The consequence of matrix dysfunction on lung immunity and the microbiome in COPD

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ABSTRACT The pulmonary extracellular matrix (ECM) is a complex network of proteins which primarily defines tissue architecture and regulates various biochemical and biophysical processes. It is a dynamic system comprising two main structures (the interstitial matrix and the basement membrane) which undergo continuous, yet highly regulated, remodelling. This remodelling process is essential for tissue homeostasis and uncontrolled regulation can lead to pathological states including chronic obstructive pulmonary disease (COPD). Altered expression of ECM proteins, as observed in COPD, can contribute to the degradation of alveolar walls and thickening of the small airways which can cause limitations in airflow. Modifications in ECM composition can also impact immune cell migration and retention in the lung with migrating cells becoming entrapped in the diseased airspaces. Furthermore, ECM changes affect the lung microbiome, aggravating and advancing disease progression. A dysbiosis in bacterial diversity can lead to infection, inducing epithelial injury and pro-inflammatory reactions. Here we review the changes noted in the different ECM components in COPD and discuss how an imbalance in microbial commensalism can impact disease development.

Introduction

The extracellular matrix (ECM) is a complex network of highly cross-linked secreted proteins that define tissue architecture, and biochemical and biophysical properties. The main structural components of the ECM, termed the "core-matrisome" include 43 types of collagen, nearly 200 glycoproteins and 40 proteoglycans [1]. The pulmonary ECM, like the ECM of all other organs, is comprised of two main structures: the interstitial matrix and the basement membrane. The interstitial matrix provides structural support to cells and tissues whilst the basement membrane is a thin ECM layer which coats the basal side of the epithelia and endothelia, surrounds muscle and fat, and provides architectural support to the tissue. In both matrices there are hundreds of non-ECM modifying factors, which include proteases, cross-linking enzymes, growth factors and cytokines, which are important for ECM remodelling and cell behaviour.

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The ECM is a dynamic structure that undergoes continuous remodelling, a process mediated by ECM degrading enzymes including matrix metalloproteinases (MMPs), serine proteinases including neutrophil elastase (NE), adamalysins and mepins, and metalloproteinase inhibitors that protect the ECM [2]. Not only do these enzymes regulate the cleavage of ECM components, they also have an important role in controlling the abundance, composition and structure of the ECM. Remodelling of the ECM is a tightly regulated endogenous process that is essential for wound healing and tissue homeostasis. However, dysregulation of this process can lead to several pathological states, including chronic obstructive pulmonary disease (COPD). The pathology of the disease is characterised by an altered expression of ECM proteins (figure 1) that contributes to remodelling and subsequent thickening of the airways and degradation of alveolar walls, causing a limitation in airflow [2].

Despite many studies investigating ECM changes in COPD, it remains poorly understood due to the heterogeneous nature of the disease, and natural variation in lung ECM composition in different regions of the airway tree. Here we summarise changes to interstitial and basement membrane matrix components in COPD in order to understand how this contributes to the disease process.

The interstitial ECM in COPD

The fibrillar collagens type I and III are upregulated in COPD interstitial matrix [3–5]. These collagens form tight right-handed triple helices of α-chains (such as COL1A1 and COL1A2), which contain repeating amino acid motifs of Gly-X-Y, where X and Y can be any amino acid, but are most commonly proline or hydroxyproline [6]. This structure interacts with other collagens, ECM components and inflammatory cell surface receptors, such as integrin α1β1 on $CD8^+$ T-cells [7]. Relative changes in collagen composition are likely to alter collagen microstructure of the lung. Second harmonic generation microscopy can be used to detect fibrillar collagens without labelling and has recently been used to demonstrate significant changes in collagen organisation of COPD lung compared to non-diseased tissue [8]. The collagen fibril cross-linking enzymes, including lysyl oxidase, are reduced in COPD [9], while transglumatminase 2 levels are increased [10], suggesting that an imbalance in enzyme cross-linking may contribute to ECM structural changes in COPD. In addition, accessory ECM cross-linking components

