The role of mucin 1 in respiratory diseases

Beatriz Ballester1,2,7, Javier Milara2,3,4,7 and Julio Cortijo2,5,6

Affiliations: 1Division of Pulmonary Sciences and Critical Care Medicine, School of Medicine, University of Colorado, Aurora, CO, USA. 2CIBERES, Health Institute Carlos III, Valencia, Spain. 3Pharmacy Unit, Consorcio Hospital General de Valencia, Valencia, Spain. 4Pharmacology Dept, University Jaume I, Castellon, Spain. 5Research and teaching Unit, Consorcio Hospital General de Valencia, Valencia, Spain. 6Dept of Pharmacology, Faculty of Medicine, University of Valencia, Valencia, Spain. 7Both authors contributed equally to this work.

Correspondence: Beatriz Ballester, Division of Pulmonary Sciences and Critical Care Medicine, School of Medicine, University of Colorado, Aurora, CO 80045, USA. E-mail: beatriz.2.ballester-llobell@cuanschutz.edu

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MUC1 is involved in most lung inflammatory, carcinogenic and fibrotic processes, thus representing a promising druggable target and useful biomarker for several lung diseases https://bit.ly/32gruZO

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ABSTRACT Recent evidence has demonstrated that mucin 1 (MUC1) is involved in many pathological processes that occur in the lung. MUC1 is a transmembrane protein mainly expressed by epithelial and hematopoietic cells. It has a receptor-like structure, which can sense the external environment and activate intracellular signal transduction pathways through its cytoplasmic domain. The extracellular domain of MUC1 can be released to the external environment, thus acting as a decoy barrier to mucosal pathogens, as well as serving as a serum biomarker for the diagnosis and prognosis of several respiratory diseases such as lung cancer and interstitial lung diseases. Furthermore, bioactivated MUC1-cytoplasmic tail (CT) has been shown to act as an anti-inflammatory molecule in several airway infections and mediates the expression of anti-inflammatory genes in lung diseases such as chronic rhinosinusitis, chronic obstructive pulmonary disease and severe asthma. Bioactivated MUC1-CT has also been reported to interact with several effectors linked to cellular transformation, contributing to the progression of respiratory diseases such as lung cancer and pulmonary fibrosis. In this review, we summarise the current knowledge of MUC1 as a promising biomarker and drug target for lung disease.

MUC1 overview
Mucin 1 (MUC1) is a large transmembrane O-glycoprotein (approximately 200 kDa) with a rigid structure that extends up to 200–500 nm from the cell surface of primarily epithelial and hematopoietic cells [1, 2]. As a tethered mucin, MUC1 is composed of dimers of two dissimilar subunits (α and β chains) held together by noncovalent, sodium dodecyl sulfate-labile bonds [1]. The larger subunit (α-chain or N-terminal subunit), which is extracellular and can be shed into the lumen, comprises a signal peptide; 25–125 tandem repeats of a 20-amino acid sequence of PTS domains (regions with high proportions of proline, threonine and serine residues) that are heavily O-glycosylated; and the sperm protein, enterokinase and agrin (SEA) domain. However, the smaller subunit (β-chain or C-terminal subunit) consists of a 58-amino acid extracellular domain, a single-pass 28-amino acid transmembrane domain and a 72-amino acid cytoplasmic tail (CT) [3], which contains 18 documented potential and putative tyrosine or serine/threonine phosphorylation sites (figure 1) [4].

At the plasma membrane, MUC1 acts as a membrane receptor, regulating several inflammatory processes through the phosphorylation and bioactivation of MUC1-CT. Nuclear translocation of bioactivated...
MUC1-CT has been demonstrated to mediate the expression of several anti-inflammatory genes, thus acting as an important mediator in respiratory diseases such as chronic rhinosinusitis [5], asthma [6] and COPD [7]. MUC1-CT has also been shown to play an anti-inflammatory role in several airway infections [8]. However, bioactivated MUC1-CT also interacts with various effectors linked to transformation [4], thus promoting carcinogenic and fibrotic processes in the lung.

The MUC1-N-terminal subunit has been shown to increase the invasive and metastatic abilities of tumour cells [1, 9, 10], as well as providing a releasable decoy barrier during airway infection [11]. Furthermore, the soluble MUC1-N-terminal subunits Krebs von den Lungen-6 (KL-6) and CA15-3 have been studied as serum biomarkers in several lung diseases [12, 13].

All these findings indicate the importance of MUC1 in the development of lung diseases and suggest that MUC1 may be useful in the diagnosis of many lung diseases, or as a therapeutic target.

MUC1 expression in lung tissue has been evaluated by immunohistochemistry analysis in several lung diseases [14–20]. However, depending on the anti-MUC1 antibody used, a large variability of MUC1 expression has been reported [21]. Therefore, immunohistochemistry analysis should be considered to be a weak point when evaluating MUC1 expression and its influence on mechanisms of disease.

MUC1 structure [22], interacting proteins [23], expression [24] and participation in several carcinogenic and inflammatory processes [4, 25], including inflammation in the respiratory tract [26], have been previously reviewed. However, the exact role of MUC1 in most of the currently known lung diseases has not been reviewed in detail yet. This review summarises the current state of MUC1 research in several respiratory diseases and provides an assessment of the potential of MUC1 as a diagnosis/prognosis tool and as a treatment target for lung disease.

MUC1 and airway inflammation and infection

MUC1 has been shown to be an important modulator of innate immunity, playing an important anti-inflammatory role during airway infection caused by bacterial and viral pathogens [27–29]. The suppressive effect of MUC1 has been attributed to Toll-like receptors (TLR) 2, 3, 4, 5, 7 and 9 signalling.
which attenuates nuclear factor (NF)-κB activation and reduce tumour necrosis factor (TNF)-α and interleukin (IL)-8 secretion [8]. Furthermore, the anti-inflammatory effect of MUC1 has been reported to require only the MUC1 cytoplasmic domain, instead of the MUC1 entire molecule [8].

