Biology of Fibroblasts and Myofibroblasts

Sem H. Phan[1]

[1]Department of Pathology, University of Michigan, Ann Arbor, Michigan

Despite their importance in fibrosis, the origin of fibroblasts and the genesis of the various subpopulations characterized by distinct phenotypes remain unclear. Various studies have described distinct and relatively stable phenotypes in fibroblasts isolated from lung tissue undergoing remodeling, which were not present in the normal intact tissue. This indicates a process by which these distinct fibroblast subpopulations could arise de novo from resident lung progenitors or precursor cells and/or be recruited from distal organs, such as the bone marrow. Evidence for these possibilities is reviewed, but there is as yet incomplete understanding of the precise precursor cells and the potential interrelationships between the various phenotypes, especially as to how they relate to the distinct myofibroblast phenotype. Moreover, the complexity of the mechanism for the genesis of these phenotypes, such as the myofibroblast, is highlighted by the multilevel regulation of the differentiation process, with evidence for the importance of multiple signaling pathways, transcription factors, and epigenetic mechanisms. Future studies into these various unsettled areas are essential to provide further insights that may help provide the pathway for novel translational approaches.

Keywords: fibroblasts; myofibroblasts; fibrosis

Fibroblasts are ubiquitous mesenchymal cells that are normally found in the stroma of many tissues. In the normal adult lung, they are present in the adventitia of vascular structures and airways. As with other tissues, they are commonly cultured as adherent cells exhibiting spindle-shape morphology and expressing interstitial collagens (types I and III), but they do not express markers of other differentiated cell types. Thus, there is no marker to indicate that this is a distinct cell type, thus accounting in part for the relative lack of information on its origins, function, and especially, whether it is part of a distinct homogeneous population of cells or a conglomeration of distinct subpopulations. Nevertheless, there is ample evidence to suggest that it is important in development (and presumably in regeneration), maintenance of stem cells, wound healing, tissue injury, and repair/remodeling/fibrosis. For the purposes of this conference, the two functions in the adult lung—namely, lung repair/fibrosis and regeneration—provide the compelling rationale for detailed studies on the origin of these cells, their phenotypic and functional characteristics, and their fate in the context of resolution versus progressive fibrosis. This article summarizes some of the evidence on aspects of these points, and is not intended to be a comprehensive review.

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Correspondence and requests for reprints should be addressed to Dr. Sem H. Phan, M.D., Ph.D., Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109-2200. E-mail: shphan@umich.edu

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FIBROBLAST HETEROGENEITY

Fibrotic lesions, including those present as "fibroblastic foci" in usual interstitial pneumonia or idiopathic pulmonary fibrosis (IPF), are highlighted by the presence of fibroblasts, or cells with morphologic characteristics of fibroblasts (1). However, by a number of different criteria, including expression of type I collagen, Thy-1, α-smooth muscle actin, cyclooxygenase (COX)-2, telomerase, and caveolin-1 (2–8), these cells appear to be heterogeneous, perhaps representing distinct subpopulations. Another key point is that these phenotypic characteristics appear only in injured lung, suggesting that these cells arise de novo from progenitor or precursor cells, perhaps by a process of differentiation in view of the relative stability of some of the phenotypes. But it is unclear if these different phenotypes are characteristics of distinct subpopulations with unique progenitors or represent the different stages of differentiation from a common progenitor. Recent evidence indicates the existence of distinct subtypes of fibroblasts in different locations of the body based on their gene expression patterns (9). This would argue for the presence of different progenitors that could potentially give rise to different activated or differentiated phenotypes in response to tissue injury. In any case, the noted differentiated subtypes in injured and fibrotic lungs have phenotypes that are consistent with their important roles in the promotion of fibrosis. Thus, the α-smooth muscle actin–expressing fibroblast, known as the myofibroblast, is shown to be the predominant source of type I collagen and fibrogenic/inflammatory cytokines in fibrotic lesions, as well as imparting altered mechanical properties to affected tissues (5, 10). Thy-1^- and caveolin-1^- fibroblasts are also associated with fibrotic lungs, and these two markers are lacking in myofibroblasts (3, 8), thus indicating some role in fibrosis. Consistent with such a role is the observation that caveolin-1 deficiency is associated with the development of lung fibrotic lesions, whereas its induced overexpression affords some protection against fibrosis (8). The significance of telomerase expression in a certain subpopulation that is distinct from myofibroblasts remains to be elucidated (7, 11, 12), and is especially intriguing in view of recent reports of telomerase mutations in certain families with IPF (13, 14). It is also unclear if some of the differentiated phenotypes, such as deficient COX-2 expression, are in any way related to the distinct myofibroblasts, which are present in virtually all fibrotic lesions. This type of analysis of fibroblast phenotypes, however, has highlighted the de novo emergence and potential pathophysiologic role of these different subpopulations of fibroblasts in pulmonary fibrosis, as well as suggesting their potential interaction. It is unclear at this time whether these different phenotypes represent various stages of differentiation that may ultimately lead to the myofibroblast or, alternatively, represent independent subpopulations arising from distinct progenitors. Therefore, a rigorous analysis and comprehensive understanding of these differentiated fibroblast subtypes or subpopulations, and their potential interrelationships and/or origins, should provide insight into the pathogenesis of progressive fibrosis in response to certain types of lung injury.

