

The function of mucins in the COPD airway

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Abstract Mucus clearance is the first defense of a normal airway against airborne pathogens and pollutants. However, mucus hypersecretion—an important feature of chronic obstructive pulmonary disease (COPD), especially the chronic bronchitis phenotype—contributes to disease pathology and mortality. Prescriptions of some mucoactive medications, e.g. *N*-acetylcysteine and carbocysteine, have proved beneficial for COPD management. Mucins are large-molecular-weight glycoproteins which constitute the major solid components of mucus, giving mucus its viscous and elastic properties and enabling its defensive function. Most-expressed in the airway are three membrane-tethered mucins (MUC1, MUC4, and MUC16) and three gel-forming secreted mucins (MUC2, MUC5AC, and MUC5B). Although over-expression of all these mucins has been observed or postulated in COPD lungs, none has been specifically evaluated as affecting COPD. Evidence regarding immunosuppressive, bacterial-adhesive, anti-inflammatory, and tumorigenic effects of MUC1 in other disease conditions suggests MUC1 may contribute to immune suppression, airway remodeling, mucus obstruction, bacterial colonization, and disruption of epithelium integrity in COPD. Regulation of mucin synthesis and secretion is increasingly well understood. Mucin over-expression in COPD is probably caused by a combination of microbial products, airborne pollutants, and mediators of inflammation. Further studies are needed to determine the individual function and regulatory signaling of each mucin in COPD airways, with the objectives of better understanding the disease mechanism and of developing novel therapeutics for COPD treatment.

Keywords COPD · Mucus · Mucins · MUC1 · MUC5AC · MUC5B

Introduction

The global initiative for chronic obstructive lung disease (GOLD, 2011) defines COPD as a complex of diseases characterized by persistent airflow limitation, which is usually progressive and associated with an enhanced chronic inflammatory response of the airways and lungs to noxious particles or gases. COPD is a leading cause of morbidity and mortality worldwide, causing a substantial and increasing economic and social burden. The COPD diagnostic criteria are progressive dyspnea, chronic cough, chronic sputum production, history of exposure to risk factors (including tobacco smoke, smoke from home cooking and heating fuels, occupational dusts and chemicals), and a post-bronchodilator FEV₁/FVC <0.70. Exacerbations of COPD airway disease may be caused by viral or bacterial infections, or by exposure to air pollutants. Bronchoalveolar lavage fluid (BALF) and induced sputum have increased neutrophil and macrophage levels. The submucosa of patients' small airways are chronically inflamed, with increased macrophages and T lymphocytes [1]. Depending on the cellular inflammatory profile, there are increased levels of IL-1 β , IL-6, IL-8, tumor necrosis factor- α (TNF- α), and monocyte chemoattractant protein-1 in induced sputum or BALF of patients with stable COPD [2]. Neutrophils and macrophages release proteases that contribute to inflammation and overwhelm anti-protease defenses. Reactive oxygen species (ROS) generated by cigarette smoke or by inflammatory cells may cause airway and parenchymal injury. Together, these inflammation mediators and products injure the airway, activating programs for structural changes to the airway wall (so-called airway remodeling) leading to airway narrowness and mucus obstruction. Structural changes to COPD airways include:

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mucus metaplasia, bronchiolar smooth-muscle hypertrophy, mural edema, peribronchiolar fibrosis, and excess mucus production in airways less than 400 μm in diameter.

Of all pathological changes associated with COPD airway disease, mucus obstruction is the most prominent cause of airflow limitation and disease mortality, particularly for elderly patients and those prone to chest infections [3, 4]. Some mucoactive medications have been proved beneficial for COPD management [5, 6•]. In this paper we review progress in understanding airway mucus and mucin biology, with emphasis on the less well-understood function of mucins in COPD airway disease.

Mucus and mucin biology in the airway

The airway epithelium is normally coated with a thin layer of airway surface liquid (ASL) and of gel-like mucus, which lies on top of the ASL. In large airways (>2 mm in luminal diameter) mucus is produced by goblet cells and by submucosal glands, whereas in small airways the only source of mucus is the goblet cell [7]. The general functions of mucus are protection, lubrication, and transport. Under healthy conditions, mucus maintains hydration in the airways and protects against inhaled pathogens, toxic gases, and other airborne pollutants via mucociliary clearance. Mucus also contributes to innate immune response in the lungs by transporting a variety of antioxidants, antiproteases, antimicrobial substances (e.g. defensins, lysozyme, and Ig A), immunomodulatory molecules (e.g. secretoglobins and cytokines), and protective molecules (e.g. trefoil proteins and heregulin) [8–10]. However, abnormal mucus production and clearance can cause airway obstruction and contribute to respiratory disease pathology [11, 12].

