Abstract

Pulmonary fibrosis is characterized by excessive deposition of extracellular matrix (ECM) and remodeling of the lung architecture, with clinically irreversible loss of lung function. The exact molecular and cellular mechanisms of pulmonary fibrosis are complicated. Many types of cells are involved in the pathogenic processes. The chemokine (C-X-C motif) ligand 12 (CXCL12) can attract circulating fibrocytes trafficking into lungs via chemokine (C-X-C motif) receptor 4 (CXCR4) to promote tissue repair or impertinently worsen fibrotic lesions. In this review, we briefly summarize the existing experimental and clinical findings about fibrocytes in pulmonary fibrosis and discuss the potential therapeutic target CXCL12/CXCR4 biological axis.

Key words: Pulmonary fibrosis; Fibrocytes; CXCL12/CXCR4.

Introduction

Pulmonary fibrosis is the common pathway of a number of lung disease that are characterized by alveolar epithelial injury, fibroblasts and myofibroblast proliferation, collagen over-production, excessive extracellular matrix (ECM) accumulation, and pulmonary neovascularization. Pulmonary fibrosis could be induced by a lot of factors such as infection, medicine, radiation, chemical agent, organic or inorganic dust and autoimmune diseases (e.g. scleroderma). Since the pathogenic mechanisms of pulmonary fibrosis are complicated, resulting in the lack of effective treatments, the identification of pathways that may provide novel therapeutic targets is absolutely necessary. This review will focus on the involvement of circulating fibrocytes in pulmonary fibrosis.

Pro-fibrotic role of circulating fibrocytes

The “fibrocyte” was firstly described in 1994 as a circulating, bone marrow-derived cell with the ability to adopt a mesenchymal phenotype, and display an adherent, spindle-shaped, fibroblast-like morphology when cultured in vitro. Fibrocytes comprise 0.1-1% of the nucleated cells in the peripheral blood of healthy hosts. These cells express markers of both hematopoietic cells (CD34, CD45, MHC II) and stromal cells (collagens I/III, fibronectin). Fibrocytes are rare in normal healthy tissue but are readily detected in fibrotic tissues including those involved the lungs, liver, kidney, autoimmunity and skin. Fibrocytes in the lung tissues and circulation were increased in both animal and human pulmonary fibrosis. Although fibrocytes are relatively small in number, these cells could traffic into injured tissue and play an important role in the process of tissue repair and fibrogenesis. Fibrocytes secrete a variety of inflammatory cytokines (IL-13, TGF-β, CTGF and TNF-α, etc.) to augment the fibrotic lesions by activating resident fibroblasts. Additionally, fibrocytes acted as a regulator that promoted progressive accumulation of Wilms’ Tumor 1 (WT1)-positive cells, a sizable subset of lung-resident mesenchymal cells, in fibrotic lesions of both human idiopathic pulmonary fibrosis (IPF) and mouse models of pulmonary fibrosis. The circulating fibrocytes secrete periostin, a matricellular...
pro-fibrotic protein that could be upregulated by TGF-β, thus promote pulmonary fibrogenesis and ECM deposition.\textsuperscript{18} Intrapulmonary TGF-β1-induced apoptosis recruited the fibrocytes accumulation and induced collagen production, which was attenuated through apoptosis inhibition.\textsuperscript{19} Fibrocytes could be a source of ECM components that restore the architecture of the damaged tissue and promote angiogenesis and wound contraction at sites of tissue injury.\textsuperscript{20,21} Fibrocyte can be recruited into the bronchial or lung tissue and transform into fibroblast or α-smooth muscle actin (α-SMA)$^+$ myofibroblasts,\textsuperscript{22-24} which are the main ECM-producing cells during the pulmonary fibrosis. More than 27% of the fibrocytes in bleomycin-induced fibrotic lung tissue expressed type I collagen (Col I). These cells constitute more than 80% of all Col I-expressing cells in the fibrotic lung.\textsuperscript{24} Interestingly, fibrocytes were present in lung tissues but are absent in bronchoalveolar lavage (BAL) of patient with IPF.\textsuperscript{14} The transformation of fibrocytes into fibroblast or myofibroblast was regulated by TGF-β1 assisted with semaphorin7a\textsuperscript{9,25,26} or endothelin-1 (ET-1).\textsuperscript{27} Th17-derived cytokines IL-17A can induce asthmatic fibrocytes to proliferate and acquire proinflammatory phenotype with increased expression of α-SMA gene and protein.\textsuperscript{28} Fibrocytes lose cell surface phenotype and chemokine receptors such as CD34, CD45, CCR2, CXCR4 during their transformation process, \textsuperscript{13,22} which may serve to trap the transformed fibroblasts and myofibroblasts within the lung and prevent their migration out of the injured lung.