Immunoprecipitation and immunofluorescence studies revealed that MUC1-CT constitutively forms a protein complex with TLR5 [30]. Since all TLR adaptor proteins (myeloid differentiation primary response gene 88 (MyD88) for TLR2, 4, 5, 7, and 9 and TIR (Toll/interleukin-1 receptor)-domain-containing adapter-inducing interferon-β (TRIF) for TLR3) share the conserved TIR domain, it has been suggested that MUC1-CT might suppress TLR signalling by associating with the TIR domains of TLRs, acting as a steric hindrance/decoy receptor and preventing recruitment of adaptor proteins to their respective receptors (figure 2) [30]. Nevertheless, further research is needed to confirm that MUC1-CT forms protein complexes with all the TLRs and, to confirm the direct interaction between MUC1-CT and TIR domains.

As an example of bacterial airway infection, MUC1 has been reported to act as an extracellular adhesion site for Pseudomonas aeruginosa, an opportunistic pathogen that colonises the lungs in several airway diseases, including bronchiectasis, cystic fibrosis, and nosocomial pneumonia [31–34]. Initially, it was suggested that during early stage infection, primary human airway epithelial cells and macrophages sense P. aeruginosa infection via TLR5 recognition of flagellin and subsequent MyD88-dependent secretion of TNF-α and IL-8. Following this, IL-8 recruits neutrophils that release neutrophil elastase (NE) into the airway lumen. TNF-α and NE secretion results in increased expression of MUC1 at the apical surfaces of lung epithelial cells. Therefore, during late-stage infection, P. aeruginosa-induced MUC1-CT tyrosine phosphorylation leads to inhibition of TLR5 signalling, downregulation of inflammation and promotion of neutrophil apoptosis, which is important for the removal of inflammatory cells from the lung (figure 2) [35, 36]. By contrast, Kato et al. [37] recently suggested that stimulation of primary human bronchial epithelial cells with P. aeruginosa or flagellin increases the release of transforming growth factor (TGF)-α, which leads to autocrine activation of epidermal growth factor receptor (EGFR) at the cell surface. Furthermore, P. aeruginosa stimulation of human or mouse macrophages has also been reported to enhance TNF-α release. Therefore, TGF-α via autocrine EGFR activation as well as TNF-α via paracrine signalling have both been reported to increase MUC1 expression in bronchial epithelial cells, thus increasing MUC1-CT/TLR5 association and inhibiting TLR5 signalling [38]. P. aeruginosa has also been shown to upregulate the expression of MUC1 in primary human alveolar macrophages via activation of TLR4 and the p38 mitogen-activated protein kinase signalling pathway, preventing hyperactivation of

![Figure 2](https://doi.org/10.1183/16000617.0149-2020)  
**FIGURE 2** The role of MUC1 in airway infection/inflammation. During early stage of infection, primary human airway epithelial cells sense *Pseudomonas aeruginosa* via TLR5 [1], *Haemophilus influenzae* (NTHi) via TLR2 [2], and RSV via TLR3 [3], which is expressed in endosomes. After recognition, MyD88/TRIF-dependent secretion of TNF-α and IL-8 is produced [4]. Following it, IL-8 and TNF-α lead to an increased expression of MUC1 at the apical surface of lung epithelial cells [5]. During the late-stage of infection, *P. aeruginosa*/NTHi/RSV-induced MUC1-CT tyrosine phosphorylation [1] leads to MUC1-CT interaction with TLR5/TLR2/TLR3 [2], leading to TLR signalling downregulation [3] and inhibition of inflammation [4]. CT: cytoplasmic tail; ERK: extracellular signal-regulated kinase; NFκB: nuclear factor-κB; IL: interleukin; MUC1: mucin 1; MyD88: myeloid differentiation primary response gene 88; RSV: respiratory syncytial virus; TIR: Toll/interleukin-1 receptor; TLR: Toll-like receptor; TNF: tumour necrosis factor; TRIF: TIR [Toll/interleukin-1 receptor]-domain-containing adapter-inducing interferon-β.
macrophages and emphasising the importance of MUC1 as a negative regulator of macrophage-generated inflammatory responses [39].

Similarly, MUC1 has been shown to be part of a feedback loop that downregulates TLR2-induced IL-8 and TNF-α production during late-stage airway epithelial cell infection with nontypeable Haemophilus influenzae (figure 2) [27].

MUC1 has been shown to also exhibit anti-inflammatory effects during TLR3 activation by respiratory syncytial virus, which is considered the leading cause of viral bronchiolitis and pneumonia, both of which represent a major health and economic burden worldwide [40, 41]. Following viral infection, TLR3, which is expressed in endosomes by airway epithelial cells, senses viral-derived double-stranded RNA, triggering TRIF-dependent inflammatory and apoptosis signalling pathways that release TNF-α, which in turn upregulate MUC1. The interaction of MUC1-CT with TLR3 suppresses TNF-α release, terminating the inflammatory response (figure 2) [28, 42].

The protective role of MUC1 during respiratory tract infections is further supported by evidence reported from research into MUC1 and certain gastrointestinal infections. Indeed, MUC1 has demonstrated to play a protective role during infection with gastrointestinal pathogens such as Helicobacter pylori [43, 44] and Campylobacter jejuni [45], which also induce inflammation via TLR signalling.

Altogether, these results suggest anti-inflammatory function of MUC1 in the host inflammatory response to microbial respiratory tract infection, specifically in the resolution phase of inflammation through its ability to inhibit TLR signalling. Therefore, genetic or epigenetic alterations of MUC1-CT might block its ability to inhibit inflammatory signalling, thus contributing to the progression of respiratory tract infections, as well as, to the pathophysiology of chronic inflammatory lung diseases.

MUC1 and chronic rhinosinusitis with nasal polyps

Chronic rhinosinusitis (CRS) encompasses a group of heterogeneous disorders associated with inflammation of the nasal and sinus mucosa with or without the presence of nasal polyps (NP). Different inflammatory phenotypes have been described in NP: NP without asthma or aspirin intolerance (NPwA), NP with aspirin-tolerant asthma (NP-ATA), and NP with aspirin-intolerant asthma (NP-AIA).

