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# Mechanisms Promoting Chronic Lung Diseases: Will Targeting Stromal Cells Cure COPD and IPF?

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## 1. Introduction

Tissue remodeling is a common pathology in many diseases. The reparative processes of wound healing result in an increase in extracellular matrix (ECM) generation, serving to restore barrier protection and normal tissue architecture. However in the lung, increased formation of ECM results in decreased lung compliance and impaired gas exchange. The chronic lung diseases COPD (chronic obstructive pulmonary disease) and IPF (idiopathic pulmonary fibrosis) both exhibit increased extracellular matrix (ECM) deposition within the lung due to increased stromal cell number and activation. The chronic nature of the disease is hypothesized to correlate with the extent of scarring and remodeling, with greater evidence being available for COPD rather than IPF. The potential underlying causes of both diseases are numerous, including direct insults to the lung such as cigarette smoke or exhaust particles; as well as underlying autoimmune conditions such as scleroderma or collagen vascular disease. Moreover, a correlation between COPD and smoking is well established; however, any causes for IPF, apart from a familial link, are currently not understood. To date there are no approved anti-fibrotic therapies that target the underlying remodeling in either disease. In order to treat this pathology, understanding the mechanisms promoting continual ECM deposition may elucidate novel therapeutic approaches that could provide clinical benefit to patients suffering from these debilitating diseases. This Chapter will focus on the cellular mechanisms and interactions within the fibrotic lung including resident fibroblast-epithelial cells as well as the cross-talk between fibroblasts and recruited bone marrow derived cells such as fibrocytes and monocytes. Also, the soluble mediators that have been associated with disease and how these can directly and indirectly modulate stromal cell activation will be discussed.

## 2. COPD and IPF disease pathogenesis

One of the common features between COPD and IPF is the heterogeneous pathology observed in the lung at the macroscopic level in both disease settings. Both diseases exhibit patchy areas of pathology, consisting of ECM proteins or inflammatory infiltrates, with

fibrotic regions being juxtaposed to regions of normal alveolar tissue or areas with interstitial leukocyte accumulation. Grossly, COPD contains regions dense in bronchiolar inflammation and consolidated lung tissue, as well as emphysematous areas, due to alveolar destruction<sup>1,2</sup>. In IPF, salient hallmarks of disease included profound collagen deposition, as well as regions of honeycombed lung due to a collapse of alveolar walls<sup>3</sup>. COPD has been clinically separated into distinct GOLD (Global initiative for chronic Obstructive Lung Disease) Stages based primarily on a key parameter of lung function, Forced Expiratory Volume in one second (FEV<sub>1</sub>). Here, patients with more significant impairment in FEV<sub>1</sub> have a higher GOLD Stage status. Supporting these changes in lung function analysis of the underlying pathologies of patients within each GOLD Stage has also highlighted that, as the disease progresses, there is an increase in airway wall thickness<sup>4</sup>. Even though the GOLD staging system is universally recognized, there are efforts underway to also separate COPD patients into fast decliners and slow decliners based on lung function parameters. Extensive work is currently underway to try and stage IPF in an equivalent manner. In this disease, retrospective analysis of patient survival has indicated that there is both a rapidly progressing phenotype and a more slowly progressing phenotype of IPF patients. In the 'rapid' IPF progressors, approximately 50% of patients will die within 6-8 months, whereas this is greatly extended in slow progressors<sup>5,6</sup>.

Chronic remodeling is a common feature between IPF and COPD. In order to understand the mechanisms underlying the maintenance, progression and staging of disease, this Book Chapter will focus on the similarities and differences between stromal cells in these patients. We will focus on the phenotypic differences in fibroblasts and myofibroblasts as well as how these cell types interact within the lung (Fig. 1). We will also describe some of the key families of mediators that are found to be elevated in disease and how their mechanism may be directly promoting chronic lung disease. Lastly, we will discuss the potential therapeutic approaches to targeting stromal cells clinically.

### 3. Profibrotic role of fibroblasts

Fibroblasts play a myriad of important roles in normal tissue function. In the lung they coordinate organogenesis and budding of the lung from the foregut through intimate bi-directional communication with adjacent epithelial cells. Myofibroblasts are "smooth muscle-like cells" that are morphologically similar to fibroblasts, but also express alpha-smooth muscle actin ( $\alpha$ -SMA)<sup>7</sup>. Fibroblasts and myofibroblasts are also key cells in the production and homeostatic maintenance of the ECM of the tissue or organ in which they reside. They are metabolically active cells, capable of synthesizing and secreting ECM components such as collagens and proteoglycans. Fibroblasts continually synthesize ECM proteins although the amount they secrete is tightly regulated, with up to 90% of all procollagen molecules being degraded intracellularly prior to secretion, depending on tissue and age. Further, fibroblasts generate both matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases, thus controlling homeostatic tissue architecture.

Myofibroblasts were first described by Gabbiani and colleagues as cells central to wound healing<sup>8</sup>. The actin filaments result in myofibroblasts having contractile properties, which, at sites of wound healing serve to close the wound. However, the presence of contractile myofibroblasts in the interstitium of the lung may cause a retraction of parenchymal tissue resulting in alveolar collapse giving the characteristic honeycombing as observed in the lungs of IPF patients, or add to the increase in alveolar size, which is characteristic of COPD<sup>9</sup>.

