CURRENT DIRECTIONS

Mutations in BMPR2 are reported for only half of the PPH families. The other families link to a similar region, and it is hoped that the molecular explanation for these other families will be recognized soon due to the dedicated efforts by many different teams. Some likely candidates are unrecognized mutations in BMPR2, perhaps in an intron or the promoter, or perhaps in another gene nearby.

Another major effort at present is to identify the determinants that modify the clinical expression of the disease when mutations of BMPR2 are present. Only 20% of those persons with a mutation actually develop PH, so there are modifying genes or exposures that have a major impact on the clinical expression when a mutation is present. It is conceivable that these two most important features (ie, the unrecognized mutation of half the families and the modifying gene for clinical expression) could be completely independent of each other or could have a single mechanistic basis.

Another recent study concluded that PPH is predominantly a genetic disease. The authors analyzed 185 relatives of 32 PPH patients by prospective assessment using stress echocardiography. Overt clinical PPH was detected in at least one relative (n = 15) of 28% of the index patients. An abnormal response in pulmonary artery systolic pressure during exercise (ie, > 40 mm Hg) was present in at least one relative of another 58% of the index patients (37 relatives). In total, 81% of the PPH patients had strong evidence of familial disease. This opinion that sporadic PPH usually has a familial basis is supported by our study, which found BMPR2 mutations in a large percentage of sporadic PPH patients described earlier.

The results of other series of studies contrasts to the opinion that PPH usually has a familial basis, and they support the opinion that sporadic PPH and FPPH are distinctly different. These investigations studied the whole lung by gene expression patterns, as well as described evidence for monoclonality and microsatellite instability in microdissected plexiform lesions. In aggregate, the studies support the opinion that sporadic PPH and FPPH are distinct and separable conditions. A definitive clarification of the differences between the molecular basis of FPPH and sporadic PPH is not possible at present but should soon be greatly facilitated by the further clarification of the molecular pathogenesis that has yet to be described for FPPH.

Interesting information is also rapidly developing relevant to the breadth of clinical pulmonary vascular disease related to mutations in members of the TGF-β superfamily, such as the recently described novel mutations in ALK1 in five families with both hereditary hemorrhagic telangiectasia and PPH. Similar new vistas of understanding are developing on the horizon for many vascular disorders.

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The Myofibroblast in Pulmonary Fibrosis*

Semin H. Phan, PhD, MD

The pathogenesis of pulmonary fibrosis remains incompletely understood. Studies of associated inflammation have led to the discovery of a number of cytokines and chemokines that are found to be important either directly or indirectly for the fibrotic process. However, the importance of inflammation in pulmonary fibrosis is unclear, and at the time of diagnosis the inflammatory component is variable and usually not responsive to anti-inflammatory therapeutic agents. Patients usually exhibit evidence
of active fibrosis with increased numbers of activated fibroblasts, many of which have the phenotypic characteristics of myofibroblasts. At these sites, increased amounts of extracellular matrix deposition are evident with effacement of the normal alveolar architecture. Animal model studies show the myofibroblast to be the primary source of type I collagen gene expression in active fibrotic sites. In vitro studies show differentiation of these cells from fibroblasts under the influence of certain cytokines but indicate their susceptibility to nitric oxide-mediated apoptosis. In addition to promoting myofibroblast differentiation, transforming growth factor-β1 provides protection against apoptosis. Thus, this well-known fibrogenic cytokine is important both for the emergence of the myofibroblast and its survival against apoptotic stimuli. This is consistent with the critical importance of this cytokine in diverse models of fibrosis in various tissues. In view of these properties, the persistence or prolonged survival of the myofibroblast may be key to understanding why certain forms of lung injury may result in progressive disease, terminating in end-stage disease.

Key words: apoptosis; collagen; fibroblasts; myofibroblasts; telomerase; transforming growth factor-β1

Abbreviations: IL = interleukin; iNOS = inducible nitric oxide synthase; TGF = transforming growth factor

Although pulmonary fibrosis has diverse etiologies, there is a common feature characteristic of this process, namely, the abnormal deposition of extracellular matrix that effaces the normal lung tissue architecture. A key cellular source of this matrix is the mesenchymal cell population that occupies much of the fibrotic lesion during the active period of fibrosis. This population is heterogeneous with respect to a number of key phenotypes. One of these phenotypes is the myofibroblast, which is commonly identified by its expression in α-smooth muscle actin and by features that are intermediate between the bona fide smooth muscle cell and the fibroblast. The de novo appearance of myofibroblasts at sites of wound healing and tissue repair/fibrosis is associated with the period of active fibrosis and is considered to be involved in wound contraction. Furthermore, the localization of myofibroblasts at sites undergoing active extracellular matrix deposition suggests an important role for these cells in the genesis of the fibrotic lesion. In recognition of the potential importance of this cell in fibrosis, and perhaps in its persistence or progression, studies have focused on the nature and precise role or roles of this cell in the context of pulmonary fibrosis.

