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EXTRACELLULAR MATRIX REMODELLING IN COPD

Gunilla Westergren-Thorsson,[1] Leif Bjermer,[2] *Oskar Hallgren[1,2]

1. Department of Experimental Medical Science, Lund University, Lund, Sweden
2. Department of Respiratory Medicine and Allergology, Skåne University Hospital, Lund, Sweden
**Correspondence to oskar.hallgren@med.lu.se*

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease that involves major remodelling of cells and tissue in the lung. There has been much focus on the inflammatory response, while less attention has been paid to structural changes and alterations of the extracellular matrix (ECM). In this review we revisit some of the latest findings on what is currently known about ECM alterations in COPD. We also discuss mechanisms involved in tissue repair and pay extra attention to the potential role of glycosaminoglycans in the disease.

Keywords: Chronic obstructive pulmonary disease (COPD), airway remodelling, extracellular matrix, collagen, proteoglycan, glycosaminoglycan, matrix metalloproteinase.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterised by a slowly progressive development of airflow limitations that have been considered to be poorly reversible.[1] This dogma has been questioned in several studies both in patients with mild disease and moderate-to-severe disease, and so has the assumption that the use of inhaled bronchodilators to measure improvement in forced expiratory volume in 1 second can differentiate COPD patients from asthma patients.[2-4] However, the airflow limitations in COPD are caused by loss of elastic recoil, degradation of alveolar walls (i.e. emphysema), and increased resistance in conducting airways. It is also associated with a wide range of phenotypical changes of structural cells and extracellular matrix (ECM) alterations. The major risk factor for developing COPD in developed countries is cigarette smoking although there are also other risk factors, including air pollution, exposure to indoor burning fuels, and occupational exposure.[5] In the lung, repeated exposure to noxious particles, such as cigarette smoke, has been suggested to trigger an immunological response elicited by the epithelial lining.[6] Epithelial cells signal to leukocytes and underlying mesenchymal cells that are recruited and activated; this starts a repair process. During normal tissue repair, the process is terminated when the initial trigger is removed; however, when the trigger remains, as is the case in smokers that are continuously exposed to cigarette smoke, the process may become pathological.[7-9] However, different repair processes may be active in different parts of the lung as the lung architecture, including the ECM scaffold, differs very much throughout the airway tree. This hypothesis was confirmed by Churg et al.[10] who suggested that genes involved in tissue repair are differentially expressed in small airways and in the lung parenchyma following exposure to cigarette smoke. Genes that promoted ECM production and deposition were upregulated in small airways but were at baseline levels or downregulated in the parenchyma. There may also be a difference between central airways and the parenchyma, as several studies have identified phenotypically unique sub-populations of fibroblasts, a cell type that is a key player in tissue repair, in central airways, and in the parenchyma.[11-14]

Moreover, in parallel with the structural remodelling of the airway tree and the subsequent airflow limitations, many patients also have remodelling of the lung vasculature, which often results in pulmonary arterial hypertension (PAH).[15] These two processes are very tightly connected and it has been shown that vascular remodelling in COPD patients is similar to what is observed in high-

altitude natives, which indicates that alveolar hypoxia can be one cause of these changes.[16] In addition, the degree of hypoxaemia has been shown to be related to the increased pulmonary arterial pressure and vascular resistance in COPD patients.[17] Although it is very probable that vascular and airway remodelling are two sides of the same coin, less attention has been paid to ECM changes in lung vasculature in COPD patients. Studies on the interplay between these two processes will therefore be important to better understand the complexity of the disease. The subject of vascular remodelling in COPD is beyond the scope of this text and has been reviewed elsewhere.[15]

The turnover of ECM is influenced by two processes: production and degradation. Mesenchymal cells, such as fibroblasts and smooth muscle cells, are the bulk producers of ECM components, but other cell types also contribute to the production of more specialised ECM molecules, including components of basement membranes. Moreover, the degradation of the ECM is mainly dictated by the delicate balance between proteases and anti-proteases, and especially matrix metalloproteinases (MMPs) and their natural inhibitors, tissue inhibitor of metalloproteinases (TIMP).[18] Since the discovery that lack of the protease inhibitor α1-antitrypsin may cause emphysema, many efforts have been focused on the influence of these molecules on the disease.[19] Clinical trials and animal models that target proteases and MMPs have so far been less successful in limiting the clinical manifestations of the disease.[20]

There have been many studies with the aim to characterise structural remodelling and ECM alterations in COPD, but many of the data generated in these papers are not consistent. One reason for this is that COPD is a very heterogeneous disease with varying degrees of emphysema, fibrosis, and small airway involvement. To further understand the dynamics of structural remodelling in COPD it is therefore crucial to further phenotype patients in the studies. Another source of variability is that immunohistochemistry has been used for identification and quantification of the ECM proteins in many studies. Since different antibodies have different binding capacity to antigens, and the preparation of the tissue may vary between different laboratories, this may contribute to the variability between studies. Herein we review some of the latest findings on what is currently known about ECM alterations in COPD.