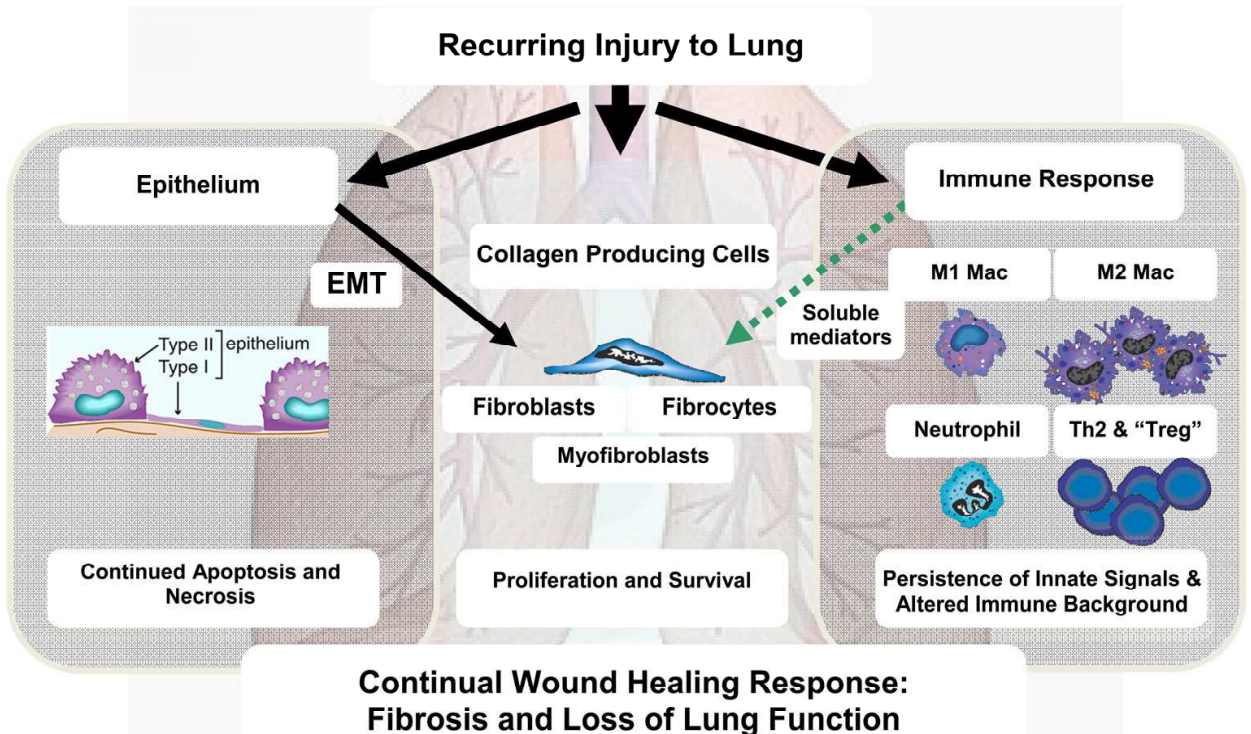


Fig. 1. *Cellular Pathways in Lung Fibrosis: Multi-Faceted Aberrant Response*

Chronic lung remodelling is hypothesized to occur following repeated trauma or injury to the lung. Multiple cellular pathways and interactions which can all promote ECM deposition have been described in lung fibrosis which promote fibroblast and myofibroblast activation, proliferation and survival. Following injury to the epithelium, apoptotic and necrotic signals stimulate collagen producing cells. These resident epithelial cells can also differentiate into a collagen-producing phenotype during EMT. The immune response in the lung is also altered in chronic lung diseases, with pro-fibrotic M2 macrophages and type 2 T cells predominating. These cells generate soluble mediators such as TGFβ, CCL2 and IL13 which directly activates collagen producing cells.

Key: EMT: epithelial to mesenchymal transition; Mac: Macrophage; Treg: regulatory T cell

Under normal conditions, myofibroblasts sequentially perpetuate and then dampen inflammation via the secretion of chemokines, cytokines, arachidonic acid metabolites and protease inhibitors. When activated, they express cell surface adhesion molecules allowing specific interactions with immune and inflammatory cells, including lymphocytes, mast cells and neutrophils. If these processes become dysregulated, fibrosis may ensue with catastrophic consequences for lung function.

Most insight into the potential role of fibroblasts at driving pulmonary remodeling, as well as phenotypic differences in fibroblasts found in fibrotic regions versus those located in normal tissue has been garnered from *in vitro* studies using fibroblasts isolated from IPF lungs and animal models. Fibroblasts isolated from fibrotic environments are phenotypically different than non-fibrotic fibroblasts<sup>10-12</sup>. Fibroblasts from a profibrotic environment exhibit altered responsiveness to growth factors, express enhanced receptor levels for chemokines, which has also been observed in murine models of pulmonary remodeling. These studies suggest a distinct heterogeneity in fibroblast function and phenotype in the fibrotic lung.



We and other investigators have reported that fibroblasts derived from IPF lungs proliferate faster than cells derived from normal lung tissue. In contrast, others have shown that the growth rate of IPF fibroblasts was significantly slower than normal fibroblasts. This discrepancy may be due to the site in the lung from which fibroblasts are harvested, since the magnitude of inflammation and fibrosis are heterogeneous in distribution. Thus, areas of active fibrosis may yield hyperproliferative fibroblasts compared to areas of established fibrosis in which cells may be hypoproliferative.

To begin to address this diversity, recent studies have used microarray technologies to profile global gene expression in pulmonary fibrosis in man and mouse models. These studies have showed expression of almost 500 genes are increased more than two-fold in fibrotic lungs, including many genes related to cytoskeletal reorganization, ECM, cellular metabolism and protein biosynthesis, signaling, proliferation and survival<sup>13,14</sup>. There was excellent concordance between gene expression in human and experimental models, giving us some confidence in the value of our efforts to model human disease. Studies examining human lung fibroblast global gene expression in response to TGF $\beta$ 1 have shown almost 150 genes upregulated, representing several functional categories described above. This included 80 genes that were not previously known to be TGF $\beta$ -responsive.

The progression and severity of many lung diseases, most notably IPF, are tightly associated with regions of fibroblast accumulation and proliferation, to the extent that these regions have become a reliable indicator of survival. The increased number of (myo)fibroblasts seen in these diseases, implies that they are either hyper-proliferative and/or resistant to apoptosis. However, most studies suggest that these cells proliferate faster than normal<sup>10,15,16</sup>

#### **4. Sources and fates of fibroblasts and myofibroblasts**

As remodeling of the lung is associated with accumulation and activation of fibroblasts and myofibroblasts, the derivation of both cell types is currently under examination. Reports indicate that there may be multiple pathways through which fibroblasts and myofibroblasts are derived<sup>17</sup>. Fibroblasts isolated from human or murine fibrotic tissue exhibit enhanced basal proliferation. Therefore an increase in the fibroblast pool could be due to local proliferation. Myofibroblasts express a panel of markers and these markers have been correlated with site of derivation. For example, myofibroblasts found in the peripheral and subpleural regions of fibrosis express  $\alpha$ -SMA, vimentin and desmin, whereas cells found in other regions of the lung do not express desmin<sup>18</sup>.

