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Review Article

The cytokines of pulmonary fibrosis: Much learned, much more to learn

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ABSTRACT

Organ fibrosis, the result of exaggerated, persistent, and often irreversible accumulation of extracellular matrix, complicates numerous diseases in all organs and tissues and has particularly serious consequences in the lungs. Abnormally accumulating scar tissue both replaces normally functioning parenchyma and distorts the architecture of unaffected tissue. In the lungs, the fibrotic process often leads to rapid and severe abnormalities in respiratory mechanics and gas exchange properties. There is no confirmed cure, and better therapies are required for treating fibrosis. The development of therapeutic strategies compels a better understanding of the cellular and molecular mechanisms of fibrosis, which are diverse, complex, and redundant. Epithelial injury, oxidative stress, coagulation disturbances, and inflammation are engaged in a complex interplay leading to augmented transformation of several cell types into myofibroblasts and prolonged survival of these extracellular matrix-producing cells. Cytokines are centrally engaged in the homeostatic and pathophysiologic regulation of connective tissue. Furthermore, it appears that identical cytokines are utilized by inflammation, profibrotic mechanisms, and the fibrotic process itself, suggesting that specific targeting or utilization of these cytokines holds therapeutic promise. In this article, we review the wealth of recent knowledge on major cytokines involved in the fibrotic process.

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1. The burden and mechanisms of fibrosis

1.1. Organ, including lung, fibrosis is a serious biomedical problem

Scarring, normally a central component of tissue healing following injury, may become exaggerated, relentless, and irreversible. The resulting accumulation of extracellular matrix (ECM), especially collagen, causes structural distortion and functional impairment of affected tissues and organs. The process of abnormal, structure-deforming, and function-impairing deposition of ECM is commonly referred to as fibrosis. It may occur in all organs and complicate a remarkably broad spectrum of diseases; its consequences are unpleasant and debilitating in the skin [1,2], and may be life-threatening in the liver [3–5], kidney [6,7], heart [8,9], or lung [10–18]. In both normal tissue healing and fibrosis, cells known as myofibroblasts are the main source of ECM accumulation, whether physiologic or pathologic [19,20]. These cells demonstrate a phenotype that is intermediate between fibroblasts and smooth muscle cells, simultaneously producing collagen and expressing α-smooth muscle actin (α-SMA) (Fig. 1). Despite the frequent occurrence of organ fibrosis, the exact epidemiologic data on fibrosis in general are not readily available, likely due to the broad diversity of conditions in which fibrosis occurs as well as the common underreporting of fibrotic complications of diseases. It is obvious, however, that fibrosis of organs poses major biomedical problems, leading to notable contributions to morbidity and mortality [1–12]. The burden of organ fibrosis is further increased by the lack of sufficiently effective therapies.

Excessive scarring of the lungs, or pulmonary fibrosis (Fig. 2), is particularly serious. Airway wall fibrosis contributes to airway remodeling and complicates several diseases, notably asthma and chronic obstructive pulmonary disease (COPD) [21,22]. Fibroproliferation is a key contributor to small airway narrowing in obliterative bronchiolitis [23]. Parenchymal fibrosis may develop in individuals exposed to radiation, chemicals, or dust (e.g., silica) [24,25]. Markedly severe outcomes of pulmonary fibrosis ensue in a spectrum of conditions known as interstitial lung diseases (ILD), characterized by various degrees of inflammation combined with stromal expansion occurring in the pulmonary interstitium, which is normally a fine framework of connective tissue fibers supporting the microarchitecture of the lungs [13]. ILD with severe pulmonary fibrosis may develop with no identifiable etiology in

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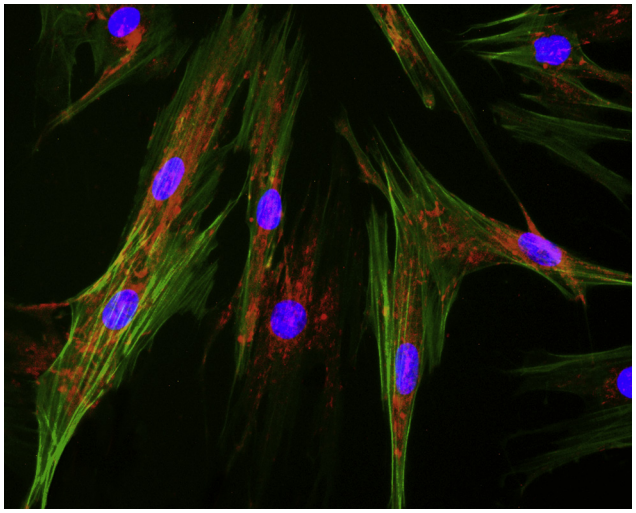

Fig. 1. Fluorescence microscopy of cultured adult primary myofibroblasts stained with fluorescently labeled antibodies against α-SMA (green) and focal adhesion kinase (FAK, red); nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, blue). For this staining, normal human lung fibroblasts were stimulated in culture with 300 ng/ml recombinant human CCL18 for 48 h, then fixed, permeablized, and incubated with the indicated antibodies. Note that, consistent with the typical fibroblast response to stimulation, even following potent activation, not all fibroblasts underwent myofibroblastic differentiation: the cell in the center bottom area expressed minimal α-SMA.

idiopathic pulmonary fibrosis (IPF) [10–13,15], as part of an autoimmune connective tissue disease such as scleroderma [13–18], or with a number of other maladies [13,14]. The societal burden of pulmonary fibrosis continues to increase rapidly, especially among older patients [10–12]. Median survival in patients with IPF is only 2–3 years [26], and the majority of patients with scleroderma die from scleroderma lung disease (SLD) [16]. Certain advancements have been made in developing therapies for lung fibrosis [18,27], but much better treatments are still needed. In the interim, lung transplantation remains the only viable option for patients with end-stage ILD.

