THE ROLE OF CIRCULATING MESENCHYMAL PROGENITOR CELLS, FIBROCYTES, IN PROMOTING PULMONARY FIBROSIS

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ABSTRACT

The resident fibroblast has been traditionally viewed as the primary cell involved in promoting pulmonary fibrosis. However, contemporary findings now support the concept of a circulating cell (fibrocyte) that also contributes to pulmonary fibrosis. Fibrocytes are bone marrow-derived mesenchymal progenitor cells that express a variety of cell surface markers related to leukocytes, hematopoietic progenitor cells and fibroblasts. Fibrocytes are unique in that they are capable of differentiating into fibroblasts and myofibroblasts, as well as adipocytes. In this review, we present data supporting the critical role these cells play in the pathogenesis of pulmonary fibrosis.

Fibrocytes were first identified in 1994 as a circulating cell that extravasated into wounds and contributed to wound repair (1). Fibrocytes are unique bone marrow-derived mesenchymal progenitor cells that are defined by their growth characteristics and surface phenotype, as they express markers compatible with leukocytes, hematopoietic progenitor cells and fibroblasts (2). In addition, fibrocytes express a number of other cell markers that include chemokine receptors and adhesion molecules (3, 4). Fibrocytes participate in tissue remodeling by producing extracellular matrix proteins (collagen I, collagen III and vimentin), and by secreting matrix metalloproteinases (5). Moreover, fibrocytes are an important cellular source of inflammatory cytokines, chemokines and growth factors that contribute to important autocrine and paracrine signals within the tissue microenvironment (4, 5). For example, fibrocytes isolated from wounds of animal models of wound repair express mRNA for IL-1β, IL-10, TNF-α, MCP, MIP-1α, MIP-1β, MIP-2, PDGF-A, TGF-β1, and M-CSF. Specific chemokine receptor/
chemokine ligand biological axes are critical to the recruitment of fibrocytes to sites of tissue injury and repair, which can contribute to propagation of the fibrotic response (5). Fibrocytes can differentiate into other mesenchymal cells, such as myofibroblasts and adipocytes (6, 7). Fibrocytes have been found to be important mediators of antigen-specific immunity via their ability to function as antigen presenting cells (6). Fibrocytes have been shown to deposit extracellular matrix in wound repair (1), and during fibroproliferative disorders in response to local inflammation (9). These unique cells have become the focus of research efforts that encompass a wide variety of focal and diffuse fibrosing disorders, including those localized to the skin, lungs, liver, kidney, pancreas and bladder; and the more diffuse involvement seen in atherosclerosis and tumors. In this review, however, we will concentrate on the pivotal role of fibrocytes in the pathogenesis of pulmonary fibrosis.

THE CIRCULATING FIBROCYTE

The circulating fibrocyte was first described in 1994 in an experimental model of wound repair (1). Within 24hrs following injury, fibrocytes represented 10% of the cells in the wound; described as spindle-shaped, they co-expressed procollagen/collagen and CD34. The concept that these cells were derived from the circulation came from the notion that their appearance in the wound occurred faster than would be expected by entry of fibroblasts from the surrounding skin into the wound chamber through the permeable plastic layer to begin collagen production (10). In addition, the CD34+ spindle-shaped cells expressed markers of connective tissue cells, not of monocytes, macrophages, endothelial cells or epithelial cells. Thus the word fibrocyte (a term combining fibroblast with leukocyte) was coined for this circulating fibroblast progenitor that produced collagen and expressed the hematopoietic marker CD34 (10). On scanning EM, fibrocytes morphologically exhibit prominent cell surface projections, making them distinct from the appearance of other leukocytes (1). In addition to promoting fibrosis, fibrocytes have been found to function as antigen presenting cells in promoting activation of T cells (8). Fibrocytes also promote angiogenesis in vivo through the generation of a variety of pro-angiogenic factors (3, 5). It has now been determined that fibrocytes comprise approximately 0.1–1% of the nucleated cells in the peripheral blood in healthy hosts (3, 4, 9, 11), and have been found in a variety of tissues under both physiologic and pathologic states (9).