##### **4.1 Epithelial to mesenchymal transition**

Another potential source of fibroblasts is by a process called epithelial to mesenchymal transition (EMT). EMT is a dynamic process by which epithelial cells undergo phenotypic transition to fully differentiated motile mesenchymal cells, such as fibroblasts and myofibroblasts. This process occurs normally during early fetal development where there is seamless plasticity between epithelial and mesenchymal cells<sup>19</sup>. Furthermore, this phenomenon is well accepted in cancer as a key mechanism that supports tumor metastases<sup>20</sup>. However, the process of EMT in development and cancer differ greatly in that, unlike developmental EMT, the tumorigenic EMT process is poorly regulated<sup>21</sup>. During the process of EMT, the downregulation of epithelial and tight junction proteins is associated with a concomitant increase in mesenchymal cell markers<sup>22,23</sup>. In chronic lung diseases, the dual expression of epithelial and mesenchymal markers in the same cells has led investigators to

postulate that EMT is a mechanism resulting in more ECM-generating mesenchymal cells in the lung <sup>24,25</sup>. The differentiation of airway epithelial cells has been previously described, for example, type I pneumocytes transitioning into goblet cells <sup>26-29</sup>. However, the switching of an epithelial cell into a phenotype that moves beyond the original cell's embryonic lineage, has only recently been suggested to be a causative factor in fibrosis <sup>23,30,31</sup>.

The initial events of EMT include the loss of cell polarity and the induction of matrix metalloproteinases (MMPs) that promote basement membrane degradation and cell detachment. The cells also undergo cytoskeletal changes, as well as altered expression of surface molecules which allow for the migration and transition of these cells to a mesenchymal phenotype <sup>32,33</sup>. The majority of the work evaluating EMT has been performed *in vitro*; however the full extent of this pathogenic pathway *in vivo* is currently being evaluated. In animal models of kidney fibrosis, it has been estimated that up to 20% of the fibroblasts found in the fibrotic lesions were derived from the epithelium through EMT <sup>30,34-37</sup>.

The idea of EMT promoting the fibrosis observed in COPD and IPF is rapidly beginning to evolve <sup>29</sup>. Several recent studies have also shown that EMT occurs in lung epithelial cells both *in vitro* and *in vivo*, supporting the concept of EMT contributing to the fibrosis observed in IPF <sup>29,38,39</sup>. Moreover, we have recently published an alteration in the microRNA regulation of genes associated with EMT in IPF<sup>40</sup>. The potential of EMT promoting COPD requires further investigation. Nevertheless, there is an emerging link between COPD and lung cancer, thus increasing the likelihood of EMT being present in the COPD lung. The link between smoking, lung cancer and COPD is also apparent, in that smoking is a risk factor for COPD and also for lung cancer <sup>41,42</sup>. An underlying response to cigarette smoke is generating an altered inflammatory environment in the lung <sup>43</sup>, which can then be susceptible to either COPD or lung cancer development <sup>44</sup>. Therefore, future work correlating the timecourse of EMT induction with COPD and IPF disease staging will be insightful to determine the extent of contribution mediated by this process.

#### 4.2 Circulating progenitor mesenchymal cells

Along with the epithelium, studies have also highlighted a role for bone marrow-derived circulating progenitor mesenchymal cells, or fibrocytes, in promoting lung fibrosis by differentiating into fibroblasts or myofibroblasts <sup>45,46,47</sup> (Fig. 1). Fibrocytes have been observed at sites of active fibrosis and increased numbers of these cells in the circulation correlate with mortality in IPF <sup>5</sup>. They are induced by pro-fibrotic mediators such as TGFβ1 and Th2 cytokines <sup>48</sup>; and the cell markers include leukocyte markers (CD45, CD34), mesenchymal markers (collagen I, fibronectin) and chemokine receptors (CCR3, CCR5, CCR7 and CXCR4) <sup>49</sup>. Human and mouse studies have demonstrated that fibrocytes from peripheral blood migrate to skin wound chambers <sup>49-51</sup> and bronchial mucosa after antigen challenge <sup>52</sup>. Furthermore, these cells have been reported in disease states with fibrotic pathologies including hypertrophic scars, asthma and IPF <sup>52-55</sup>.

Fibrocytes are pleiotropic and may contribute to fibrogenesis by directly producing collagen, as well as inflammatory cytokines, hematopoietic growth factors, and chemokines <sup>54-58</sup> (Fig. 2). In a study performed in collaboration between AstraZeneca and Malmö University Hospital, increased fibrocyte numbers were observed in the circulation of COPD patients, where patients with mild COPD had the most elevated number of fibrocytes in comparison to moderate COPD, severe COPD or healthy patients <sup>59</sup>. Although there was no correlation of fibrocyte number with any lung function parameter, inhaled glucocorticosteroid use

