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Bone Marrow-Derived Cells in the Pathogenesis of Lung Fibrosis

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Abstract

Progressive pulmonary fibrosis is characterized by failed alveolar reepithelialization and fibroblast/myofibroblast accumulation, with deposition of extracellular matrix. This results in loss of lung elasticity, alveolar collapse and fibrosis, impaired gas exchange and progressive decline in pulmonary function. Myofibroblasts represent an activated, contractile cellular phenotype that are potent producers of collagen and other extracellular matrix proteins. It is generally thought that myofibroblasts derive from local tissue fibroblasts. However, recent evidence suggests a portion of the progenitors for these cells may arise from the bone marrow. Fibrocytes, which share both leukocyte and mesenchymal markers, are found in increased numbers in bone marrow and lung of injured mice. Fibrocytes circulate in blood and are recruited to injured sites via chemotactic signals. Studies with bone marrow chimeric and parabiotic mice suggest that fibroblasts (and in some cases myofibroblasts) arise from circulating bone marrow precursors. Chemokine and chemokine receptor interactions are critical for the recruitment of bone marrow-derived progenitors. Once fibrocytes arrive in injured tissues, local factors induce their differentiation into fibroblasts/myofibroblasts. This review will summarize the experimental findings, supporting a role for the participation of bone marrow-derived cells in animal models of lung fibrosis, and potential implications for the pathogenesis of fibrotic lung diseases.

Keywords

Fibrosis; bone marrow; fibroblasts; fibrocytes; chemokines; lung

PULMONARY FIBROSIS: DESCRIPTION AND CLASSIFICATION

The lung is an organ, uniquely designed for the exchange of gas across thin layers of epithelium and endothelium, separated by a normally thin mesenchyme. This specialized anatomy, along with the elastic properties of the lung are crucial for sustaining gas exchange and ventilation, essential for aerobic life. For this reason, fibrotic diseases of the lung are especially devastating. Pulmonary fibrosis is characterized by epithelial cell injury and hyperplasia, variable degrees of inflammatory cell infiltrate, fibroblast proliferation and accumulation, and the relentless deposition of extracellular matrix (ECM) [1]. The end results of this process are a loss of lung elasticity and loss of alveolar surface area, leading to

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severe compromises in pulmonary function and respiratory failure [1–5]. In humans, there are many types of fibrotic lung diseases [6]. Among the diffuse parenchymal lung diseases (DPLDs) are the diseases of known cause (e.g. drug-related or those associated with collagen vascular disease), the idiopathic interstitial pneumonias (IIPs), granulomatous DPLDs (e.g. sarcoidosis) and non-categorized diseases, such as lymphangioleiomyomatosis (LAM). Within the classification of the IIPs, are diseases such as idiopathic pulmonary fibrosis (IPF), which is histopathologically identified as usual interstitial pneumonia (UIP). Additional diseases within the IIP category are desquamative interstitial pneumonia (DIP), respiratory bronchiolitis interstitial lung disease (RBILD), acute interstitial pneumonia (AIP), cryptogenic organizing pneumonia (COP, also known as bronchiolitis obliterans organizing pneumonia or BOOP), lympohocytic interstitial pneumonia (LIP) and nonspecific interstitial pneumonia (NSIP). UIP is the most common form of IIP, and carries the worst prognosis, with a mean survival time, following diagnosis of less than 5 years [4, 7–9]. The fact that biopsies from a single patient can show patterns consistent with both UIP and NSIP [8, 10, 11], have led to a further reevaluation of the pathobiologic mechanisms of pulmonary fibrosis [12], and the suggestion that NSIP, may be a precursor to UIP.

In addition to the DPLDs, there are other diseases of the lung in which fibroproliferative changes have been described. These include late stages of acute respiratory distress syndrome (ARDS), airway remodeling associated with asthma and vasculopathies, such as primary pulmonary hypertension.