In the context of physiologic and pathologic fibrosis, tissue fibro-
blasts and myofibroblasts are historically thought to be derived from resident fibroblasts that migrate, proliferate and express constituents of the extracellular matrix in response to tissue injury (12–14). However, two contemporary theories have been proposed that have added complexity to the concept that fibroblast-like cells are only derived from local fibroblasts. The first theory states that tissue injury induces epithelial cells to transition to a mesenchymal phenotype (the fibroblast/myofibroblast concept of epithelial mesenchymal transition), that subsequently contributes to the fibroproliferative process (1, 13, 15, 16). The second theory is that circulating fibrocytes (bone marrow-derived progenitor cells) home and extravasate into sites of tissue injury, differentiate into fibroblasts/myofibroblasts and contribute to the generation of extracellular matrix during the fibroproliferative process in response to injury (1, 3, 9, 13, 15, 17).

**THE ORIGIN OF FIBROCYTES APPEARS TO BE THE BONE MARROW**

Fibrocytes express markers of hematopoietic cells (CD45, major histocompatibility complex II and CD34) and stromal cells (collagens I and III, and fibronectin) (1, 4, 9, 15, 17–21). Human fibrocytes from peripheral blood (type I collagen-positive by FACS analysis) express the common leukocyte antigen, CD45, greater than CD34 (100% positive vs only 10% positive, respectively; unpublished observation). They do not express T cell markers (CD3, CD4 and CD8), B cell markers (CD19), the IL-2 receptor chain CD25, the low affinity Fc gamma receptor III (CD16) or myeloid markers (CD14 and non-specific esterase) (1, 3, 4, 9, 15, 22). Since the expression of CD34 by the fibrocyte has been shown to decrease over time (both in culture and in vivo) depending on the inflammatory milieu (9, 10, 22), the co-expression of collagen production and the other hematologic markers (such as CD45) are frequently used to identify fibrocytes. For example, fibrocytes early in culture are associated with expression of CD34, CD45, collagen I and vimentin; however, after exposure to TGF-β or endothelin, fibrocytes differentiate into myofibroblast-type cells resulting in expression of α-smooth muscle actin and loss of CD34 and CD45 expression (4, 9, 22, 23). Thus, the classic markers for circulating fibrocytes identified in peripheral blood by FACS analysis and newly isolated fibrocytes in culture are CD45, CD34 and type I collagen (collagen I).

Some studies suggest that fibrocytes may differentiate from CD14+ peripheral blood monocytes that express the receptors for the Fc portion of IgG, CD64 and CD32 (19–21, 24). Circulating fibrocytes may be
present in a subset of CD14+ CD16− monocytes that carry the chemokine receptor, CCR2, on their surface (25, 26). At the time of tissue injury, this monocyte subset is released from the bone marrow into the peripheral blood and migrates to inflamed sites via a CCR2-mediated pathway (25, 26). Other studies have suggested that human fibrocytes may represent an intermediate stage of differentiation of a monocyte subset into mature fibroblasts and myofibroblasts in tissue (27). This hypothesis is supported by the fact that fibrocytes express the major histocompatibility complex class I and class II, CD80, CD86 (1, 8, 20, 21, 28), exhibit antigen-presenting activity (8) and activate CD4+ and CD8+ lymphocytes (8, 28), but do not express markers of monocyte-derived dendritic cells such as CD1a, CD10, and CD83. However, when you use quantitative FACS analysis of freshly isolated human fibrocytes from peripheral blood, the majority of these fibrocytes are CD14− and CXCR4+, not CD14+ and CCR2+ (unpublished observation). While it is increasingly clear that fibrocytes are most likely myeloid lineage in character, it remains to be fully elucidated whether they are truly derived from a CD14+ progenitor cell.

FIBROCYTES CAN UNDERGO DIFFERENTIATION TO OTHER MESENCHYMAL LINEAGE CELLS

In long-term cell culture and in tissue in vivo, fibrocytes lose their expression of CD34 and CD45 (6, 15, 20, 24, 29). While isolated fibrocytes in cell culture (i.e., presence of serum) spontaneously differentiate into myofibroblasts (9, 21, 22), this process is augmented in the presence of TGF-β or endothelin-1 (3, 4, 7, 9, 15, 22), resulting in cells that produce fibronectin and collagen and express the myofibroblast marker, α-smooth muscle actin. For example, using a wound repair model, bone marrow transplantation from GFP-transgenic animals to wild-type animals showed that the cells in the wound co-expressed GFP and α-smooth muscle actin, suggesting that the myofibroblasts present in the wound were from the bone marrow (30). Fibrocytes have also been found to respond to type 2 cytokines and more readily differentiate into myofibroblast-like cells. The pro-fibrotic cytokines, IL-4 and IL-13, promote fibrocyte differentiation to α-smooth muscle actin positive cells (i.e., myofibroblasts) from peripheral blood mononuclear cells without inducing proliferation, whereas the anti-fibrotic cytokine, IFN-γ, inhibites fibrocyte differentiation (31). In addition to fibrocytes differentiating into α-smooth muscle actin positive cells (i.e., myofibroblasts), fibrocytes have been found to differentiate into adipocytes in vitro and in vivo, which appears to be a process that is PPAR-γ-
dependent and inhibited by TGF-β (6, 7). These findings taken together with the previous studies suggest that fibrocyte differentiation is influenced by a complex profile of cytokines within the local microenvironment of tissue injury.