Recently, it has been recognized that deregulated vascular remodeling is one of the most critical biological processes of pulmonary fibrosis. The increased circulating and intrapulmonary fibrocytes secreted pro-angiogenic factors (VEGF, PDGF, b-FGF, HGF, GM-CSF, CTGF) that promote an angiogenic phenotype in endothelial cells.\textsuperscript{29,30} Moreover, fibrocytes may ease cell migration and further facilitate the angiogenesis process by secreting a variety of matrix metalloproteinases (MMPs).\textsuperscript{31}

### Differentiation regulation of circulating fibrocyte

The exact origin of fibrocytes was not identified clearly so far, most studies revealed that fibrocytes were differentiated from a subpopulation of CD14$^+$ peripheral blood monocytes.\textsuperscript{32,33} Circulating monocytes from patients with IPF and connective tissue disease interstitial lung disease (CTD-ILD) displayed enhanced differentiation into fibrocytes compared with normal healthy controls.\textsuperscript{19} Different culture condition such as blood collection, substrates, media, and cell density all influence in vitro fibrocytes differentiation.\textsuperscript{33} The differentiation of monocytes into fibrocytes was affected by resident fibroblasts. TNF-α-stimulated fibroblasts secreted lumican (a small leucine-rich proteoglycan), one of the main component of ECM, and lumican potentiated fibrocytes differentiation via α2β1, αMβ2, and αβ2 integrins\textsuperscript{34}. Whereas, the neuronal guidance protein Slit2 secreted by fibroblast inhibited human fibrocytes differentiation.\textsuperscript{35}

Niedermeier and colleagues reported that differentiation of fibrocytes was critically dependent on CD4$^+$ T cells. In the model of unilateral ureteral obstruction (UUO) in mice, different way of T cell activation determines whether development of fibrocytes is supported or blocked. Polyclonal activation of CD4$^+$ T cells induced the release of soluble factors (IL-2, TNF, IFN-γ and IL-4), and that completely prevented the outgrowth of fibrocytes and reduced the severity of fibrosis. In contrast, activation of CD4$^+$ T cells in the presence of calcineurin inhibitors (Cyclosporine A) markedly enhanced the outgrowth of fibrocytes and renal deposition of collagen I.\textsuperscript{36} It was found that IL-4 and IL-13 promote fibrocyte differentiation, whereas IL-12 and IFN-γ inhibit fibrocyte differentiation. These cytokines have different ways to effect monocyte-to-fibrocyte differentiation. IL-4, IL-13 and IFN-γ appear to act directly on monocytes, whereas IL-12 acts indirectly, possibly through NK cells. IL-4 and IL-13 increased the number of precursor cells to differentiate into fibrocytes rather than inducing fibrocyte proliferation, as well as without altering the morphology or phenotype of these cells.\textsuperscript{37} Pilling \textit{et al.} reported that only heat-aggregated IgG but not monomeric IgG or monomeric or heat-aggregated IgA, IgE or IgM could inhibit fibrocyte differentiation, which suggest that ligation and cross-linking of FcγRs are inhibitory signals for monocyte-to-fibrocyte differentiation.\textsuperscript{38} In addition, fibrocyte differentiation could be mediated by epidermal growth factor receptor (EGFR) pathway,\textsuperscript{39} lumican,\textsuperscript{34} low molecular weight hyaluronic acid (LMWHA),\textsuperscript{40} Toll-like receptor 2 (TLR2) agonists.\textsuperscript{41}

### CXCL12/CXCR4 axis mediated fibrocyte trafficking in pulmonary fibrosis

Human fibrocytes express several chemokine receptors, including CCR2, CCR5, CCR7, and CXCR4. Fibrocyte traffic into wound site in response to chemokines such as CXCL12, CCL21. Among those mentioned above, the CXCL12/CXCR4 biological axis is the most important one in mediating fibrocyte influx to the fibrotic lung, and this ligand/receptor
has received a great deal of attention in this regard.\textsuperscript{4,42} CXCR4 is an important chemokine receptor in cell trafficking, and the differential expression of its ligand CXCL12, also known as stromal cell-derived factor 1 (SDF-1), in tissues creates the chemotactic gradient required for trafficking of CXCR4\textsuperscript{+} cells. CXCR4 are widely expressed in hematopoietic progenitor cells, white blood cells, lungs, and liver.\textsuperscript{33,44} The binding of CXCL12 to CXCR4 are able to activate various downstream signaling that promote cell proliferation, migration, and fibrotic lesions. The downstream intracellular signaling transduction pathways including Phosphatidylinositol 3-kinase (PI3K)/Akt/ mammalian target of rapamycin (mTOR), Rac1/extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK), p38MAPK, stress-activated protein kinase (SAPK), c-Jun N-terminal kinase (JNK)/ activator protein-1 (AP-1), Janus kinase/signal transducers and activators of transcription (JAK/STAT).\textsuperscript{45,46}

