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Small airway fibrosis in COPD

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is characterised by an accelerated decline in airway function with age compared to age-matched non-smokers. There is increasing evidence that this is due to small airway disease rather than from emphysema, especially in the early stages of the disease. Small airways (< 2 mm internal diameter) are narrowed in COPD with thickening and distortion of the airway wall and peribronchiolar fibrosis. In addition, loss of elasticity in alveolar attachments and mucus hypersecretion contribute to the airway narrowing and closure, leading to air trapping. The mechanisms of peribronchiolar fibrosis are poorly understood and small airway fibroblasts have not been characterised. In small airways of COPD patients the fibroblasts are profibrotic, pro-inflammatory and senescent. There is a reduction in the anti-ageing molecules sirtuin-1 and -6, which are regulated by specific microRNAs that are increased in COPD cells. It is plausible that extracellular vesicles from senescent airway epithelium transmit senescent signals to airway fibroblasts to stimulate fibrosis and inflammation. Small airways fibrosis is a target for new drug development that inhibit growth factor receptors, new antioxidants and particularly those that are targeted to mitochondria and inhibitors of cellular senescence or senolytic therapies.

1. Introduction

Professor Geoff Laurent, previous editor of this journal, was an outstanding respiratory scientist, who provided important new insights into chronic lung diseases, and particularly those that involve fibrosis of the lungs (Laurent et al., 2007). His research laid the foundations for current understanding of fibrosing lung diseases, particularly idiopathic pulmonary fibrosis (IPF), which has now become a major focus for research (Maher et al., 2007). Chronic obstructive pulmonary disease (COPD) is not usually regarded as a fibrotic lung disease and the main abnormality in the lung parenchyma is destruction of alveoli (emphysema) rather than fibrosis, although is some patients emphysema and lung fibrosis may occur together –the rare combined pulmonary fibrosis emphysema (CPFE) patients (Alsumrain et al., 2019). However, fibrosis appears to be a major mechanism for the narrowing of small airways, which is now recognised as probably the earliest and most important mechanism for COPD progression (Hogg et al., 2017). For this reason, I have focussed on what is known about small airway fibrosis in COPD, although this area of research has been surprisingly neglected, so there are no relevant animal models and little is understood about the mechanisms of small airway fibrosis.

COPD has now become a global epidemic and affects over 300 million people worldwide. It is now the third most prevalent cause of death globally, a leading cause of hospital admission and the fifth ranked cause of disability (Barnes et al., 2015; Global Burden of Disease Collaborators, 2017). COPD is increasing even in high income countries as populations age and mortality from other common causes of death decrease. The increasing prevalence of COPD in low-to-middle income countries is even greater as smoking is increasing, especially in women, and a large proportion of the population is exposed to household air pollution and traffic pollution (Salvi and Barnes, 2009; Sood et al., 2018). COPD is difficult to manage as long-acting bronchodilators, which are the mainstay of current management, do not treat the underlying progressive disease process and corticosteroids, which are highly effective anti-inflammatory treatments in asthma, provide little clinical benefit for most patients. It has proved difficult to develop effective and safe anti-inflammatory or disease-modifying treatments as underlying disease mechanisms are not well understood and the heterogeneity of the disease makes it difficult to select the right patient population (Barnes, 2013). More research is needed on underlying disease mechanisms and a molecular and cellular level in order to identify new therapeutic targets for future drug development.

