New Light Is Shed on the Enigmatic Origin of the Lung Myofibroblast

Therapeutically targeting myofibroblasts that synthesize excessive quantities of extracellular matrix may be an essential component of treating progressive human pulmonary fibrosis; however, the origin of myofibroblasts in solid organs remains controversial because a number of well-designed studies have identified multiple potential cellular origins and generated conflicting results (1).

Fibroblasts exposed to fibrogenic cytokines, mediators, and matrix signals *in vitro* can differentiate into myofibroblasts, and resident fibroblasts in the lung are a likely source of myofibroblasts. The role of epithelial-to-mesenchymal transition (EMT) has generated much interest and debate. A number of recent studies have suggested that up to 50% of stromal cells are derived from alveolar epithelial cells (AECs) that undergo EMT (2, 3). Rock and colleagues found no evidence at the cellular or molecular level for EMT of labeled cells into myofibroblasts; rather, the authors observed proliferation of pericyte-like interstitial cells in response to experimental injury (*see below*) (4). Furthermore, lung injury accelerated the transition of type I to type II AECs and gave rise to alveolar cell lineages derived from an epithelial progenitor, which contributed to alveolar repair. These findings are consistent with a second study by Chapman and colleagues, which showed that a subpopulation of mouse laminin receptor $\alpha 6\beta 4^+$ /pro-SPC⁺-expressing AECs are potential progenitor cells during lung repair (5). Thus there is controversy on whether AECs are a significant source of lung myofibroblasts.

Circulating bone marrow-derived fibrocytes are a third potential source of lung myofibroblasts (6, 7). Fibrocytes home to the lungs of mice injured with bleomycin, and the number of fibrocytes found in the bone marrow, blood, and lung correlates with lung collagen I and III expression (8). Moreover, in patients with idiopathic pulmonary fibrosis (IPF), a positive correlation between numbers of lung fibrocytes, extent of lung fibrosis, and survival has been shown (9). These and other studies completed in the last decade demonstrate that elevated circulating levels of fibrocytes are associated with increased fibrosis and adverse clinical outcomes; however, additional studies are required to determine the therapeutic potential of targeting fibrocytes in organ fibrosis.

In this issue of the *Journal*, Hung and colleagues (pp. 820–830) describe the impact of pericytes and other mesenchymal cells on the development of bleomycin-induced pulmonary fibrosis in mice (10). Using elegant lineage fate mapping, the authors identify pericytes as significant contributors to the production of extracellular matrix proteins in lung tissue damaged by bleomycin. To date, pericytes have been implicated in the pathogenesis of renal (11), dermal (12), and spinal cord (13) fibrosis, but their role in lung disease, especially scarring, has yet to be defined. Previously, Rock and colleagues studied the populations of stromal cells present in fibrotic lung tissue in patients with IPF and in the lung tissue from the bleomycin mouse model of pulmonary fibrosis (4). The authors discovered that pericytes were abundantly present in the fibrotic lesions of both IPF and bleomycin-induced mouse lung fibrosis. However, in their report, the majority of the cells defined as pericytes did not express α -smooth muscle actin, a protein closely associated with myofibroblasts. The current article by Hung and colleagues further explores the populations of stromal cells found in bleomycin-induced mouse lung fibrosis. They identified populations of cells that contain markers for pericytes, myofibroblasts, or both. Furthermore, they discovered that the population of cells

identified as pericytes significantly contributed to extracellular collagen production and concluded that pericytes were a major effector in lung scarring.

Although pericytes are found in fibrotic areas and there is increasing evidence that supports their direct contribution to pulmonary fibrosis, pericytes are also present in healthy lung tissue. Importantly, several studies have reported that “normal” pericytes aid in the resolution of myocardial infarct and limit the extent of scar formation (14). Furthermore, there is increasing literature that supports the protective effects of perivascular pericytes in acute cerebrovascular ischemic injury (15). This raises the strong possibility that there are different populations of pericytes in the body and/or that there are different states of pericyte activation/differentiation. Similarly, myofibroblasts, although implicated in the pathogenesis of numerous types of fibrosis, are themselves an important part of normal wound healing. As a scientific community, we have learned much about the mechanisms of myofibroblast differentiation and survival, and investigators are now pursuing the concept that myofibroblast can in fact de-differentiate. The extent of pericyte plasticity remains to be fully elucidated. Important questions that can be addressed include not only defining the mechanisms and extent of pericyte activation/differentiation and/or de-activation/-differentiation, but the role of pericytes in normal wound healing and the signals that promote their survival or death. Just as we have learned that there is much heterogeneity in resident fibroblasts and myofibroblasts, we can assess the heterogeneity of pericytes and their similarities and differences across the organ systems (16). The local cytokine and matrix milieu in each tissue/organ varies in both health and disease and impacts resident fibroblast, epithelial, and myofibroblast behavior, and it would be interesting to assess how this local milieu modulates pericyte behavior.

