# Potassium Channels, Renal Fibrosis, and Diabetes

**Paolo Menè and Nicola Pirozzi**

Progressive renal fibrosis is a common outcome of almost all forms of renal damage, including diabetic nephropathy. Fibrosis leads to chronic kidney failure and, eventually, dialysis or transplantation (1–3). Although much is known about the molecular background and mediators that prompt fibroblasts or transdifferentiated kidney cells to release collagen and matrix (4–6), the search for a unique event that initiates the process remains inconclusive. Targeting this putative key signal would enable researchers to "switch off" fibrosis and, perhaps, the progressive loss of renal function that plagues millions of individuals worldwide (1–6). The intermediate/small-conductance $Ca^{2+}$-activated $K^+$ channel (KCa3.1; KCNN4; SK4) is one intriguing candidate for such function because it promotes fibrogenesis in target tissue by altering the membrane potential of cells, thus enhancing extracellular $Ca^{2+}$ entry (7,8). Subsequent Smad2/3 or mitogen-activated protein kinase (MEK)–dependent phosphorylation upregulates profibrotic genes and collagens in human and animal fibroblasts (7–9).

## BACKGROUND

KCa3.1 $Ca^{2+}$-activated channels regulate $K^+$ outflow, increasing the driving force for $Ca^{2+}$ entry through hyperpolarization of the plasma membrane (7,10). In KCa3.1, four identical subunits are gathered as a symmetric homotetramer. Six hydrophobic α-helical domains are inserted into the cell membrane in each subunit (Fig. 1). A five-residue loop between the fifth and sixth transmembrane domain confers $K^+$ selectivity. $K^+$ channels are usually tightly associated with calmodulin, the regulatory protein that accounts for the $Ca^{2+}$ sensitivity of these channels, usually activated by $[Ca^{2+}]_i$ slightly below 1 μmol/L (7–10) (Fig. 1). In turn, KCa3.1-mediated $Ca^{2+}$ influx has been linked to vascular inflammation, atherogenesis, and proliferation of several cell types, including endothelial cells, T lymphocytes, macrophages, vascular smooth muscle cells, and fibroblasts (7–10). Cell proliferation is believed to result from $Ca^{2+}$-dependent growth factor gene expression, along with activation of cyclins and kinases involved in cell division. However, proliferation is limited by enhanced apoptosis, resulting from cell shrinkage upon $K^+$ efflux (apoptotic volume decrease), and by activation of caspases, directly inhibited by cytosolic $K^+$ (7–10). KCa3.1 have also been implicated in transcellular chloride secretion and cyst growth in autosomal-dominant polycystic kidney disease (11).

In addition to diabetes, mouse unilateral ureteral obstruction (UUO) has often been used as a model of renal tubulointerstitial fibrosis (12). Robust upregulation of KCa3.1 was always detectable in ligated kidneys. KCa3.1 knockout mice showed reduced expression of fibrotic markers, less chronic tubulointerstitial damage, collagen deposition, and α-smooth muscle$^+$ cells after UUO, with better preservation of functional tissue. The selective KCa3.1 blocker, TRAM-34, attenuated progression of UUO-induced renal fibrosis in wild-type mice and rats (12,13).

## OVERVIEW

In this issue, Huang et al. (14) provide multiple lines of evidence that KCa3.1 triggers renal scarring in diabetes. First, they evaluated the role of KCa3.1 in mice rendered diabetic by streptozotocin, showing that gene knockout/silencing or TRAM-34 suppressed development of renal fibrosis. Second, they reported increased expression of KCa3.1 in humans with diabetic nephropathy as well as in diabetic mice. Third, several genes linked to inflammation (monocyte chemoattractant protein-1, intracellular adhesion molecule-1, the macrophage marker F4/80) or fibrosis (plasminogen activator inhibitor 1, collagen types III and IV, transforming growth factor-β1 [TGF-β1], TGF-β receptor II) were turned off upon KCa3.1 silencing or knockdown. In other models, blocking gene expression of TGF-β1, bone morphogenic protein-1, or platelet-derived growth factor similarly blunted fibrosis and tissue scarring (12,13,15–17). Finally, in human proximal tubular cells, inhibition of KCa3.1 also suppressed fibrotic markers and Smad2/3 phosphorylation.

## IMPORTANCE AND DISCUSSION

That KCa3.1 is involved in renal fibrosis, and particularly diabetic nephropathy, has been extensively proven in earlier studies. Besides UUO, angiotensin II (ANG II) enhances KCa3.1 expression and proliferation of rat cardiac fibroblasts, an effect blocked by TRAM-34, silencing RNAs, kinase inhibitors, and ANG II receptor antagonists (RA) (12,18). Recent work focused on advanced glycation end products (AGE), an in vitro model of the diabetic milieu, which also upregulate KCa3.1 in cardiac fibroblasts and vascular smooth muscle, once again triggering cell proliferation and migration (19,20). These effects of AGE were blocked by anti-AGE receptor antibodies, KCa3.1 gene silencing, and pharmacologic inhibitors (19,20).