THE ROLES OF DIFFERENTIATED FIBROBLAST SUBPOPULATIONS

The various differentiated fibroblast subpopulations described above could contribute to the fibrotic response by their respective characteristic phenotype(s). Thus, the myofibroblast, by virtue of its ability to express high levels of cytokines, extracellular matrix, and α-smooth muscle actin, is expected to have key roles in inflammation, connective tissue deposition, and lung tissue mechanics, respectively (10). The Thy-1–expressing fibroblast has more recently been reported to have less fibrogenic properties than its Thy-1–negative counterpart (3). Interestingly, the myofibroblast phenotype is associated with absence of Thy-1 expression (4), similar to that observed for the telomerase as well as caveolin-1– expressing fibroblast phenotypes (7, 8). In the case of telomerase, its induction in fibrotic lung fibroblasts may have survival advantages for these cells, but these could differentiate to myofibroblasts, which are associated with loss of the induced telomerase expression (11, 12). Thus, the increased survival of these cells may result in an expanded precursor population with the potential to differentiate to myofibroblasts under the influence of transforming growth factor (TGF)-β, which is highly expressed in fibrotic lesions. Alternatively or additionally, the increased survival of the telomerase-positive cells may contribute to the production of fibrogenic cytokines or mediators that could then stimulate myofibroblast differentiation in susceptible progenitors. A similar situation is noted with respect to caveolin-1 expression, namely its association with decreased myofibroblast differentiation (8). Conversely, reduced caveolin-1 expression is reported in IPF lung tissue and fibroblasts relative to that in normal lungs. This antifibrotic role of caveolin-1 is confirmed by evidence that overexpression of this molecule suppresses myofibroblast differentiation and bleomycin-induced pulmonary fibrosis, whereas deficiency in its expression results in pulmonary fibrosis. Decreased expression of COX-2 is also characteristic of lung fibroblasts from patients with IPF. In this case, COX-2 expression is also serving an antifibrotic role via elaboration of prostanoids, which are known to inhibit collagen production as well as fibroblast proliferation (6). Thus, in three of these phenotypes, namely those expressing low levels (or none) of Thy-1, caveolin-1, or COX-2, their differentiation to a fibrotic phenotype(s) is associated with loss of antifibrotic phenotypes, rather than a gain or activation of fibrotic phenotypes. Beyond these common characteristics, it is unclear if these are manifestations of the same cell, or of different cell subpopulations whose interrelationships, if any, remain unclear. The fact that fibrosis may be due to loss of antifibrotic properties rather than activation of fibrotic processes suggests that, in normal tissues, active mechanisms to suppress fibrosis may be constitutively important in maintaining tissue homeostasis. Consequently, loss or dysregulation in this active homeostatic control mechanism would be expected to contribute to the pathogenesis of fibrosis. It appears, therefore, that the active fibrotic phenotype embodied in the myofibroblast may be the result of a differentiation mechanism that inactivates normally or homeostatically inhibitory pathways. This issue is further discussed below in the section addressing regulatory mechanisms in myofibroblast differentiation.

The function of fibroblasts in fibrosis has been viewed primarily in the narrow context of their ability to elaborate extracellular matrix, and perhaps in elaboration of cytokines and regulation of tissue mechanical properties. However, in the context of fibroblast–epithelial cross-talk, as postulated for cellular components of the fibroblastic foci, there is recent evidence that the fibroblastic elements underlying epithelium have considerable influence on the epithelial phenotype. Thus, the different anatomic localization of dermal fibroblasts can determine the overlying keratinocyte phenotype—for example, in terms of pigmentation (9, 15). Extrapolating from these findings to the lung suggests that emergence of de novo differentiated fibroblast phenotypes in injured lungs could have a profound effect on the neighboring alveolar epithelial cell phenotype in a manner conducive to promotion of fibrosis.