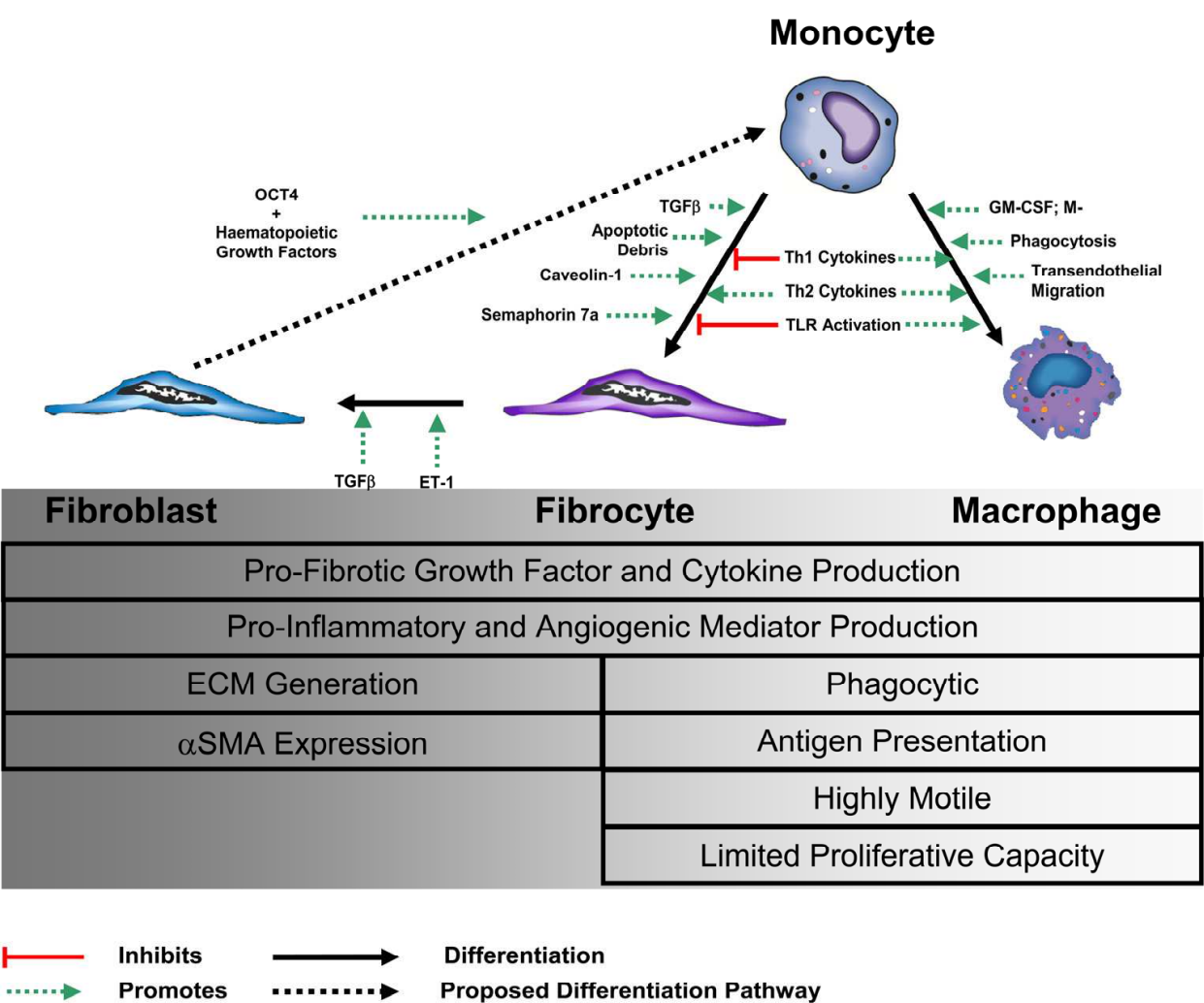


Fig. 2. Overlapping Differentiation Pathways and Functions of Fibroblasts, Fibrocytes and Macrophages

Fibroblasts, fibrocytes and macrophages share many *in vitro* and *in vivo* functions such as mediator production, host defence and extracellular matrix deposition. Monocytes can differentiate into fibrocytes or macrophages depending on the environment and the nature of the stimulus. *In vitro* studies have highlighted a role for various mediators such as cytokines, TLR signals, cellular debris and cell function at promoting differentiation. However, it is likely a combination of these pathways *in situ* which dictates the fate of the monocyte. Fibrocytes have been shown to differentiate into fibroblasts, in part through losing CD45 expression but retaining collagen I expression. Moreover *in vitro*, fibrocytes stimulated with TGFβ or ET-1 can promote fibrocyte to fibroblast differentiation. Recent evidence has indicated that fibroblasts can differentiate into haematopoietic cells when stimulated with appropriate growth factors.

Key: TGFβ: transforming growth factor β; GM-CSF: granulocyte macrophage colony-stimulating factor; M-CSF: macrophage colony-stimulating factor; TLR: toll-like receptors; ET-1: endothelin-1; OCT4: octamer-binding transcription factor 4); ECM: extracellular matrix; αSMA: α-smooth muscle actin.

significantly inhibited circulating fibrocyte numbers, corroborating the pro-inflammatory association of fibrocytes <sup>59</sup>. While it was originally thought that fibrocytes promote fibrosis through production of ECM components, it is becoming increasingly hypothesized that their primary role in tissue remodelling may be through secretion of soluble factors.

Fibrocytes have pleuripotent potential to differentiate into other cell lineages, as has been demonstrated with fibrocyte-derived adipocytes <sup>60</sup>. Furthermore, the extreme plasticity of these cells *in vitro*, makes both the derivation and characterization of these cells difficult. Exposure of fibrocytes to TGF $\beta$ 1 *in vitro* results in the cells transitioning into a myofibroblast phenotype that expresses both fibronectin and type III collagen <sup>52</sup>. Using an adoptive transfer model of bone marrow cells from green fluorescent protein (GFP) transgenic mice into mice challenged with intratracheal bleomycin to initiate lung injury, recruited GFP+ fibrocytes were shown to differentiate into fibroblasts while resident lung fibroblasts differentiated into myofibroblasts <sup>56</sup>.

At sites of normal wound healing, once sufficient ECM has been deposited, fibroblasts and myofibroblasts undergo apoptosis <sup>61,62</sup>. This serves to limit the excessive deposition of ECM and also dampen the pro-inflammatory and pro-fibrotic milieu. However, myofibroblasts persist in fibrotic conditions. Moreover, IPF fibroblasts are relatively resistant to apoptosis *in vitro*, with IPF cells inducing a different pattern of pro-apoptotic enzymes in response to Fas-L stimulation compared to normal fibroblasts <sup>63</sup>.

A recently described study showed an alternate potential fate of fibroblasts in that they can de-differentiation or transition into haematopoietic cells<sup>64</sup>. Here the investigators showed that, in the presence of haematopoietic transcription factors, fibroblasts can convert into haematopoietic progenitor cells, indicating a stem-like potential of cells that have traditionally thought to be resident to the lung <sup>64</sup>. Therefore, expanding our understanding on the pathways that control fibroblast to myofibroblast fate, as well as fibroblast to non-fibroblast cell fate, particularly in disease, will greatly expand our understanding of disease.