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PULMONARY FIBROSIS AND THE MYOFIBROBLAST

The presence of myofibroblasts in patients with pulmonary fibrosis is amply documented in both lung tissues taken from patients with pulmonary fibrosis as well as in those taken from animal models of the disease.1-4 Since fibroblasts in fibrotic lesions are considered historically to be the cells responsible for the deposition of the matrix that constitutes the scar, early studies focused on the importance of the myofibroblast in this capacity. Thus, localization at sites undergoing active matrix deposition and the elevated collagen synthetic capacity of granulation tissue myofibroblasts strongly suggest such a role for the myofibroblast in fibrosis.3 Direct confirmation that these cells are key sources of collagen gene expression is provided by the use of combined in situ hybridization for procollagen type I messenger RNA and immunostaining for α-smooth muscle actin.5 Using similar approaches, however, these cells were additionally found to be significant sources of several cytokines, including transforming growth factor (TGF)-β1, a well-established key fibrogenic mediator, and the CC chemokine, monocyte chemotactic protein-1.6,7 Given the inflammatory properties of both of these cytokines, the myofibroblast appears to play additional potentially important roles beyond the deposition of extracellular matrix. By elaborating on these mediators, they have the potential of contributing to the recruitment of inflammatory cells, and thus of intensifying or prolonging the inflammation that is often associated with fibrosis. Such amplification of the inflammatory response may result in a positive feedback loop resulting in the intensification and progression of fibrosis.

An additional property of the myofibroblast is its contractility. This is thought to be important in wound contraction and perhaps to contribute to the altered mechanical characteristics of the fibrotic lung.1,2 This property can be observed in vitro as the contraction of myofibroblast-populated collagen gels, which positively correlates with increased α-smooth muscle actin expression, a marker of myofibroblast differentiation.8 Thus, this cell appears to have the capability of reproducing important features of fibrotic lung tissue.

From these studies, it appears that the myofibroblast has the potential to play important roles in the pathogenesis of pulmonary fibrosis. Its dual role, as a key source of extracellular matrix and as an inflammatory cell, makes it the key cell in two processes that represent the hallmark of pulmonary fibrosis. Its importance in altering the compliance of the lung in this disease provides additional support for its role in pathogenesis. Further support for these roles is provided by the correlation between the appearance of the myofibroblast and active fibrosis, as manifested by increased cellularity, and heightened collagen and cytokine gene expression. The distinct kinetics of the appearance and disappearance of the myofibroblast in normal wound healing and self-limiting models of pulmonary fibrosis parallels the initiation of active fibrosis and its resolution or termination.5,8 The failure of the disease to resolve, as seen in patients with progressive disease, correlates with the persistence of the myofibroblast.3 These characteristics are reminiscent of the behavior of...
inflammatory cells at sites of tissue injury and inflammation, thus further supporting the designation of the myofibroblast as an inflammatory cell. In view of this similarity to the initiation and termination of inflammation, plus the evidence for the importance of the myofibroblast, recent studies have focused on the mechanisms underlying its de novo appearance and its disappearance.

**Myofibroblast Differentiation**

Since there are significant parallels between inflammatory cells in inflammation and myofibroblasts in fibrosis, it is not surprising to note that fibroblasts can be chemotactically recruited. Additionally recruited inflammatory cells undergo distinct phenotypic changes, as exemplified by the differentiation of monocytes to macrophages. With respect to the myofibroblast, a key marker of its differentiation is the expression of α-smooth muscle actin accompanied by heightened collagen and cytokine gene expression, increased contractility, and reduced motility and proliferative capacity. Morphologically, this cell contains dense aggregates of microfilaments and exhibits specialized contacts with adjacent myofibroblasts and extracellular matrix components.\(^{10-12}\) The cytoskeletal composition is somewhat variable, depending on the tissue localization. Thus, myofibroblasts in the fibrotic lung express α-smooth actin and vinculin, but do not express desmin, except for those myofibroblasts that are localized in more peripheral and subpleural areas of fibrosis.\(^{5}\) In a self-limiting model of lung injury and fibrosis, they emerge during the proliferative and active phase of fibrosis, and subsequently gradually disappear.\(^{5}\) The origin of these myofibroblasts is controversial, but kinetic studies suggest that the myofibroblast in pulmonary fibrosis is derived from preexisting peribronchial and perivascular adventitial fibroblasts.\(^{5}\) This observation is supported by \textit{in vitro} biochemical and morphologic evidence of the fibroblastic origin of the myofibroblast.\(^{13-15}\) Furthermore, cultured fibroblasts \textit{in vitro} can be induced to differentiate into myofibroblasts by treatment with cytokines, such as TGF-β and interleukin (IL)-4.\(^{16,17}\) This, with respect to pulmonary fibrosis, the presumed mechanism for the emergence of the myofibroblast is its cytokine-induced differentiation from fibroblasts, the population of which has been expanded under the influence of growth factors secreted by inflammatory and other lung cells.