PATHOGENESIS OF FIBROSIS

The pathogenesis of all fibrotic lung diseases involves the accumulation and activation of fibroblasts [13]. Fibroblasts are mesenchymal cells, capable of synthesizing collagen types I and III and fibronectin, which are predominant components of ECM proteins, deposited in IPF lung. Fibroblasts which actively secrete ECM proteins can be termed “effector” fibroblasts. Effector fibroblasts can acquire the expression of α-smooth muscle actin (α-SMA) and non-muscle myosins. The expression and organization of these proteins into stress fibers confers, contractile properties to these cells, referred to as myofibroblasts [14–18]. Myofibroblasts are the most potent producers of ECM proteins. A distinguishing feature of UIP histopathology, is the presence of fibrotic foci, which are clusters of myofibroblasts that represent sites of active collagen synthesis [8, 11, 19]. Myofibroblasts are considered to be the principal collagen-producing cells within fibrotic lesions [14, 15]. However, the origins of effector fibroblasts and myofibroblasts in pulmonary fibrosis are uncertain.

ORIGINS OF EFFECTOR FIBROBLASTS/MYOFIBROBLASTS IN PULMONARY FIBROSIS

There are at least three potential sources for effector fibroblasts/myofibroblasts that are seen in fibrotic lung disease. These cells could arise from: 1) resident tissue-specific precursors; 2) epithelial to mesenchymal transition (EMT); or 3) circulating bone marrow (BM)-derived progenitors. The traditional view of progressive fibrosis suggests that, the elaboration of local profibrotic factors such as transforming growth factor beta-1 (TGF-β1) induces differentiation of resident fibroblasts into myofibroblasts [14, 16, 17, 20, 21]. Moreover, it

has been suggested that fibroblasts may also arise from EMT. EMT has not been demonstrated in the lung, but it has been observed in other organ systems [22]. Recently, there has been increasing interest in the possibility that effector fibroblasts in fibrotic lesions may arise from circulating BM-derived precursors [23–25]. These findings raise new questions regarding: 1) the origins of these cells; 2) the signals that recruit BM progenitors to the injured lung; 3) the role of injury in the recruitment and differentiation of BM progenitors; 4) the local factors that induce differentiation of these cells into effector fibroblasts and/or myofibroblasts; 5) the contribution of these cells to fibrotic progression; 6) the role of proliferation and apoptosis in the accumulation of these cells; and 7) implications for new therapeutic strategies to treat pulmonary fibrosis.

EVIDENCE THAT MESENCHYMAL CELLS ARISE FROM CIRCULATING CELLS

Mesenchymal cells are structural cells that provide architectural support to tissues and have not traditionally been recognized to derive from circulation. Yet, several studies suggest that these cells might circulate in blood [26–29]. Initial evidence for circulating mesenchymal cells came from biopsies taken from sex-mismatched kidney allograft recipients who were undergoing chronic rejection. Thirty-38% of the infiltrating mesenchymal cells in male recipients of female kidneys contained the Y chromosome [30]. These studies documented that a circulating population of mesenchymal cells could migrate and engraft at the sites of chronic rejection. Similarly, myocytes, arterioles and capillaries that were of host origin (male) could be identified in female donor heart biopsies [31]. In aggregate, these studies document that, circulating mesenchymal cells can be recruited to sites of injury or inflammation.

Most of the clinical evidence for circulating mesenchymal cells has come from organ transplant situations. In such cases, there are many factors, such as immunologic differences and vascular injury that may influence the accumulation of circulating mesenchymal cells in tissue. More recent studies with parabiotic mice created by surgically joining a wild-type mouse and a transgenic mouse, which ubiquitously express green fluorescent protein (GFP) such that, shared circulatory systems were created [32], support the concept that circulating mesenchymal cells are recruited to sites of lung injury. In this study, when the lungs of the wild-type parabiotic mice were injured, 5–20% of the fibroblasts, that were cultured from the injured wild-type lungs expressed GFP, suggesting that they were recruited to the injured lungs from the circulation [32].

