Renal Fibroblasts: Origins, Activation and Their Role in Renal Fibrosis

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Please be noted that this syllabus is largely based on my recent review (Nat Rev Nephrol 7: 684-696, 2011).
Introduction

Although many types of cells in renal tubulointerstitium, such as fibroblasts, tubular epithelial cells and subsets of macrophages, are capable of producing extracellular matrix (ECM), fibroblasts are commonly regarded as the principal matrix-producing cells that produce a large amount of interstitial matrix components including fibronectin, type I and type III collagens. Activated fibroblasts in diseased kidneys often express a molecular signature, α-smooth muscle actin (α-SMA), and are also referred as myofibroblasts. In this context, one of the fundamental issues in renal fibrosis field is to delineate the origin, activation and regulation of these matrix-producing myofibroblasts.\(^1\)\(^4\)

There are at least 5 different sources have been proposed to contribute to myofibroblast pool in diseased kidneys. These include activation of interstitial fibroblasts and pericytes, phenotypic conversion of tubular epithelial and endothelial cells, and recruitment of circulating fibrocytes.\(^5\) The relative contribution, and even the very existence, of each particular myofibroblast-generating pathway to renal fibrosis is a matter of intense debate and highly controversial. This is largely due to the inherent difficulty in identifying and tracking fibroblasts owing to the lack of specific markers for this cell type. Another major problem is that fibroblasts exhibit enormous phenotypic heterogeneity, probably reflecting their diverse origins, and change their phenotypes over the activation status, localization and times in renal fibrogenesis.

Activation of interstitial fibroblasts and pericytes

In normal adult kidneys, fibroblasts situate in the interstitial space between the capillaries and the epithelia.\(^6\) Morphologically, these cells are stellate shaped and exhibit abundant rough endoplasmic reticulum, collagen-secreting granules and actin filaments. They possess multiple cell processes, which connect to the tubular and capillary basement membranes.\(^6\) In the resting, quiescent state, interstitial fibroblasts express CD73, also known as ecto-5’-nucleotidase, in their plasma membrane, and produce erythropoietin.\(^6,7\) They also express platelet-derived growth factor receptor β (PDGFR-β),\(^8\)\(^-\)\(^10\) and fibroblast-specific protein-1 (Fsp1), a small cytoskeleton-associated and calcium-binding protein also
known as S100A4. Upon activation, fibroblasts acquire a myofibroblast phenotype by expressing α-SMA and producing a large amount of ECM components.

Several markers have been used to characterize fibroblasts and myofibroblasts in the kidneys such as α-SMA, desmin, FSP1, CD73, PDGFRβ. Unfortunately, none of these markers is specific. In addition, they are rarely expressed by all fibroblasts/myofibroblasts or hardly ever present all the times. Even α-SMA, a classic hallmark for myofibroblast activation in organ fibrosis of all types, is not without problem. The expression of α-SMA is not exclusive for myofibroblasts, as it is also present in vascular smooth muscle cells. In addition, not all activated fibroblasts express α-SMA all the time.

Activated fibroblasts are often characterized by two key features: proliferation and myofibroblastic activation. The latter is illustrated by α-SMA expression and matrix production. Both fibroblasts and myofibroblasts have the capacity to proliferate in response to cytokine cues, leading to the expansion of fibroblast population and interstitial space in diseased kidneys. Several fibrogenic growth factors including PDGF, transforming growth factor-β (TGF-β), basic fibroblast growth factor (FGF-2) and connective tissue growth factor (CTGF) are the well-known mitogens for fibroblasts. In addition to these classic cytokines, tissue-type plasminogen activator (tPA) is another critical player promoting fibroblast survival, proliferation and myofibroblastic activation.

Studies suggest that vascular pericytes are a major source of myofibroblasts in fibrotic kidneys. Pericytes are a subset of stromal cells that partially cover capillary walls, thereby stabilizing endothelium. Following kidney injury, pericytes are detached from the endothelium, undergo migration and proliferation, and differentiate into myofibroblasts. The story of pericytes is quite interesting, because pericyte detachment and differentiation into myofibroblasts under pathological conditions not only result in destabilization of microvasculature, but also contribute to myofibroblast activation, leading to interstitial fibrosis.

