Hierarchy of molecules in TGF-β₁ signaling relevant to myofibroblast activation and renal fibrosis

Ming Zhan and Yashpal S. Kanwar

Department of Pathology and Medicine, Northwestern University, Chicago, Illinois

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A NUMBER OF MOLECULES using different transforming growth factor (TGF)-β₁-induced signaling pathways are involved that activate a wide variety of cells leading to a terminal event known as “scarring or fibrosis” in various organ systems. Likewise, renal fibrosis is an inevitable consequence of various types of progressive chronic kidney diseases (CKD), and it is characterized by continued exacerbation of glomerulosclerosis, tubular atrophy, and interstitial fibrosis, which leads to irreversible kidney scarring. In the clinical setting, CKD patients with recalcitrant renal fibrosis manifest with progressive renal function loss, and they ultimately develop end-stage renal disease (ESRD), which requires renal replacement therapy (10). This Editorial Focus underscores the conundrum of intricate signaling pathways alluded to in a recent article in the American Journal of Physiology-Renal Physiology by Manickam et al. (13) with the end point being renal fibrosis.

In renal fibrosis, there is excessive amassing of the extracellular matrix (ECM) under the influence of profibrogenic cytokines, which initiate a myriad of signaling cascades. Under such stimuli, certain renal mesenchymal cells, such as mesangial cells and fibroblasts, differentiate upon activation into a profibrogenic myofibroblast phenotype and synthesize excessive amounts of the ECM (1, 10). TGF-β₁, a prototype of the TGF-β superfamily, is widely considered to be the major profibrogenic cytokine that is responsible for the myofibroblast differentiation with ECM synthesis along with the suppression of ECM-degrading enzymes (11). TGF-β₁ first binds to a type II serine/threonine kinase receptor, which transphosphorylates and activates a type I receptor followed by the commencement of a series of downstream signaling events. In the canonical pathway, the activated TGF-β₁ receptor interacts with Smad2/3 to form a heterodimeric complex, which translocates into the nucleus and regulates the transcription of TGF-β₁-targeted genes, such as, collagen type I, c-Jun, and fibronectin (7). Along with Smads, ERK1/2 and Akt/PKB also modulate the TGF-β₁-initiated signaling cascade, and cross-talk occurs between the Smads and ERK signaling pathways that are central to ECM pathobiology in renal fibrosis.

Another set of molecules that are involved in the TGF-β₁-initiated events include ROS, which are derived from both NADPH oxidase and the mitochondrial respiratory chain, and they play a critical role in the pathogenesis of various CKDs, including diabetic kidney disease, culminating in renal fibrosis (1, 5, 15). It is known that ROS can be induced after various stimuli, including angiotensin II, PKC, and TGF-β, which ultimately affect the synthesis of the ECM. Lee et al. (9) showed that in response to angiotensin II, NADPH-dependent ROS interact with mitochondrial ROS to promote ECM accumulation and the fibrogenic response through modulation of endothelial nitric oxide synthase dysfunction in mesangial cells. In addition, after TGF-β₁ induction, generated ROS have been reported to promote cardiac myofibroblast activation and differentiation (3). However, the role of ROS in TGF-β₁ signaling and the cross-talk between these two profibrogenic signals during kidney myofibroblast activation are still not very well defined. One of the major facets that are highlighted by Manickam et al. in their study (13) relates to the involvement of NADPH oxidase-derived ROS and a third set of molecules, i.e., the Rho family of small GTPases, in the TGF-β₁ signaling pathway; these molecules form an intricate signaling complex that exerts a stimulating effect on myofibroblast activation. In an earlier work by these authors, they demonstrated in vitro that TGF-β₁-induced renal myofibroblast activation is regulated through NADPH oxidases of the Nox family, whereby this profibrogenic cytokine stimulates an increase in Nox4 expression and generation of NADPH oxidase-derived ROS. The downstream effect of Nox4 included the acquisition of myofibroblast markers, e.g., α-smooth muscle actin and increased synthesis of fibronectin (2). Thus, Nox4-derived ROS were considered as second messengers in the TGF-β₁-signaling pathway, where, in a hierarchical order, they are placed downstream of Smad3 but upstream of ERK1/2 during the transformation of fibroblasts to myofibroblasts (2). Furthermore, another interesting aspect of their study relates to the inclusion of another unique molecule, i.e., Nox4 enhancer protein, polymerase (DNA-directed) δ-interacting protein 2 (Poldip2), in the TGF-β₁-induced signaling pathway. It seems that during redox signaling with myofibroblast activation of kidney fibroblasts, Poldip2 serves as an upstream regulatory factor, whereby it interacts with Nox4 and enhances NADPH oxidase activity, and, in turn, Nox4 reciprocally upregulates the expression of this interacting protein: a unique reciprocal cooperative relationship between these two putative second messengers (13).