FIBROCYTES ARE CIRCULATING MESENCHYMAL PRECURSORS

A population of circulating cells termed “fibrocytes” that express both fibroblast and leukocyte markers has been isolated from human and murine blood [33–38]. Fibrocytes were originally reported, as a population of adherent cells that may be cultured from the peripheral blood [34]. Later it was reported that these cells could migrate to subcutaneously implanted wound chambers in response to injury [34, 36]. Ten to 15% of the inflammatory cells recruited to these wound chambers on day 10 had spindle-shaped morphology, typical of cultured primary fibroblasts [34]. The kinetics of recruited fibrocytes suggested

emigration from the blood, rather than slow migration from wound margins. Fibrocytes synthesize the ECM proteins, collagen I (col I), collagen III (col III), and fibronectin [34]. In addition, fibrocytes express a variety of leukocyte markers such as the common leukocyte antigen (CD45), the pan-myeloid antigen, CD13, the hematopoietic stem cell antigen, CD34, and the class II MHC antigens. Fibrocytes do not express epithelial or endothelial markers, and are negative for non-specific esterases and the monocyte/macrophage markers CD14 and CD16 [34]. In addition, they are negative for the Langerhan's cell marker CD1a, dendritic-cell-associated CD25, CD10 and CD38 and the pan B cell antigen CD19 [34]. It has been estimated that this population comprises about 0.5% of human peripheral blood leukocytes in healthy volunteers [34].

EVIDENCE FOR A BM ORIGIN OF FIBROCYTES IN INJURED LUNG

The origin of fibrocytes are controversial. The original description of this cell type suggested that, when female mice received male BM transplants following 800 rads of total body irradiation (TBI), fibrocytes bearing significant levels of the male SRY gene were not noted, leading to the conclusion that, "blood-borne fibrocytes do not originate from radiosensitive hematopoietic stem cells, but arise instead, from either a radio resistant BM progenitor cell, or from other tissue sources [34]." More recent studies using GFP BM-chimeric mice have provided evidence that pulmonary fibroblasts can arise from radiosensitive bone marrow precursors [23, 24]. In GFP-BM chimeric mice (wild-type mice that have received a BM transplant from a GFP transgenic mouse), GFP-expressing fibroblasts have been identified in fibrotic regions of lungs exposed to irradiation [23], and in fibrotic regions of bleomycin-exposed lungs [24]. Similarly, fibrocytes are increased in number, in BM of bleomycin-treated mice [25]. The reason for the discrepancy regarding a BM origin for fibrocytes is not entirely clear, but may be related to the dose of irradiation used. The original studies which used 800 rads (approximately 8Gy) of TBI did not find evidence of BM-derived fibrocytes in circulation [34]. In more recent studies, injection of GFP+ BM cells into mice that received 7 Gy of TBI did not result in BM-derived cells being incorporated into lung tissue [23]. However, mice that received both 20 Gy of lung irradiation, and 7 Gy of TBI did show evidence of BM-derived fibroblasts in fibrotic lesions [23]. Similarly, chimeric mice generated by injecting GFP+ BM cells into mice, which had received 10 Gy of TBI also showed evidence of BM-derived fibroblasts following 20 Gy of lung irradiation [23] or bleomycin inoculation [24]. These studies suggest that the progenitor that gives rise to fibrocytes/fibroblasts is indeed a relatively radio-resistant cell, which is eliminated by high levels of irradiation (~10 Gy of TBI).

EVIDENCE FOR THE ROLE OF FIBROCYTES IN FIBROTIC DISEASE

Although fibrocytes were described in 1990, evidence for their involvement in fibrotic disease did not come until recently. Fibrocytes are present in biopsies taken from remodeled airways of human asthmatic patients [39]. In this immunohistochemical study CD34 positive cells co-localized with cells expressing col 1 mRNA. Fibrocytes, characterized by this dual expression of CD34 and col 1 mRNA, were seen in areas of collagen deposition and basement membrane thickening, suggesting a pathogenic role for these cells [39]. Using an animal model of allergen challenge, these investigators showed that fibrocytes could be

isolated from the peripheral blood and fluorescently labeled *ex vivo*. When injected into allergen-primed mice intravenously, labeled fibrocytes were able to home to bronchial mucosa [39]. Interestingly, the phenotype of fibrocytes recovered from the bronchial mucosa were different than the phenotype of circulating fibrocytes recovered from peripheral blood, suggesting local differentiation events within the lung. Fibrocytes have also been shown to accumulate in the lungs of bleomycin-exposed mice [25]. The kinetics of fibrocyte recruitment to the lung correlates with the development of fibrosis. Additionally, fibrocytes have been demonstrated in nephrogenic fibrosing dermopathy [40], and hypertrophic scarring [41], again suggesting a pathogenic role for this cell type.