Mucus is approximately 97 % water and 3 % solids. Besides salts, lipids, polypeptides, cells, and cell debris, mucins are the chief constituents of the solid part of mucus [13]. Mucins are defined by their serine and/or threonine and/or proline-rich tandem repeat (TR) domains. They are very high-molecular-weight glycoproteins (~3 MDa) [12], 50–90 % carbohydrate by mass and with dozens to several hundreds of O-glycosylation sites per molecule, the result of the large number of TRs in the protein backbone. On the basis of their cellular location, mucins are classified into two groups: membrane-tethered or secreted mucins. A subset of secreted mucins, the gel-forming mucins, have large, heavily O-glycosylated apoprotein cores, and N and C-terminal, cysteine-rich, von-Willebrand factor-like domains that participate in disulfide bond-mediated oligomerization. Once secreted, gel-forming mucins form very large (1 to >10 MDa) viscoelastic macromolecular complexes, contributing to the elastic and viscous properties of mucus [14]. Membrane-tethered mucins may behave as receptors and participate in

cell adhesion, glycocalyx generation, and airway defense; they may also be secreted into the mucus layer as the result of shedding or synthesis of splice variants lacking transmembrane domains [15, 16].

More than 22 mucin genes have been identified in the human and murine genome [12, 17]. All but MUC2, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC9, and MUC19 are membrane-tethered mucins [15, 16]. At least 14 mucin genes (MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC7, MUC8, MUC11, MUC13, MUC15, MUC16, MUC19, MUC20, and MUC21) are present at protein or mRNA levels in normal airways (Table 1) [12, 18–21]. MUC3 and MUC6 are expressed in well-differentiated NHBE cells cultured *in vitro*; their expression in normal bronchi is controversial [18, 21–23]. In normal sputum secreted mucins, mainly MUC5AC and MUC5B, comprise approximately 90 % of mucin content, the remaining 10 % mainly consisting of the three membrane-tethered mucins MUC1, MUC4, and MUC16 [24, 25].

In airway tissues from healthy individuals, mucins are produced by ciliated, basal, goblet, and submucosal-gland secretory cells in the upper and lower airway epithelia [12]. MUC1, MUC4, and MUC16 are the best-characterized of the membrane-associated mucins, and are mostly found at the apical surfaces of ciliated cells and Clara cells [21, 25]. MUC1 has also been detected in alveolar type-II epithelial cells [26, 27]. MUC2 mRNA and protein are weakly expressed in the surface epithelium, in some goblet cells, within submucosal glands, in mucus cells, and in some serous cells; because of either its low expression or its insolubility, it is barely detectable in sputum [14, 21]. MUC5AC is usually expressed by goblet cells, with some expression in the submucosal glands. The mucus cells of submucosal glands mainly produce MUC5B, with some expression of MUC8 and MUC19 [28, 29]. MUC6 is local to the bronchial and bronchiolar epithelium [23]; MUC7 is local to the serous cells of submucosal glands [30].

Table 1 Mucins expressed in the airway [9]

Airway mucin	
Membrane tethered	Gel-forming
MUC1	MUC2
MUC3A/B	MUC5AC
MUC4	MUC5B
MUC11	MUC6
MUC13	MUC7
MUC15	MUC8
MUC16	
MUC20	MUC19
MUC21	

Regulation of airway mucus secretion

Mucin macromolecules in secretory cells (i.e. goblet cells) form secretory granules or vesicles and undergo a multi-step process of exocytic secretion, via intercompartmental vesicular trafficking. They progress from the ER, where they are synthesized, to the cis-Golgi, where they undergo core glycosylation; then through the Golgi, where they undergo further post-translational processing and sorting; and finally from the trans-Golgi to the cell surface. The secretory granules move from the trans-Golgi region to the cell periphery along microtubules and then along actin filaments. Only a few of the proteins that regulate the secretory process in airway goblet cells have been identified. Rab GTPases regulate the transition from microtubules to actin filaments. SNARE proteins, including vesicular-associated membrane protein 8 (VAMP8), regulate mucin granule exocytosis [31]. Deletion of Munc 13-2, a priming protein for mucin granule exocytosis that is activated by diacylglycerol during agonist stimulation, affects tonic mucin secretion in mouse airways [32]. Synaptotagmin 2, a low-affinity Ca^{2+} sensor, mediates acute agonist-stimulated mucin secretion in mouse airway goblet cells [33]. Myristoylated, alanine-rich C-kinase substrate (MARCKS) protein regulates mucin granule release via linking of mucin granules to the contractile cytoskeleton; the activity of MARCKS is regulated by protein kinase C and protein kinase G [34]. An inhibitory MARCKS peptide has been shown to reduce airway mucin secretion in a mouse model of asthma [35]. The identities and precise functions of many other molecular components of the goblet-cell secretory pathway are yet to be defined.

