coupling of airway ciliary activity and mucin secretion to mechanical stresses by purinergic signaling. Respir Physiol Neurobiol. 2008; 163(1–3):208–13. [PubMed: 18635403]
81. Hayashi T, et al. ATP regulation of ciliary beat frequency in rat tracheal and distal airway epithelium. Exp Physiol. 2005; 90(4):535–44. [PubMed: 15769883]
82. Korngreen A, Priel Z. Purinergic stimulation of rabbit ciliated airway epithelia: control by multiple calcium sources. J Physiol. 1996; 497 (Pt 1):53–66. [PubMed: 8951711]

83. Lazarowski ER, Boucher RC. Purinergic receptors in airway epithelia. Curr Opin Pharmacol. 2009; 9(3):262–7. [PubMed: 19285919]
84. Lieb T, et al. Prolonged increase in ciliary beat frequency after short-term purinergic stimulation in human airway epithelial cells. J Physiol. 2002; 538(Pt 2):633–46. [PubMed: 11790825]
85. Jain B, et al. Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. Biochem Biophys Res Commun. 1993; 191(1):83–8. [PubMed: 7680560]
86. Jiao J, et al. Regulation of ciliary beat frequency by the nitric oxide signaling pathway in mouse nasal and tracheal epithelial cells. Exp Cell Res. 2011; 317(17):2548–53. [PubMed: 21787770]
87. Li D, et al. Regulation of ciliary beat frequency by the nitric oxide-cyclic guanosine monophosphate signaling pathway in rat airway epithelial cells. Am J Respir Cell Mol Biol. 2000; 23(2):175–81. [PubMed: 10919983]
88. Yang B, Schlosser RJ, McCaffrey TV. Dual signal transduction mechanisms modulate ciliary beat frequency in upper airway epithelium. Am J Physiol. 1996; 270(5 Pt 1):L745–51. [PubMed: 8967508]
89. Schmid A, et al. Nucleotide-mediated airway clearance. Subcell Biochem. 2011; 55:95–138. [PubMed: 21560046]
90. Salathe M. Regulation of mammalian ciliary beating. Annu Rev Physiol. 2007; 69:401–22. [PubMed: 16945069]
91. Wyatt TA, et al. Sequential activation of protein kinase C isoforms by organic dust is mediated by tumor necrosis factor. Am J Respir Cell Mol Biol. 2010; 42(6):706–15. [PubMed: 19635931]
92. Frizzell RA. Role of absorptive and secretory processes in hydration of the airway surface. Am Rev Respir Dis. 1988; 138(6 Pt 2):S3–6. [PubMed: 2849352]
93. Donaldson SH, Boucher RC. Sodium channels and cystic fibrosis. Chest. 2007; 132(5):1631–6. [PubMed: 17998363]
94. Thibodeau PH, Butterworth MB. Proteases, cystic fibrosis and the epithelial sodium channel (ENaC). Cell Tissue Res. 2013; 351(2):309–23. [PubMed: 22729487]
95. Com G, Clancy JP. Adenosine receptors, cystic fibrosis, and airway hydration. Handb Exp Pharmacol. 2009; (193):363–81. [PubMed: 19639288]
96. Blouquit-Laye S, Chinet T. Ion and liquid transport across the bronchiolar epithelium. Respir Physiol Neurobiol. 2007; 159(3):278–82. [PubMed: 17433793]
97. Ferrera L, Zegarra-Moran O, Galietta LJ. Ca2+-activated Cl− channels. Compr Physiol. 2011; 1(4):2155–74. [PubMed: 23733701]
98. Riordan JR. Assembly of functional CFTR chloride channels. Annu Rev Physiol. 2005; 67:701–18. [PubMed: 15709975]
99. Bear CE, et al. Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). Cell. 1992; 68(4):809–18. [PubMed: 1371239]
100. Tabcharani JA, et al. Phosphorylation-regulated Cl− channel in CHO cells stably expressing the cystic fibrosis gene. Nature. 1991; 352(6336):628–31. [PubMed: 1714039]
101. Gray MA, Greenwell JR, Argent BE. Secretin-regulated chloride channel on the apical plasma membrane of pancreatic duct cells. J Membr Biol. 1988; 105(2):131–42. [PubMed: 2464063]
102. Cheng SH, et al. Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. Cell. 1991; 66(5):1027–36. [PubMed: 1716180]
103. Hollenstein K, Dawson RJ, Locher KP. Structure and mechanism of ABC transporter proteins. Curr Opin Struct Biol. 2007; 17(4):412–8. [PubMed: 17723295]
104. Canessa CM, et al. Amiloride-sensitive epithelial Na+ channel is made of three homologous subunits. Nature. 1994; 367(6462):463–7. [PubMed: 8107805]
105. Jasti J, et al. Structure of acid-sensing ion channel 1 at 1.9 A resolution and low pH. Nature. 2007; 449(7160):316–23. [PubMed: 17882215]
106. Hughey RP, et al. Maturation of the epithelial Na+ channel involves proteolytic processing of the alpha- and gamma-subunits. J Biol Chem. 2003; 278(39):37073–82. [PubMed: 12871941]
107. Kleyman TR, Carattino MD, Hughey RP. ENaC at the cutting edge: regulation of epithelial sodium channels by proteases. J Biol Chem. 2009; 284(31):20447–51. [PubMed: 19401469]