POTENTIAL PHENOTYPES AND DIFFERENTIATION OF LUNG FIBROCYTES

Fibrocytes have been identified in circulation, skin wounds, bronchial mucosa and bleomycin-injured lungs [25, 32–34, 37, 39]. Thus, it is possible that fibrocytes contribute directly to fibrotic processes. Alternatively, fibrocytes might differentiate into effector fibroblasts or myofibroblasts. There are several potential phenotypes associated with lung fibrocytes. 1) Lung fibrocytes could remain unchanged from their phenotype in circulation. They would retain CD34, CD45, CD13, MHC class II, and would synthesize modest levels of ECM proteins. It is likely that CD34 expression could be lost on this population [39], but other markers may remain. 2) Fibrocytes may acquire α-SMA expression, but retain CD45, CD13 and MHC class II, antigens and secrete modest to moderate amounts of ECM. This population may be referred to as myofibrocytes. 3) Fibrocytes could differentiate into effector fibroblasts. This would result in the loss of CD45, CD13 and MHC class II antigens from the cell surface, and would be associated with moderate amounts of ECM synthetic capacity. 4) Fibrocytes could differentiate into myofibroblasts. The phenotype of myofibroblasts is CD45−, CD13−, MHC class II−, α-SMA+ and is associated with exuberant synthesis of ECM proteins. It is likely, that fibrocytes may first differentiate into effector fibroblasts before ultimately differentiating into highly activated myofibroblasts.

In vitro experimental evidence supports the hypothesis that, fibrocytes can differentiate into myofibrocytes, effector fibroblasts, and myofibroblasts. Human fibrocytes express high levels of CD34 and low levels of α-SMA upon isolation [39]. The maintenance of the cells in serum-free culture conditions results in loss of the CD34 expression, but no expression of α-SMA. This would support a transition from the fibrocyte, to effector fibroblast phenotype. The culture of the fibrocytes in the presence of endothelin-1 or TGF-β1 for 6 days induces the loss of CD34 expression and the gain of α-SMA expression [39], indicating a functional transition to the myofibroblast phenotype. These experiments replicate earlier work showing TGF-β1 stimulation induces α-SMA expression in cultured human fibrocytes, and augments the synthesis of collagen by these cells in a dose-dependent manner [33]. This suggests that under profibrotic stimulation, fibrocytes can transition all the way from fibrocytes to myofibroblasts. Kinetic studies further support the hypothesis of fibrocyte differentiation. Cultured human fibrocytes express both CD45 and CD34 at high levels within the first two weeks, after isolation from blood. By the third week post-isolation, expression of CD34 is dramatically reduced by culture in complete media. By the fourth week post-isolation, CD45 expression is diminished. Both CD34 and CD45 expression continue to diminish by the 5th week post-isolation. In contrast, α-SMA expression increases by the 4th week post-culture

indicating an *in vitro* transition from the, fibrocyte to the effector fibroblast and myofibroblast phenotypes [25].