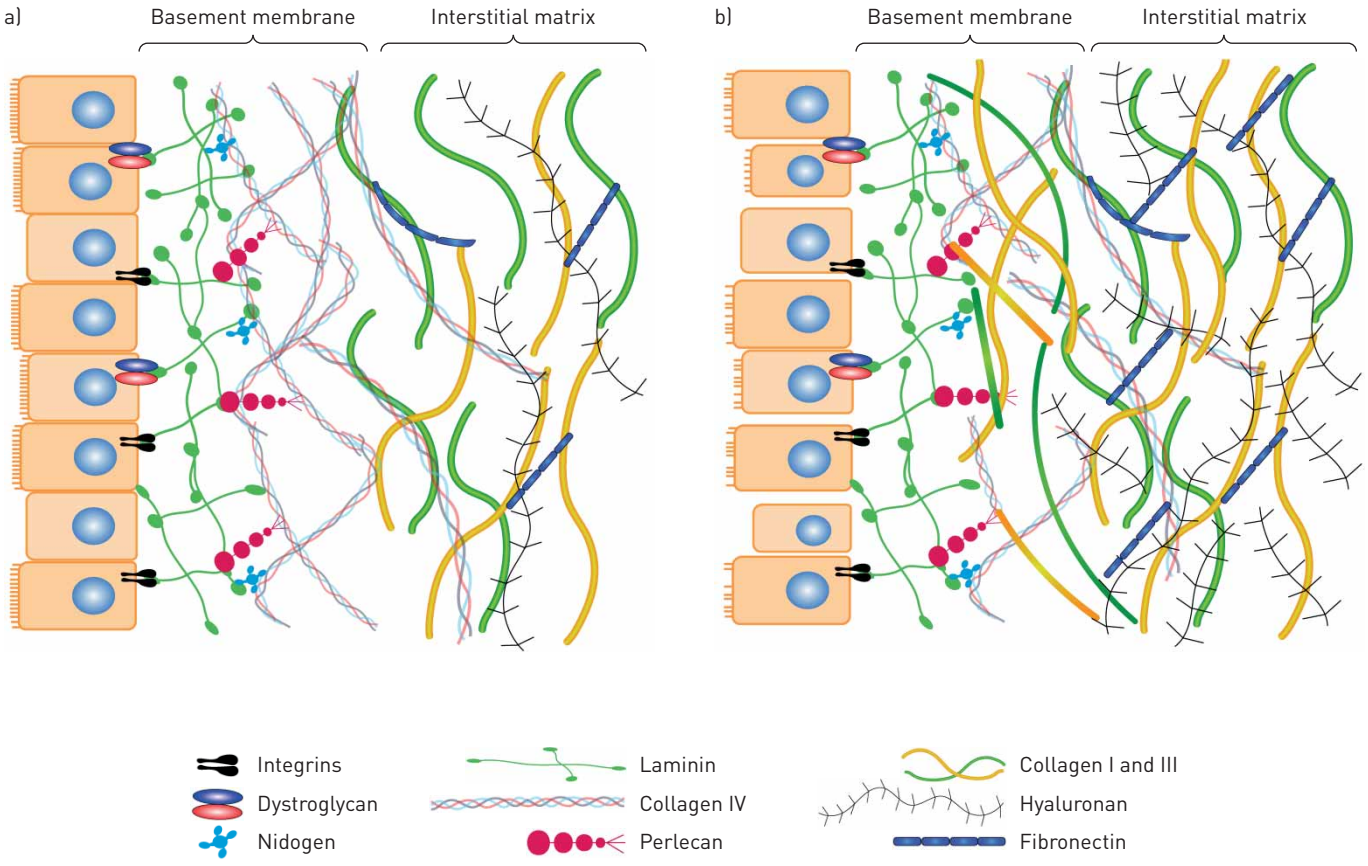

FIGURE 1 Schematic illustration of the dominant changes in extracellular and basement membrane matrix from a) the healthy steady state to b) the development of chronic obstructive pulmonary disease.

including decorin, biglycan and lumican modulate collagen fibril assembly, determining size, shape and collagen content [11]. Decorin and biglycan are abundant in the lung, but are reduced in human COPD [3, 12, 13], whereas versican is increased [14]. Similar changes are observed in the elastase murine model of emphysema [15]. The precise effects of these changes are unclear but are likely to affect collagen tensile strength or the binding of proteins such as Wnt, transforming growth factor (TGF)-β and epidermal growth factor receptor, which modify cell fate or function [16].