108. Staub O, et al. Regulation of stability and function of the epithelial Na+ channel (ENaC) by ubiquitination. The EMBO Journal. 1997; 16(21):6325–6336. [PubMed: 9351815]
109. Kimura T, et al. Deletion of the ubiquitin ligase Nedd4L in lung epithelia causes cystic fibrosis-like disease. Proc Natl Acad Sci U S A. 2011; 108(8):3216–21. [PubMed: 21300902]
110. Guggino WB. Cystic fibrosis and the salt controversy. Cell. 1999; 96(5):607–10. [PubMed: 10089875]
111. Hobbs CA, Da Tan C, Tarran R. Does epithelial sodium channel hyperactivity contribute to cystic fibrosis lung disease? J Physiol. 2013; 591(Pt 18):4377–87. [PubMed: 23878362]
112. Horisberger JD. ENaC-CFTR interactions: the role of electrical coupling of ion fluxes explored in an epithelial cell model. Pflugers Arch. 2003; 445(4):522–8. [PubMed: 12548399]
113. Boucher RC. Human airway ion transport. Part one. Am J Respir Crit Care Med. 1994; 150(1): 271–81. [PubMed: 8025763]
114. Knowles MR, et al. Ion composition of airway surface liquid of patients with cystic fibrosis as compared with normal and disease-control subjects. J Clin Invest. 1997; 100(10):2588–95. [PubMed: 9366574]
115. Coakley RD, Boucher RC. Regulation and functional significance of airway surface liquid pH. Jop. 2001; 2(4 Suppl):294–300. [PubMed: 11875275]
116. Garcia-Caballero A, et al. SPLUNC1 regulates airway surface liquid volume by protecting ENaC from proteolytic cleavage. Proc Natl Acad Sci U S A. 2009; 106(27):11412–7. [PubMed: 19541605]
117. Lazarowski ER, et al. Nucleotide release provides a mechanism for airway surface liquid homeostasis. J Biol Chem. 2004; 279(35):36855–64. [PubMed: 15210701]
118. Button B, et al. Mechanosensitive ATP release maintains proper mucus hydration of airways. Sci Signal. 2013; 6(279):ra46. [PubMed: 23757023]
119. Button B, Boucher RC. Role of mechanical stress in regulating airway surface hydration and mucus clearance rates. Respir Physiol Neurobiol. 2008; 163(1–3):189–201. [PubMed: 18585484]
120. Gaillard EA, et al. Regulation of the epithelial Na+ channel and airway surface liquid volume by serine proteases. Pflugers Arch. 2010; 460(1):1–17. [PubMed: 20401730]
121. Matsui H, et al. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell. 1998; 95(7):1005–15. [PubMed: 9875854]
122. Kunzelmann K, et al. Purinergic inhibition of the epithelial Na+ transport via hydrolysis of PIP2. FASEB J. 2005; 19(1):142–3. [PubMed: 15504951]
123. Pochynyuk O, Bugaj V, Stockand JD. Physiologic regulation of the epithelial sodium channel by phosphatidylinositides. Curr Opin Nephrol Hypertens. 2008; 17(5):533–40. [PubMed: 18695396]
124. Yue G, Malik B, Eaton DC. Phosphatidylinositol 4,5-bisphosphate (PIP2) stimulates epithelial sodium channel activity in A6 cells. J Biol Chem. 2002; 277(14):11965–9. [PubMed: 11812779]
125. Hobbs CA, et al. Identification of SPLUNC1's ENaC-Inhibitory Domain Yields Novel Strategies to Treat Sodium Hyperabsorption in Cystic Fibrosis Airway Cultures. Am J Physiol Lung Cell Mol Physiol. 2013
126. Rollins BM, et al. SPLUNC1 expression reduces surface levels of the epithelial sodium channel (ENaC) in Xenopus laevis oocytes. Channels (Austin). 2010; 4(4):255–9. [PubMed: 20519934]
127. Garland AL, et al. Molecular basis for pH-dependent mucosal dehydration in cystic fibrosis airways. Proc Natl Acad Sci U S A. 2013; 110(40):15973–8. [PubMed: 24043776]
128. Cho DY, et al. Acid and base secretion in freshly excised nasal tissue from cystic fibrosis patients with DeltaF508 mutation. Int Forum Allergy Rhinol. 2011; 1(2):123–7. [PubMed: 22034590]
129. Tarran R, Redinbo MR. Mammalian short palate lung and nasal epithelial clone 1 (SPLUNC1) in pH-dependent airway hydration. Int J Biochem Cell Biol. 2014; 52:130–5. [PubMed: 24631954]
130. Di YP, et al. Molecular cloning and characterization of spurt, a human novel gene that is retinoic acid-inducible and encodes a secretory protein specific in upper respiratory tracts. J Biol Chem. 2003; 278(2):1165–73. [PubMed: 12409287]
131. Berdiev BK, Qadri YJ, Benos DJ. Assessment of the CFTR and ENaC association. Mol Biosyst. 2009; 5(2):123–7. [PubMed: 19156256]