**Phenotypic conversion of epithelial and endothelial cells**
Another source of matrix-producing cells could come from tubular epithelium through EMT, a cell phenotypic conversion process occurs in embryonic development, tumor metastasis and organ fibrosis.\textsuperscript{28-32} Similarly, fibroblasts/myofibroblasts may derive from capillary endothelium by endothelial to mesenchymal transition (EndoMT).\textsuperscript{33,34} The contribution of EMT to renal fibrosis is controversial and is the focus of several recent reviews and debates.\textsuperscript{26,35-41}

There is a broad agreement that tubular epithelial cells \textit{in vitro} can undergo EMT, characterized by loss of epithelial feature and acquisition of mesenchymal markers, under the bombardment of various pro-fibrotic cytokines, particularly TGF-\(\beta\). However, whether this merely represents an \textit{in vitro} artifact or it does occur \textit{in vivo} is the center of argument. Using a genetic lineage-tracking, fate mapping technique, an early study demonstrates that more than one third of the Fsp1\(^+\) interstitial fibroblasts are derived from tubular epithelia in obstructive nephropathy.\textsuperscript{42} Likewise, two independent studies also show significant contribution of endothelial cells to the generation of fibroblasts/myofibroblasts via EndoMT in a variety of CKD.\textsuperscript{33,34} However, these results are challenged by a number of similar cell fate mapping studies in which no epithelial or endothelial origin of fibroblasts is evident.\textsuperscript{27,43} Thus far, the reason behind these discrepancies is unsettled.

EMT is a dynamic program in which epithelial cells and fibroblasts represent two extremes of a continual spectrum of a variety of intermediate cell phenotypes. The frequency that epithelial cells that complete the entire EMT course and ultimately become fibroblasts probably is limited, and heavily depends on the disease models, stages and persistence of elevated cytokine pressure in the inflamed milieu.\textsuperscript{44} In most circumstances, tubular cells undergo a partial EMT, in which epithelial cells only change one or two phenotypic markers, while the transcriptional program of EMT is activated. Such a partial EMT, however, is closely associated with poor outcomes and predicts the progression toward interstitial fibrosis in humans.\textsuperscript{45} Not surprisingly, blockade of EMT by a variety of agents ameliorates renal fibrosis and preserves kidney function.

**Recruitment of circulating fibrocytes**
Fibrocytes are a subset of bone marrow-derived, circulating monocytes with fibroblast-like feature in the peripheral blood. They are spindle-shaped, express hematopoietic cell marker CD45, and are capable of producing type I collagen. Fibrocytes also express certain chemokine receptors such as CCR7. In response to kidney injury, fibrocytes mobilize and infiltrate into renal parenchyma and participate in fibrogenesis.

The relative importance of fibrocytes in renal fibrogenesis is another area full of controversy. Because of the lack of specific markers for these cells, clear discrimination of them from monocytes, macrophages, fibroblasts and myofibroblasts is a great challenge. In addition, fibrocytes appear to exhibit different subpopulations. Thus far, experimental results on the involvement of fibrocytes, and bone marrow-derived cells in general, in renal fibrosis are inconsistent. Clarification of this issue needs more investigations.

The different origins of fibroblasts likely contribute to their phenotypic heterogeneity. The relative contribution of each lineage to the myofibroblast pool may depend on the disease model and specific stages. It is conceivable that myofibroblast activation from fibroblasts, pericytes or fibrocytes is an early event, while EMT often takes place at a late stage after a sustained injury (Figure 2). The pathological impact of the early activation of fibroblasts versus EMT to renal fibrosis may be different. While fibroblast activation is important for the onset of renal fibrosis, EMT could be a major determinant for fibrosis progression and irreversibility (Figure 2).