The proposed CRS aetiology includes both host and environmental factors that activate the host innate and adaptive immune responses involved in pathogenesis [46, 47]. However, depending on the ethology, CRS immune responses might be different.

Cigarette smoke (CS) has been identified as a significant risk factor for the development of CRS [48]. Cigarette smoke-associated pathological phenotype includes globet cell metaplasia (GCM) and overproduction of mucus among others [49]. Recently, it has been reported that in response to cigarette smoke exposure, total protein levels of MUC1 are not altered in the lung tissue [50]. However, an expansion of basal/intermediate cells enriched with MUC1 in the intracellular compartment is apparent following exposure to cigarette smoke, leading to GCM and MUC5AC overproduction [50].

Eosinophilic inflammation is another hallmark of chronic rhinosinusitis with nasal polyps (CRSwNP). Staphylococcus aureus exotoxins are among the best characterised elicitors of eosinophilic inflammation and are thought to be heavily involved in the pathogenesis of CRSwNP through their behaviour as superantigens [51]. S. aureus has been observed to promote the production of IL-22 by NP cells. In this context, IL-22 regulates chronic inflammation and has been shown to significantly enhance the levels of MUC1 expression in NP cells, which are significantly and positively correlated with IL-22 receptor mRNA levels [52].

Oral systemic corticosteroids are the first-line therapy for patients with CRSwNP [53]. Among responders, corticosteroids have been shown to upregulate membrane-tethered mucins (MUC1 and MUC4) while downregulating secreted mucins (MUC5AC and MUC5B) in NP [54]. No differences for MUC1 expression have been observed among responders in the NPwA, NP-ATA and NP-AIA groups [5]. However, a large number of patients show resistance to the effects of systemic corticosteroids (CR-CRSwNP).

Dexamethasone has been shown to induce the formation of a protein complex between MUC1-CT and glucocorticoid receptor α (GRα), which results in translocation of GRα into the nucleus to exert its anti-inflammatory effects (figure 3) [5]. However, since patients with CR-CRSwNP exhibit significantly lower expression of MUC1 in the NP epithelium, as well as upregulation of TLR2 and TLR5 [5], it results in overactivation of TLR signalling and subsequent inflammation, contributing to the loss of corticosteroid efficacy in these patients. The same pattern of MUC1 expression was also observed between patients with CR-CRSwNP showing NPwA, NP-ATA and NP-AIA [5].

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Patients with CR-CRSwNP show significantly higher extracellular signal-regulated kinase (ERK) 1/2 phosphorylation than responders [5]. Similarly, these patients also show higher glucocorticoid receptor (GR)-Ser226 phosphorylation [5], which has been reported to enhance GR nuclear export, thus contributing to termination of GR-mediated transcription [55]. It suggests that GR-Ser226 hyperphosphorylation is induced by ERK1/2 hyperphosphorylation, leading to an inhibition of corticosteroids-induced GRα nuclear translocation and GR anti-inflammatory mediated effects [56]. Therefore, MUC1 downregulation in patients with CR-CRSwNP has been suggested to contribute to the loss of corticosteroid efficacy, by GR-Ser226 hyperphosphorylation, which is consequence of the enhanced TLR-induced ERK1/2 phosphorylation and by the reduced formation of the MUC1-CT-GRα transcription complex [5]. Furthermore, in the absence of ligand, GR resides predominantly in the cytoplasm, sequestered in a multimeric chaperone complex comprising heat shock protein (hsp) 90, hsp70, and other factors that prevent its degradation and assist in its maturation [57]. MUC1-CT has been shown to form a complex with hsp90 [58]. Therefore, MUC1-CT may bind the GRα-chaperone complex, leading to nuclear translocation of the mature complex (figure 3).

Overall, these findings suggest that increased or altered localisation of MUC1 expression might be a defence mechanism to compensate for excessive inflammation in patients with CRSwNP, modulating the anti-inflammatory effects of the corticosteroid therapy used and inhibiting overactivation of TLR-triggered inflammation.

MUC1 and asthma

Bronchial asthma is a chronic airway inflammatory disease whose strongest risk factors are a combination of genetic predisposition with environmental exposure to inhaled substances and particles that may provoke allergic reactions or irritate the airways.

The symptoms of patients with mild to moderate asthma are often well controlled with low doses of inhaled corticosteroids (ICS). However, the symptoms of most patients with severe asthma are not well controlled with ICS [59].

![Figure 3](https://doi.org/10.1183/16000617.0149-2020)
Four distinct asthma inflammatory phenotypes can be identified based on the presence (or absence) of increased levels of eosinophils and neutrophils. These phenotypes are termed eosinophilic (increased eosinophils); neutrophilic (increased neutrophils); granulocytic asthma (MGA) (increased both eosinophils and neutrophils); and paucigranulocytic (normal levels of eosinophils and neutrophils). Up to now, any association between MUC1 expression and the different asthma inflammatory phenotypes has not been reported yet. However, similar to patients with CR-CRSwNP, corticosteroid resistance in patients with uncontrolled severe asthma has been associated with MUC1 downregulation in human bronchial epithelial cells (HBECs) and blood neutrophils, as compared with patients with mild asthma and healthy controls [6]. Indeed, neutrophils and bronchial epithelial cells from patients with severe asthma are less sensitive to the anti-inflammatory effects of corticosteroids [6]. Similar results have been reported in an ovalbumin-induced inflammatory asthma model, in which wildtype (WT) mice, but not MUC1 knockout (KO) mice, responded to the anti-inflammatory effects of corticosteroids [6].