**THE CHEMOKINE RECEPTOR/CHEMOKINE (CXCR4/CXCL12) BIOLOGICAL AXIS PLAYS A CRITICAL ROLE IN TRAFFICKING OF CIRCULATING FIBROCYTES INTO THE LUNG DURING THE PATHOGENESIS OF PULMONARY FIBROSIS**

Fibrotic lung diseases are a large group of disorders characterized by varying degrees of inflammation and fibrosis of the lung parenchyma (32). The clinical course is usually one of progressive replacement of lung tissue with scar tissue, and concomitant clinical deterioration. In some of these disorders the underlying etiology is known, while in others, the etiology is still unknown. Among these fibrotic lung disorders, idiopathic pulmonary fibrosis is the most common and is defined as the histopathologic finding of usual interstitial pneumonia in the absence of other recognizable causes (32).

Several lines of evidence support the role of circulating fibrocytes in the development of lung fibrosis (33). In general, interest in studying the potential role of fibrocytes in the pathophysiology of lung fibrosis stems from the well-known characteristics of fibrocytes themselves that include: 1) fibrocytes can differentiate into fibroblasts and myofibroblasts (3, 4, 9, 15, 22); 2) fibrocytes can produce cytokines that induce collagen deposition (3–5, 9, 15, 22); 3) fibrocytes can produce pro-angiogenic mediators and promote angiogenesis (34); and 4) fibrocytes are potent antigen presenting cells that can recruit and activate T cells (8).

Human fibrocytes express several chemokine receptors, including CCR3, CCR5, CCR7, and CXCR4; in contrast, mouse fibrocytes express predominately CXCR4, CCR2, and CCR7 (4, 9, 15, 35). Fibrocyte migration into wound sites has been previously quantified by *ex vivo* labeling cells with a fluorescent dye followed by intravascular injecting and monitoring their trafficking to intradermal sites of CCL21 (chemokine ligand to CCR7) and CXCL12 (chemokine ligand to CXCR4) injections (15). The trafficking of CXCR4+ fibrocytes in response to CXCL12 is especially apropos to the importance of chemokine signaling during pulmonary fibrosis.

The CXCR4/CXCL12 biological axis plays an important role in the homing of bone marrow-derived progenitor cells (36). CXCR4 is an important chemokine receptor in stem cell trafficking, and the differ-
ential expression of CXCL12 in tissues creates the chemotactic gradient required for trafficking of CXCR4+ cells. In a mouse model of bleomycin-induced pulmonary fibrosis, fibrocytes have been shown to home to the lungs and contribute to fibrosis (9). In initial studies, isolated human fibrocytes were found to have the increased capacity to home to the lungs of bleomycin exposed SCID mice during the pathogenesis of pulmonary fibrosis (9). Similarly, in immunocompetent bleomycin-treated mice, the magnitude of lung pro-collagen I and III expression correlated with the number of CD45+ collagen I+ CXCR4+ fibrocytes in the bone marrow, peripheral blood and lung (9). In addition, CXCL12 was found to be significantly increased in the lungs of mice that were treated with bleomycin, supporting the notion that a CXCL12 gradient between the lungs and the plasma promoted the recruitment of the CD45+ collagen I+ CXCR4+ fibrocytes to the fibrotic lung. In subsequent experiments, the administration of specific neutralizing anti-CXCL12 antibodies to bleomycin-treated mice resulted in markedly reduced fibrocyte extravasation into the lung, reduced pulmonary collagen deposition and reduced morphometric expression of collagen deposition and α-smooth muscle actin. However, the effect of blocking CXCL12 did not alter the magnitude of infiltration of other leukocyte populations in the lungs under these conditions (9). Using mice that had been transplanted with bone marrow from GFP+ mice, studies have confirmed that fibrocytes that extravasate into the lung during bleomycin-induced pulmonary fibrosis are bone marrow derived (unpublished observation). Moreover, these same studies have confirmed that the bone marrow, in part, is a source for α-smooth muscle actin positive cells in the lung under the same conditions.