ECM

ECM consists of a large number of fibrous proteins, including collagens and elastin, but also glycoproteins (GPs) and proteoglycans (PGs). Fibrous collagens, such as collagen Type 1 and 3 form rope-like fibril bundles that give the tissue stability and strength. Elastin, the most abundant protein in elastic fibres, contributes to the elastic properties of the lung. Other important players of the ECM are the PGs that are found in the ECM, in basement membranes and on cell membranes. They have long unbranched polysaccharide side-chains, glycosaminoglycan (GAG), attached to the core protein.[21,22] GAG chains are negatively charged at neutral pH due to uronic acid groups and varying amounts of sulphate bound to the polymer which makes them very bioactive. However, it is not only the degree of sulphation, but also the location of sulphate groups that dictates the bioactivity of GAGs.

Depending on the content of the polysaccharide chain there are several types of GAGs: heparan sulphate (HS), heparin, chondroitin sulphate (CS), dermatan sulphate (DS), keratan sulphate (KS), and hyaluronic acid (HA).[23] They serve as a tissue reservoir that binds and immobilises growth factors, cytokines, and proteases that can be released and form local gradients within the tissue, and may contribute to diverse functions such as chemotaxis, proliferation, and differentiation. Due to their negative charge, they bind water and thereby influence the viscosity of the tissue.[24,25] Moreover, GAGs have been shown to interact with and influence the bioactivity of several mediators involved in the COPD pathogenesis. Growth factors involved in tissue repair that may induce fibroblast differentiation and altered production of ECM proteins, including tumour growth factor-beta, connective tissue growth factor, fibroblast growth factor, hepatocyte growth factor, and platelet-derived growth factor, have been shown to bind to GAGs.[26-28]

In COPD, activated epithelial cells and macrophages release chemotactic mediators such as tumour necrosis factor-alpha, chemokine (C-C motif) ligand 2, chemokine (C-X-C motif) ligand 1 (CXCL1), CXCL8, CXCL9, CXCL10, and CXCL11 that form gradients to attract leukocytes and lymphocytes to the site of inflammation. To retain gradients and protect mediators from proteolysis they readily bind to HS and/or CS/DS-GAGs in the

ECM.[29-32] Importantly, this interaction depends on the disaccharide composition within the GAG sequence in addition to merely the charge. GAGs also bind and regulate the activity of many extracellular proteases. One example is HS which has been demonstrated to be a natural inhibitor of neutrophil elastase.[33] Furthermore, the activity of MMP-2 and MMP-9 has been shown to be influenced by GAG. While some GAGs - HS, DS, and CS - increase the activity of MMP-2 and MMP-9, KS decreases the activity.[34]

The view of proteins belonging to the ECM is normally limited to collagens, elastin, PGs, and GPs. However, Naba and co-workers[35-37] suggested that a wider range of proteins should be sorted into this compartment. By mass spectrometry and a subsequent *in silico* bioinformatic approach to analyse the non-cellular part of tissue, i.e. the ECM, they identified the proteins mentioned above but also additional groups of proteins, including ECM-associated proteins and secreted factors, which they collectively called the matrisome. In total, 1,050 proteins were predicted to belong to the matrisome including cytokine growth factors and proteases. Importantly, the additional proteins that are resident in the matrisome may also contribute to ECM property and function, but have so far been overlooked in this context in most studies.

ECM ALTERATIONS IN COPD

Bronchus and Bronchioles

Thickening of the reticular basement membrane in central airways is an established part of the histopathology in asthma, but lack of unequivocal data makes it less clear in COPD.[38-40] In studies by Sohal and co-workers[41] it was shown that the bronchial and bronchiolar reticular basement membrane is fragmented in current smokers, and especially in COPD patients. In the fragmented regions cells positive for both epithelial and mesenchymal markers, the hallmark for epithelial mesenchymal transition (EMT), were observed.[42,43] These data were confirmed in a study showing that freshly isolated primary bronchial epithelial cells show an increase in the EMT expressing profile. In central airways in patients with mild-to-moderate disease, collagen Type 1 and 3 is increased in basement membranes, lamina propria, and in the adventitia.[39,44,45] These changes were negatively correlated to lung function parameters. An increase in total collagen has been recorded also in bronchiolar tissue in moderate and severe disease.[46,47] The increase was due to an increased staining area of collagen Type 1 and 3 at the expense of other collagens. In parallel, the total amount of bronchiolar tissue was increased in moderate COPD patients while, in more severe disease, the amount is comparable to control subjects. In severe disease the level of total collagen decreases to levels lower than control subjects, suggesting that there may be different processes active at different parts of the disease.