132. Kunzelmann K, Schreiber R. Airway epithelial cells--hyperabsorption in CF? Int J Biochem Cell Biol. 2012; 44(8):1232–5. [PubMed: 22542896]
133. Boucher RC, et al. Na+ transport in cystic fibrosis respiratory epithelia. Abnormal basal rate and response to adenylate cyclase activation. J Clin Invest. 1986; 78(5):1245–52. [PubMed: 3771796]
134. Kunzelmann K, et al. Inhibition of epithelial Na+ currents by intracellular domains of the cystic fibrosis transmembrane conductance regulator. FEBS Lett. 1997; 400(3):341–4. [PubMed: 9009227]
135. Stutts MJ, et al. CFTR as a cAMP-dependent regulator of sodium channels. Science. 1995; 269(5225):847–50. [PubMed: 7543698]
136. Lazrak A, et al. Enhancement of alveolar epithelial sodium channel activity with decreased cystic fibrosis transmembrane conductance regulator expression in mouse lung. Am J Physiol Lung Cell Mol Physiol. 2011; 301(4):L557–67. [PubMed: 21743028]
137. Dransfield MT, et al. Acquired cystic fibrosis transmembrane conductance regulator dysfunction in the lower airways in COPD. Chest. 2013; 144(2):498–506. [PubMed: 23538783]
138. Vallet V, et al. An epithelial serine protease activates the amiloride-sensitive sodium channel. Nature. 1997; 389(6651):607–10. [PubMed: 9335501]
139. Caldwell RA, Boucher RC, Stutts MJ. Neutrophil elastase activates near-silent epithelial Na+ channels and increases airway epithelial Na+ transport. Am J Physiol Lung Cell Mol Physiol. 2005; 288(5):L813–9. [PubMed: 15640288]
140. Tan CD, et al. Cathepsin B contributes to Na+ hyperabsorption in cystic fibrosis airway epithelial cultures. J Physiol. 2014; 592(Pt 23):5251–68. [PubMed: 25260629]
141. Alli AA, et al. Cathepsin B is secreted apically from Xenopus 2F3 cells and cleaves the epithelial sodium channel (ENaC) to increase its activity. J Biol Chem. 2012; 287(36):30073–83. [PubMed: 22782900]
142. Butterworth MB, et al. Activation of the epithelial sodium channel (ENaC) by the alkaline protease from Pseudomonas aeruginosa. J Biol Chem. 2012; 287(39):32556–65. [PubMed: 22859302]
143. Hoenderdos K, Condliffe A. The neutrophil in chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol. 2013; 48(5):531–9. [PubMed: 23328639]
144. Borgerding M, Klus H. Analysis of complex mixtures--cigarette smoke. Exp Toxicol Pathol. 2005; 57(Suppl 1):43–73. [PubMed: 16092717]
145. Hoffmann D, Djordjevic MV, Hoffmann I. The changing cigarette. Prev Med. 1997; 26(4):427–34. [PubMed: 9245661]
146. Talhout R, et al. Hazardous compounds in tobacco smoke. Int J Environ Res Public Health. 2011; 8(2):613–28. [PubMed: 21556207]
147. Scian MJ, et al. Chemical analysis of cigarette smoke particulate generated in the MSB-01 in vitro whole smoke exposure system. Inhal Toxicol. 2009; 21(12):1040–52. [PubMed: 19772483]
148. Fowles J, Dybing E. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. Tob Control. 2003; 12(4):424–30. [PubMed: 14660781]
149. Dye JA, Adler KB. Effects of cigarette smoke on epithelial cells of the respiratory tract. Thorax. 1994; 49(8):825–34. [PubMed: 8091331]
150. Yaghi A, et al. Ciliary beating is depressed in nasal cilia from chronic obstructive pulmonary disease subjects. Respir Med. 2012; 106(8):1139–47. [PubMed: 22608352]
151. Agius AM, Smallman LA, Pahor AL. Age, smoking and nasal ciliary beat frequency. Clin Otolaryngol Allied Sci. 1998; 23(3):227–30. [PubMed: 9669071]
152. Wyatt TA, et al. Acetaldehyde-stimulated PKC activity in airway epithelial cells treated with smoke extract from normal and smokeless cigarettes. Proc Soc Exp Biol Med. 2000; 225(1):91–7. [PubMed: 10998203]
153. Tamashiro E, et al. Cigarette smoke exposure impairs respiratory epithelial ciliogenesis. Am J Rhinol Allergy. 2009; 23(2):117–22. [PubMed: 19401033]
154. Lam HC, et al. Histone deacetylase 6-mediated selective autophagy regulates COPD-associated cilia dysfunction. J Clin Invest. 2013; 123(12):5212–30. [PubMed: 24200693]