**Molecular machinery that integrates fibrogenic signals and orchestrates matrix production**

The expression and synthesis of ECM proteins by activated fibrobleasts is primarily controlled at the gene transcriptional levels in response to various extracellular fibrogenic cues. Key fibrogenic factors include TGF-β1, PDGF, FGF-2, CTGF and angiotensin II, while HGF and BMP-7 inhibit matrix production primarily by antagonizing TGF-β1 action. Through their respective receptors and specific downstream intracellular signal cascades, these fibrogenic cytokines activates a host of transcription factors that act on the cognate elements in the promoter regions of the collagen and
fibronectin genes to activate their transcription. Such signal transduction cascades and expression of matrix genes are also regulated by a variety of microRNA.56-60

Activated fibroblasts contain stress fibers and display abundant transmembrane connections, also known as fibronexus,61 between extracellular fibronectin-containing matrix and actin microfilaments, suggesting that an increased interaction of ECM and integrins.9 Integrins transmit their signals by activating the downstream effector kinase, FAK and ILK, as they possess no enzymatic or actin-binding activity. Extensive studies indicate that ILK, a scaffolding/adaptor protein and a serine/threonine protein kinase, is especially suited to serve as a molecular platform that integrates various fibrogenic signals.62

It becomes increasingly clear that integrins/ILK and their associated proteins constitute a fibrogenic molecular machinery that orchestrates matrix production and its extracellular assembly. Analogous to the concept of inflammasome,63 we propose this multi-component, integrin-associated protein complex as ‘matrisome’, which is molecular platform activated upon injury that integrates various fibrogenic signal inputs and triggers the production and assembly of matrix components. It appears that almost all key fibrogenic cues promote matrix production somehow by regulating, directly or indirectly, this molecular machinery.

Summary

Renal fibrogenesis is an enormously complex, dynamic process in which activation of fibroblasts is arguably the most important event leading to excessive ECM production and scar formation. We now have a better appreciation for the diverse origins and phenotypic heterogeneity of the matrix-producing fibroblasts, as well as their roles in renal fibrogenesis. As a variety of strategies targeting fibroblast activation are effective in animal models, we are optimistic that some of these remedies will become clinically relevant for CKD patients in the future.
References


SAM Questions

1) There is no specific marker that is exclusively expressed in activated fibroblasts. But investigators often use all of the following to identify interstitial fibroblasts, except:
   a. α-smooth muscle actin
   b. Desmin
   c. CD31
   d. PDGFRβ

Correct answer: CD31. Fibroblasts do not express CD31 in any circumstances. CD31 is a marker for endothelial cells, although it is also found on platelets, macrophages, granulocytes, T / NK cells, lymphocytes, megakaryocytes, osteoclasts, neutrophils.

2) Several different sources have been proposed to contribute to (myo)fibroblast pool in diseased kidneys. During renal fibrogenesis, an activated fibroblast can, in theory, come from:
   a. Interstitial fibroblasts and vascular pericytes
   b. Tubular epithelial cells and endothelial cells
   c. Circulating fibrocytes
   d. All of them

Correct answer: all of them. Based on published literature, activated fibroblasts (myofibroblasts) can come from a wide variety of cells, including quiescent interstitial fibroblasts, vascular pericytes, tubular epithelial cells, endothelial cells and circulating fibrocytes.

3) All of the following statements regarding matrix production in renal fibrosis are true, except:
   a. The expression of ECM proteins by activated fibrobleasts is primarily controlled at the gene transcriptional levels in response to various extracellular fibrogenic cues.
b. All growth factors such as TGF-β1, PDGF, CTGF, HGF and BMP-7 are fibrogenic and promote matrix production by fibroblasts.

c. Matrix genes are also regulated by a variety of microRNA

d. Integrins and its downstream ILK signaling are important for matrix production and deposition.

Correct answer: b. While TGF-β1, PDGF and CTGF are well known fibrogenic cytokines, HGF and BMP-7 have been demonstrated to be anti-fibrotic and inhibit matrix production.