As it was described above, corticosteroids mediate some of their anti-inflammatory effects by forming MUC1-CT/GRα nuclear transcription complexes (figure 3) [5]. HBECs from patients with severe asthma exhibit increased GR-Ser226 phosphorylation compared with cells from patients with mild asthma and healthy controls. Furthermore, immunoprecipitation experiments revealed that the expression levels of GRα/MUC1-CT complexes are decreased in HBECs from severe asthmatics [6]. In this context, in an ovalbumin-induced inflammatory model of asthma, corticosteroids have been shown to promote bronchial epithelial nuclear translocation of the MUC1-CT/GRα complex in WT mice, inducing the expression of corticosteroid-inducible anti-inflammatory genes. By contrast, GRα nuclear translocation is not observed in MUC1-KO mice [6]. Corticosteroids also inhibit inflammatory cell extravasation to lung tissue. When pulmonary artery endothelial cells (HPAECs) are transfected with small interfering RNA targeting MUC1, they become resistant to the effects of corticosteroids inhibiting neutrophil adhesion and downregulation of intracellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM) and E-selectin. These results suggest that MUC1 not only mediates corticosteroid regulation of anti-inflammatory gene expression but also mediates the inhibitory effects of corticosteroids on neutrophil migration and adhesion to HPAECs [6].

Viral or bacterial infections are observed in 70% of inpatients with an asthma exacerbation [60]. Therefore, MUC1-CT downregulation in severe asthma patients may enhance viral and bacterial-induced TLR signalling (figure 3) [30], triggering sustained inflammation in the pathogenesis of asthma [61]. However, elevated KL-6 serum levels have been reported in acute exacerbations of paediatric patients with bronchial asthma [62], suggesting that KL-6 may become a useful biomarker for the diagnosis and prognosis of asthma.

The death of epithelial cells in asthmatic airways also plays a regulatory role in severe asthma [63]. Indeed, multiple mechanisms have been implicated in mediating epithelial apoptosis in asthma, including viral infection [64]. It has been demonstrated that asthmatic patients have an impaired antiviral response, and it has been hypothesised that this may allow certain viruses to proliferate in epithelial cells with consequential necrosis, the release of damage-associated molecular patterns (DAMPs), and persistent inflammation [65, 66]. In this context, MUC1 downregulation in HBECs has been shown to impair the antinecrotic (necrosis regulated by orchestrated pathways) effects of glucocorticoids in these cells [67].

In summary, these findings suggest that MUC1 expression mediates the corticosteroids effects in patients patients with severe asthma.

**MUC1 and COPD**

COPD is a chronic inflammatory, progressive, and debilitating lung disease mainly caused by chronic exposure to cigarette smoke [68]. Anti-inflammatory options for COPD are based on corticosteroids. However, similar to severe asthma [6] and CRSwNP [5], the loss of corticosteroid anti-inflammatory efficacy is common in patients with COPD [69].

MUC1-CT has been shown to be downregulated in lung tissue, isolated bronchial epithelial cells and sputum neutrophils from smokers and corticosteroid resistant patients with COPD defined according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) as GOLD II (moderate disease) and GOLD III (severe disease), without recent exacerbations [7]. Additionally, the forced expiratory volume (FEV1,%) of smokers and patients with COPD without recent exacerbations has been directly correlated with MUC1 lung tissue expression [7].

Similar to the mechanism of action of corticosteroids in asthma [6] and CRSwNP [5], it has been suggested that corticosteroids given to patients with COPD also partially mediate their anti-inflammatory effects by forming MUC1-CT-GRα complexes that translocate to the nucleus to increase the expression of anti-inflammatory genes [7]. Furthermore, TLR4, whose inflammatory downstream signalling is activated...
by cigarette smoke [70], is chronically elevated in bronchial epithelial cells and neutrophils from patients with COPD [7, 71]. Therefore, the increased expression of TLR4 and decreased expression of MUC1-CT could explain the greater inflammatory response in patients with COPD, as well as the lack of corticosteroid responsiveness (figure 3). Indeed, HBECs transiently transfected with siRNA-MUC1 have been reported to be resistant to the anti-inflammatory effects of corticosteroids in response to cigarette smoke extract [7]. Consistent with this, corticosteroids are unable to exert anti-inflammatory effects in lungs from a MUC1-KO model of acute cigarette smoke inflammation [7]. Nevertheless, the loss of MUC1 expression in the lungs of patients with COPD is still controversial because there is evidence of bronchial/alveolar epithelium of patients with COPD which display highly positive areas of KL-6 staining in contrast to the situation in nonsmokers and smokers [72]. Furthermore, as it was previously mentioned, it has been also reported that oxidative stress upregulates MUC1 expression [73] or modifies the localisation of MUC1 to the intracellular compartment of hyperplastic intermediate cells [50].

MUC1 downregulation is not the only cause for the lack of corticosteroid responsiveness in COPD. Indeed, the endogenous glucocorticoid hydrocortisone and the synthetic glucocorticoids administered to patients with COPD function via histone deacetylase 2 (HDAC2)-regulated epigenetic modifications to suppress pro-inflammatory gene transcription. Therefore, changes in the histone acetylation/deacetylation balance due to oxidative stress induced by cigarette smoke may also be an important factor in inducing glucocorticoid resistance in patients with COPD [74].

Similar to asthma, respiratory virus infections are a major cause of COPD exacerbations, sometimes resulting in secondary bacterial infections. In this context, MUC1-CT expression in sputum has been reported to be increased in acute phase COPD exacerbations (AECOPD) compared with sputum from the remission phase [75], thus suggesting a protective role of MUC1 during AECOPD. Furthermore, linear regression analysis has shown that, during COPD acute exacerbation, the increased levels of sputum MUC1-CT fragments are positively correlated with sputum neutrophil number [75].

The extracellular domain (EC) of MUC1 has also been shown to be elevated in sputum not only from patients with AECOPD, but also patients with GOLD I–III COPD [72, 75, 76]. The contribution of MUC1 shedding to mucous biology is not fully understood. Nevertheless, it might be hypothesised that the untethered MUC1-N-terminal subunit may contribute to mucus hypersecretion and increased amount of sputum in patients with COPD, likely serving as a protective barrier against invading pathogens. However, long-term mucous plugging, particularly of the small airways, might also contribute to airflow limitation. Therefore, elevated MUC1-EC levels in sputum can have beneficial and nonbeneficial effects in patients with COPD.

Elevated KL-6 serum levels have been also detected in patients with COPD, although elevated levels have also been observed in smokers regardless of COPD diagnosis [72].