155. Verra F, et al. Ciliary abnormalities in bronchial epithelium of smokers, ex-smokers, and nonsmokers. Am J Respir Crit Care Med. 1995; 151(3 Pt 1):630–4. [PubMed: 7881648]
156. Auerbach O, Hammond EC, Garfinkel L. Changes in bronchial epithelium in relation to cigarette smoking, 1955–1960 vs. 1970–1977. N Engl J Med. 1979; 300(8):381–5. [PubMed: 759914]
157. Leopold PL, et al. Smoking is associated with shortened airway cilia. PLoS One. 2009; 4(12):e8157. [PubMed: 20016779]
158. Lungarella G, Fonzi L, Ermini G. Abnormalities of bronchial cilia in patients with chronic bronchitis. An ultrastructural and quantitative analysis. Lung. 1983; 161(3):147–56. [PubMed: 6876878]
159. Sisson JH, et al. Smoke and viral infection cause cilia loss detectable by bronchoalveolar lavage cytology and dynein ELISA. Am J Respir Crit Care Med. 1994; 149(1):205–13. [PubMed: 8111584]
160. Kensler CJ, Battista SP. Components of Cigarette Smoke with Ciliary-Depressant Activity. Their Selective Removal by Filters Containing Activated Charcoal Granules. N Engl J Med. 1963; 269:1161–6. [PubMed: 14061124]
161. Cantin AM, et al. Cystic fibrosis transmembrane conductance regulator function is suppressed in cigarette smokers. Am J Respir Crit Care Med. 2006; 173(10):1139–44. [PubMed: 16497995]
162. Xu X, et al. Cigarette smoke exposure reveals a novel role for the MEK/ERK1/2 MAPK pathway in regulation of CFTR. Biochim Biophys Acta. 2015
163. Rasmussen JE, et al. Cigarette smoke-induced Ca2+ release leads to cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction. J Biol Chem. 2014; 289(11):7671–81. [PubMed: 24448802]
164. Gelman MS, Kannegaard ES, Kopito RR. A principal role for the proteasome in endoplasmic reticulum-associated degradation of misfolded intracellular cystic fibrosis transmembrane conductance regulator. J Biol Chem. 2002; 277(14):11709–14. [PubMed: 11812794]
165. Johnston JA, Ward CL, Kopito RR. Aggresomes: a cellular response to misfolded proteins. J Cell Biol. 1998; 143(7):1883–98. [PubMed: 9864362]
166. Sharma M, et al. Misfolding diverts CFTR from recycling to degradation: quality control at early endosomes. J Cell Biol. 2004; 164(6):923–33. [PubMed: 15007060]
167. Okiyoneda T, et al. Peripheral protein quality control removes unfolded CFTR from the plasma membrane. Science. 2010; 329(5993):805–10. [PubMed: 20595578]
168. Younger JM, et al. Sequential quality-control checkpoints triage misfolded cystic fibrosis transmembrane conductance regulator. Cell. 2006; 126(3):571–82. [PubMed: 16901789]
169. Stevens JF, Maier CS. Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. Mol Nutr Food Res. 2008; 52(1):7–25. [PubMed: 18203133]
170. Wang HT, et al. Mutagenicity and sequence specificity of acrolein-DNA adducts. Chem Res Toxicol. 2009; 22(3):511–7. [PubMed: 19146376]
171. Phillips DH. Smoking-related DNA and protein adducts in human tissues. Carcinogenesis. 2002; 23(12):1979–2004. [PubMed: 12507921]
172. Raju SV, et al. Cigarette smoke induces systemic defects in cystic fibrosis transmembrane conductance regulator function. Am J Respir Crit Care Med. 2013; 188(11):1321–30. [PubMed: 24040746]
173. Moran AR, et al. Aqueous cigarette smoke extract induces a voltage-dependent inhibition of CFTR expressed in Xenopus oocytes. Am J Physiol Lung Cell Mol Physiol. 2014; 306(3):L284–91. [PubMed: 24318115]
174. Ballard ST, et al. CFTR involvement in chloride, bicarbonate, and liquid secretion by airway submucosal glands. Am J Physiol. 1999; 277(4 Pt 1):L694–9. [PubMed: 10516209]
175. Hug MJ, Tamada T, Bridges RJ. CFTR and bicarbonate secretion by [correction of to] epithelial cells. News Physiol Sci. 2003; 18:38–42. [PubMed: 12531931]
176. Pryor WA, Prier DG, Church DF. Electron-spin resonance study of mainstream and sidestream cigarette smoke: nature of the free radicals in gas-phase smoke and in cigarette tar. Environ Health Perspect. 1983; 47:345–55. [PubMed: 6297881]