The population of mesenchymal cells isolated from lung mince of unchallenged mice and maintained in complete media are a mixture of both fibrocytes (identified as CD45+, col I+ cells) and effector fibroblasts [CD45−, col I+; (see Fig. 1). Interestingly, the expression of col I, as indicated by flow cytometry or western blotting demonstrates that fibrocytes produce lesser amounts of col I; whereas, effector fibroblasts produce col I with greater abundance. Additionally, both myofibrocytes and myofibroblasts (cells that express α-SMA) can be identified when lung minces are cultured in complete media (BBM, unpublished observation). Thus, it may be possible for fibrocytes to contribute directly to the deposition of extracellular matrix, and contractile properties of the lung in fibrotic processes. Given the limited ECM synthetic capacity of fibrocytes, however, it is more likely that transition of fibrocytes into effector fibroblasts/myofibroblasts occurs rapidly *in vivo,* and the enhanced collagen synthetic capacity of effector fibroblasts and myofibroblasts contribute significantly to the fibrotic deposition of ECM. In this regard, it is interesting to note that the fibrocytes themselves can produce TGF-β1 [36], and thus, differentiation to the effector fibroblast/ myofibroblast phenotype may indicate an autocrine regulation pathway.

ORIGIN OF MYOFIBROBLASTS IN INJURED LUNG

Myofibroblasts have long been the hallmark of fibrotic lung disease [3, 14, 15]. Their increased capacity for collagen synthesis and contractile properties make them likely accomplices in the process of alveolar collapse and fibrosis that can occur in diseases like UIP [15]. They are clearly present in patients with UIP, where they are most prominent in fibrotic foci [10, 19]. The role of BM-derived cells in the generation of myofibroblasts is controversial. Studies in GFP-BM chimeric mice demonstrated that, more than 80% of the collagen-producing cells that accumulated in the lungs of bleomycin-treated mice were BM-derived, as evidenced by their expression of GFP [24]. Interestingly, however, when fibroblasts were cultured from bleomycin-treated lungs of GFP-BM chimeric mice, there was no evidence of BM-derived (GFP+), col 1+, α-SMA+ cells [24]. These data would seem to imply that these BM-derived fibroblasts do not develop into myofibroblasts. Furthermore, this study suggested that the majority of the extracellular matrix deposited in murine bleomycin-induced lesions derived from BM-derived effector fibroblasts, and not from myofibroblasts. It is possible that the GFP-expressing fibroblasts arose from BM-derived fibrocyte precursors that were recruited to the lung. However, the surface phenotype of the GFP-expressing cells in either the circulation, or the lung was not determined. Similar to the studies of Hashimoto *et al.*, early studies on peripheral blood fibrocytes suggested that, they are also negative for α-SMA [34]. Thus, it is possible that some effector fibroblasts that are α-SMA negative may arise from fibrocytes; further differentiation to the myofibroblast phenotype is blocked, perhaps as a result of TGF-β1 insensitivity, as proposed in the Hashimoto study [24].

Several studies support the concept that, fibrocytes can give rise to myofibroblasts *in vivo* [25, 33, 39]. CD34-positive, α-SMA-expressing cells were noted in the bronchial mucosa of patients with allergic asthma, suggesting that myofibrocytes exist *in vivo*. Murine cells

recruited to bronchial airways post-allergen challenge were CD34-positive, α-SMA expressing myofibrocytes [39]. The reasons for the differences regarding the potential of BM-derived cells to fully differentiate into myofibroblasts are unclear. One potential explanation is that there may be different BM populations that can differentiate into pulmonary fibroblasts. For instance, mesenchymal stem cells (MSCs) or CD34− stem cells in the BM may be able to give rise to fibroblasts and myofibroblasts under certain conditions [42–44]. Thus, compared to fibrocytes, these two populations may differentially reconstitute effector fibroblasts and myofibroblasts following BM transplantation and lung injury. These hypotheses will require more experimental testing before we know, whether different BM cell progenitors give rise to effector fibroblasts and myofibroblasts, as well as the conditions that determine such cell fates.