2. Pathophysiology of COPD

There are three major pathological mechanisms in the lungs of

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COPD patients, although their relative proportions may vary between patients (Hogg and Timens, 2009). Chronic bronchitis involves goblet cell hyperplasia with increased mucus secretion which may predispose to bacterial colonisation and acute exacerbations (Boucher, 2019). Emphysema is due to destruction of alveolar walls, resulting in reduced gas diffusion and appears to be a late feature of the disease in most patients. Although associated with cigarette smoking, it is rarely seen in COPD associated with exposure to biomass smoke exposure in developing countries (Zhao et al., 2018). By contrast, small airway disease is an early feature of COPD and is linked to disease progression in both smoking and biomass exposure COPD. This has focussed attention on the mechanisms of small airway obstruction in COPD (Hogg et al., 2017). Serial measurements of small airway obstruction using high resolution computerised tomography (HRCT) scans suggest that small airway disease is the earliest feature of COPD and is the main determinant of progressive airways obstruction (Galban et al., 2012). Histological studies show that there is widespread narrowing and fibrosis of small airways in COPD patients and, even in early COPD, there is a marked loss of small airways, which further emphasises that small airway obstruction is an early feature of the disease (Hogg et al., 2004; McDonough et al., 2011). Detailed analysis of lung sections from COPD patients in combination with micro-CT has shown a 40% loss of terminal bronchioles in patients with mild COPD accompanied by a 33% reduction in alveolar surface area, both increasing with disease severity. However much of the small airway disease occurs in areas of lung unaffected by emphysema. The remaining small airways are narrowed with thickened walls, which become more obstructed with increasing disease severity, so that 100% of terminal bronchioles are obstructed in very severe COPD (Koo et al., 2018). These obstructed airways show a marked increase in fibrosis with collagen deposition and infiltration with inflammatory cells. Thus, evidence now strongly suggests that small airway disease may be an early feature of COPD and precedes emphysema. In order to prevent progression of COPD in the future it will be necessary to target small airway disease.

3. Small airway obstruction in COPD

Small airways of less than 2 mm internal diameter offer less than 10% of airway resistance in normal lungs due their very high number, but become a major site of airway obstruction in COPD patients. This has been confirmed by measurement of airway resistance by direct measurements of intrabronchial pressure (Yanai et al., 1992). Several ways of measuring small airway obstruction in COPD patients have now been developed (Ostridge et al., 2019). The forced oscillation technique (FOT) using impulse oscillometry is relatively easy for patients to perform, as it is measured during tidal breathing and shows evidence of marked small airway disease, with an increase in peripheral airway reactance (AX) (Lipworth and Jabbal, 2018). Multiple breath nitrogen washout measures peripheral airway inhomogeneity (Sacin) and this is more closely linked to emphysema (Bell et al., 2018). Closure of small airways on expiration results in gas trapping which can be measured using HRCT with inspiratory and expiratory scans and correlates with the FOT measurements (Ostridge et al., 2019). Longitudinal analysis of parametric response mapping of the difference in inspiratory and expiratory HRCT scans suggests that small airway disease is a transitional phase between normal airways and the development of emphysema in severe disease (Boes et al., 2015; Galban et al., 2012).

There are several mechanisms that may contribute to obstruction of small airways in COPD (Fig. 1) (Barnes, 2004). Goblet cell hyperplasia may lead to occlusion of the lumen with tenacious mucus that is difficult to clear, particularly as they may be dysfunction of cilia and replacement of ciliated small airway epithelial cells with goblet cells (Saetta et al., 2000). Cilia may be shortened and dysfunctional in the peripheral airways of COPD patients and this may result in mucus retention in peripheral airways (Leopold et al., 2009). In COPD there is an increase in MUC5AC and MUC5B mucins which makes the mucus more viscous and difficult to clear (Boucher, 2019; Fahy and Dickey, 2010; Kesimer et al., 2017) This mucus hypersecretion may involve activation of epidermal growth factor receptors (EGFR), which are activated in small airway epithelial cells in COPD patients (Takeyama et al., 2001). More importantly, the wall of the airway is thickened and encroaches on the lumen as a result of inflammatory cell infiltration and fibrosis. There are increased numbers of macrophages, CD4+ and CD8+ lymphocytes and B-lymphocytes in small airways of COPD patients, (Hogg et al., 2004). The increase in B-lymphocytes is correlated with a loss of alveolar attachments in terminal bronchioles (Tanabe et al., 2018). B-cells, T-cells sand dendritic cells are organised into well-defined lymphoid follicles which increase with disease severity (Seys et al., 2015). Peribronchiolar fibrosis is a typical feature of the remaining small airways in COPD patients (Hogg et al., 2004; Koo et al., 2018). Another contributory mechanism to gas trapping is the loss of alveolar attachments to small airways and to alveolar elasticity as a result of emphysema, which is associated with the breakdown of elastin fibres and destruction of alveolar walls. Gas trapping worsens with exercise, leading to dyspnoea on exertion, the major symptom of COPD patients.