CXCR4 is the predominant chemokine receptor on human fibrocytes. More and more evidence have indicated that substantial numbers of CXCR4\textsuperscript{+} fibrocytes can migrate and accumulate in lung in response to CXCL12 during pulmonary fibrosis.\textsuperscript{24,47} CXCR4 levels increase in the lungs after intratracheal bleomycin, and the synthetic specific CXCR4 antagonist TN14003 attenuated bleomycin-induced pulmonary fibrosis in mice.\textsuperscript{48} CXCR4 expression is upregulated by hypoxia inducible factor-1\textalpha\ (HIF-1\textalpha), vascular endothelial growth factor (VEGF), PDGF, NF-\kappaB, Wnt/\beta-catenin, bradykinin, IL-1\beta, TNF-\alpha, IFN-\gamma, TGF-\beta1 and drastically downregulated by the Th2 cytokines IL-5 and IL-4, IL-3, GM-CSF in inflammation or hypoxia conditions.\textsuperscript{52,49-54} It should be noticed that treatment with IL-5, but not IL-4, apparently decreased the level of CXCR4 mRNA in eosinophils, although both cytokines suppressed surface protein expression of CXCR4. It might be possible that each cytokine may regulate CXCR4 expression via different pathways or mechanisms, and surface CXCR4 expression is modulated not only at the level of post-transcription but also at least in part at the level of transcription.\textsuperscript{50} Both hypoxia-induced and PDGF-induced CXCR4 expressions could be attenuated by specific inhibition of PI3 kinase and mTOR.\textsuperscript{42}

Phillips et al. firstly reported circulating fibrocytes migrated in response to CXCL12 and trafficked into the lungs in a murine model of bleomycin-induced pulmonary fibrosis, and specific neutralization of CXCL12 inhibited intrapulmonary recruitment of fibrocytes and attenuated lung fibrosis.\textsuperscript{15} The expression of CXCL12 increased significantly under ischemia and hypoxia conditions.\textsuperscript{55,56} The CXCL12 levels in the lungs, bronchoalveolar lavage and serum were found significantly elevated associated with increased circulating fibrocytes in mice and human pulmonary fibrosis.\textsuperscript{13,42,48,57} Rapamycin treatment resulted in a 50% decrease of CXCL12 expression in lungs.\textsuperscript{42} Regulatory T cells (Tregs) could reduce lung fibrocyte recruitment by inhibiting the CXCL12/CXCR4 axis after acute lung injury.\textsuperscript{58}

**Clinical pathogenic significance of circulating fibrocyte**

Researches from patients showed that circulating fibrocyte increased in various lung disease including chronic obstructive asthma\textsuperscript{25}, acute asthma exacerbation without airflow obstruction\textsuperscript{59}, acute exacerbation of chronic obstructive pulmonary disease (AECOPD)\textsuperscript{60}. Fibrotic interstitial lung diseases\textsuperscript{57}, IPF\textsuperscript{61}, AECOPD\textsuperscript{60} chronic hypersensitivity pneumonitis\textsuperscript{62}, obliterative bronchiolitis (OB)\textsuperscript{63}, cystic fibrosis patients\textsuperscript{64}, rheumatoid arthritis-usual interstitial pneumonia\textsuperscript{65}, pulmonary hypertension (PE)\textsuperscript{66}, sickle cell lung disease\textsuperscript{67}. Blood fibrocytes are recruited in lungs during COPD exacerbations and related to mortality and low lung function.\textsuperscript{60} The total, activated, and differentiated fibrocytes were increased in asthmatic patients experiencing an asthma exacerbation in the preceding 12 months. The \alpha-SMA\textsuperscript{+} fibrocyte was able to differentiate into myofibroblast thus contribute to the airway remodel and pulmonary fibrosis.\textsuperscript{68}