The secretion of mucus in the surface epithelium is controlled by a range of neurohumoral mediators, including tobacco smoke, SO_2 , NH_3 , leukotrienes C_4 and D_4 , neutrophil elastase, histamine, platelet-activating factor (PAF), substance P, reactive oxygen species, ATP, TNF- α , NO, and extremes of pH [36]. Of these mediators ATP, possibly released into the airway lumen via mechanical stretching, is believed to be the most potent secretagogue of goblet cells. ATP and its analogue UTP cause rapid mucin-granule discharge by interacting directly with P2Y_2 purinoceptors on airway goblet cells. They also stimulate Cl^- and fluid secretion [37, 38], ciliary activity [39], and, importantly, mucociliary clearance [40].

Airway gland secretion is regulated by neural actions [36]. In humans, the submucosal glands are richly innervated by the parasympathetic, sympathetic, and nonadrenergic, noncholinergic (NANC) nervous systems [41]. Muscarinic, α -adrenergic, β -adrenergic, and peptidergic receptors have all been observed on airway gland cells. Cholinergic agonists are the most potent releasers of mucus from the airway glands of humans and other mammals. In cats, α -adrenergic agents and cholinergic agents stimulate approximately equal

volume flow. β -Adrenergic agents induce lower volumes of secretions than either cholinergic or α -adrenergic agents.

The intracellular second messengers of mucus secretion may involve inositol 1,4,5-trisphosphate (IP_3), DAG, and Ca^{2+} . The PLC-specific inhibitor U73122 and loading of cells with the calcium chelator BAPTA inhibit agonist-stimulated secretion [42, 43], whereas secretion is stimulated by:

1. the DAG-mimic PMA;
2. increasing intracellular Ca^{2+} with the ionophore ionomycin;
3. permeabilizing the cells into an extracellular Ca^{2+} EGTA-based buffer with streptolysin-O; and
4. applying IP_3 to permeabilized cells [44].

Purinergic activation led to intracellular Ca^{2+} mobilization in human goblet cells, with a classic peak-and-plateau waveform. This mobilization was inhibited by U73122 or by BAPTA loading [45]. Ca^{2+} may regulate mucin granule exocytosis by activating actin filament disruption and remodeling (scinderin), priming (Munc13), and exocytic fusion (synaptotagmin) [44]. PLC is believed to be important in regulating mucin secretion. It is probable that multiple inputs (purinergic agonists and inflammatory mediators) converge via PLC to generate two cellular messengers, DAG and Ca^{2+} , which then lead to exocytosis.

Mucus hypersecretion in COPD airway

Mucus hypersecretion is a prominent feature of COPD, manifested as increased sputum. Excess mucus has been associated with several pathological features of COPD:

- increased frequency and duration of infection;
- reduced lung function; and
- increased morbidity and mortality [46].

In the small airways of healthy individuals goblet cells are absent or sparse [1, 11] whereas in COPD patients elevated numbers of goblet cells (goblet cell metaplasia (GCM)) are found, with excessive mucus production [47]. The components of sputum are derived mainly from the central airways, with some contribution from the peripheral airways; however, over-production of mucus in the peripheral airways is the main cause of airflow obstruction in patients with COPD [48].

In COPD, airway mucus hypersecretion is often associated with changes to both location and profile of mucin expression in epithelium and submucosal glands. MUC1 is the first cloned and best-understood membrane-tethered mucin. Our unpublished data indicate that its extracellular part may be released into the airway lumen, because the level was increased in the BALF of mice with cigarette-smoke-induced COPD. Ishikawa et al. recently reported that the sputum level