There may be organ- and model-specific differences in the role of pericytes. The role of pericytes in renal fibrosis is under active investigation and debate. For example, Humphreys and colleagues have shown that during nephrogenesis, FoxD1-positive mesenchymal cells give rise to pericytes ($CD73^+$ and $Pdgfrb^+$), which expand and differentiate into myofibroblasts, accounting for a significant part of the myofibroblast pool (17). In contrast, Lebleu and colleagues recently completed a comprehensive functional analysis of myofibroblast origin in a kidney fibrosis model using fate-mapping experiments (18). Although they confirmed that EMT and endothelial-mesenchymal transition contribute modestly (5 and 10%, respectively) to the myofibroblast pool, the authors conclude that pericytes ($NG2$ -YFP⁺ and $Pdgfrb$ -RFP⁺ cells) do not expand the myofibroblast population after experimental injury. Furthermore, specific deletion of pericytes did not significantly impact recruitment of myofibroblasts or modulate the development of kidney fibrosis. Interestingly, the two major sources of myofibroblasts were proliferating resident interstitial fibroblasts (~65%) and nonproliferating bone marrow-derived mesenchymal cells (~35%); deletion of proliferating renal interstitial myofibroblasts reduced the development of kidney fibrosis. Although differences in genetic tagging, cell depletion methods, and mouse strains (C57BL/6 vs. BALB/c) limit comparisons between studies, the different findings compel us to further investigate the potential contribution of lung pericytes to the development of pulmonary and other organ fibrosis.

In summary, three main sources of lung myofibroblasts have been extensively studied over the last decade. The development

of novel imaging and genetic tools has increased our ability to determine the cellular origin and functional contribution of the heterogeneous group of cells that populate fibrotic lesions. The study of Hung and colleagues takes us one step further in our quest to identify lung stromal cell progenitors; these new findings, taken together with our collective knowledge of other mesenchymal cells, can help us rapidly learn more of the role of pericytes in pulmonary fibrosis, perhaps identifying novel therapeutic targets.

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Modeling Respiratory Syncytial Virus Cytopathogenesis in the Human Airway

Respiratory syncytial virus (RSV) is a worldwide cause of respiratory tract infections in young children, the elderly, and immune suppressed with hospitalizations from bronchiolitis around 30% and intensive care admission for ventilatory assistance approximately 2% (1–3). Despite our increasing understanding of RSV disease in humans, the mechanisms underlying disease remain rudimentary because most findings have been limited to animal models and continuous cell lines (4, 5). Although these studies have provided a number of important insights into RSV–host interactions, animal models, with the exception of chimpanzees, are semipermissive for RSV infection and do not reproduce the range of pathologies described in RSV-infected humans (6). Continuous cell lines, such as HEP-2 and A549 cells, have been used extensively to study RSV–host interactions; however, they inadequately reveal the complexities of RSV infection and the host cell response in the human respiratory tract. Therefore, the need for

models of RSV infection that elucidate RSV–host interactions is compelling. In this regard, the advent of technology to culture primary human airway epithelial cells and differentiate these cells into physiologically authentic pseudostratified airway epithelial cultures has provided a path forward.

RSV is a descending infection that commences in the upper respiratory tract and progresses to the lower respiratory tract. The principal targets for RSV infection in human airways are apical ciliated epithelial cells (7–10). However, there is very little information about the cytopathogenesis of RSV in the nasal tract (8). There are a number of groups now exploiting fully differentiated primary normal human bronchial epithelial cells to study RSV–host interaction as these cells emulate aspects of airway epithelium morphology and physiology (11). In an earlier study by Villenave and colleagues, the authors described a model of RSV infection based