177. Cantin AM, et al. Oxidant stress suppresses CFTR expression. Am J Physiol Cell Physiol. 2006; 290(1):C262–70. [PubMed: 16162662]

178. Hassan F, et al. MiR-101 and miR-144 regulate the expression of the CFTR chloride channel in the lung. PLoS One. 2012; 7(11):e50837. [PubMed: 23226399]

179. Hassan F, et al. Accumulation of metals in GOLD4 COPD lungs is associated with decreased CFTR levels. Respir Res. 2014; 15:69. [PubMed: 24957904]

180. Sloane PA, et al. A pharmacologic approach to acquired cystic fibrosis transmembrane conductance regulator dysfunction in smoking related lung disease. PLoS One. 2012; 7(6):e39809. [PubMed: 22768130]

181. Astrand AB, et al. Linking increased airway hydration, ciliary beating, and mucociliary clearance through ENaC inhibition. Am J Physiol Lung Cell Mol Physiol. 2015; 308(1):L22–32. [PubMed: 25361567]

182. Tyrrell J, et al. Roflumilast Combined with Adenosine Increases Mucosal Hydration in Human Airway Epithelial Cultures after Cigarette Smoke Exposure. Am J Physiol Lung Cell Mol Physiol. 2015 p. ajplung 00395 2014.

183. Andersen I, et al. Nasal clearance in monozygotic twins. Am Rev Respir Dis. 1974; 110(3):301–5. [PubMed: 4472209]

184. Stanley PJ, et al. Effect of cigarette smoking on nasal mucociliary clearance and ciliary beat frequency. Thorax. 1986; 41(7):519–23. [PubMed: 3787531]

185. Rubin BK, et al. Respiratory mucus from asymptomatic smokers is better hydrated and more easily cleared by mucociliary action. Am Rev Respir Dis. 1992; 145(3):545–7. [PubMed: 1546833]

186. Hill DB, et al. A biophysical basis for mucus solids concentration as a candidate biomarker for airways disease. PLoS One. 2014; 9(2):e87681. [PubMed: 24558372]

187. Baltimore RS, Christie CD, Smith GJ. Immunohistopathologic localization of Pseudomonas aeruginosa in lungs from patients with cystic fibrosis. Implications for the pathogenesis of progressive lung deterioration. Am Rev Respir Dis. 1989; 140(6):1650–61. [PubMed: 2513765]

188. Matsui H, et al. A physical linkage between cystic fibrosis airway surface dehydration and Pseudomonas aeruginosa biofilms. Proc Natl Acad Sci U S A. 2006; 103(48):18131–6. [PubMed: 17116883]

189. Coles SJ, Levine LR, Reid L. Hypersecretion of mucus glycoproteins in rat airways induced by tobacco smoke. Am J Pathol. 1979; 94(3):459–72. [PubMed: 426035]

190. Lamb D, Reid L. Goblet cell increase in rat bronchial epithelium after exposure to cigarette and cigar tobacco smoke. Br Med J. 1969; 1(5635):33–5. [PubMed: 5761894]

191. Basbaum C, et al. Control of mucin transcription by diverse injury-induced signaling pathways. Am J Respir Crit Care Med. 1999; 160(5 Pt 2):S44–8. [PubMed: 10556169]

192. Borchers MT, Carty MP, Leikauf GD. Regulation of human airway mucins by acrolein and inflammatory mediators. Am J Physiol. 1999; 276(4 Pt 1):L549–55. [PubMed: 10198352]

193. Borchers MT, Wert SE, Leikauf GD. Acrolein-induced MUC5ac expression in rat airways. Am J Physiol. 1998; 274(4 Pt 1):L573–81. [PubMed: 9575876]

194. Churg A, Cosio M, Wright JL. Mechanisms of cigarette smoke-induced COPD: insights from animal models. Am J Physiol Lung Cell Mol Physiol. 2008; 294(4):L612–31. [PubMed: 18223159]

195. Hughes JR. Effects of abstinence from tobacco: etiology, animal models, epidemiology, and significance: a subjective review. Nicotine Tob Res. 2007; 9(3):329–39. [PubMed: 17365765]

196. Johnson JD, et al. Effects of mainstream and environmental tobacco smoke on the immune system in animals and humans: a review. Crit Rev Toxicol. 1990; 20(5):369–95. [PubMed: 2202327]

197. Liu C, Russell RM, Wang XD. Exposing ferrets to cigarette smoke and a pharmacological dose of beta-carotene supplementation enhance in vitro retinoic acid catabolism in lungs via induction of cytochrome P450 enzymes. J Nutr. 2003; 133(1):173–9. [PubMed: 12514286]

198. Hautamaki RD, et al. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. Science. 1997; 277(5334):2002–4. [PubMed: 9302297]