Proteolysis of the interstitial collagens (type I and III) is important for normal development, homeostasis and wound healing but has also been linked to COPD pathogenesis. Though resistant to proteolysis by most enzymes, specialised MMPs including MMP1, MMP8, MMP13, membrane-type (MT) 1-MMP1 and MT3-MMP hydrolyse into 1/4 and 3/4 length fragments at conserved protease cleavage sites within the collagen triple helix [17, 18]. Initial cleavage destabilises the helical structure allowing unwinding and further collagenolysis by enzymes including neutrophil derived gelatinases, which are upregulated in COPD [17, 19]. The proteolytic processing of interstitial collagens liberates bioactive fragments (matrikines) such as the acetylated tripeptide Pro-Gly-Pro (acetyl-PGP) by MMP8 or MMP9, promoting further lung neutrophil recruitment [20].

Fibronectin and tenascin C are also increased in COPD [3]. Fibronectin is a high molecular weight ECM protein that aids the initial orientation of new collagen fibres. In turn, mature collagen I provides the tensile strength for fibronectin conformational maturation and elongation [21]. Fibronectin contains RGD motifs (Arg-Gly-Asp amino acid sequence) recognised by RGD-dependent integrins such as αVβ6 and β8 for interaction with epithelial cells, fibroblasts and macrophages. Fibronectin is also a substrate for a number of proteases including MMPs, adamlysins including disintegrin and metalloproteinase domain-containing proteins (ADAM) 9 and 12, and meprin α [22]. Tenascin C levels increase in response to lung infection or injury and can interact with fibronectin [23], which may modify cell–matrix interactions.

Hyaluronan is a large molecular weight (>2500 kDa), unsulfated glycosaminoglycan and an abundant component of lung interstitial matrix [24]. Hyaluronan is synthesised on the cell surface by hyaluronan synthases and forms a long cable-like polysaccharide structure, which is integral to cell and matrix organisation, and bound by various hyaluronan binding proteins expressed on cell surfaces [24]. Hyaluronan is involved in a diverse range of functions including cell migration, inflammation and matrix interaction. High-molecular weight hyaluronan is thought to be anti-inflammatory, whereas degradation leads to bioactive fragments that are proinflammatory [24]. In severe disease, hyaluronan may also enter the alveoli and bronchioles [4] and can be sampled in sputum and the bronchoalveolar lavage fluid [25].

Elastin is a major constituent of elastic fibres in the lung and provides recoil tension to bronchioles *via* their attachment to the alveolar epithelium [4, 26]. There have been contradicting reports on the content of elastin in severe COPD. Staining of tissue sections revealed decreased elastin in both the alveolar and small airway walls of COPD patients [4]; however, elastin mRNA expression has been reported to be significantly increased in the lungs of severe Global Initiative for Chronic Obstructive Lung Disease IV patients [27]. In addition to increased elastin expression, severe COPD lungs displayed an increase in alveolar elastic fibre density [27]. Destruction of elastin contributes to the development of emphysema where alveoli are over-inflated trapping air in the lungs (for a review see [28]), whilst in animal models, elastin administration recapitulates the features of severe COPD including the development of emphysema [29]. Elastin is highly resistant to proteolysis, but its breakdown can be mediated, primarily, by neutrophil elastase and MMP12, but also *via* MMP9, MMP7 and ADAM9, resulting in the generation of bioactive fragments (matrikines) that are chemotactic for inflammatory cells in chronically inflamed lungs [18, 30–33]. Furthermore, interaction studies have revealed that several MMPs [18] and NE bind to elastin [34], supporting early work that NE is bound to elastin in the lungs of patients with emphysema [35].