4. Small airway fibrosis in COPD

The mechanisms of small airway fibrosis in COPD are poorly understood. Cigarette smoke extract induces oxidative stress and apoptosis of human lung fibroblasts (Carnevali et al., 2003). COPD lung fibroblasts show defective repair functions and contraction of collagen gels (Togo et al., 2008). Very little is known about the function of fibroblasts in small airways or whether these differ from interstitial lung fibroblasts which have been extensively investigated. There is increasing evidence for fibroblast heterogeneity, even within the same tissue and this has important implications for targeting fibroblast subtypes in future therapy (Lynch and Watt, 2018). It is not yet known whether small airway fibroblasts (SAF) differ from interstitial lung fibroblasts and lung fibroblast cell lines that have been characterised to date.

SAFs from COPD patients showed reduced proliferation and increases expression of senescence markers compared to age-matched normal smokers and non-smokers (Wrench et al., 2018). In addition, these cells secrete increased collagens 1A1 and 3A1 and increased matrix metalloproteinases (MMP2, MMP9). Oxidative stress induces cellular senescence markers in SAFs from COPD patients with release of transforming growth factor-β (TGFβ) and COL3A1 (but not COL1A1) and proinflammatory mediators, such as CXCL8, whereas there is a reduction in the antioxidants superoxide dismutase(SOD)-2 and -3 (Wrench et al., 2019).

5. Mechanisms of small airway fibrosis

Oxidative stress is increased in the lungs of COPD patients and persists on smoking cessation due to the production of reactive oxygen species by inflammatory cells. It drives many of the inflammatory mechanisms in COPD and is a stimulus for the release of profibrotic mediators, such as TGF-β from airway epithelial cells (Fig. 2) (Kirkham and Barnes, 2013). Airway epithelial cells may release several profibrotic mediators. Increased expression of TGF-β has been reported in small airway epithelial cells of COPD patients compared to control subjects (de Boer et al., 1998). In small airway epithelial cells sampled by fine bronchoscopy TGF-β1 was increased in COPD and normal smokers compared to non-smoking control subjects (Takizawa et al., 2001). Cultured airway epithelial cells from COPD patients showed increased epithelial-mesenchymal transition and increased release of TGF-β, which was correlated with the degree of peribronchiolar fibrosis and airway obstruction (Gohy et al., 2015). However, a more detailed study recently shown that there is a surprising reduction in expression of TGF-β1 protein and the downstream cytokine connective tissue growth factor (CTGF) in small airway epithelial cells of COPD patients

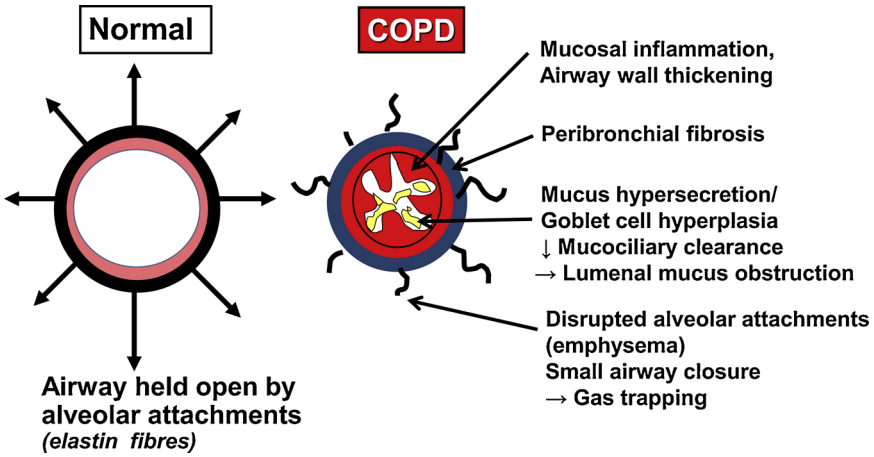

Fig. 1. *Mechanisms of small airway narrowing in COPD.* Normal small airways have a thin wall and are held open by alveolar attachments that contain elastin fibres. In COPD small airways are narrowed as the airway wall is thickened as a result of inflammation and there is progressive peribronchiolar fibrosis. In addition, the lumen is occupied by tenacious mucus as a result of goblet hyperplasia, mucus hypersecretion and impaired mucociliary clearance, as well as loss of alveolar attachments due to emphysema, which results in airway closure on expiration and gas trapping in the lungs.

(Di Stefano et al., 2018). In this study there appears to be increased expression of TGF-β3 in the lamina propria in COPD patients, suggesting that it has been released into the small airways. In pulmonary fibroblasts from COPD patients there is a reduced response of downstream SMAD3 in response to TGF-β which is consistent with the loss of connective tissue in the lung parenchyma, but as discussed above, SAFs may differ from interstitial lung fibroblasts (Zandvoort et al., 2008). It is possible that TGF-β is released early in the course of the disease or that other fibrogenic mediators are playing a more important role.