The finding of the importance of CXCR4/CXCL12 in the mouse model of pulmonary fibrosis has also been found to be important in human pulmonary fibrosis. Recently, a study of patients with idiopathic fibrotic interstitial lung disease demonstrated that the numbers of circulating CD45+, collagen I+, CXCR4+ fibrocytes were an order of magnitude higher than in healthy control subjects and accounted for approximately 6% to 10% of their circulating nucleated cell population. In addition, this study demonstrated that the expression of CXCL12 was markedly elevated in the lung and plasma of the patients with pulmonary fibrosis (11). The predominate cell in the lung that appeared to express CXCL12 was the hyperplastic type II pneumocyte (11). This study has been confirmed in a recent study of patients with idiopathic pulmonary fibrosis (37). In lung tissue from patients with idiopathic pulmonary fibrosis, morphometric analysis by immunofluorescence
and confocal microscopy demonstrated the presence of CD34+ or CD45+ CXCR4+ fibrocytes that co-expressed markers of collagen production and α-smooth muscle actin \(^{(37)}\). In addition, CXCL12 was found to be significantly increased in the plasma of patients with idiopathic pulmonary fibrosis, as compared to healthy controls; and CXCL12 was detectable in the bronchoalveolar lavage fluid of 40% of the patients with pulmonary fibrosis, but not in control subjects \(^{(37)}\). Similar to the above study, this study demonstrated that CXCL12 was strongly expressed by hyperplastic type II pneumocytes within the lung tissue \(^{(37)}\). To extend the above studies further, Moeller and colleagues have found in patients with idiopathic pulmonary fibrosis that the magnitude of circulating fibrocytes directly correlated with their exacerbations of the disease, and with resolution of the exacerbation, the elevated levels of fibrocytes were shown to return to a persistently elevated baseline \(^{(38)}\). Furthermore, this study demonstrated that patients with greater than 5% circulating levels of fibrocytes had a worse prognosis than patients with circulating level of fibrocytes less than 5% of their circulating nucleated cell population \(^{(38)}\). Results of the above studies, underscore the importance of chemokine-mediated fibrocyte influx during the pathogenesis of pulmonary fibrosis, and indicate that circulating fibrocytes, likely recruited through the CXCR4/CXCL12 axis, may contribute to the expansion of the fibroblast/myofibroblast population in idiopathic pulmonary fibrosis.

**CONCLUSION**

The fibrocyte is a recently described unique mesenchymal progenitor cell. Increasing evidence points to a pivotal role of these cells as an important source of fibroblasts and myofibroblasts during both physiologic and pathologic remodeling and repair processes. Fibrocytes have been detected in the lung during the pathogenesis of pulmonary fibrosis, and attenuation of their trafficking in mouse models directly correlates with a reduction in pulmonary fibrosis. Furthermore, circulating levels of fibrocytes in patients with idiopathic pulmonary fibrosis may represent a biomarker that indicates greater risk of mortality. Additional data are needed to ultimately translate these experimental and clinical findings to the development of novel therapeutic tools to selectively manipulation these cells and attenuate pulmonary fibrosis.

**REFERENCES**

22. Schmidt M, Sun G, Stacey MA, Mori L, Mattoli S. Identification of circulating...
27. Bellini A, Mattoli S. The role of the fibrocyte, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses. Lab Invest 2007;87:858–70.

DISCUSSION

Rounds, Providence: Beautiful presentation and very interesting work. I noticed that in your human studies you were studying both patients with IPF and with non-
specific interstitial pneumonitis and found increased circulating fibrocytes as well as lung fibrocytes; and those two diseases are very different in their response to steroids, which in fact, may be distinct entities. I am curious as to whether that begs the question of whether these fibrocytes are, in fact, contributing to the poor prognosis of patients with IPF.

**Strieter, Charlottesville:** Sharon, thank you very much. Actually the patients with NSIP were the variety with NSIP with fibrosis, and this patient population typically does not respond to steroids. In other words, this was not your usual patient with cellular NSIP.

**Metcalfe, Bethesda:** So it would seem, if you wanted to stop the trafficking, you would want to shut off whatever is producing the chemotactic ligands that are bringing in the mesenchymal cells. I wonder if you would comment on what you think bleomycin induces or whatever stimulants you are talking about induces what cell is producing what and what you need to shut off besides the steroids. The second part of that question would be, could you comment on other fibrotic diseases and whether or not you think it’s similar mechanisms?