The basement membrane matrix in COPD

The basement membrane primarily consists of collagen IV, laminins, proteoglycans (such as decorin, biglycan, aggrecan and versican), the heparan sulfate proteoglycans (HSPGs) perlecan and agrin, and proteins including nidogen [2]. Changes in basement membrane structure and composition are less well studied than the interstitial matrix. Reduced expression of decorin and biglycan occurs in patients with severe COPD [12, 13], whereas in mild-to-moderate disease their expression is comparable to healthy controls [3]. Decorin and biglycan are critical collagen cross-linking molecules which bind TGF-β1 [36]. Notably, TGF-β1 deposition and potential bioavailability is altered in the COPD lung. Increased staining of TGF-β1 is observed in the basement membrane of healthy smokers, and in both current and ex-smokers with COPD [37]. However, it has also been reported that TGF-β1 expression is decreased in patients with moderate COPD [13]. A reduction of HSPGs, including perlecan, agrin and collagen type 18, also occurs in the alveolar basement membranes in COPD [12], whereas versican is increased. Increased

versican is linked to a decrease in elastin [14] and elastic fibres in COPD [26]. Collagen IV is a major component of the basement membrane and its alteration in COPD is less clear with studies reporting an increase or decrease [3, 5]. In contrast collagen I and collagen III increase in basement membranes of bronchial tissue samples from patients with COPD [5].

In addition to changes in proteins that make up the basement membrane, alterations in its structure in COPD are also evident. Bronchial biopsies from smokers with or without COPD reveal hyper-vascularity of the basement membrane, which is linked to an increase in vascular endothelial growth factor [38]. Furthermore, the basement membranes of airway biopsies from smokers and COPD patients are highly fragmented [39]. Fragmented areas are positive for both epithelial and mesenchymal markers, suggesting active epithelial mesenchymal transition; a process that involves epithelial cell differentiation and migration through the basement membrane that may contribute to the pathological re-modelling observed in COPD [39].

The influence of ECM alterations on immune cell positioning and retention

ECM, its composition and cross-linking also affects cell migration and positioning. During an inflammatory response in the lung large numbers of cells are recruited *via* extravasation from the pulmonary vasculature. What happens to migrating cells then is an area of intense research with some studies revealing stochastic, random migration and others promoting a more orderly process determined by mechanical guidance scaffolds [40]. Intravital microscopy shows lymphocytes crawling along components of the ECM [41, 42], a process that may be facilitated by chemokines deposited along their structures [43]. However, matrix may also provide the signals that cease cell movement. Matrix scaffolds alter cell adhesion and retention [44] by binding chemokines, growth factors and receptors on immune cells [45–47].

Persistence of such stop signals may, however, result in the retention of excessive cell numbers and impairment of organ primary function. Furthermore, the natural process for cell migration and clearance may be impaired due to matrix deposition forming a physical object that infiltrating cells cannot navigate around, as shown for T-cells in lymph nodes [48]. There are few studies addressing the pathological role of retention *versus* recruitment in COPD. In the bleomycin and elastase models of pulmonary inflammation, versican accumulates [15, 49] and an increase has been observed in COPD [50, 51]. Versican causes the retention of regulatory T-cells and macrophage aggregation during lung inflammation [52]. This role of the ECM and its cross-linking is clearly important and may even represent a novel therapeutic area in the future [53].

The retention of cells may be even more prominent when the role of the cell is to recognise and clear matrix turnover products. We have discovered an imbalance in hyaluronan production following resolution of a severe lung influenza virus infection, driven by hyaluronan synthase 2 from epithelial cells, endothelial cells and fibroblasts (unpublished data). In conditions of endoplasmic stress, viral infections, hyperglycaemia and adrenergic receptor stimulation, hyaluronan can form cable-like structures that disrupt tissue architecture and are more adhesive to inflammatory cells [54]. Airway hyaluronan is elevated in patients with COPD and associated with poor lung function, asthma, idiopathic arterial pulmonary hypertension and acute respiratory distress syndrome amongst others (for a review see [55]). Low-molecular hyaluronan interacts with CD44 and RHAMM [56], and this interaction may cause macrophage retention in the airspaces. Similarly, activated T-cells induce hyaluronan production by human fibroblasts that results in cable-like structures of matrix that trap monocytes [57]. IL-1β also induces a hyaluronan-rich ECM from fibroblasts that promotes monocyte binding [58].

The retention of cells by altered matrix architecture raises two important considerations: 1) is chronic lung disease a truly inflammatory scenario or simply a reflection of cell retention; and 2) does an exacerbation represent excessive cell recruitment during infection or is activation of immune cells already present?