1.2. Collagen-producing myofibroblasts originate from numerous precursors

Homeostatic balance between ECM deposition and turnover is central to maintaining tissue integrity. During healing from injury, this balance is temporarily shifted toward ECM deposition in a precisely controlled fashion. Scar-forming, ECM-depositing myofibroblasts are generated in the course of the tissue repair process and are then removed through apoptosis as the scar is formed. Fibrosis is thought to result from regulatory disturbances leading to excessive generation and activation of myofibroblasts combined with their slowed functional suppression and removal. This combination results in a potentiated and prolonged shift toward ECM deposition and away from ECM turnover.

It is intuitive and has been extensively confirmed in detail that resident tissue fibroblasts become collagen-producing myofibroblasts following injury. This notion can explain the source of ECM in normal scarring as well as in pathologic fibrosis in fibroblast-rich organs, such as skin or lung, but not in organs in which fibroblasts are normally rare, such as kidney or liver. Thus, it appears that myofibroblasts may arise from a number of non-fibroblast precursor cells, and these precursors may be of resident or non-resident (bone marrow) origin (Table 1). The epithelial–mesenchymal transition (EMT) is a possible source of myofibroblasts [28,29], although the extent of its contribution to real-life mechanisms of human fibrotic diseases and animal models of fibrosis remains to be established [30–33]. Similarly, the endothelial–mesenchymal transition (EndoMT) [34] and the mesothelial–mesenchymal transition (MMT) [35–37] may contribute to fibrosis. Pericytes have gained attention as a source of myofibroblasts in major organ fibroses [38–41], although some controversy remains about the myofibroblastic conversion of pericytes in pulmonary fibrosis [31], and myoblasts may also transdifferentiate into myofibroblasts [42,43]. Fibrocytes are circulating cells of bone marrow origin that express CD45, CD34, and collagen; home to sites of injury; differentiate into myofibroblasts; and contribute to the pool of ECM-producing cells [44]. They also contribute to fibrosis indirectly, by producing a spectrum of cytokines and immune cell surface molecules, and thus act as immunomodulators and regulators of connective tissue homeostasis [45].

Overall, it appears that myofibroblasts arise from a variety of cellular sources. Such redundancy of sources ensures generation of myofibroblasts, which are critical for normal tissue healing following injury. One could argue that, in light of the multiple possible sources of myofibroblasts, broad therapeutic targeting of the myofibroblastic conversion in fibrosis may not be a promising therapeutic approach at present. Adding to this concern, not only is the production of myofibroblasts exaggerated in fibrosis, but these cells are also resistant to apoptosis and thus fail to stop depositing excess ECM; such resistance is controlled by multiple factors [46–48], further increasing the complexity of the process. Possible therapeutic targeting is further complicated by the finding that the hallmark of the myofibroblastic phenotype, the expression of α-SMA, is not necessary for normal repair and fibrosis [49,50]. Consistent with this notion, both TGF-β and PDGF induce transformation of pericytes into ECM-producing connective tissue cells [51], but the resulting phenotypes are much different in their expression levels of numerous genes; among these differences, TGF-β induces expansion of myofibroblasts, whereas PDGF induces a fibroblast phenotype negative for α-SMA. These findings suggest that our traditional approach to identifying ECM-producing cells may not be fully reliable and that a more thorough understanding of such cells is needed.

1.3. Multiple mechanisms propel fibrosis

The main profibrotic mechanisms appear to be shared among major organs, but there are also substantial dissimilarities. Parenchymal fibroblasts are not as abundant in normal liver and kidney tissues as in skin or lung, suggesting that activation of resident fibroblasts is an unlikely source of hepatic or renal fibrosis and that other cell types serve as sources of ECM-producing myofibroblasts (see above). In addition to differences in cellular composition, there are distinct organ-specific tissue architectures, patterns of vascularization, and differences in regenerative capacity, all of which contribute to distinctive patterns of fibrosis, depending on the organ affected. In this review, we focus predominantly on pulmonary fibrosis—perhaps the most severe of organ fibroses (see above)—while including relevant mechanistic observations from research on fibroses of other organs.

There are several major pathways implicated in fibrosis (Table 2). The earliest theory that propelled identification of pro- and antifibrotic factors was the inflammation theory of fibrosis. Inflammatory cells, including lymphocytes, macrophages, plasma cells, eosinophils, and neutrophils, consistently accumulate, albeit in variable amounts, in association with histologic fibrosis in all organs, prompting the notion that these cells activate myofibroblasts and induce ECM accumulation through their soluble and cell-surface factors. Fibroblasts, in turn, take an active part in the immune response in their own right as sentinel cells [52,53]. While intuitively plausible, this theory by itself fails to explain fibrosis.

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