Endothelin may be released from human airway epithelial cells and is increased in the sputum of COPD patients (Chalmers et al., 1999; Mattoli et al., 1990). Endothelin may stimulate epithelial cells to produce fibronectin and activates fibroblasts via receptor ET_A receptors. Mechanical stress of airway epithelial cells may release endothelin and TGF-β and could be relevant in peripheral airways of COPD patients due to the mechanical stress of airway closure on expiration (Tschumperlin and Drazen, 2006; Tschumperlin et al., 2003). If this were the case, long-acting bronchodilators, such as long-acting muscarinic antagonists (LAMA) and long-acting β_2-agonists (LABA) could theoretically reduce small airway fibrosis through reducing the mechanical stress on small airway epithelial cells. There is some evidence that bronchodilators may reduce airway wall remodelling, including collagen deposition under the epithelium, independent of any anti-inflammatory effects (Grainge et al., 2011).

6. Cellular senescence in COPD

There is increasing evidence that COPD represents accelerated ageing of the lung, with the accumulation of senescent cells (Barnes, 2017; Barnes et al., 2019). Small airway epithelial cells from COPD patients show increased senescence with reduced cellular growth and increased expression of senescence markers such as the cyclin-dependent kinase inhibitors $p16^{INK4a}$ and $p21^{CIP/WAF}$ with a reduction in the anti-ageing molecules sirtuin-1 and sirtuin-6 (Baker et al., 2019, 2016). These senescent cells also release a spectrum of inflammatory proteins known as the senescence-associated secretory profile (SASP), including IL-6, CXCL8, MMP2 and MMP9, all of which are known to be increased in COPD lungs. Plasminogen activator inhibitor-1 (PAI-1) is a characteristic SASP molecule that is also increased in sputum and peripheral lung of COPD patients (To et al., 2013). PAI-1 has been recruited in local lung fibrosis through the recruitment and binding of vimentin but its role in small airways has not been studied (Schuliga et al., 2018).

MicroRNAs play a key role in linking oxidative stress to senescence through inhibiting sirtuins-1 and -6. In COPD small airway epithelial cells there is an increase in miR-34a via activation of the phosphoinositide-3 kinase (PI3K)-mTOR (mammalian target of rapamycin), with parallel reduction of sirtuins-1 and -6. MiR-517 is also increased in COPD lungs via p38 MAP kinase and activator protein-1 signalling and reduced sirtuin-1. Specific antagomirs that block these miRNAs are able to restore sirtuins, reduce markers of senescence and the secretion of SASP mediators and also restores cellular growth (rejuvenation effect)