199. Clarke LL, et al. Relationship of a non-cystic fibrosis transmembrane conductance regulator-mediated chloride conductance to organ-level disease in Cftr(−/−) mice. Proc Natl Acad Sci U S A. 1994; 91(2):479–83. [PubMed: 7507247]
200. Snouwaert JN, et al. An animal model for cystic fibrosis made by gene targeting. Science. 1992; 257(5073):1083–8. [PubMed: 1380723]
201. Majima Y, et al. Mucociliary clearance in chronic sinusitis: related human nasal clearance and in vitro bullfrog palate clearance. Biorheology. 1983; 20(2):251–62. [PubMed: 6603238]
202. Mall MA. Role of cilia, mucus, and airway surface liquid in mucociliary dysfunction: lessons from mouse models. J Aerosol Med Pulm Drug Deliv. 2008; 21(1):13–24. [PubMed: 18518828]
203. Mall M, et al. Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. Nat Med. 2004; 10(5):487–93. [PubMed: 15077107]
204. Donaldson SH, et al. Mucus clearance and lung function in cystic fibrosis with hypertonic saline. N Engl J Med. 2006; 354(3):241–50. [PubMed: 16421365]
205. Elkins MR, et al. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. N Engl J Med. 2006; 354(3):229–40. [PubMed: 16421364]
206. Taube C, et al. Airway response to inhaled hypertonic saline in patients with moderate to severe chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2001; 164(10 Pt 1):1810–5. [PubMed: 11734428]
207. Angle N, et al. Hypertonic saline resuscitation diminishes lung injury by suppressing neutrophil activation after hemorrhagic shock. Shock. 1998; 9(3):164–70. [PubMed: 9525322]
208. Lansdell KA, et al. Regulation of murine cystic fibrosis transmembrane conductance regulator Cl − channels expressed in Chinese hamster ovary cells. J Physiol. 1998; 512 (Pt 3):751–64. [PubMed: 9769419]
209. Gray MA, et al. Anion selectivity and block of the small-conductance chloride channel on pancreatic duct cells. Am J Physiol. 1990; 259(5 Pt 1):C752–61. [PubMed: 1700622]
210. Illek B, et al. Defective function of the cystic fibrosis-causing missense mutation G551D is recovered by genistein. Am J Physiol. 1999; 277(4 Pt 1):C833–9. [PubMed: 10516113]
211. Van Goor F, et al. Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. Proc Natl Acad Sci U S A. 2009; 106(44):18825–30. [PubMed: 19846789]
212. Dalemans W, et al. Altered chloride ion channel kinetics associated with the delta F508 cystic fibrosis mutation. Nature. 1991; 354(6354):526–8. [PubMed: 1722027]
213. Van Goor F, et al. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. Proc Natl Acad Sci U S A. 2011; 108(46):18843–8. [PubMed: 21976485]
214. Cholon DM, et al. Potentiator ivacaftor abrogates pharmacological correction of DeltaF508 CFTR in cystic fibrosis. Sci Transl Med. 2014; 6(246):246ra96.
215. Lambert JA, et al. Cystic fibrosis transmembrane conductance regulator activation by roflumilast contributes to therapeutic benefit in chronic bronchitis. Am J Respir Cell Mol Biol. 2013; 50(3): 549–58. [PubMed: 24106801]
216. Kew KM, Dias S, Cates CJ. Long-acting inhaled therapy (beta-agonists, anticholinergics and steroids) for COPD: a network meta-analysis. Cochrane Database Syst Rev. 2014; 3:CD010844. [PubMed: 24671923]
217. Boucher RC, et al. Chloride secretory response of cystic fibrosis human airway epithelia. Preservation of calcium but not protein kinase C- and A-dependent mechanisms. J Clin Invest. 1989; 84(5):1424–31. [PubMed: 2478586]
218. O'Riordan TG, et al. Acute hyperkalemia associated with inhalation of a potent ENaC antagonist: Phase 1 trial of GS-9411. J Aerosol Med Pulm Drug Deliv. 2014; 27(3):200–8. [PubMed: 23905576]
219. Kleyman TR, Cragoe EJ Jr. The mechanism of action of amiloride. Semin Nephrol. 1988; 8(3): 242–8. [PubMed: 2849182]
220. Grasemann H, Ratjen F. Emerging therapies for cystic fibrosis lung disease. Expert Opin Emerg Drugs. 2010; 15(4):653–9. [PubMed: 20812885]