**Strieter, Charlottesville:** I think, indeed, what is intriguing about the expression of SDF-1 is hypoxia in and of itself. HIF-1, for an example, is a very important transactivating event for not only the expression of the receptor in these fibrocytes (CXCR4) but also the ligand SDF-1. If you use biochemical markers of hypoxia in the lungs of patients with pulmonary fibrosis, you actually see significant areas of hypoxia. Even under conditions that are relatively normoxic, activating any receptor tyrosine kinase will ultimately lead to downstream activation of mTOR, and that leads to increased HIF-1, which results in further upregulation of both SDF-1 and CXCR4. So targeting that particular pathway, PI3 kinase, AKT and mTOR may very well be very important for attenuating the expression of this process. Now in the context of other fibrotic processes, what I didn’t have time to show you here is actually that we have an ongoing collaboration with the NIH in regards to Hermansky-Pudlak syndrome. These are patients that have genetic abnormalities of the Hermansky-Pudlak gene that is associated with the development of usual interstitial pneumonitis-like pattern in their third and fourth decade of life, which is the reason they die. As it turns out, patients with Hermansky-Pudlak syndrome that have pulmonary fibrosis have elevated levels of fibrocytes that rival what we see in patients with idiopathic pulmonary fibrosis. We have also seen this in other fibroproliferative processes, for an example, in patients with scleroderma.

**Quesenberry, Providence:** Very nice talk. We have been working on the ability of injured lung to transmit genetic phenotype via microvesicles, and it’s a very robust phenomenon in multiple different marrow cell types—stem cells and monocytes, etc. Just as one possibility is whether there is an ongoing flow of genetic information from an injured lung to your blood cells.

**Strieter, Charlottesville:** I think that is an absolutely interesting and intriguing concept, and certainly, we know, in some of model systems, that not so much specific genetic material but signals from the lungs such as MCSF, which plays a very important role to talk to the bone marrow to not only expand but also to begin mobilizing these cells.

**Boxer, Ann Arbor:** Are the circulating fibrocytes amenable to treatment with myelosuppressive drugs?

**Strieter, Charlottesville:** Very nice question. We have a significant interest in mTOR inhibition from the standpoint of regulation of specific chemokine receptors. Targeting mTOR with an mTOR inhibitor, such as rapamycin, plays an incredibly important role for attenuating the circulating levels of these cells, as well as the extravasation of these
cells in the lung under conditions of bleomycin-induced pulmonary fibrosis. The effect is a reduction in pulmonary fibrosis.

**Brenner, San Diego:** I wanted to make sure that I understood that according to the bleomycin model, it looked like the majority of alpha-smooth muscle actin positive myofibroblasts were fibrocytes originally by bone marrow transfer. Is that correct?

**Strieter, Charlottsville:** What we had found by FACS analysis using a chimeric wild-type mouse with GFP bone marrow was the presence of a significant population of bone marrow-derived (GFP+) fibrocytes (Col1+) that were alpha-smooth muscle actin positive in the lungs of animals exposed to bleomycin.

**Runge, Chapel Hill:** I thought your data on the fibrocytes was really interesting, and my question is whether you think there are tissue-specific signals that these same fibrocytes are the same ones that are involved in fibrotic processes and other organs and potentially in reparative processes, because they have a lot of similar characteristics.

**Strieter, Charlottesvile:** These cells are not unique to idiopathic pulmonary fibrosis. In fact, they have actually been found in airway remodeling in the context of asthmatic patients and mouse models of asthma. They have been found in the context of fibroproliferative disorders of the liver. They have been found in fibroproliferative disorders in the kidney. These cells have the ability to migrate into any organ and, in fact, are essentially a salient feature of essentially all fibroproliferative disorders.

**Neilson, Nashville:** I was interested in the beneficial effects of interrupting the CXCL12 signaling pathway in the circulation. I wonder if you have any information about its ability to disrupt movement in other fluid compartments, for example the interstitial fluid compartment where all the resident fibroblasts also reside, likely move based on some signaling property; and I wonder if that was also affected by the disruption experiments.

**Strieter, Charlottesvile:** In regards to resident fibroblasts that we have isolated from the lung, their expression of CXCR4 is relatively low. I can’t comment *in vivo* whether or not there is a change in a resident fibroblast phenotype in terms of regulation of CXCR4. In addition, we don’t see significant expression of CXCR4 *in vitro* in culture on the surface of conventional fibroblasts.