The amount of the small PGs decorin and biglycan has been shown to decrease in the adventitia of bronchioles and in alveolar walls in patients with COPD, and especially in more severe disease.[48,49] Biglycan and decorin play an important role in cross-linking collagen fibrils, and this finding may, therefore, indicate loss of tissue integrity. These data do not agree with a study by Annoni et al.[50] who did not see any difference in the staining of decorin and biglycan in central or small airways, but the patients had milder disease in this study. The volume fraction of the GPs tenascin and fibronectin were, on the other hand, elevated in bronchi and bronchioles, which has also been shown by others.[39,51] These proteins have demonstrated to be elevated following tissue injury and inflammation, regulating cell adhesion and differentiation.[52] The non-sulphated GAG HA has been shown to increase in both alveolar tissue and in bronchioles in COPD.[46] HA has, in an animal model, been shown to have a protective role in bronchial tissue by blocking bronchial obstruction caused by inhaled elastase.[53,54] In a study by Kunz and co-workers[55] the effect of inhaled corticosteroids on expression of ECM proteins in COPD patients was examined. Tissue staining for collagen Type 3 and versican increased in bronchial biopsies following corticosteroid treatment for 18 months. The data suggest that long-term treatment with steroids can influence the remodelling process in the airways.

Alveolar Tissue

Elastic fibres in the alveoli of COPD patients have been demonstrated to be abnormal and partly fragmented.[56] In addition it has also been shown that the volume fraction of elastic fibres is decreased in alveolar tissue and bronchioles in COPD patients.[57-59] This has been confirmed in other studies that show a decrease in elastin, the major component in elastic fibres, in alveoli and small airways in COPD patients of varying severity.[46,60] In agreement with these findings, fibroblasts isolated

from distal lung from COPD patients have impaired capability to produce elastin in response to TGF-β1.[61] Furthermore, a decreased amount of elastin has been suggested to be linked to an increase in the PG versican in alveolar walls. The increased versican is associated with a decrease in elastin binding protein that is required to assemble *de novo* produced elastin and other components of elastic fibres.[60] These data are in agreement that fibroblasts isolated from alveolar tissue from COPD patients have increased production of versican.[11] This is an example of the impaired repair function of fibroblasts in COPD that have been described by many groups.

Togo and colleagues[62] have shown that fibroblasts from mild-to-moderate COPD patients have impaired potential to migrate and contract. However, fibroblasts from severe COPD patients have been shown to be more contractile, suggesting that there may be different processes in different severities of the disease.[12] The levels of the eicosanoid prostacyclin have been shown to be elevated in COPD, and fibroblasts from severe COPD patients respond improperly to iloprost, a prostacyclin analogue, which could lead to impaired collagen network fibrillogenesis.[63] In line with these results, it has been shown by several groups that fibroblasts isolated from COPD patients show markers of senescence and are less prone to proliferate.[61,64-66] Van Straaten and coworkers[49] have also found diminished staining of HS PGs (perlecan, agrin, and collagen Type 18) in alveolar basement membranes, which may be an indication of alveolar damage.[67] So far, less is known about the GAG moiety of PGs in COPD patients, although both the amount of GAGs and the degree of sulphation are increased in other chronic airway diseases, including asthma and idiopathic pulmonary fibrosis. In addition, patients with idiopathic PAH have been shown to have increased levels of HA.[68,69]

MMPs in COPD

Since the breakdown of alveolar tissue and ECM is one of the major causes of airflow limitations in COPD, much effort has been made to examine the role of proteases and MMPs in particular. MMPs do not only contribute to the breakdown of the ECM in alveolar tissue but also to the remodelling process in bronchi and bronchioles, including to goblet cell metaplasia.[70] MMP-2 has been shown to increase in bronchioles and in surrounding parenchyma in COPD patients.[71,72] Increased levels of MMP-9 have been observed in bronchoalveolar lavage fluid from patients with emphysema.[73,74] Mercer and co-workers[75] have shown that the ratio between MMP-9 and its natural inhibitor, TIMP-1, becomes skewed in the direction of MMP-9 during an exacerbation, but during stable disease the relative level of MMP-9 decreases and the level of TIMP-1 increases. MMP-1 and MMP-8 have also been found to be elevated in COPD.[76-78] Further supporting the role of MMPs in COPD is that single nucleotide polymorphisms in the genes of MMP-1, MMP-2, MMP-9, and MMP-12 have been shown to be linked to disease.[79]

CONCLUSION

Many of the airflow limitations in COPD are caused by structural remodelling resulting from an imbalance between synthesis and breakdown of ECM proteins. However, the involvement of these two counteracting processes is different throughout the airways as there is often excessive deposition of ECM in bronchi and bronchioles and loss of alveolar tissue. In addition, the involvement of these processes varies between different patients because of the great heterogeneity of the disease. To deepen the understanding of the dynamics and impact of these changes, there is therefore a great need to develop better tools for clinical and molecular phenotyping of patients. Moreover, since structural remodelling is an endogenous physiological response, it cannot be excluded that it may not always be detrimental but also protective, at least in some windows of disease progression. These possibilities should be considered in the development of new therapeutic approaches. One group of molecules that has so far been neglected in the exploration of structural remodelling in COPD are GAGs, the carbohydrate moiety of PGs. These molecules have been shown to interact with many cells and mediators that drive the disease, and should therefore be given more attention in the future.

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