This paradigm is complicated by the discovery of an additional activated lung fibroblast phenotype in a model of lung injury and fibrosis. This phenotype is characterized by the expression of telomerase activity, which does not localize to cells expressing α-smooth muscle actin (\textit{i.e.}, in nonmyofibroblasts).\(^{18}\) This seems to suggest that the telomerase-positive cells represent an intermediate activated phenotype between the quiescent fibroblast and the highly activated myofibroblast. However, whether this telomerase phenotype is an obligatory intermediate step in myofibroblast differentiation remains to be determined.

Myofibroblast differentiation can be defined on the basis of the induction of α-smooth muscle actin expression. The significance of the expression of this actin isoform \textit{vis-à-vis} the other more functionally relevant (to fibrogenesis) phenotypic features of the myofibroblast are unclear. However, given the unique nature of this induction of α-smooth muscle actin as a differentiation marker, studying the mechanism regulating the induction of this gene may provide insight into the means of clarifying the differentiation process. Analysis of the effects of the differentiation-promoting cytokine TGF-β on the α-smooth muscle actin promoter shows some unique features that are distinguishable from those seen in smooth muscle cells.\(^{19}\) In contrast to smooth muscle cells and certain cell lines, the induction of α-smooth muscle actin expression in myofibroblast differentiation requires only a TGF-β control element, while the CArG elements present in the promoter are unnecessary. A requirement for additional elements and regulatory factors requires further investigation. The distinction from smooth muscle and other cells of this unique regulatory mechanism in myofibroblast differentiation suggests a potential significance for future attempts at arresting the progression of fibrosis by the inhibition of this process.

**Myofibroblast Disappearance**

In normal wound healing, the number of myofibroblasts gradually declines as the healing process is successfully completed.\(^{9,11,12}\) Similarly, in a self-limiting model of pulmonary fibrosis, myofibroblasts gradually disappear as the active fibrotic phase is terminated.\(^{5}\) In contrast, these cells persist and can be found in various stages of human pulmonary fibrosis where the disease is progressive.\(^{5}\) Thus, the mechanism of the myofibroblast disappearance is of potential interest since it can provide insight into the basis for its persistence and hence into the maintenance or progression of the fibrosis.

Studies of wound healing\(^{20}\) suggest that myofibroblast disappearance occurs via apoptosis. Support for a similar mechanism in studies of self-limiting pulmonary fibrosis in rodents is provided by \textit{in vitro} evidence showing increased susceptibility of lung myofibroblasts for undergoing apoptosis relative to fibroblasts.\(^{21}\) Apoptosis induced by IL-1β is dependent on the induction of inducible nitric oxide synthase (iNOS) expression and nitric oxide production by fibroblasts exclusively, while the target myofibroblasts themselves do not express iNOS.\(^{22}\) Furthermore, the apoptotic pathway involves a reduction in the antiapoptotic protein Bcl-2 without affecting the proapoptotic protein Bax. In addition to inducing myofibroblast differentiation from fibroblasts, TGF-β1 promotes myofibroblast survival by affording protection against IL-1β apoptosis by inhibiting iNOS induction and the apoptosis-associated reduction in Bcl-2 expression.\(^{22}\) Despite this evidence of \textit{in vitro} susceptibility to IL-1β-induced apoptosis, the actual \textit{in vivo} signal for myofibroblast apoptosis is unknown. A possible clue is provided by the parallels between inflammatory cells and myofibroblasts as noted earlier. In the case of inflammatory cells, it appears that the loss of growth factor signaling represents a key trigger for apoptosis. In the case of eosinophils, the loss of IL-5 signaling induces apoptosis.\(^{23}\) In view of the multiple functions of TGF-β in promoting myofibroblast differentiation and maintaining its survival, a loss of TGF-β signaling may represent a signal for myofibroblast apoptosis.
sis. This possibility has some support in vivo, wherein the gradual decline in myofibroblast numbers occurs at a period when TGF-β expression is also declining in cases of bleomycin-induced pulmonary fibrosis.5,6

CONCLUSION

The emergence and disappearance of the myofibroblast appears to correlate with the initiation of active fibrosis and its resolution, respectively. In addition, the myofibroblast has many phenotypic features, which embody much of the pathologic alterations in fibrotic lung tissue. These features would seem to argue for an important role for the myofibroblast in the pathogenesis of pulmonary fibrosis. Furthermore, the persistence of the myofibroblast may herald progressive disease, and, conversely, its disappearance may be an indicator of resolution. This in turn suggests that future therapeutic strategies targeting the myofibroblast may be productive. Clearly, more studies are needed to uncover the regulatory mechanisms involved in myofibroblast differentiation and apoptosis before such strategies can be evaluated.

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Roles for Insulin-Like Growth Factor I and Transforming Growth Factor-β in Fibrotic Lung Disease*

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Idiopathic pulmonary fibrosis (IPF) is a lung disease that is characterized by epithelial cell damage and areas of denuded basement membrane resulting in...

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