LUNG FIBROBLAST PROGENITORS

Recent interest in stem cell plasticity has engendered great interest in the possibility that mesenchymal cells, as reported for epithelial and other differentiated cells, can arise from bone marrow progenitors or adult bone marrow stem cells. Although marrow-derived mesenchymal stem cells have been reported to be protective (16), other studies have provided evidence of fibroblasts or fibroblast-like cells derived from the bone marrow or the circulation that appear to promote fibrosis in the lung (17). Studies using bone marrow chimera mice to trace migration of bone marrow progenitors indicate significant infiltration of bone marrow–derived fibroblast-like cells in remodeling tissues (18–20). Consistent with this finding is the presence of fibroblasts derived from circulating fibrocytes in animal model studies (21–23). There is, however, some controversy with respect to the phenotype of the fibroblast-like cell that is recruited to the injured lungs undergoing fibrosis. Although some studies using certain fibrocyte markers (CD34, CD45, collagen I) and, in some cases, CXCR4 expression suggest that the fibrocytes represent a significant source of myofibroblasts in the lung undergoing fibrosis (22, 23), other studies cannot demonstrate the ability of bone marrow–derived fibroblast-like cells to differentiate to myofibroblasts (18–20, 24). Given that the fibrocytes can only elaborate less than 10% of the level of collagen production in tissue-derived fibroblasts, it has been suggested that the fibrocyte may play an indirect role by secretion of fibrogenic mediators, such as TGF-β, to promote myofibroblast differentiation in locally derived tissue fibroblasts (25). In any case, the evidence with bone marrow–derived fibroblast-like cells appears to support a profibrogenic role for these cells, regardless of whether they could give rise to the myofibroblast.

The resident tissue fibroblast as a source of myofibroblasts has been documented extensively in multiple tissues, primarily by studies of these cells in tissue culture, wherein myofibroblast differentiation can be induced by treatment with TGF-β and other cytokines (26). This implies the presence of myofibroblast progenitors in the normal lung, either from adventitial fibroblasts (5) and multipotent mesenchymal progenitor cells (27) or epithelial and perhaps endothelial cells via epithelial and endothelial–mesenchymal transitions (28–31). The relative contributions by these mechanisms to the overall myofibroblast population remain uncertain, especially *in vivo*.

MYOFIBROBLAST DIFFERENTIATION

The myofibroblast embodies the key features of active fibrosis by its ability to express high levels of extracellular matrix and fibrogenic cytokines, and to contribute to the altered mechanical properties of affected tissues. There is evidence that TGF-β stimulation of fibroblast collagen production is a consequence of myofibroblast differentiation—that is, that acquisition of the myofibroblast phenotype is necessary for the increased collagen production (32). Moreover, this effect on collagen production is irreversible, persisting even after the removal of TGF-β. This indicates that the heightened matrix gene expression is a phenotypic feature of the myofibroblast that is manifested on complete and perhaps terminal differentiation. It is noteworthy that suppression of α-smooth muscle actin expression results in

reduction in collagen gene expression (33), thus affirming the concept that enhanced collagen gene expression is manifested only in the fully differentiated phenotype. More recently, similar suppression of α-smooth muscle actin expression inhibits connective tissue growth factor (CTGF) promoter activity, which is associated with reduced nuclear factor (NF)-κB nuclear translocation (34). Thus, the manifestation of the α-smooth muscle actin–expressing phenotype may be central to acquisition of many of the notable characteristics of the fully differentiated myofibroblast, which may represent a key event in induction and progression of fibrosis. Previous paragraphs have summarized recent evidence of the potentially diverse cellular origins of the myofibroblast, whereas this section summarizes recent progress on the mechanisms involved in myofibroblast differentiation.