221. Chiu TF, et al. Rapid life-threatening hyperkalemia after addition of amiloride HCl/hydrochlorothiazide to angiotensin-converting enzyme inhibitor therapy. Ann Emerg Med. 1997; 30(5):612–5. [PubMed: 9360571]
222. O'Riordan TG, et al. GS-5737, A Novel Epithelial Sodium Channel (ENaC) Inhibitor: Results of a Phase 1 Safety and Pharmacodynamic (PK) Study. Pediatric Pulmonology. 2013; S36:290.
223. Terryah S, et al. A SPLUNC1-Derived Peptide Reduces Lung Disease in SCNN1B Mice. Pediatric Pulmonology. 2014; S38:285.
224. Lee YO, et al. Multiple tobacco product use among adults in the United States: cigarettes, cigars, electronic cigarettes, hookah, smokeless tobacco, and snus. Prev Med. 2014; 62:14–9. [PubMed: 24440684]
225. Benowitz NL, Goniewicz ML. The regulatory challenge of electronic cigarettes. JAMA. 2013; 310(7):685–6. [PubMed: 23856948]
226. McAuley TR, et al. Comparison of the effects of e-cigarette vapor and cigarette smoke on indoor air quality. Inhal Toxicol. 2012; 24(12):850–7. [PubMed: 23033998]
227. Wu Q, et al. Electronic cigarette liquid increases inflammation and virus infection in primary human airway epithelial cells. PLoS One. 2014; 9(9):e108342. [PubMed: 25244293]
228. Chen Y, et al. Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. J Biol Chem. 2003; 278(19):17036–43. [PubMed: 12624114]
229. Jensen RP, et al. Hidden formaldehyde in e-cigarette aerosols. N Engl J Med. 2015; 372(4):392–4. [PubMed: 25607446]
230. Brown JE, et al. Candy flavorings in tobacco. N Engl J Med. 2014; 370(23):2250–2. [PubMed: 24805984]
231. Carpenter CM, et al. New cigarette brands with flavors that appeal to youth: tobacco marketing strategies. Health Aff (Millwood). 2005; 24(6):1601–10. [PubMed: 16284034]
232. Regan AK, Dube SR, Arrazola R. Smokeless and flavored tobacco products in the U.S.: 2009 Styles survey results. Am J Prev Med. 2012; 42(1):29–36. [PubMed: 22176843]
233. Willis DN, et al. Menthol attenuates respiratory irritation responses to multiple cigarette smoke irritants. FASEB J. 2011; 25(12):4434–44. [PubMed: 21903934]
234. Uhl GR, et al. Menthol preference among smokers: association with TRPA1 variants. Nicotine Tob Res. 2011; 13(12):1311–5. [PubMed: 21719896]
235. Shibamoto T. Diacetyl: occurrence, analysis, and toxicity. J Agric Food Chem. 2014; 62(18): 4048–53. [PubMed: 24738917]
236. Kreiss K, et al. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. N Engl J Med. 2002; 347(5):330–8. [PubMed: 12151470]
237. McMillen R, Maduka J, Winickoff J. Use of emerging tobacco products in the United States. J Environ Public Health. 2012; 2012:989474. [PubMed: 22654922]
238. Rath JM, et al. Patterns of tobacco use and dual use in US young adults: the missing link between youth prevention and adult cessation. J Environ Public Health. 2012; 2012:679134. [PubMed: 22666279]
239. Terchek JJ, et al. Measuring cigar use in adolescents: inclusion of a brand-specific item. Nicotine Tob Res. 2009; 11(7):842–6. [PubMed: 19474182]
240. Hentschel J, et al. Dynamics of soluble and cellular inflammatory markers in nasal lavage obtained from cystic fibrosis patients during intravenous antibiotic treatment. BMC Pulm Med. 2014; 14:82. [PubMed: 24885494]
241. Tsoumakidou M, Tzanakis N, Siafakas NM. Induced sputum in the investigation of airway inflammation of COPD. Respir Med. 2003; 97(8):863–71. [PubMed: 12924512]