Based on previous work, Barnes and colleagues further explored and confirmed the regulatory roles of the Rho family of small GTPases, RhoA and its downstream effector Rho kinase (ROCK), during renal myofibroblast activation as well as their interrelationship with Poldip2/Nox4 and NADPH oxidase-generated ROS in the TGF-β₁-induced signaling pathway (13). Rho GTPases are a family of small GTP-binding proteins that belong to the Ras superfamily. They cycle between an active GTP-bound and inactive GDP-bound form to act as “molecular switches” that regulate complex cellular processes and signaling pathways (4). Three key members of the family (Rac1, RhoA, and Cdc42) have been widely studied in the regulation of cytoskeleton and actin dynamics. While
both Rac1 and RhoA have been reported to be involved in ECM pathobiology and fibrogenesis, Rac1 is activated in response to TGF-\(\beta_1\) and promotes mesangial cell collagen accumulation through phosphatidylinositol 3-kinase/Akt pathways (6). RhoA kinase-ROCK inhibition has been widely confirmed to be beneficial in the amelioration of diabetic nephropathy and renal fibrosis, partly by preventing ECM accumulation and antioxidative effects (8, 14). Recent accumulated evidence has also highlighted the convergent role of Rho signaling in response to profibrogenic mediators such as TGF-\(\beta_1\), endothelin-1, and integrins that result in myofibroblast activation (14, 16).

Using targeted gene knockdown techniques and chemical inhibitors in rat kidney fibroblast cells, the authors demonstrated that TGF-\(\beta_1\) rapidly induces the activation of both RhoA GTPase and ROCK but not Rac1, another member of the Rho family. Rho/ROCK can modulate the expression of Nox4 and the Nox4 enhancer protein Poldip2, but the latter two have no effect on RhoA, indicating that Nox4 is “downstream” of Rho/ROCK (13). In this regard, the importance of Rho/ROCK regulation on TGF-\(\beta_1\)-induced NADPH oxidase-derived ROS signaling during myofibroblast activation has been clearly verified by Barnes and colleagues, at least in cell types such as kidney fibroblasts. Intriguingly, in vascular smooth muscle cells, RhoA has been reported to be “downstream” of Poldip2/Nox4 in response to TGF-\(\beta_1\) induction (12). The authors explained these peculiar differences, i.e., “reverse order of signaling” as due to possible heterogeneity in cellular signaling in different cell types, and it is also conceivable that Rho/ROCK-induced Poldip2 and Nox4-dependent ROS may reciprocally modulate each other to amplify the TGF-\(\beta_1\) signaling cascade, resulting in myofibroblast activation. Taken together, the authors’ current and previous works collectively delineate a “unique hierarchy” in the RhoA/ROCK/Poldip2/Nox4/ERK signaling cascade that is initiated by TGF-\(\beta_1\) in kidney myofibroblast differentiation in renal fibrosis (Fig. 1).

The elucidation of this unique signaling cascade raises one or two important issues that need further investigation: 1) whether other cell types, such as pericytes, circulating bone marrow-derived fibrocytes, tubular epithelial cells, orendothe-
lial cells (amenable to epithelial or endothelial-mesenchymal transitions), also undergo a transformation to acquire a myo-fibroblast phenotype similar to kidney fibroblasts following TGF-β1-induced and RhoA and NADPH oxidase-dependent signaling pathway, and 2) whether mitochondrial-derived ROS, another major source of ROS, have any effect on TGF-β1-dependent renal fibrosis. Finally, further in vivo investigations on the specific roles of small GTPase and NADPH oxidase-derived ROS in TGF-β1-induced myofibroblast activation during renal fibrosis would be helpful to verify the possible therapeutic targets alluded to by the authors in their in vitro investigations.

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