The potential influence of ECM alterations on the lung microbiome

The lower respiratory tract maintains diverse communities of bacteria, and colonisation does not always induce infection as host–microbiome interactions play a role during health and disease [59]. A dysbiosis in bacterial diversity, however, is recognised to be a major contributor to infection, inducing epithelial injury and pro-inflammatory reactions and aggravating chronic diseases, including COPD. Altered matrix is likely to impact on the lung microbiome. Bacteria have an affinity for different substrates for optimal growth in *in vitro* culture conditions, and changes in lung matrix mirror such an environment supporting an imbalance in microbiota. Normal microbial samples vary widely in bacterial density and diversity. However, samples from sputum, lung tissue, bronchial brushing and bronchoalveolar lavage commonly reveal the presence of *Pseudomonas, Streptococcus, Prevotella, Fusobacterium, Haemophilus, Veillonella* and *Porphyromonas* [60–62], and other potentially pathogenic microorganisms (*Enterobacteriaceae,*

Haemophilus, Methylobacterium, Ralstonia and *Tropheryma*) [59, 61]. In mild-to-moderate COPD, 16S rRNA qPCR and sequencing show the *Streptococcus* genus is common in oral, nasal and different lung sites. Though aspiration seeds some of the microbiota in the lung, it accounts for 30% of the bacterial microbiome [63].

Whether the diversity of microorganisms in the lung increases or decreases in COPD is a matter of debate and may reflect differences in disease severity, age and sex [61, 62]. Changes to the ECM and effects on the composition of the microbiome are unknown. Classifying the bacterial microbiome alone is complex due to the differences in taxa between individuals and the overlap with health, COPD and COPD with disease progression. Bacterial diversity is further complicated by distinct heterogeneity in microbiota between lobes of the lungs [60, 63], and by more profound alterations in the oral bacterial community [59]. Despite the complexity in defining healthy bacterial taxa, dysbiosis of microbial commensalism can significantly impact on the overall health and progression of disease as demonstrated in other sites rich in microbiota, such as the gut. Bacteria and bacterial products induce phenotypic and functional changes in immune pro-inflammatory gene expression, and also modify cellular adhesion, migration and cell death [64]. Binding affinity to different components of the ECM allows bacteria to adhere to host tissue. An alteration of matrix may therefore increase bacterial virulence through host-pathogen accessibility. For example, *S. aureus* expresses fibronectin binding proteins that facilitate binding to fibronectin [65] and fibrinogen [66]. *S. aureus* also expresses bacterial elastin binding proteins, a matrix protein increased in lung tissue [67]. A number of bacteria have genes for elastase [67], which increases elastin availability and consequently bacterial binding. Bacteria also express collagen receptors that facilitate host–pathogen binding [65].

Though not discussed in detail in this short review, glycosaminoglycans (GAGs) are another common ECM component dysregulated in COPD. GAGs are widely distributed and function in cellular and extracellular signalling in all biological processes. Bacteria express GAGs and are used to inhibit phagocytosis by increasing molecular weight; for example, *Streptococcus* has a cellular coating of high-molecular weight hyaluronan [68]. Manipulation of GAGs, by removal of heparin sulfate or reducing its synthesis, inhibits attachment of *S. aureus* and *S. pneumonia* in lung epithelial cells and fibroblasts [69]. GAGs may act a possible binding substrate, increasing vulnerability during chronic inflammation and disease. Though there are studies on changes in the ECM and microbiome during disease, the interaction between the two is unclear and warrants further study. Changes in matrix composition and the altered immunological environment promote vulnerability towards adverse microflora.

Conclusion

Aberrant repair is a common feature of many lung pathologies and is equally as detrimental as inadequate repair. Aberrant repair alters the physical properties of the lung and limits airflow and, therefore, therapeutics specifically targeting repair could restore lost lung function. Much emphasis has been placed on targeting MMPs and other proteins that regulate the breakdown of ECM to prevent the production of inflammatory products [70]. Another strategy, however, would be to prevent the excessive production of high-molecular weight matrix in the first place. To target this aspect, a detailed knowledge of matrix production, the molecular processes leading to its cross-linking, and the relative importance of different matrix components are required. To capture the complexity of the lung microenvironment candidate-based investigations are limiting. Recent proteomic studies using label-free mass spectrometry has enabled characterisation of mouse lung ECM [71], an approach that is now being applied to the human lung [72].

Conflict of interest: None declared.

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