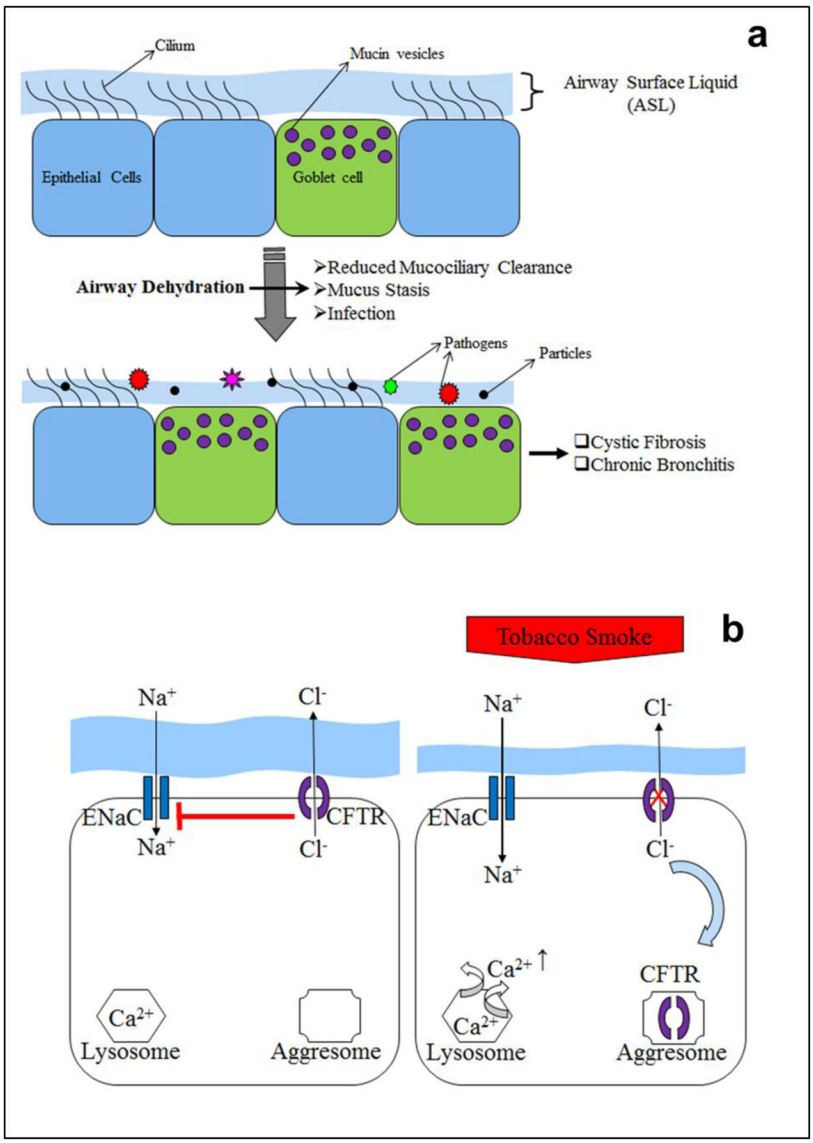

Figure 1. Overview of airway dehydration in the pathogenesis of chronic bronchitis

A, The compromised airway hydration status leads to defective mucociliary clearance leading to goblet cell hyperplasia, mucus stasis and infection with subsequent manifestations of diseases like cystic fibrosis and chronic bronchitis. **B,** Exposure to tobacco smoke leads to relocation of surface CFTR to aggresome and release of Ca^{2+} from the lysosome resulting in augmented absorption of Na^{+} through ENaC and ASL height decrease.

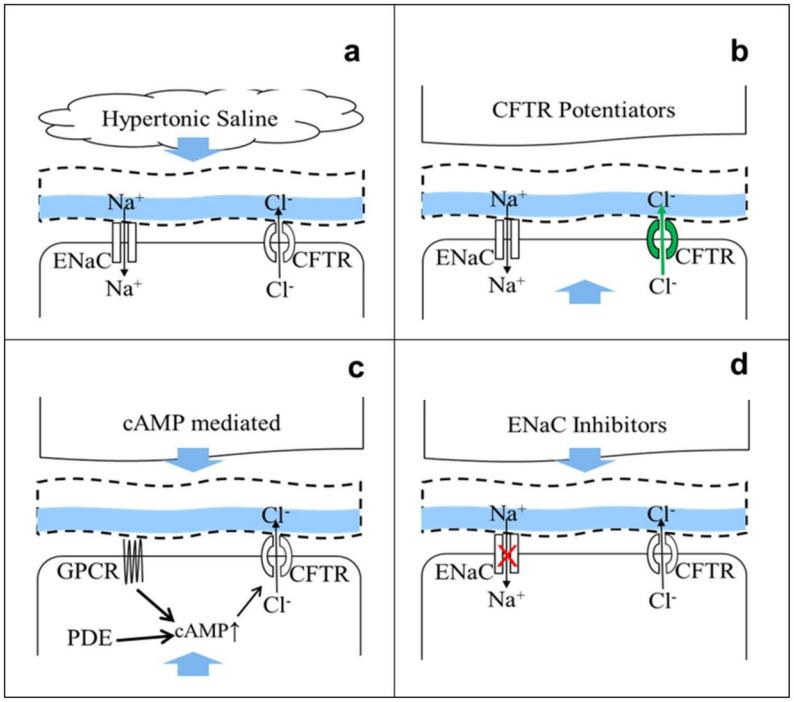

Figure 2. Strategies for treating airway dehydration in CB/COPD

Schema showing a typical airway epithelial cells and different therapies for ASL rehydration. Therapies may be inhaled or taken orally, as indicated by blue arrows. **a**, Inhaled hypertonic saline directly rehydrates the airway surface, which helps facilitate mucociliary clearance. **b**, CFTR corrector or potentiator drugs enhances CFTR channel activity and can be taken orally. **c**, cAMP mediated CFTR enhancement is achieved either through phosphodiesterase (PDE) inhibition or β2-adrenergic receptor (GPCR) activation. **d**, Inhaled ENaC inhibitors reduce Na^+ absorption and would enhance the driving force for CFTR-mediated Cl^- secretion.