The KCa3.1/fibrosis theory has some drawbacks, however. First, is the uniqueness of the fibrotic stimulus. A recent review by Boor et al. (5) identified no less than 17 distinct mechanisms involved in kidney fibrosis, successfully counteracted by nearly 80 different experimental approaches, including blocking antibodies, inhibitors, and RAs. The likelihood of a "master switch" mechanism is low,

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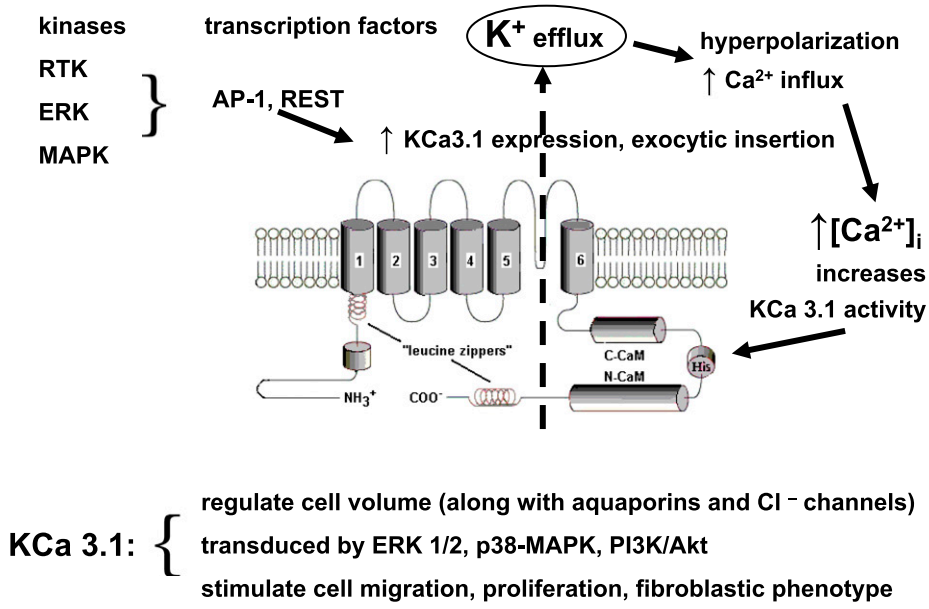


**FIG. 1. Diagram shows structure and function of a typical intermediate-/small-conductance KCa3.1 channel. A single subunit (out of four identical, forming a homotetramer) is depicted, with a pore region between transmembrane-spanning segments 5 and 6. A rise of cytosolic $Ca^{2+}$, resulting from agonist-mediated release of inositol 1,4,5-trisphosphate or $Ca^{2+}$ influx from plasma membrane channels (receptor-operated, voltage-operated, transient receptor potential) increases KCa3.1 open probability via constitutively bound calmodulin (calmodulin C-domain [C-CaM], calmodulin N-domain [N-CaM]), hyperpolarizing the cell. Subsequent $Ca^{2+}$ influx creates a positive feedback, activating several signaling kinase cascades (extracellular signal–related kinase [ERK 1/2], p38-mitogen-activated protein kinase [MAPK], phosphatidylinositide 3 kinase [PI3K]/Akt, Smad2/3, mitogen-activated protein kinase, etc.). Further downstream events include expression of monocyte chemoattractant protein-1, intracellular adhesion molecule-1, plasminogen activator inhibitor, and several collagen isoforms, with resulting matrix deposition and fibrosis. Channel expression and/or surface translocation are controlled by kinases and transcription factors as well (*upper left*). AP-1, activator protein 1; RTK, receptor-tyrosine kinase.**

based on current understanding of the complexity of tissue healing (1,2,13).

Second, blocking fibrosis might not suffice to rescue a kidney from progressive failure. Unless the cause of renal cell apoptosis or necrosis is reversed, tissue disruption would tend to progress, with collagen just "filling in the void." Diabetes is a typical example of continuing injury to vascular and tubulointerstitial components of the kidney. Similarly, in immune/inflammatory forms of renal injury, such as glomerulonephritis, cutting off the deposition of collagen would leave untouched the primary process that yields nephron loss over months to years.

Finally, because the kidney of higher mammals has a much longer lifespan and a slower rate of collagen fiber deposition, compared with the rodents in this study, inhibition of KCa3.1 would have to last for years to ward against fibrosis in diabetic subjects, with unpredictable effects on excitable tissue and extrarenal organs expressing this channel.

In conclusion, it seems that the time is yet to come for clinical nephrologists to selectively suppress the fibrosclerotic evolution of renal diseases (5,13). To date, the only proven approach to slow the decay of renal function in diabetes is blockade of the renin-angiotensin system with ACE inhibitors, ANG II-RA, or renin inhibitors (5), possibly in combination. An alternative option would be welcome, in view of the known adverse effects of renin-angiotensin system blockers, including acute impairment of renal hemodynamics and hyperkalemia, among others. Work like this by Huang et al. (14) could eventually shed light on novel mechanisms of diabetic injury to the kidney and vasculature that may be eventually targeted to prevent or delay progression of renal failure (21).

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