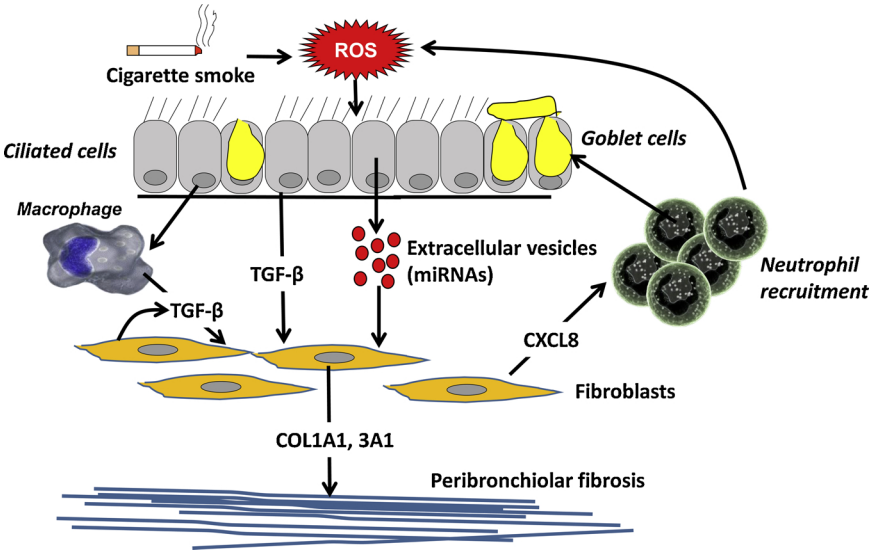

Fig. 2. *Mechanisms of fibrosis in small airways of COPD.* Cigarette smoke and reactive oxygen species (ROS) stimulate airway epithelial cells to release fibrogenic mediators, such as transforming growth factor(TGF)-β, which stimulates small airway fibroblasts to produce collagens, such as collagen(COL)1A1 and 3A1 (and other matrix proteins), resulting in peribronchiolar fibrosis. Fibroblasts also release inflammatory mediators, such as CXCL8, which recruits neutrophils to the airways, which then stimulate mucus secretion from goblet cells and provide additional ROS. Epithelial cells release extracellular vesicles, which contain RNA species such as microRNAs that may activate and induce senescence in fibroblasts.

(Baker et al., 2019, 2016). In SAFs from COPD patients there is also increased expression of miR-34a, increased markers of senescence and reduced sirtuin-1, which is restored by an antagomir of miR-34a (Wrench et al., 2017). MiRNAs are exported from cells in extracellular vesicles, which include exosomes and microvesicles (Kadota et al., 2018), so it is feasible that senescent small airway epithelial cells exposure to oxidative stress in the airway lumen release vesicles containing miR-34a and -507, which are taken up by fibroblasts to induce senescence and fibrosis. (Fujita et al., 2015).

7. Therapeutic implications

In view of the key role of small airway fibrosis in disease progression, particularly in the early stages in COPD, it is surprising that there have been so few studies in COPD patients. The mechanisms of peribronchiolar fibrosis remain to be elucidated, but it now seems likely that SAFs may play a key role through the release of profibrotic mediators, inflammatory proteins and MMPs (Kadota et al., 2018). It is possible that small airway epithelial cells that are exposed to an oxidative stress in the airways from cigarette smoking or biomass smoke exposure may release inflammatory signals that activate fibroblasts and these signals may include miRNAs released in extracellular vesicles that are taken up by local fibroblasts, including the miRNAs 34a and -570 that promote cellular senescence. This has suggested that antagomirs of these miRNA might be effective against cellular senescence in peripheral lung of COPD patients, especially if they can be derived by inhalation with sequences that promote cell penetration or in the form of therapeutic exosomes (Zhang et al., 2018).

It is likely that oxidative stress is an important driving mechanism of small airway fibrosis and may further enhance fibroblast senescence (Wrench et al., 2019). There is a need for more effective antioxidants, and particularly mitochondria-targeted antioxidants as mitochondrial dysfunction in COPD cells is largely due to reactive oxygen species derived from dysfunctional mitochondria (Murphy, 2014; Zinovkin and Zamyatnin, 2019). It has been difficult to find drugs that target fibrosis and several studies have shown that anti-TGF-β antibodies and inhibitors of TGF-β signalling are so far ineffective in fibrotic diseases.

A promising approach is to target cellular senescence, either by inhibiting pro-senescence signalling pathways such as PI3K-mTOR inhibitors or to induce apoptosis and cell removal with so called senolytic therapies (Barnes et al., 2019; Kirkland et al., 2017). Using the inhaled approach it may be possible to focus these treatments in small airways and to inhibit small airway epithelial cells and fibroblasts. The long-term safety of these approaches has not yet been explored, however.

As discussed above, mechanical stress may be an important driving mechanisms leading to local fibrosis, implying the long-acting bronchodilators might reduce small airway fibrosis, particularly if used in early disease. Treatment with the long-acting muscarinic antagonist tiotropium bromide appears to have a small effect on disease progression in moderately severe COPD (GOLD2), but not in more severe disease (GOLD3,4) (Decramer et al., 2009). Tiotropium and other LAMA may have direct effects on airway fibrosis. In a guinea pig model of COPD involving repeated lipopolysaccharide challenge inhaled tiotropium and reduce collagen deposition in the airways while having no effect on emphysema (Pera et al., 2011). This may reflect an inhibitory effect of tiotropium on muscarinic M_3-receptors that are expressed on fibroblasts (Meurs et al., 2013).

Clinical assessment of small airway fibrosis is challenging, especially for new drugs. At present there is no way of imaging small airway fibrosis, but it is possible that techniques to quantify collagen may be developed in future. Any effect on fibrosis would be expected to slow progression of COPD, but this can currently only be measured with serial FEV_1 measurements over 3 years. More sensitive measurements of small airway function may show more rapid changes that could be assessed over shorter periods.

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