## 5. Fibroblast cell: Cell interactions

In the lung, fibroblasts are found in the greatest number in the subepithelial layer of the conducting airways and the interstitium of the lung parenchyma (Fig. 1). Here they are in a prime location to interact with the epithelial and endothelial cells, as well as leukocytes in the airspaces, interstitium and vasculature. One of the key inflammatory cells that is found in the lung, and one that is becoming more associated with the maintenance and progression of fibrosis, is the alternatively activated (M2) macrophage. The M2 macrophage is the predominant macrophage found in the lungs of IPF patients <sup>65</sup>. Moreover, M2 macrophages have been associated with COPD <sup>66,67</sup>. In healthy tissue, alveolar macrophages are known to remove apoptotic and necrotic debris and pathogens via phagocytosis in a non-phlogistic mechanism, in that downstream inflammation is limited and the inflammatory process is attenuated <sup>68</sup>.

In chronic lung disease, the predominant M2 macrophage phenotype is inefficient at clearing debris. M2 macrophages are defective in phagocytosis and do not dampen the inflammatory response <sup>69</sup>. These macrophages are capable of synthesizing pro-fibrotic mediators, which supports their role in wound healing, yet this cell type is inefficient at supporting host defense <sup>69</sup>. This may explain why COPD and IPF patients are susceptible to repeated bouts of pulmonary infections or exacerbation of disease. These cells express elevated levels of scavenger receptors such as macrophage scavenger receptor (MSR) and



mannose receptor C (MRC/ CD206) <sup>69,70</sup>. Assessing circulating primary cells from IPF patients, we have determined an elevation of CD163+CD14+ cells and M2-associated soluble mediators in the circulation, which was more pronounced in progressive IPF patients, suggestive of an overall elevated M2 background in these patients <sup>71</sup>. We have also shown that peripheral blood monocytes from patients with scleroderma-related lung disease display a pro-fibrotic phenotype, characterized by increased CD163 expression and CCL18 production <sup>72</sup>. Interestingly, macrophages in the lungs of COPD patients, as well as smokers with or without COPD, also demonstrate a skewing towards an M2 phenotype, with a deactivated M1 phenotype <sup>73,74</sup>. Moreover *in vitro*, cigarette smoke induces a M2-type phenotype and this result was also seen in an *in vivo* cigarette smoke model in mice <sup>73</sup>. *In vitro*, macrophage polarization to an M2 phenotype has only been shown robustly with mouse cells. Here the differentiation of a monocyte to an M2 phenotype requires a cocktail of cytokines including IL13 and CCL17/TARC<sup>75</sup>. Therefore the Th2 cytokine profile observed in remodeled lungs contributes to the appearance of M2 macrophages.

Studies of bleomycin-induced fibrosis have assessed either M2 macrophage or fibrocyte number <sup>71,76</sup>. It is increasingly recognized that there is some overlap between these cell subsets in terms of both function and markers <sup>76</sup>. However, although both fibrocytes and M2 macrophages can be derived from monocytes, they are not completely redundant in function. Recently, using a lung-specific TGF $\beta$ 1 over-expression model of lung fibrosis, we determined that depletion of lung monocyte/macrophages using liposomal clodronate reduced collagen accumulation, but this had no effect on the TGF $\beta$ -induced fibrocyte recruitment <sup>77</sup>. Therefore, for novel therapeutic approaches such as cell depletion or specific targeting, the M2 macrophage may be a more compelling target for chronic lung remodeling.

In COPD, alveolar macrophages have been hypothesized to have reduced anti-inflammatory properties, as well as a reduced capacity to turn over matrix <sup>78</sup>. However a recent study looking at macrophage number, MMP expression and emphysema determined a correlation between a greater infiltration of macrophage to the lung and emphysema in COPD patients <sup>79</sup>. *Ex vivo*, cigarette smoke induces a wide array of changes in the inflammatory profile and the host defence potential of COPD alveolar macrophages <sup>80</sup>. Moreover, microarray analysis indicated a correlation between alveolar macrophage gene expression, circulating monocyte gene expression and lung function <sup>81</sup>. The investigators hypothesized that there is a “COPD-related gene expression pattern” as many genes differentially expressed in COPD alveolar macrophages were also expressed in COPD circulating monocytes <sup>81</sup>.

## 6. Activators of fibroblasts/ mesenchymal cells

Fibroblasts are activated by numerous signals including: mechanical forces such as those imposed during bronchoconstriction; matrix interactions; hypoxia and resultant changes in pH levels; and soluble mediators (Fig. 1). The large variety of soluble mediators capable of activating mesenchymal cells are produced by many different cell types found in fibrotic regions. Furthermore, proteases of the coagulation cascade and other serum factors also promote fibroblast proliferation, collagen synthesis, migration and differentiation.

### 6.1 Growth factors

Growth factors are the most recognized mediators that activate mesenchymal cells. Transforming growth factor  $\beta$  (TGF $\beta$ ) is one of the most potent profibrotic mediators *in vitro* and is a central driver in the remodeling process. TGF $\beta$ 1 regulates numerous biologic

activities such as proliferation, apoptosis, and differentiation<sup>82,83</sup>. TGF $\beta$ 1 is upregulated in the lungs of IPF patients and patients with other chronic lung diseases<sup>84-89</sup>. Interestingly, expression of TGF $\beta$ 1 is nearly absent in the bronchial epithelial cells but is highly expressed in inflammatory cells beneath the basement membrane where subepithelial fibrosis predominates<sup>90</sup>. In COPD, TGF $\beta$  has also been reported to be produced by circulating and interstitial T cells and monocytes<sup>91</sup>. Polymorphisms in the promoter region of TGF $\beta$ 1 have been reported in COPD patients<sup>92</sup>. However, TGF $\beta$  SNPs have been associated with protection in COPD<sup>93</sup> or have been linked to COPD but not related to worsening of disease<sup>94</sup>. Transient overexpression of TGF $\beta$ 1, or pulmonary delivery of this cytokine to mouse lungs, induces a pronounced interstitial fibrosis mediated by excess ECM generation and deposition, as well as the presence of myofibroblasts<sup>95</sup>. Using a transgenic mouse model of SMAD-3 deficiency, TGF $\beta$ /SMAD-3 signaling has been shown to be required for alveolar integrity and ECM homeostasis and that this pathway is involved in pathogenic mechanisms mediating both tissue destruction and fibrogenesis.