In summary, these findings suggest that MUC1 expression levels in patients with COPD are dependent on the nature of patients with COPD, with differences reported between AECOPD and patients with stable (without recent exacerbations) COPD. It is possible that the different MUC1 subunits play different roles in COPD, as MUC1-CT has been shown to participate in modulation of the anti-inflammatory effects of corticosteroids in patients with stable COPD. However, the significance of the elevated levels of MUC1-EC/KL-6 in the development and progression of COPD is still unknown.

**MUC1 and lung cancer**

MUC1 basal expression is generally restricted to the apical/luminal side of cells [3]. However, MUC1 has long been viewed as a tumour-associated molecule due to its frequent overexpression and aberrant glycosylation in many carcinomas [3], in which there is loss of polarity and aberrant cell surface distribution [77]. In this context, several clinical studies based on immunohistochemical staining [14–19] and meta-analysis studies [78, 79] have demonstrated a negative prognostic association of tumour MUC1 overexpression and depolarised cellular distribution in patients with nonsmall cell lung cancer (NSCLC). Nevertheless, some contradictory findings have also been reported, although they represent minor contributions [80–82].

Elevated levels of MUC1 have been detected more frequently in adenocarcinoma (86.3%) and other NSCLCs (74.1%) compared with squamous cell carcinoma tissue (39.1%) [15]. However, some studies have shown that MUC1 expression is increased during the progression of pre-malignant lung lesions to invasive carcinoma; this increase is significant for squamous cell carcinoma but not for adenocarcinoma [83].

CA15-3 serum levels have also been reported to be significantly increased and correlated with metastasis in patients with lung cancer [84, 85]. Otherwise, preoperative serum KL-6 levels have been identified as a simple and novel predictor of recurrence following curative surgery in patients with NSCLC [86–88], while...
postoperative serum KL-6 levels have been identified as a prognostic factor for resected patients with NSCLC [89]. In this context, the degree of the decrease of natural autoantibody to KL-6/MUC1 has been correlated with impaired NSCLC prognosis [90]. By contrast, recent findings have reported no significant difference in serum MUC1 levels between patients with NSCLC and healthy individuals, although higher plasma exosomal MUC1 levels have been detected in patients with NSCLC [91].

In lung cancer cells, STAT3 [92] and 14-3-3ζ [93] oncogenes have been shown to regulate the expression of MUC1 in a promoter-dependent manner. Overexpressed and depolarised MUC1 has been shown to inhibit lung cancer cell apoptosis and to induce proliferation, the epithelial–mesenchymal transition (EMT), tumour growth and angiogenesis and metastasis [92–95]. The MUC1-N-terminal subunit has been shown to increase the invasive and metastatic capability of tumour cells by different mechanisms: 1) reducing cell–cell adhesion by disturbing E-cadherin interactions between adjacent cells [9]; 2) reducing cell–extracellular matrix adhesion through inhibition of integrin-mediated cell adhesion to extracellular matrix components [10]; and 3) interacting with adhesion molecules such as endothelial ICAM-1, which triggers invasion of MUC1-expressing tumour cells into the endothelium and reattachment at distant sites of metastasis (figure 4) [1].

MUC1-C-terminal subunit has been reported to interact with the β-galactosidase binding lectin galectin-3 (figure 1), increasing MUC1 association with EGFR and leading to EGFR activation and signalling, which mediates epithelial cell growth and survival [96]. Independently of EGFR, galectin 3 binding to MUC1-C has also been shown to promote MUC1-CT phosphorylation, activating mitogen-activated protein kinase (MAPK) and PI3K/AKT signalling pathways, resulting in cell proliferation, motility, and tumour angiogenesis [25, 97, 98]. Similarly, other transmembrane receptors such as platelet-derived growth factor receptor (PDGFR)-β [99], fibroblast growth factor receptor (FGFR) 3 [58] and Met [23] and ErbB1–4 receptors [77, 100] have also been shown to phosphorylate MUC1-CT (figure 1), promoting cell proliferation, migration, and transformation [4, 23]. Furthermore, β-catenin preferentially binds the

![Diagram of MUC1 and its interactions](https://doi.org/10.1183/16000617.0149-2020)

**FIGURE 4.** The role of MUC1 in lung cancer. (1) MUC1 is overexpressed and depolarised in lung cancer. Therefore, MUC1 interactions with potential binding partners are increased under these circumstances, thus increasing the invasive and metastatic capability of tumour cells by different mechanisms: (2) reducing cell–cell adhesion through disturbing E-cadherin interactions between adjacent cells, (3) reducing cell–ECM adhesion through inhibition of integrin-mediated cell adhesion to ECM components, and (4) interacting with adhesion molecules such as the endothelial ICAM-1. (5) MUC1-C-terminal subunit interacts with galectin-3, increasing MUC1 association with the EGFR and leading to EGFR activation and signalling, which mediates epithelial cell proliferation and survival. (6) Galectin-3 binding to MUC1-C also phosphorylates MUC1-CT, activating mitogen-activated protein kinase and PI3K/AKT signalling pathways, which lead to cell proliferation and motility. (7) β-Catenin preferentially binds to the phosphorylated intracellular tail of MUC1, thus also contributing to the decrease of cell–cell adhesions through E-cadherin. (8) MUC1-CT/β-catenin complex translocates into the nucleus to regulate genes involved in cell proliferation and differentiation. (9) MUC1-N (KL6) is shed from the cell surface and has demonstrated to be a prognostic and treatment responsiveness serum biomarker in lung cancer. AKT: protein kinase B; ECM: extracellular matrix; EGFR: epidermal growth factor receptor; ERK: extracellular signal-regulated kinase; ICAM: intracellular adhesion molecule; KL-6: Krebs von den Lungen-6; LEF: lymphoid enhancer factor; MUC1: mucin 1; MUC1-CT: MUC1 cytoplasmic domain; TCF: T-cell factor protein.
phosphorylated intracellular tail of MUC1 (figure 1), also contributing to the decrease in cell–cell adhesions through E-cadherin [25]. MUC1-CT/β-catenin complexes translocate to the nucleus and regulate genes involved in cell proliferation and differentiation (figure 4) [101].