The higher quantity of circulating fibrocytes indicated a poor prognosis in IPF\textsuperscript{61}, and increased mortality in patients with acute respiratory distress syndrome (ARDS)\textsuperscript{69}. The counts of circulating fibrocytes were significantly elevated in the blood of patients with stable IPF (2.72±0.34\%) compared with healthy control subjects (1±0.12\%). During episodes of acute exacerbation, fibrocyte counts increased substantially up to 10-fold above the values measured during stable disease (14.51±2.53\%).\textsuperscript{61} Fibrocyte numbers were not correlated with lung function or radiologic severity scores, but they were an independent predictor of early mortality. The mean survival of patients with fibrocytes higher than 5\% of total blood leukocytes was 7.5 months compared with 27 months for patients with less than 5\%.\textsuperscript{61} Fujiwara et al. further confirmed a correlation between increased numbers of circulating fibrocytes and activity and progression of interstitial lung diseases (ILD). The number of circulating fibrocytes was significantly and inversely correlated with measurable clinical lung functions parameters including vital capacity (VC)
and diffusing capacity of the lung for carbon monoxide/alveolar ventilation (DLco/VA), and positively correlated with serum levels of sialylated carbohydrate antigen (KL-6, an inflammatory biomarker of disease activity)\textsuperscript{70}. In addition, there are also positive correlation between the amount of circulating fibrocytes and severity of COPD\textsuperscript{71}, asthma\textsuperscript{68}, and bronchiolitis obliterans syndrome (BOS) development in lung transplant patients\textsuperscript{72}. Taken together, these studies indicated that fibrocytes may be used as an indicator or a clinical biomarker for respiratory fibrogenic activity, disease progression, and prognosis.

### Potential therapy targeted on circulating fibrocyte and CXCR4

Based on these findings that CXCL12/CXCR4 axis mediated recruitment of fibrocytes served as a main pathogenic resource of pulmonary fibrosis. The promising therapeutic targets of fibrocytes differentiation or CXCR4 have received considerable attention. Several small molecule CXCR4 antagonists (e.g. AMD3100, MSX-122, TN14003) have been developed and some of them significantly reduced bleomycin or radiation-induced pulmonary fibrosis in mice\textsuperscript{48,73-75}. Plerixafor (solution of AMD3100 for subcutaneous injection, brand name: Mozobil\textsuperscript{TM}, Genzyme Corporation) has been approved by the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) for use in hematopoietic stem cells (HSC) mobilization for autologous transplant for patients with non-Hodgkin’s lymphoma and multiple myeloma (MM)\textsuperscript{76}. So, it is also worth to perform clinical trial to evaluate the therapeutically potential of Plerixafor in pulmonary fibrosis patient population in future.

Another selective and potent antagonist of CXCR4 which developed by the protein epitope mimetics technology, named protein epitope mimicetic (PEM) POL5551, could inhibit vascular accumulation of CXCR4-expressing smooth muscle cells\textsuperscript{77}. Compared with Plerixafor, POL5551 showed a superior ability to block CXCL12-induced responses and mobilize hematopoietic stem and progenitor cells more effectively\textsuperscript{78}. In clinical trials, a new small molecule AMD11070 has been reported as an orally bioavailable inhibitor of CXCR4 with safe, well-tolerated, and more favorable pharmacokinetic profile when administered to healthy volunteers\textsuperscript{79, 80}. A serial of novel small molecule modulators of the CXCR4 receptor have been developed these years. These new compounds may potentially open up new therapeutics for CXCR4 related pulmonary fibrosis\textsuperscript{81,82}. Serum amyloid P (SAP) is a highly phylogenetically conserved, naturally circulating plasma protein in human being, which has been proven to inhibit the monocyte-to-fibrocyte differentiation as an endogenous inhibitor by binding to FcγRs. Preclinical cell and animal experiments suggested that SAP could reduce bleomycin-induced lung fibrosis\textsuperscript{38, 83-85}. Intravenous administration of recombinant form of human SAP (PRM-151) resulted in a 30-50\% decrease in fibrocyte numbers 24 h post-dose in pulmonary fibrosis patients\textsuperscript{86}. This first-in-class modulator of the fibrosis pathway has been granted orphan drug designation for myelofibrosis by FDA, and the phase 2 clinical trials ongoing (NCT01981850)\textsuperscript{87}. A phase 1b study of intravenous (IV) PRM151 in patients with IPF has been completed and it has advanced to phase 2 clinical testing (NCT01254409). These studies showed that PRM-151 was well tolerated at all dose levels, with no serious adverse reactions. Some clinical efficacy markers including pulmonary function tests, George’s Respiratory Questionnaire (SGRQ), blood biomarkers (multiple matrix metalloproteinases, cytokines and chemoattractants, etc.) showed trends towards improvement in the PRM-151 dose groups\textsuperscript{88}.

In summary, some potential agents have definitively demonstrated the efficacy and safety, but the amounts of subjects and population included were limited. More randomized, double-blind, multiple-center clinical research with larger amounts of subjects are needed to support these innovative therapeutic approaches that may one day offer the clinical benefits to all pulmonary fibrosis patients.

### Competing Interests

The authors have declared that no competing interest exists.

### References


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