Due to TGF $\beta$ 1 being such a potent growth factor, the release and activation of this growth factor is tightly regulated. TGF $\beta$  is released in a latent complex, associated with LAP (latency activated peptide) and LTBP. There are several mechanisms by which TGF $\beta$  is then activated and these include integrin-mediated cell cytoskeletal rearrangement in the case of integrin  $\alpha$ v $\beta$ 6<sup>96</sup>; cell membrane MMP recruitment for enzymatic cleavage in the case of integrin  $\alpha$ v $\beta$ 8<sup>97</sup> and other protease-related mechanisms exhibited by components of the coagulation cascade<sup>98</sup> (discussed later). The integrin  $\alpha$ v $\beta$ 6 is upregulated in IPF<sup>99</sup> and neutralization of this integrin *in vivo* reduces fibrosis in various experimental models<sup>99-101</sup>.

There are also endogenous inhibitors of TGF $\beta$ , the largest being the BMP (bone morphogenic protein) family. The BMP family is structurally and functionally related to the TGF $\beta$  superfamily<sup>102</sup>. BMPs inhibit TGF $\beta$  signaling through either inhibiting Smad 2/3 phosphorylation or competing for Smad 4 or both<sup>103,104</sup>. Recombinant BMP7 has been shown to inhibit TGF $\beta$ -induced EMT *in vitro*, as well as reducing renal fibrosis<sup>105</sup>. However, we have shown that BMP7 had no anti-fibrotic effect in models of lung fibrosis, nor were there effects on lung epithelial cells *in vitro*<sup>106</sup>.

## 6.2 Th2-associated mediators

Interleukin 13 (IL13) and IL4 are two pleiotropic, Th2-associated cytokines, with numerous distinct and overlapping functions. They share overlapping roles due to the shared IL13R $\alpha$ 1 receptor subunit. However they also have unique subunits that confer the distinct functions. IL13 activates epithelial cells and goblet cells causing mucous production, goblet cell hyperplasia and EMT<sup>107,108</sup>. Various animal models of pulmonary fibrosis have indicated a more pro-fibrotic role for IL13 than IL4. Indeed it has been hypothesized that IL4 is involved in the initiation of fibrosis whereas IL13 is central to the maintenance of the fibrotic response.

We have published that IL-13 is elevated in the lungs of IPF patients<sup>71</sup> and this protein is associated with fibrotic pathologies and aberrant remodeling at various tissue sites<sup>65,109</sup>. There is also elevated expression of the two IL13 receptor subunits IL13R $\alpha$ 1 and IL13R $\alpha$ 2 which are prominent on fibroblasts<sup>110,111</sup>. More recently we have shown that IPF fibroblasts are hyper-responsive to IL13 in comparison to non-fibrotic fibroblasts<sup>58</sup>. It was originally hypothesized that IL13R $\alpha$ 2 is a decoy receptor as it has a short cytoplasmic tail and although it is the higher affinity receptor subunit, it is found at high levels in a soluble form, but only

in murine models of fibrosis<sup>112</sup> and not in humans<sup>113</sup>. However, recent data has suggested that signaling of IL13 through IL13R $\alpha$ 2 is pro-fibrotic, resulting in TGF $\beta$ 1 production<sup>114</sup>.

Lung-specific over-expression of IL13 in mice results in remodeling and emphysema<sup>115</sup>. Moreover, various MMPs and cathepsins that are associated with COPD<sup>1</sup> are also induced in the IL13-transgenic lung<sup>116</sup>. However robust detection of IL13 has not been consistently reported. IL13 producing macrophages and NKT cells have been detected in the COPD lung<sup>117</sup>. Also, the presence of the cytokine and IL13 positive cells have been shown in the bronchial epithelium of smokers with chronic bronchitis<sup>118</sup>. In contrast, decreased IL13 has been measured in emphysema compared to non-emphysematous lungs<sup>119</sup>. An inverse correlation between plasma IL13 and the lung diffusion capacity for carbon monoxide (DLCO) parameter, used to determine the efficiency of gas exchange in COPD has been reported<sup>120</sup>.

Another Th2-associated mediator that is being actively researched in fibrosis is the chemokine CCL18/ PARC. In IPF, CCL18 has been associated with poor outcome, namely, patients with CCL18 levels greater than 150ng/ml in the circulation will typically have more progressive disease, compared to those with CCL18 levels below 150ng/ml<sup>6</sup>. CCL18 expression is also a marker for M2 macrophages<sup>121</sup>, therefore linking the profibrotic cell phenotype with IPF progression. In the recent ECLISPE study, elevated CCL18 levels in COPD patients has been associated with increased chance of COPD exacerbation<sup>122</sup>. This association of CCL18 with COPD exacerbations was in stark contrast to TNF $\alpha$ , IL6 or CXCL8/ IL8 levels, or the number of pack years of smoking<sup>122</sup>. These observations may change the understanding of the pleiotropic nature of the underlying mechanisms for COPD, as CCL18 is not associated with smoking or with neutrophil accumulation and activation.

### 6.3 Coagulation cascade

Thrombin is a serine protease generated during activation of the coagulation pathway<sup>123</sup>. Thrombin has been implicated in a number of pulmonary fibrotic diseases such as acute lung injury (ALI)<sup>124,125</sup> acute respiratory distress syndrome (ARDS)<sup>126</sup>, interstitial lung disease (ILD)<sup>127,128</sup> and IPF<sup>127,129-132</sup> and IPF BAL fluid thrombin has been shown to promote fibroblast proliferation<sup>131</sup>. In COPD, elevated procoagulant activity has been observed in the serum of patients with moderate to severe disease<sup>133</sup>. Animal models of fibrosis have strengthened the connection between thrombin and fibrosis. Increased thrombin is found in the lungs of mice challenged with bleomycin and pharmacological inhibition of thrombin significantly reduced the collagen deposition<sup>130</sup>.