of KL-6 (MUC1) was significantly higher for COPD patients than for non-smokers, smokers, and prolonged coughers with normal lung function. The increases positively correlated with smoking history, aging, sputum macrophages, and eosinophils, and MUC1 was more prominently expressed in the bronchiolar and/or alveolar epithelium in COPD than in control lungs [49, 50]. The predominant gel-forming mucins in the airways, MUC5AC and MUC5B, are present at lower levels in mucus from non-diseased airways [11]; in COPD airways their expression is much increased and their expression patterns altered compared with those of smokers and normal subjects. Studies analyzing the mucin components of airway mucus in COPD patients have revealed that MUC5AC and MUC5B are the major mucins in sputum, with MUC5B being the predominant form [51, 52]. Caramori et al. reported that in peripheral COPD airways COPD is associated with increased expression of MUC5B in the bronchiolar lumen and with increased MUC5AC in the bronchiolar epithelium [23]. In COPD patients, expression of MUC5AC is increased not only in surface epithelium but also in submucosal glands, and the increases are correlated with smoking history and inversely correlated with FEV1 (% of predicted) [53]. MUC5B, normally expressed in the submucosal glands of the bronchials, was also present in the surface epithelium [52]. The expression and location of MUC2, MUC4, and MUC6 in peripheral airways were not affected by smoking history or by COPD [23, 51, 54]. Other mucins have not been evaluated, but MUC7 and MUC8 are postulated to change during COPD. This assumption is made because cigarette smoke extract and LPS exposure induced MUC7 in human airway epithelial cells *in vitro* and in mouse cells *in vivo* [55], and because MUC8 was induced in airway epithelial cells by high-mobility-group box-1 protein (HMGB1), a recently-identified pro-inflammatory mediator in a variety of inflammatory diseases [56].

In addition to the altered expression profile and increased synthesis of mucins, COPD airway mucus is usually dehydrated and more viscous, impeding mucus clearance. Recent studies suggest that the dehydrated mucus is probably the result of defects in activity or expression of CFTR, aquaporins, and other ion channels, leading to reduced ion and water secretion. CFTR functions both as a Cl^- channel and as an important anti-inflammatory molecule [57]. Moreover, CFTR can mediate secretion of bicarbonate (HCO_3^-), which is believed to enhance mucin swelling and hydration by reducing Ca^{2+} cross-linking in mucins, thereby reducing mucus viscosity and probably increasing its transportability [58]. Consequently, mutations in the CFTR gene are associated with mucus dehydration and airway inflammation, as seen in cystic fibrosis (CF). Cigarette smoke exposure rapidly impairs CFTR function by internalizing CFTR, leading to ASL dehydration. This promotes mucus stasis and failure of mucus clearance,

leaving smokers at risk of developing chronic bronchitis (CB) [59]. AQP₅ may affect mucus secretion by regulating fluid and mucin secretion [60]. Wang and associates [61] showed that airway expression of AQP₅ was reduced in COPD patients compared with control subjects. Attenuated expression of AQP₅ was related to severity of airflow obstruction and negatively correlated with expression of MUC5AC in the airway epithelium and of mucins in the submucosal glands. Chen et al. [62] found that the AQP₅ gene could negatively regulate expression of the MUC5AC gene and proteins in the human airway submucosal gland-cell line SPC-A1.

Function of mucins in COPD airway

The general functions of mucins

Normally mucus floats like a raft above the cilia, capturing foreign bodies including microbes, particles, inflammatory cells, and cell debris by means of its gel-like structure and adhesive properties, and transporting them to locations where they can be expelled from the body via ciliary beating. The mucus layer may also provide a physical barrier to the epithelium against microorganisms and insoluble material, thereby maintaining the local molecular environment's hydration, ionic composition and concentration, and accessibility to macromolecules. The best-known function of mucus is this first innate defense of the respiratory tract [63]. The function of mucus is believed to be primarily conferred by mucins. In addition to defending the respiratory tract, functions of those mucins that have been described include [15]: capturing, holding, and releasing biologically-active molecules; signal transduction; regulation of inflammation and immune responses; and regulation of differentiation and proliferation.

Mucins have been shown to capture, hold, and release biologically active molecules, including cytokines, growth factor, and trefoil factors (TFFs). [15, 64]. Via these molecules, mucins may participate in regulation of inflammation and immune responses, and regulation of the repair of the injured airway. Besides capturing inflammatory cells, mucins have been found to capture interleukins, including IL-1, IL-4, IL-6, and IL-7, by means of interactions with the specific lectin moieties on these molecules [15, 64]. Thus, mucins may enable inflammatory mediators and effectors to interact with pathogens, enabling resolution of inflammation. TFFs are expressed by most mucin-producing epithelia, including those of the respiratory tract. They bind to mucins, interact with them [65, 66] and regulating mucus viscosity [67], and via these actions may enhance the protective capability of the mucosal defense barrier. Studies of trefoil peptides in gastrointestinal epithelial cells found TFFs can enhance cell migration *in vitro*