EVIDENCE THAT CHEMOKINES MEDIATE FIBROCYTE RECRUITMENT TO SITES OF INJURY

In order for BM-derived cells to participate in fibrosis, several conditions must be met. First, the BM must receive a signal(s) for the amplification and release of precursor cells. Second, the BM precursors must exit the BM and enter the circulation. Third, the circulating cells must receive instructive signals to direct their migration to sites of injury. Chemokines likely play central roles in these events as they do in the migration of leukocytes to areas of inflammation. Chemokines are *chemo*tactic cyto*kines* that are released from inflammatory and structural cells, in response to a wide variety of stimuli [45]. It is possible that chemokines are responsible for both sending the message to the BM to amplify and release progenitors, as well as in directing the egress of progenitors from the blood to injured tissue. Chemokines are classified on the basis of their primary amino acid structure into CXC, C, CX_3C, and CC families, based on the relative positions of the two conserved cysteine residues found in the N-terminus of all chemokine proteins. A subcommittee recently adopted a new nomenclature system for chemokines and their receptors [46]. The CXC chemokines bind to CXCR chemokine receptors, and are well known for their roles in neutrophil recruitment and angiogenesis [47, 48]. The CC chemokines are generally noted for recruitment of monocytes and lymphocytes [48]. Additionally, two chemokine receptors, the CXCR4 and CCR5 receptor have been identified as co-receptors for HIV-1 infection and are targets for anti-viral therapy [49].

The chemokine receptor expression profile of human circulating fibrocytes is an area of active investigation. Human fibrocytes express CCR3, CCR5, CCR7 and CXCR4, but not CCR4, CCR6 or CXCR3 [33]. CCR2 expression has not been evaluated. Murine fibrocytes have been reported to express CCR7 and CXCR4 [25, 33]. Interestingly, these same two receptors were reported on BM-derived fibroblasts [24]. Cultured murine fibrocytes express CCR1, CCR2, CCR5, CCR7 and CXCR4 (BBM, unpublished observation). Thus, fibrocytes/fibroblasts have chemokine receptors necessary to respond to chemokine-mediated recruitment signals.

Experimental evidence suggests that fibrocytes, and fibroblasts which bear chemokine receptors can migrate in response to appropriate chemokine ligands. CCR7- and CXCR4-

expressing BM-derived fibroblasts migrated to CCL21 (a CCR7 ligand) and also to CXCL12 (a CXCR4 ligand) [24]. Phillips *et al.* [25] demonstrated that subsets of human fibrocytes express CXCR4, and that the CXCR4+ fibrocytes migrate in response to CXCL12 *in vitro.* Furthermore, administration of anti-CXCL12 antisera to bleomycin-treated mice reduces the accumulation of CXCR4+ fibrocytes in the lung [25]. Similarly, CCR7+ fibrocytes were shown to migrate to the lung at day 8 post-bleomycin; a time when the expression of the CCR7 ligand, CCL21 was elevated [25].

There are several potential sources for the chemokine ligands that recruit fibrocytes and fibroblasts to the lung. It has been suggested that inflammatory monocytes first migrate to sites of tissue injury, and the subsequent release of chemokines by these inflammatory cells may signal fibrocyte recruitment [23]. However, structural cells, such as alveolar epithelial cells may also release chemokine mediators in response to injury. In this regard, alveolar epithelial cells are known to secrete CCL2 (a ligand for CCR2), in response to numerous stimuli [50–52]. We have reported that murine fibrocytes are recruited to the airspaces of injured wild-type, but not CCR2−/− mice [53], and the absence of fibrocyte recruitment to injured airspaces of CCR2−/− mice correlates with their protection from fibrosis [54]. Unlike the expression of CXCR4, expression of CCR2 was expressed in all fibrocytes in our studies (BBM, unpublished observation).

Based on these observations, we speculate that injury signals in the lung result in the elaboration of chemokines, and the stimulation of fibrocyte proliferation and release from the BM. Fibrocyte subsets can be classified on the basis of their chemokine receptor expression. Some chemokine receptors may be ubiquitously expressed on all fibrocyte subsets (such as CCR2), whereas others may mark unique subsets (such as CXCR4). The fibrocyte subsets circulate in the blood stream, and are recruited to sites of tissue injury by the temporal release of chemokines that bind specifically to the chemokine receptors expressed on that fibrocyte subset (Fig. 2). Once *in situ*, the autocrine production of TGF-β1 by the fibrocytes, and the elaboration of profibrotic factors within the local microenvironment likely drive the differentiation of fibrocytes into effector fibroblasts and myofibroblasts (Fig. 1).