Myofibroblast differentiation is commonly induced by treatment of fibroblasts or other susceptible precursor cells with TGF-β. Thus, most of the studies focused on aspects of TGF-β signaling that gives rise to the differentiated phenotype, with primary focus on the expression of the marker gene, α-smooth muscle actin. In addition to a basic requirement for mechanical stress, presence of a soluble stimulus such as TGF-β, found in inflammatory zone 1 (FIZZ1), and other cytokines results in complete differentiation (26). Evidence for various kinase pathways, including Jun (JNK) and p38 mitogen-activated protein (MAP) kinases, has been reported, although not necessarily in agreement in all studies (26, 35). In addition to being a key marker of myofibroblast differentiation and its role in regulation of collagen and CTGF gene expression, α-smooth muscle actin has also been implicated in interactions with signaling components, including transcription factors with different target genes (34, 36, 37). This interaction with signaling components and/or transcription factors may facilitate nuclear translocation of factors as well as compartmentalization or localization of signaling components for optimal activity. Indeed, p38 kinase activation induced by mechanical stress on the cell requires the presence of α-smooth muscle actin, and the interaction between these two components facilitates access to p38 substrates (36). Similar to mediation of TGF-β signaling by p38 (38), the aforementioned regulation of CTGF expression by α-smooth muscle actin is also dependent on p38 (34). These p38-mediated mechanisms promoting myofibroblast differentiation may be the basis for the ability of p38 inhibitors to suppress pulmonary fibrosis in animal model studies (39).

The well-known effect of TGF-β on α-smooth muscle actin expression and myofibroblast differentiation suggests the importance of the canonical TGF-β–associated Smad pathway. *In vitro* evidence indicates the importance of Smad3 in α-smooth muscle actin expression in lung fibroblasts (40), and Smad3 deficiency *in vivo* results in a significant reduction in pulmonary fibrosis (41). However, additional mechanisms regulating expression of this gene may be operative as evidenced by studies showing a multitude of factors that could regulate its promoter activity. Four areas in the promoter appear to be of predominant importance: namely, a Smad binding element (SBE), a TGF-β hypersensitivity region (THR), a TGF-β control element (TCE), and a C/EBP binding element, which are activated by Smad3, SP1/SP3, Krüppel-like factors, and C/EBPβ, respectively (26). The totality of the factors that could interact with these sites on the promoter, both directly and indirectly via interactions with directly bound factors, remains to be identified. Moreover, both stimulatory and inhibitory factors are involved in regulating these sites. For example, the inhibitory effects of gut Krüppel-like factor (GKLF) can be mediated directly at the TCE and by binding interaction with the MH2 domain of Smad3, reducing its binding to the SBE (41, 42). With respect to C/EBPβ, its predominant isoform, liver-enriched activating protein (LAP), activates myofibroblast differentiation, whereas the truncated isoform, liver-enriched inhibitory protein (LIP), inhibits differentiation (43). However, C/EBPβ–deficient mice exhibited significant reduction in pulmonary fibrosis associated with diminished myofibroblast presence (44). Additional factors that may play a role include the Notch signaling pathway, which appears to be important in epithelial–mesenchymal transition (45), whereas YB-1 (Y-box binding protein-1), NF-κB, and PPARγ (peroxisome proliferator activated receptor-γ) may be important in suppressing differentiation (46, 47). Thus, relief from inhibition as well as activation by stimulatory transcription factors may be operative in myofibroblast differentiation.

An additional level of complexity is suggested by evidence that epigenetic regulation may also be important. For instance, inhibition of histone deacetylase (HDAC) or DNA methylation suppresses myofibroblast differentiation (47, 48). In the latter case, evidence is presented that this may be indirectly mediated by derepression of suppressors of α-smooth muscle actin expression, rather than via direct effects on the methylation of the actin gene promoter (47). However, direct analysis of methylation status of the α-smooth muscle actin gene, as well as modification of histones closely associated with this gene, has not been systematically undertaken. Thus, this brief overview has highlighted the complexity of the mechanisms underlying just one key component of the myofibroblast differentiated phenotype. Future studies into these areas are necessary to shed more light on their feasibility as targets for controlling fibrosis.

CONCLUSIONS

Several distinct fibroblast phenotypes have been recovered from tissues undergoing remodeling or fibrosis, many with properties that suggest their contribution to the fibrotic process. Their origins, potential interrelationships, interactions, and the mechanisms that gave rise to these phenotypes have been characterized to a limited extent in a compartmentalized manner that prevents full appreciation of their precise roles in the overall pathogenesis of progressive fibrotic lung diseases. More coordinated work needs to be done in the future to more systematically uncover key mechanisms involved in genesis of these various phenotypes, and their relationship to the myofibroblast.

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