and promote epithelial restitution and mucosal repair in vivo [68, 69]. In a murine asthma model it was discovered that trans-differentiating Clara cells express TFF1, which is stored in a subset of secretory granules [70]. Royce et al. reported that TFF2 regulates airway remodeling in animal models of asthma: TFF2-deficient mice developed asthma and had increased goblet-cell hyperplasia and sub-epithelial collagen layer thickness [71]. Lung transcript profiling of mice identified TFF2 as one of the candidate genes determining lung function [72]. Oertell and colleagues revealed that human recombinant TFF2 and TFF3 both stimulate migration of human airway epithelial cells in chemotactic and two-dimensional wound repair assays, either alone or in concert with EGF [73]. TFF3 was shown to promote airway epithelial ciliated cell differentiation, and induced expression of TFF3 was associated with differentiation of in-vivo humanized tracheal xenograft and in-vitro air-liquid-interface culture models. Furthermore, exogenous TFF3 promoted differentiation of ciliated cells in an EGF-receptor-dependent manner [74]. In a recent study, Wiede and associates found TFF3, but not TFF1 and TFF2, in airway mucosa and in the sputum of subjects with chronic bronchitis [75]. Reduced lung TFF3 mRNA expression was found in rats with COPD induced by passive smoking and LPS [76]. The function of TFFs in normal airways and in the repair of injured COPD epithelium is unknown.

Descriptions of mucins' functions in mediating signal transduction and in regulating differentiation and proliferation are mostly derived from studies of cancerous cells, with some already proved to apply to individual mucins in the airway epithelium. Below we summarize the known functions of the best-characterized airway mucins.

Membrane-tethered mucins

Of the membrane-tethered mucins MUCs 1, 4, 11, 15, 16, and 20 which have been identified in the lung, MUC1, MUC4, and MUC16 are the main three produced and released by airway surface-epithelial cells [77]. Understanding of the functions of MUC4 and MUC16 is still limited. MUC4 is broadly expressed in the small intestine, colon, stomach, cervix, and lung [78]. It is an inter-membrane ligand for ErbB2 [79]: binding of MUC4 to ErbB2 may block access of ErbB2 to its soluble ligands, thereby affecting regulation of cell proliferation and growth. In COPD the airway epithelium is chronically exposed to neutrophil elastase, a highly inflammatory protease released by infiltrated neutrophils. Neutrophil elastase has been shown to up-regulate MUC4 mRNA and protein expression in normal human bronchial epithelial cells in vitro [80], suggesting MUC4 may affect lung inflammation. MUC16 has been mostly studied in ovarian cancer cells, and is also expressed in normal airway epithelium and submucosal gland cells [19]. The MUC16 (CA-125) blood level of COPD patients

is significantly higher than that of control subjects, and is correlated with systolic pulmonary artery pressure [81]. The function of MUC16 in the airway is unknown.

MUC1 is the first cloned and best-understood mucin. MUC1 is expressed on the surface of most epithelial cells and some hematopoietic cells [82, 83]. It is a type I transmembrane protein with three domains: a highly glycosylated large extracellular (EC) domain made up of 25 to 125 twenty-amino-acid tandem repeats, a hydrophobic transmembrane (TM) domain, and a cytoplasmic tail (CT) [82]. The glycosylated tandem repeat of MUC1 is an extended rod-like structure that can extend 200–500 nm above the cell surface. Both anti-adhesive and adhesive properties of MUC1 have been proposed on the basis of this large, extended conformation of the EC domain and the sialyl Lewis^x and sialyl Lewis^a carbohydrate found on it. MUC1 may also adhere, with ICAM-1, on endothelial cells and antigen-presenting dendritic cells (APC), facilitating tumor-cell metastasis or promoting T-cell and APC interaction [82]. The CT domain of MUC1 contains functional binding sites for numerous signaling molecules, including c-Src, ErbB family members, GSK3 β , PKC δ , β -catenin, Grb-2, p53, p120 catenin, HSP70, and HSP90, and can be phosphorylated in response to a variety of extracellular stimulations, suggesting an important function of MUC1 CT in signal transduction [25, 84].

It has been suggested MUC1 is an oncoprotein because functions which include:

1. stimulating cell proliferation via beta-catenin, ErbB and ER α -dependent mechanisms;
2. facilitating cell survival via regulation of FOXO3a and p53 [25];
3. facilitating PyV MT-1 and Wnt-1-mediated oncogenesis [85]; and
4. facilitating tumor metastasis by interacting with endothelial intercellular adhesive molecule-1 (ICAM-1) [86].

Interaction with β -catenin and nuclear translocation of MUC1 CT initiates epithelial-to-mesenchymal transition (EMT) of pancreatic cancer cells, resulting in increased invasiveness and metastasis [87]. The secreted form of MUC1 (shed MUC1) seems also to be required for EMT [87, 88].