Recently, it has been shown that an AUG codon downstream to the MUC1 initiation codon initiates an alternate reading frame (ARF) thereby generating a novel protein, MUC1-ARF (figure 1). The amino acid sequence of the MUC1-ARF variable number tandem repeat (VNTR) as well as N- and C-sequences flanking it differ entirely from those of MUC1-transmembrane and it has been shown that MUC1-ARF protein mainly localises in the nucleus of malignant epithelial cells of pancreatic cancer and breast cancer. In this context, further research would be needed to address if MUC1-ARF is also expressed in lung cancer, as well as, to describe the link between MUC1-ARF and malignancy.

In the clinic, paclitaxel (PTX) is an important first-line chemotherapeutic agent against lung cancer [102, 103]. However, its treatment can generate resistance, which has been associated with enhanced MUC1-C expression in NSCLC [104]. Alternatively, among agents for the treatment of lung cancer, the EGFR tyrosine kinase inhibitors (EGFR-TKIs) gefitinib and erlotinib have been established as options for first-, second-, or third-line, or maintenance, treatment. Nevertheless, EGFR activating mutations in NSCLC cells frequently develop, resulting in resistance to EGFR-TKIs. In this context, targeting the oncogenic protein MUC1-C has been shown to inhibit mutant EGFR-mediated signalling and survival of NSCLC cells, suggesting a potential approach for the treatment of EGFR-TKI-resistant NSCLC [105]. Monitoring of circulating KL-6 levels in patients with NSCLC prior to and 2 weeks after the start of EGFR-TKI treatment revealed that serum KL-6 levels are increased in EGFR-TKI no-responder patients, which is accompanied by disease progression and reduced survival. By contrast, in partial-response patients, KL-6 serum levels significantly reduced after 2 weeks of EGFR-TKI treatment [106]. Furthermore, it has been suggested that patients with NSCLC with high serum KL-6 levels [106, 107] and MUC1 mRNA positivity in blood have unfavourable responses and poor clinical outcomes when treated with gefitinib [108]. Therefore, KL-6 serum levels and/or MUC1 mRNA blood levels may be promising noninvasive and repeatable markers for predicting the efficacy of EGFR-TKIs, which could help in the choice of treatment for different patients. By contrast, other findings have demonstrated that monitoring of KL-6 levels in the intrabronchial epithelial lining fluid near the tumour before and 2 weeks after the start of gefitinib treatment can predict tumour response at 4 weeks, whereas serum levels do not. However, the median KL-6 level near the tumour is significantly higher in treatment responders than in nonresponders prior to treatment [109].

Importantly, aberrant overexpression of MUC1-C in NSCLC has been linked to the induction of programmed death ligand 1 (PD-L1) [110], which promotes the evasion of NSCLC cells from immune recognition [111]. Therefore, several vaccines using MUC1 as an antigen have been developed and investigated as an immunomodulatory approach to the treatment and prevention of lung cancer. TG4010 (a modified vaccinia virus expressing MUC1 and IL-2) has been studied in a phase II trial in combination with first-line chemotherapy in patients with stage IV untreated NSCLC. This trial demonstrated a small but statistically significant improvement in progression-free survival [112]. Another vaccine, tecemotide (L-Blp25; a liposome-based vaccine consisting of a synthetic 25-amino acid lipopeptide derived from the tandem repeat region of MUC1, together with the nonspecific adjuvant monophosphoryl lipid A and three different lipids [113]), has been administered in a phase III study as maintenance therapy for unresectable stage III/IV NSCLC following treatment with platinum-based chemotherapy and radiation. Results from this clinical trial have already shown an improved overall survival [114, 115]. Additionally, two clinical trials are currently investigating the combination of MUC1 targeting vaccines with anti-PD1 therapy in advanced NSCLC: one using the above mentioned TG4010 vaccine (NCT02823990), and another using the CV301 vaccine (NCT02840994), which targets both MUC1 and carcinoembryonic antigen (CEA). Finally, preliminary studies have reported that the MUC1-targeted dendritic cell-based vaccine also significantly prolongs the survival of patients with MUC1-expressing lung cancer [116].

In summary, MUC1 is considered a lung cancer-associated molecule, since its overexpression and depolarisation have been shown to be linked to lung cancer progression. Therefore, part of the clinical efforts are currently focused on targeting MUC1 to treat lung cancer. Furthermore, numerous studies have demonstrated that CA-15-3 and KL-6 are reliable lung cancer prognostic and treatment responsiveness biomarkers, thus highlighting the potential use of MUC1 in the treatment of lung cancer.

**MUC1 and interstitial lung disease**

Elevated KL-6/MUC1 serum levels have been observed in >70% of patients with interstitial lung diseases (ILDs) [117, 118]; serum levels are negatively correlated with the diffusing capacity of lungs for carbon monoxide, as well as positively correlated with the high-resolution computerised tomography (HRCT) score [119]. Additionally, serum levels of KL-6 have been reported to be significantly correlated with KL-6 levels in bronchoalveolar lavage fluid (BALF), which are significantly correlated with the numbers of
total cells, lymphocytes, and neutrophils in BALF from patients with ILD [120] and KL-6 expression in regenerating alveolar type II (ATII) cells from ILD tissue [117]. Therefore, KL-6 serum levels are useful for evaluating the extent of ILDs, predicting disease outcome, monitoring the clinical course, and identifying patients with ILDs who are at increased risk for subsequent mortality [117–119, 121]. Nevertheless, ILD is a term that comprises the pathologies described below.

**Hypersensitivity pneumonitis**
Measurement of serum KL-6 concentrations is useful to screen for hypersensitivity pneumonitis (HP) and to detect HP activity [122]. Indeed, bird-related and house-related HP show seasonal serum KL-6 variations, showing a significant increase in serum KL-6 concentrations in bird-related HP during the winter and in house-related HP during the summer. Furthermore, serum KL-6 variations have been reported to be significantly greater in acute HP than chronic HP [123].