At the cellular level, thrombin has numerous biologic effects that are in addition to its role as a coagulation pathway proteinase. It has been shown to promote inflammation and fibrosis through inducing chemokine and growth factor production from fibroblasts. Many of the cell-based activities of thrombin are mediated through a family of receptors termed proteinase-activated receptors (PARs). PARs are G-protein coupled receptors (GPCR), with 4 known subtypes identified to date. A defining feature of these receptors is that they are activated by proteases that cleave a portion of the extracellular amino terminus to unmask a new N-terminal sequence, which then functions as a tethered ligand that autoactivates the receptor. PAR-1-deficient mice are protected from bleomycin induced lung fibrosis<sup>134</sup>. Further, PAR-2-deficient mice had decreased eotaxin/CCL11 and reduced eosinophilia in the lungs following antigen challenge in an allergen sensitization and challenge model of asthma<sup>135,136</sup>. Furthermore, thrombin and other PAR1 agonists promote the integrin-mediated activation latent TGF $\beta$  in a model of acute lung injury<sup>98</sup>. Thus, the local activation of TGF $\beta$  at sites of fibrosis may be enhanced by the coagulation cascade in both IPF and COPD.

#### 6.4 Pentraxins

Pentraxins comprise a highly conserved superfamily of cyclic pentameric proteins. These proteins interact with numerous ligands, including selected pathogens and apoptotic cells<sup>137</sup>, and are recognized by macrophages via mannose 6P and Fc gamma ( $\gamma$ ) receptors, ultimately leading to complement activation, pathogen recognition, and apoptotic cell clearance<sup>138-140</sup>. Pentraxins are subdivided into the short pentraxins that include C-reactive protein (CRP) and serum amyloid P component (SAP, PTX2), and the long pentraxin 3 (PTX3)<sup>141,142</sup>. SAP appears to be uniquely involved in the resolution or repair phase of tissue injury via its modulator effects on resident and bone marrow derived collagen producing cells<sup>76,143</sup>. SAP binds to Fc $\gamma$  receptors<sup>144</sup> and the anti-fibrotic activities of SAP have been shown to be mediated through Fc $\gamma$  receptors<sup>143</sup> which affect peripheral blood monocyte differentiation and activation states.

SAP promotes the differentiation of M1 classically activated macrophages in a tuberculosis model of lung infection<sup>145</sup>. We have recently demonstrated that SAP has prominent immunomodulatory effects on mouse macrophages, thereby providing a mechanism for its ability to prevent the development and reverse established experimental fungal airway disease<sup>75</sup>. Moreover, human SAP has been shown to potently inhibit the differentiation of monocytes into fibrocytes<sup>146</sup> and it has consequently been used therapeutically in animal models to inhibit lung fibrosis and fibrosis in a number of organ sites. In addition, SAP causes an inhibition of the differentiation of peripheral blood mononuclear cells into CD45<sup>+</sup>/collagen I<sup>+</sup> cells called fibrocytes<sup>76,146,147</sup>, as well as reducing M2 macrophage number<sup>75,77</sup>.

#### 6.5 Matrix Metalloproteinases and Chitinases

Active remodeling of the ECM is dependent on the coordinated activities of proteases and protease inhibitors<sup>148,149</sup>. Fibroblasts generate metalloproteinases (MMPs), which are elevated in asthma and COPD (reviewed in<sup>150</sup>). MMPs are a family of proteins that exert proteolytic activities on various proteins including ECM components and are thus central to ECM formation and organization<sup>150</sup>. Tissue inhibitors of metalloproteinases (TIMP)s are endogenous inhibitors of MMPs that bind to the catalytic site on these proteinases<sup>151</sup>. In IPF, MMP1, 2 and 9 were co-localized to the epithelium surrounding fibrotic lesions, whereas increased TIMP2 was also observed suggesting that the MMP activity may be inhibited and that the fibrotic region not degraded<sup>152</sup>.

Another family of enzymes associated with fibrosis and remodeling is the chitinase family. Chitinases are proteolytic enzymes that bind, but do not cleave chitin<sup>153</sup>. Chitin is the main component of the insect exoskeleton, thought to be absent in human tissue. However, these proteins are postulated to play important, yet currently undefined roles in biology. The prototypic chitinase-like protein is derived from the chitinase 3 like 1 (*Ch3l1*) gene, called YKL40 in humans and BRP39 in mice. YKL40/BRP39 is a circulating regulator of apoptosis, and shown to have a role in M2 macrophage activation, TGF $\beta$ 1 induction and tissue fibrosis<sup>154</sup>. An early report in asthmatics indicated that YKL40 is elevated in the circulation and is associated with asthma severity<sup>155</sup>. Chitinolytic activity has also been demonstrated in COPD patients<sup>156,157</sup>, as well as in models of cigarette smoke induced emphysema<sup>157</sup>. Interestingly, BRP39 gene deficient mice have increased epithelial cell apoptosis and alveolar destruction in response to cigarette smoke, but worsened pathology in hyperoxia-induced lung injury which is a more acute ARDS-like model<sup>157,158</sup>. Increased YKL40 has also been detected in the lungs and circulation of IPF patients, with levels correlating with survival<sup>159,160</sup>. Thus, there is clear disease association with chitinase activity, however because of discrepancies in the different



animal models, whether these chitinases are promoting disease or uniquely serve as a marker of disease activity still requires further elucidation.

## 7. Therapeutic options for targeting pro-fibrotic cells

IPF is a disease that is driven by continual parenchymal ECM deposition, which reduces lung compliance and effective gas exchange. The fibrosis observed in the lungs of COPD patients predominates around small airways<sup>4,161</sup> and may not be the main driving factor causing the loss in lung function and death. Emphysema and lung tissue destruction is often observed in COPD patients and honeycombing is a salient feature in IPF patient lungs<sup>162</sup>. Overall this suggests an imbalance in repair processes within the lung and targeting the underlying pro-fibrotic mechanisms may impact COPD and IPF in a positive way. In IPF, the capacity of the lungs (FVC, forced vital capacity); as well as the efficiency of gas exchange (DLCO) may improve with a reduction in fibrosis. Both of these parameters are used clinically in IPF patients to measure lung function and monitor disease progression<sup>162</sup>. In COPD, anti-fibrotic strategies directed at the same cells and/or mediators may attenuate the obstructive nature in the airways and this may be the most discernible improvement in lung function. This would translate to an improvement in forced expiratory volume in a short time frame (FEV<sub>1</sub>). Again, FEV<sub>1</sub> is commonly used in COPD patient management and is used to segregate patients into the various GOLD stages of disease<sup>4</sup>.