Investigating the function of MUC1 in the airway, our research group revealed that both hamster Muc1 and human MUC1 can provide a binding site on cells for *Pseudomonas aeruginosa* (PA), via interaction with the flagellin [89–91]. Binding of PA or flagellin to Muc1 induces tyrosine phosphorylation on the CT domain of MUC1 and resulting activation of ERK2 [84]. Later, using Muc1 null mice (Muc1^{-/-}), we found airway challenge from either PA or its major pathogenic protein flagellin caused exaggerated lung inflammation for Muc1^{-/-} mice compared with that for Muc1 wild-type mice (Muc1^{+/+}), indicating that Muc1

has an anti-inflammatory effect on airway PA infection, probably via interference with TLR5-mediated signaling [92••]. In HEK193 cells simultaneously over-expressing MUC1 and TLR5, we revealed that the mechanism of the inhibitory function of MUC1 in TLR5 signaling involves its association with TLR5, with subsequent inhibition of MyD88 recruitment to TLR5 in response to inflammatory stimulus [93]. In addition, we found neutrophil elastase can up-regulate MUC1 via a signaling pathway involving PKC δ →dual oxidase 1→reactive oxygen species→TNF- α -converting enzyme→TNF- α →TNFR1→ERK2→Sp1 [94, 95]. After PA infection, Muc1 levels in the lungs of Muc1^{+/+} mice steadily increased. The inflammatory responses of TNFR^{-/-} mice were similar to those of Muc1^{-/-} mice. After PA infection, both Muc1^{-/-} mice and TNFR-knockout mice (TNFR^{-/-}) failed to up-regulate Muc1 and failed to resolve PA-induced inflammation [96]. These results indicate that TNF- α production is required for PA-induced Muc1 up-regulation: during airway infection, the feedback loop involving TNF- α (pro-inflammatory) and Muc1 (anti-inflammatory) is crucial for resolution of inflammation. As well as TLR5-mediated inflammation during PA infection or inhalation of flagellin, we found Muc1 also inhibits other TLR signaling (TLR2, 3, 4, 7, and 9) [97], and suppresses non-typeable *Hemophilus influenzae* [98] and respiratory syncytial virus-induced [99] airway inflammation, suggesting that MUC1 and Muc1 greatly affect infection and inflammation of the airway by various pathogenic bacteria and viruses.

It has been shown that MUC1 can be shed by means of proteolytic cleavage, both spontaneously and upon stimulation from, for example, TNF- α and PMA [100]. Proteases responsible for MUC1 shedding include neutrophil elastase [101, 102], TACE [100], MT1 MMP [103], MMP-14 [104], and gamma-secretase [105]. The function of MUC1 shedding is not fully understood. It has been suggested that shed MUC1, and possibly other membrane-tethered mucins, may form a gel in the immediate vicinity of the apical cell surface, probably serving as a protective barrier against invading pathogens and chemicals [24]. How and when membrane glycoproteins are cleaved is largely unknown, and are important questions to address in the context of airway infection and inflammation. Given the ability of the MUC1 ectodomain to bind invading bacteria [89, 91, 104], it is possible that shed MUC1 may serve as a decoy receptor [104], preventing direct interaction of bacteria with the epithelial cell surface and facilitating bacterial clearance during infection. CF mice had mucus accumulation in the small intestine, which was not the result of Muc2, Muc3, and Muc5ac: protein expression of these mucins was similar, whereas there was a moderate increase in Muc1 protein [106]. Making CF mice Muc1 deficient (CF/Muc1^{-/-} mice) prevented mucus accumulation, and these mice survived better on solid food [106]. Shed

Muc1 may therefore form part of mucus and contribute to mucus obstructions. In addition, accumulating evidence indicates shed MUC1 may also suppress immune responses by:

1. affecting leukocyte motility;
2. providing an impenetrable barrier for immune effector cells, thereby preventing an anti-tumor or anti-bacterial response;
3. inactivating immune effector cells via receptor–ligand interactions; and
4. sequestering cytokines (i.e. transforming growth factor (TGF)- α and TGF- β) or other compounds that suppress immune response.

Besides its predominant expression on epithelial cells, MUC1 is also expressed by subsets of naïve and activated T cells (CD4+, CD8+, Th17) [83, 107–110], dendritic cells [110, 111], monocytes [112], and macrophages (unpublished results). In immune cells of Muc1-deficient mice, MUC1 causes defects in T cell development and in natural killer and dendritic cells [82]. Dendritic cells lacking expression of Muc1 were constitutively activated and were more responsive to TLR signaling [113]. MUC1 inhibited development of myeloid-derived suppressor cells (MDSCs) from bone marrow (BM) progenitor cells, via regulation of beta-catenin stability [114].