**Sarcoidosis**
In sarcoidosis, serum levels of KL-6 have been reported to vary according to the genotypic distribution of rs4072037 [124], a single nucleotide polymorphism in exon 2 of the MUC1 gene that has been linked with MUC1 VNTR size class [125]. In this context, the large molecular size of KL-6/MUC1 has been associated with the high levels of serum KL-6 reported in sarcoidosis [126, 127]. However, these results are based on an exceptionally small case–control study subject to type 1 error. Therefore, further research would be needed to confirm rs4072037 MUC1 genetic linkage with sarcoidosis.

**Radiation pneumonitis**
Patients lung cancer who have received thoracic radiotherapy (TRT) with or without chemotherapy are at risk of developing radiation pneumonitis (RP). In this context, serum KL-6 levels have shown a consistent tendency to increase following clinical diagnosis of severe RP. By contrast, in patients with localised (within the irradiated field) RP, serum levels do not show a tendency to increase during or after TRT [128].

**Drug-induced interstitial pneumonitis**
Interstitial pneumonitis can be drug-induced and is characterised by different histological patterns. In the context of KL-6 serum levels, only diffuse alveolar damage (DAD) and chronic interstitial pneumonia (CIP) patterns exhibit KL-6 increased serum levels, which are closely correlated with their clinical course. By contrast, serum KL-6 levels in bronchiolitis obliterans organising pneumonia or eosinophilic pneumonia (BOOP/EP) patterns are within the normal range [129].

**Collagen vascular disease-associated interstitial pneumonia**
Collagen diseases are also frequently associated with interstitial pneumonia (IP); the mean serum KL-6 level of patients with both diseases is significantly higher than that of patients with collagen diseases and inactive IP. Furthermore, serum KL-6 levels have been shown to increase with the deterioration of IP, while successful treatment of IP results in a significant decrease in KL-6 serum levels [130].

**Idiopathic interstitial pneumonias**
Idiopathic pulmonary fibrosis (IPF) and patients with nonspecific interstitial pneumonia (NSIP) have also shown higher BALF KL-6 levels than healthy controls. These levels positively correlate with oxygen demand at rest and during a 6 min walk test [131], as well as with the extent of fibrotic abnormalities on HRCT [132].
In the clinic, initial evaluation of serum KL-6 levels has been suggested to predict survival in patients with IPF [133]. Furthermore, several studies have reported increased serum KL-6 levels during IPF acute exacerbations, correlating with a rapid decline in forced vital capacity (FVC) predicted and lower survival rates [134]. Interestingly, MT1-MMP or MMP14 proteins are an alternative mechanism to the MUC1 SEA self-cleaving domain and shedding to the plasma membrane [135, 136]. Thus, the elevated levels of MT1-MMP under lung fibrotic conditions [137] are hypothesised to mediate the release of the extracellular MUC1 domain (MUC1-N/KL-6) in many biological fluids in IPF. Recently, KL-6 serum levels have been considered a reliable prognostic biomarker indicative of the response to nintedanib treatment in patients with IPF [138].

There is evidence of weak MUC1/MUC1-CT expression mainly localised to the membrane of ATII cells and alveolar macrophages in control lung sections [139, 140]. However, under fibrotic conditions, MUC1/MUC1-CT is overexpressed and located mostly in hyperplastic ATII cells and fibroblasts of fibrotic areas, being mainly distributed in the cell cytoplasm and nucleus [117, 140] and contributing to IPF pathology. Furthermore, two phosphorylated/bioactivated forms of MUC1-CT (MUC1-P/T-1224 and MUC1-P/Y-1229)
have been shown to be overexpressed in IPF lung tissue, with a similar pattern of distribution to that of nonphosphorylated MUC1-CT [140].

In IPF, MUC1-CT has been reported to collaborate with transforming growth factor (TGF)-β1, promoting disease progression [140]. Following MUC1-CT phosphorylation/activation via TGF-β1-induced Smad3 phosphorylation, MUC1-CT increases the active form of β-catenin, forming a MUC1-CT/phospho-Smad3/active-β-catenin nuclear protein complex that promotes fibrotic processes such as the EMT, the fibroblast–mesenchymal transition (FMT), fibroblast proliferation and ATII and fibroblast senescence [140]. Otherwise, MUC1-CT has also been shown to be activated by galectin 3 [97, 140], whose expression has been observed to be increased in BALF and serum of patients with stable IPF, as compared with those with nonspecific interstitial pneumonitis and controls [141]. Furthermore, galectin-3 has been shown to interact directly with MUC1-C (figure 1), functioning as a bridge between MUC1-C and EGFR, as well as other cell surface receptors [142]. Indeed, galectin-3 has been reported to promote IPF through stabilisation of the TGF-β1 receptor (TβR) [141]; it has been hypothesised that galectin-3 might also serve as a bridge between MUC1-C and TβR, suggesting MUC1-CT might be activated indirectly via p-Smad3 and directly via galectin 3 (figure 5). Importantly, a phase I/II clinical trial (NCT02257177) to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of TD139, a galectin 3 inhibitor, in patients with IPF has been successfully conducted.

Additionally, the N-terminal binding of MUC1-N to ICAM-1 has also been related to IPF progression; ICAM-1 serum levels are elevated in patients with IPF [143] and the MUC1/ICAM-1 association results in a rapid increase of intracellular calcium in MUC1-expressing cells [144], inducing cytoskeletal changes and motility.