As has been highlighted in this Chapter, monocytes and mesenchymal cells express a variety of receptor that can promote recruitment, proliferation or activation, as well as differentiation into other phenotypes (Fig. 2). In experimental mouse models of lung fibrosis, blocking fibrocyte recruitment through chemokine ligand/ receptor blockade significantly attenuated ECM deposition<sup>54-56,163</sup>. However, these chemokine receptors are not uniquely expressed on fibrocytes<sup>164</sup>, so the anti-fibrotic effects observed following blockade of these G-protein coupled receptors might extend beyond impaired fibrocyte recruitment. Also, we have demonstrated that depletion of lung monocyte/ macrophages inhibits TGF $\beta$ -induced lung fibrosis, but has no effect on lung fibrocyte number, suggesting a redundant role for fibrocyte recruitment in promoting TGF $\beta$ -induced lung fibrosis<sup>77</sup>.

One other mechanism to consider in any therapeutic approach in pulmonary fibrosis is fibrocyte activation. A prototypic activator of fibrocytes, fibroblasts and myofibroblasts is TGF $\beta$ . Adenoviral-mediated over-expression of TGF $\beta$  in the lungs of mice or rats, or lung-specific transgenic over-expression in mice induces significant lung pathology<sup>95,165,166</sup>. In the lung specific TGF $\beta$  over expression, active TGF $\beta$  is expressed by airway epithelial cells. At early timepoints following over-expression, apoptosis is observed, followed by interstitial leukocyte accumulation and then increased collagen deposition, predominating around the airways and then gradually increasing in the parenchyma<sup>77,165,167</sup>. At later stages of lung-specific TGF $\beta$  over-expression, alveolar collapse is observed<sup>77</sup> whereas adenoviral TGF $\beta$  expression results in a prolonged, severe fibrosis<sup>95</sup>. These apparent chronic differences may be due to the initial extent of acute lung injury immediately following TGF $\beta$  over-expression, in that transgenic mediated TGF $\beta$  induces extensive apoptosis, whereby blocking apoptosis inhibits subsequent fibrosis<sup>165</sup>. Blockade of TGF $\beta$  in experimental models of lung fibrosis through either antibody neutralization, receptor inhibition or TGF $\beta$  activation has been shown to attenuate lung remodelling<sup>99,100,168-170</sup>. However, TGF $\beta$ 1 gene deficient mice die within 3-4 weeks of age and demonstrate significant autoimmune pathologies, potentially due to the lack of immunoregulation in the absence of TGF $\beta$ <sup>171,172</sup>.

Therefore direct neutralization of this target might have significant safety concerns in IPF or COPD patients.

Other factors that work in concert with TGF- $\beta$  include IL4 and IL13. In order to examine the role of IL4- and IL13-responsive cells in pulmonary fibrosis, we have conducted preclinical studies using IL13 conjugated to a Pseudomonas exotoxin, IL13-PE<sup>173</sup>, which targets cells expressing the type 2 IL4 receptor and IL13R $\alpha$ 2. This protein-toxin conjugate selectively targets IPF fibroblasts because they express these receptor subunits, resulting in specific cell death. Moreover, *in vivo* use in a murine model of bleomycin-induced lung fibrosis showed that selective targeting of fibroblasts at the end stages or maintenance phase of fibrosis, attenuated remodelling. In contrast, IL13-PE delivered to the lung during the onset or initiation of disease had no therapeutic benefit<sup>173</sup>. This suggests that targeting these cells in established disease may allow for the resolution of lung pathology that is observed in chronic lung disease patients.

Taken together, targeting stromal cells to dampen the extent of activation and the amount of ECM deposition, regardless of disease, or region within the lung may have an impact in lung function that can either halt disease progression or promote resolution and restoration of lung function.

## 8. Acknowledgements

We also thank Dr. Judith Connett for her critical reading of the manuscript.

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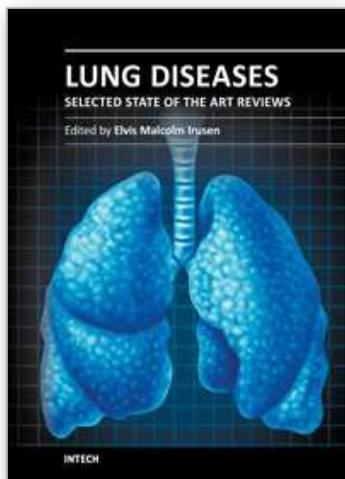
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## **Lung Diseases - Selected State of the Art Reviews**

Edited by Dr. Elvisgran Malcolm Irusen

ISBN 978-953-51-0180-2

Hard cover, 690 pages

**Publisher** InTech

**Published online** 02, March, 2012

**Published in print edition** March, 2012

The developments in molecular medicine are transforming respiratory medicine. Leading clinicians and scientists in the world have brought their knowledge and experience in their contributions to this book. Clinicians and researchers will learn about the most recent advances in a variety of lung diseases that will better enable them to understand respiratory disorders. This treatise presents state of the art essays on airways disease, neoplastic diseases, and pediatric respiratory conditions. Additionally, aspects of immune regulation, respiratory infections, acute lung injury/ARDS, pulmonary edema, functional evaluation in respiratory disorders, and a variety of other conditions are also discussed. The book will be invaluable to clinicians who keep up with the current concepts, improve their diagnostic skills, and understand potential new therapeutic applications in lung diseases, while scientists can contemplate a plethora of new research avenues for exploration.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Lynne A. Murray and Cory M. Hogaboam (2012). Mechanisms Promoting Chronic Lung Diseases: Will Targeting Stromal Cells Cure COPD and IPF?, Lung Diseases - Selected State of the Art Reviews, Dr. Elvisgran Malcolm Irusen (Ed.), ISBN: 978-953-51-0180-2, InTech, Available from: <http://www.intechopen.com/books/lung-diseases-selected-state-of-the-art-reviews/mechanisms-promoting-chronic-lung-diseases-will-targeting-stromal-cells-cure-copd-and-ipf->

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