In conclusion, MUC1 expression in the airway may greatly affect mucociliary clearance, regulation of immune response, and lung inflammation resolution. The function of MUC1 in COPD airway disease is unknown. Because MUC1 expression increases in COPD lungs, we assume over-expression of MUC1 may contribute to COPD lung pathology in the following ways [49]:

1. causing immunosuppression in the systemic and lung immune system via its EC and CT domain;
2. promoting airway remodeling and mucus differentiation, via its action in causing EMT;
3. masking bacteria, helping them escape killing by immune cells;
4. participating in mucus components, or acting as a tether for mucus gel, thus contributing to mucus obstruction [106]; and
5. disrupting intra-epithelial adhesion via c-Src and GSK3 β -dependent interaction with β -catenin [115].

These hypotheses must be proved in future studies.

Secreted mucins

As described above, there are four main secreted mucins in the lung: MUC2, MUC5AC, MUC5B, and MUC19. MUC2 is the main intestinal mucin and is expressed by the goblet cells of the colon and small intestine, although expression may be detected in diseased lungs of humans and rats [116]. Muc2-knockout mice have defects of goblet cell development in the colon, with absence of the protective colonic mucus layer

leading to colonic inflammation and to spontaneous development of colitis [117] and of colorectal cancer [118]. The anti-inflammatory and tumor-suppressive functions of MUC2 in the colon are not fully understood. MUC2 is expressed in the lungs of humans with chronic bronchitis and cystic fibrosis [119], but immunoassay and proteomics analysis of normal and COPD sputum indicate that it may not be a major component of mucins [54, 77, 120]. MUC19, the main salivary glandular mucin, has also been identified in the tracheolarynx [121] and found to be expressed in mouse lungs [122]. The functions of MUC2 and MUC19 in the lung are unknown.

MUC5AC and MUC5B are the main components of gel-forming mucins in normal airways and are believed to contribute to both the defensive barrier function and the rheology of airway mucus. Their biochemistry was recently reviewed by Thornton et al. [123]. MUC5AC has been shown to be the goblet cell mucin [124] and MUC5B the submucosal gland mucin [125]. MUC5AC is widely used as a marker for goblet cell metaplasia [126]. MUC5B is the main mucin type in the apical secretions of air–liquid-interface-cultured NHBE cells and in induced sputum [77]. MUC5AC is up-regulated in a variety of pathologies, including COPD [53, 127]. MUC5AC expression is increased in the bronchial submucosal glands of stable COPD patients [53]. During allergic airway inflammation MUC5AC expression greatly increases, whereas MUC5B expression remains constant [122, 128, 129]. By use of the western blotting assay, Thornton et al. measured levels of MUC2, MUC5AC, and the different glycoforms of MUC5B mucin directly in sputum [14, 51]. Comparing secretions from normal subjects and from individuals with asthma, CF, and COPD, they found more MUC5B mucin, particularly the low-charge form, in CF and COPD sputum with possible infection. Therefore, MUC5AC is believed to be more related to asthma [128, 130, 131] and MUC5B to COPD [52]. A significant association was found between the overall distribution of MUC5AC variable number tandem repeat (VNTR) length and CF lung disease severity; there was also a strong association of the 6.4 kb *HinfI* VNTR fragment with severity of lung disease [127]. It is suggested that MUC5AC may function by facilitating ciliary clearance of mucus, whereas MUC5B may form the basis of a gel to facilitate clearance of specific pathogens or other irritants. Recently, an MUC5B-promoter polymorphism has been associated with pulmonary fibrosis [132]. The comprehensive functions of MUC5AC and MUC5B in COPD are yet to be discovered; it is expected this will happen very soon now that knockout mice are available.

Regulation of mucus hypersecretion related to COPD

Growing evidence indicates that mucus hypersecretion in COPD is probably induced by microbial products, airborne pollutants, and mediators of inflammation. Both virus and