The effect of MUC1 targeting in the development of pulmonary fibrosis has been studied by using the MUC1-C peptide inhibitor GO-201 in vitro and in vivo, which has been shown to inhibit the targeting of

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**FIGURE 5** The role of MUC1 in IPF. Transforming growth factor β1 [TGF-β1] receptor (TβR) activation induces Smad3 phosphorylation (1), which phosphorylates MUC1-CT (2). (3) Bioactivated MUC1-CT has demonstrated to increase the active form of β-catenin, forming a MUC1-CT/phospho-Smad3/active-β-catenin nuclear protein complex that promotes fibrotic processes such as epithelial to mesenchymal transition, fibroblast to mesenchymal transition (FMT), fibroblast proliferation and alveolar type II and fibroblast senescence. (4) MUC1-CT is also activated by galectin 3, which has demonstrated to directly interact with MUC1-C, functioning as a bridge between MUC1-C and the epidermal growth factor receptor [EGFR], as well as, other cell surface receptors such as TβR. (5) MUC1-N-terminal binds to intracellular adhesion molecule-1 [ICAM-1] and the MUC1/ICAM-1 association results in a rapid increase of intracellular calcium in MUC1-expressing cells, inducing cytoskeletal changes and motility. (6) MUC1-N (KL6) is shed from the cell surface and has demonstrated to be a diagnosis and prognostic serum biomarker in idiopathic pulmonary fibrosis KL-6: Krebs von den Lungen-6; LEF: lymphoid enhancer factor; MUC1: mucin 1; SBE: Smad binding element; MUC1-CT: MUC1 cytoplasmic domain.
| Disease | MUC1 expression | Role of MUC1 |
|---------|-----------------|--------------|
| **Infectious diseases** | | |
| *Pseudomonas aeruginosa* | Upregulated in lung epithelial cells [35, 36] and alveolar macrophages [39] | During late-stage infection: Downregulation of TLR5 (epithelial cells)-induced [35, 36] and TLR4 (macrophages)-induced [39] inflammation Promotion of neutrophil apoptosis [35, 36] |
| *Haemophilus influenzae* | Upregulated in airway epithelial cells [27] | Downregulation of TLR2-induced inflammation [27] |
| Respiratory syncytial virus | Upregulated in airway epithelial cells [28, 42] | During last stage infection: Downregulation of TLR3-induced inflammation [28, 42] |
| **CRSwNP** | | |
| Corticosteroid responders | Upregulated in NP epithelium [54] | MUC1-CT/GRα nuclear translocation: anti-inflammatory effects [5] |
| Corticosteroid resistant | Downregulated in NP epithelium [5] | Overactivation of TLR2/5-induced inflammation [5] Reduced formation of the MUC1-CT/GRα nuclear transcription complex [5] |
| **Asthma** | | |
| Mild asthma (corticosteroid responders) | Normal expression levels in HBECs and neutrophils | MUC1-CT/GRα nuclear translocation: anti-inflammatory effects [5] |
| Severe asthma (corticosteroid resistant) | Downregulation in HBECs and neutrophils [6] | GR-Ser226 hyperphosphorylation [6] Reduced formation of the MUC1-CT/GRα nuclear transcription complex [6] Impairment of glucocorticoid antinecroptotic effects [67] Enhancing of viral and bacterial-induced TLR signalling [30] Biomarker |
| **Paediatric acute exacerbation** [62] | Elevated KL-6 serum levels | |
| **COPD** | | |
| Corticosteroid responders | Normal expression levels in lung tissue, bronchial epithelial cells and sputum neutrophils | MUC1-CT/GRα nuclear translocation: anti-inflammatory effects [5] |
| Corticosteroid resistant without recent exacerbation | Downregulated in lung tissue, bronchial epithelial cells, and sputum neutrophils [7] | GR-Ser226 hyperphosphorylation [6] Reduced formation of the MUC1-CT/GRα nuclear transcription complex Biomarker |
| Acute exacerbation | Upregulated MUC1-CT in sputum [75] Elevated MUC1-EC levels in sputum [72, 76] | Protective role Biomarker |
| **Lung cancer** | | |
| Paclitaxel no responders | Overexpression and depolarisation in epithelial cells [77] Increased CA15-3 serum levels [84, 85] | Inhibition of lung cancer cell apoptosis [92] Induction of cell proliferation [93] Induction of EMT [93] Induction of PD-L1 expression: evasion from immune recognition [110] Biomarker for predicting the efficacy of lung cancer treatments |
| EGFR-TKI no responders | Enhanced MUC1-C expression [104] High serum KL-6 levels prior to treatment [106, 107] Increased serum KL-6 levels after EGFR-TKI treatment [106] Reduced serum KL-6 levels after EGFR-TKI treatment | Biomarker |
| EGFR-TKI partial responders | | |
| **ILDs** | | |
| Hypersensitivity pneumonitis | Elevated KL-6 serum levels [123, 127–132] | Biomarker [122, 124, 128–132] |
| Sarcoidosis | | |
| Radiation pneumonitis | | |
| Drug-induced interstitial pneumonitis | | |
| Collagen vascular disease-associated IP | | |
| Nonspecific IP | | |

Continued
MUC1-C to the nucleus and to attenuate the development of pulmonary fibrosis in a bleomycin-induced model [140]. Furthermore, antibodies against KL-6 have been shown to attenuate bleomycin-induced lung fibrosis [145], and MUC1-KO mice have been shown to exhibit resistance to bleomycin-induced pulmonary fibrosis, with improvements in lung function, survival, and fibrotic lung tissue remodelling [140]. By contrast, in a silica-induced in vivo model of lung fibrosis, it has been observed that MUC1-KO mice have increased lung fibrosis [146].

Previously to this paper, higher expression levels of MUC1 in MUC5B+ cells were already registered in the IPF Cell Atlas (www.ipfcellatlas.com/). MUC5B had been largely reported as a proven lung fibrosis mediator [147, 148]. Therefore, it further supports the participation of MUC1 in IPF.

In summary, these findings suggest that KL-6 may be a novel, noninvasive marker for the diagnosis and evaluation of severity in patients with ILD. Furthermore, the detection of activated MUC1-CT in lung tissue and cells from patients with IPF may be of scientific value in the development of new targets to treat this devastating disease.

Conclusions

Respiratory diseases include a group of complex diseases with diverse backgrounds. Therefore, diagnosis and/or treatment are often challenging. As MUC1 is broadly associated with airway defence, airway inflammation, cell growth, and tissue remodelling processes compatible with the processes observed in most respiratory diseases (table 1), analysing the expression, distribution, activation and function of MUC1 in lung pathological conditions may be a useful tool for lung disease diagnosis, follow-up and treatment. However, mucin biology is complex; in spite of numerous pre-clinical and clinical studies focused on MUC1, further in-depth studies are required before MUC1 can be robustly used as a biomarker for diagnosis, prognosis or treatment responsiveness or as a new therapeutic approach in lung diseases.

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