THE POTENTIAL FOR BM-DERIVED CELLS TO PARTICIPATE IN EMT

Recent studies have indicated that interstitial fibroblasts can arise from BM migration, but can also be derived from EMT in murine models of kidney fibrosis, following unilateral ureteral obstruction. Interestingly, the most potent inducer of EMT is the pro-fibrotic cytokine, TGF-β1 [55]. Given the fact that lung epithelial cells have been shown to arise from BM, or circulating precursors following injury [28, 32, 56, 57], it is theoretically possible that BM-derived epithelial cells may undergo EMT, contributing to the effector fibroblast population in fibrotic lungs. Because fibrocytes themselves are sources of TGF-β1, it is also possible that the recruitment of fibrocytes to injured lung may influence this process. EMT is believed to occur when epithelial cells lose cell-cell adherens junction complexes, and select mesenchymal cell fates under the influence of local factors [55]. The current literature regarding BM-derived mesenchymal cells in fibrosis has not addressed this possibility.

DIRECTIONS FOR FUTURE RESEARCH

The data we have reviewed are compelling to suggest, that BM-derived circulating cells contribute to animal models of fibrosis, following a diverse set of injuries [23–25, 32, 39, 53]. Furthermore, the data suggest that, chemokine-mediated recruitment of these cells are critical for their migration from the BM, via the circulation and into the injured lung. Phillips *et al.* noted that there were distinct subsets of CXCR4+ and CXCR4− human fibrocytes [25]. The specific roles that these subsets play are unclear, but one possibility is that different chemokine receptors are necessary for the recruitment of fibrocytes to distinct anatomic locations within the lung. For instance, CCR2 expression may be necessary for alveolar homing, whereas CXCR4 may direct interstitial accumulation. CCR7 may mediate recruitment to lymph nodes, in a manner analogous to the role it plays in dendritic cell homing (reviewed in [58]). Additionally, temporal expression of chemokine ligands may regulate the sequential recruitment and activation of fibrocytes. Expression of CCL2 (a CCR2 ligand) is known to occur early (within 24 h) after injury, whereas expression of CXCL12 (a CXCR4 ligand) appears to peak after the first week [24, 25]. CCL21 (a CCR7 ligand) is not elevated until 2 weeks post-injury [24]. Thus, further research will be needed to determine which chemokine/chemokine receptors regulate specific fibrocyte subsets, crucial for the localization of effector cells and development of fibrosis.

In order for these observations to have meaning in human disease, there are still many unanswered questions. 1) Are BM-derived mesenchymal cells present in human fibrotic lung disease? 2) Which subsets of these cells are present? 3) Where are they located (blood, alveolar space, interstitium)? 4) Do different subsets of cells correlate with different diseases or disease progression? 5) What are the chemokine/cytokine networks required for recruitment and differentiation of these cells? 6) Can we utilize the “fibrotic homing potential” of fibrocytes to target therapeutics?

The ultimate goal of therapy would be to block the chemokine/chemokine receptor interactions that are required for fibrotic progression, without severely compromising the ability of the host to mount inflammatory responses to infection. If this can be done, then small molecule chemokine receptor antagonists may become effective tools for anti-fibrotic therapy. Alternatively, the ability to isolate these cells from the blood of patients with pulmonary fibrosis may represent an opportunity for targeted gene therapy. Fibrocytes proliferate *in vitro* and may be amenable to genetic manipulation. Adoptively transferred fibrocytes home to areas of fibrosis [39]. As such, it may be possible to engineer fibrocytes to produce anti-fibrotic factors, and use them as an autologous, targeted delivery system for these agents. The findings that BM-derived cells contribute to the pathogenesis of fibrosis have opened up new avenues for investigation, have given us a broader understanding of disease pathogenesis, and may offer new therapeutic strategies for patients with fibrotic lung disease.