bacterial products have been shown to directly up-regulate mucin expression [133]. Instillation of lipopolysaccharide (LPS) and inhalation of sulfur dioxide, ozone, cigarette smoke, and acrolein can all induce GCM and up-regulate MUC5AC expression in rat or mouse airway epithelia [30, 134–138]. Exposure to these airway irritants leads to GCM and to neutrophilia inflammation in animals. It is noteworthy that this associated inflammation also seems to mediate development of GCM. Instillation of neutrophil elastase stimulates bronchial GCM in hamsters and mice [139, 140]. TH1 and TH2 cytokines shown to regulate mucin gene expression and GCM in vivo include IL-9 and IL-13 (the predominant TH2 cytokines), IL-1 β , tumor necrosis factor- α (TNF- α), TGF- α , IL-6, and IL-17 [141]. Downstream signaling mechanisms of mucin up-regulation and GCM probably involve epidermal growth factor receptor (EGF-R), STAT6, FOXA2, SAM-domain-containing prostate-derived Ets factor (SPDEF), and NF-kappaB [141]. In addition, activation of hypoxia-inducible factor-1 signaling was recently found to contribute to the mechanism of GCM in COPD patients [142]. Notch signaling is critical to negative regulation of Muc5ac expression and GCM in postnatal mouse lungs [143]. Of these known signaling mechanisms for mucin and GCM regulation, EGF-R and Th2 cytokine pathways may have the greatest potential for inhibiting excessive mucus production [144].

Management of COPD airway mucus

Mucus retention in COPD is associated with disease exacerbation [145], accelerated decline in FEV₁ [146], and inflammatory cell infiltration [145]. Mucus retention usually results from a combination of hypersecretion and impaired clearance. Therefore, mucoactive medications used to enhance secretion removal or reduce hypersecretion are important in managing COPD. Mucoactive agents are classified, on the basis of their pharmacological functions, as [36]:

1. expectorants, e.g. guaifenesin, which induces a “vagal gastric” reflex, and hypertonic saline and mannitol, which stimulate epithelial water secretion;
2. ion-transport modifiers, e.g. CPX, genistein, and phenylbutyrate, CFTR activators, which improve the ability of abnormal CFTR to transport Cl⁻, and UTP, which increases non-CFTR Cl⁻ and water transport;
3. mucolytics, including *N*-acetylcysteine (NAC), heparin, and low-molecular-weight dextran, which disrupt mucus or sputum polymers;
4. mucokinetics, e.g. beta-agonists and surfactants, which increase clearance of sputum in coughs; and
5. mucoregulatory agents, including atropine and glucocorticosteroids, which reduce mucin production and/or secretion.

The efficacy of mucoactive agents for COPD management is shown in a well-summarized systemic review by Poole and Black [147]. This review included 26 randomized, placebo-controlled studies recruiting 7,335 participants with COPD. Their data showed that treatment with mucolytics for at least two months significantly reduced the occurrence of exacerbation, and suggested longer-term treatment with mucolytics might achieve even better results.

Of the mucoactive agents, NAC is the most commonly used and cost-effective. Several clinical studies found that administration of NAC to COPD patients reduced acute exacerbation. The exception is the BRONCHUS study [148], in which significant reduction in the occurrence of exacerbation by use of NAC 600 mg day⁻¹ was shown only for patients without concomitant use of inhaled corticosteroids. According to studies by Gerrits [149] and by Zuin et al. [150], the effectiveness of NAC in reducing risk of COPD exacerbation could be dose-dependent. Therefore, a higher dose of NAC (1200 mg day⁻¹) was used in our PANTHEON study trial [151].

Carbocysteine is an analogue of NAC, and is more metabolically stable and lower in cost. Our recent clinical trial revealed that oral administration of 1,500 mg day⁻¹ for one year resulted in significant reduction of the annual occurrence of exacerbation for COPD patients (risk ratio 0.75 (95 % CI 0.62–0.92, $p=0.004$)) [6••], irrespective of disease severity, smoking status, and concomitant use of inhaled corticosteroids. Quality of life, determined by use of St George's Respiratory Questionnaire (SGRQ), also improved significantly. The efficacy of carbocysteine may be attributable to its mucolytic, anti-inflammatory, and anti-oxidation activity. First, carbocysteine improves removal of mucus via activating Cl⁻ conductance in human respiratory cells, correcting the balance between sialo and fuco-mucins, and modulating Cl⁻ and water secretion. Second, carbocysteine is able to stimulate secretion of glutathione (GSH) [152]. A promoter of mucolysis because of its ability to cleave disulfide bonds in mucus, GSH is also one of the most important defense mechanisms against oxidative stress in COPD. Third, it is possible that carbocysteine performs this function via inhibiting mucin production, reducing MUC5AC production and inflammation induced by influenza viruses A and B and by respiratory syncytial virus [133].

Conclusions

Mucus hypersecretion is an important pathological feature of COPD airway disease. Knowledge of mucus and mucin biology, and of the mechanisms of their regulation, has greatly increased. Understanding the function of mucins in COPD airway disease may result in development of novel therapeutic approaches for COPD; the lack of current knowledge in this area justifies more studies.

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Conflict of Interest Wenju Lu declares that she has no conflict of interest.

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