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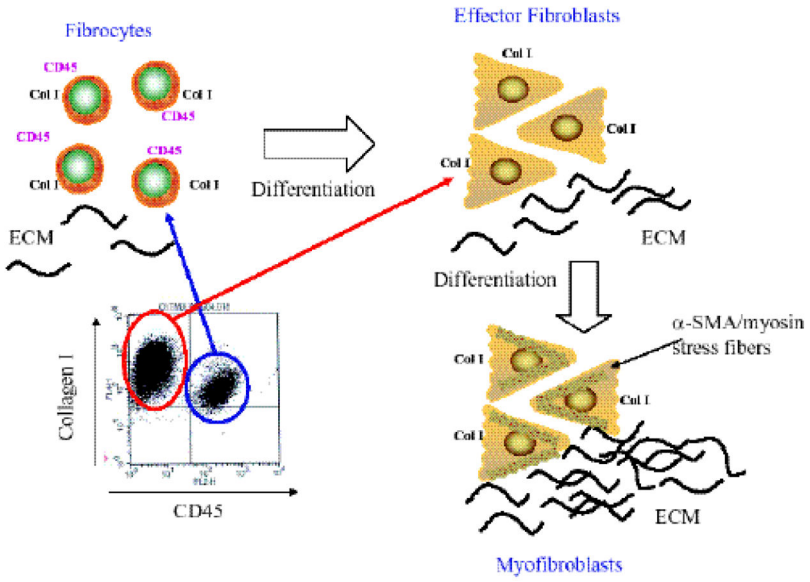

Fig. 1. Differentiation of Fibrocytes to Effector Fibroblasts and Myofibroblasts

Fibrocytes in the lung are characterized by the dual expression of leukocyte markers (CD45, CD13) and mesenchymal markers (col I, col III and fibronectin). For simplicity, this diagram depicts only CD45 and col I expression. The flow cytometry diagram demonstrates that, both fibrocytes and effector fibroblasts can be cultured from unchallenged murine lungs. The production of ECM by fibrocytes is modest, compared to that of effector fibroblasts which are characterized by an absence of the leukocyte markers (CD45 and CD13), and the upregulation of ECM synthesis. Further differentiation to the myofibroblast phenotype is associated with the appearance of α-SMA expression, the formation of stress fibers within the cell, the acquisition of a contractile phenotype, and further upregulation of ECM production.

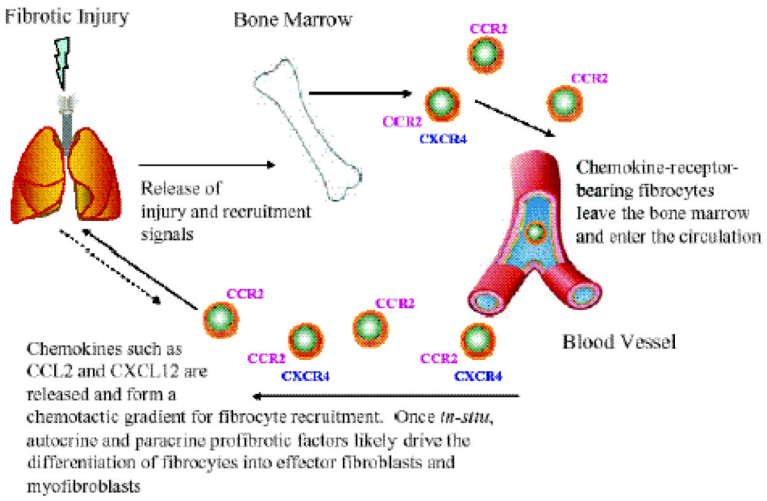

Fig. 2. Mobilization of Stem Cells from BM to Circulation in Response to Injury Signals from the Lung

Fibrotic injury induces the release of recruitment signals involving chemokines that stimulate the amplification and release from the BM of fibrocyte subsets, bearing unique chemokine receptor profiles. Once released from the BM, these subsets travel via the circulation, and are recruited to sites of injury via specific chemokine/chemokine receptor interactions. Once *in situ*, fibrocytes are stimulated to differentiate into effector fibroblasts and myofibroblasts, via local profibrotic factors.