Blockade of KCa3.1 Ameliorates Renal Fibrosis Through the TGF-β1/Smad Pathway in Diabetic Mice

Chunling Huang,1,2 Sylvie Shen,1 Qing Ma,1 Jason Chen,3 Anthony Gill,3 Carol A. Pollock,1 and Xin-Ming Chen1

Diabetic nephropathy is a major cause of end-stage renal failure and premature mortality. Although strategies such as glycemic control, blood pressure control, and inhibition of the renin-angiotensin-aldosterone system have been shown to be effective to a limited extent, the number of patients with diabetes that ultimately develop progressive renal damage remains high (1,2). Therefore, it is of utmost importance to identify novel interventions for mitigating the progression of diabetic nephropathy.

Transforming growth factor-β1 (TGF-β1) has been identified as a key regulator of extracellular matrix (ECM) protein synthesis and degradation in diabetic nephropathy (3). TGF-β1 promotes renal fibrosis by upregulating genes encoding ECM proteins, leading to their increased synthesis, and at the same time enhances the production of inhibitors of ECM-degrading enzymes, such as plasminogen activator inhibitor type 1 (PAI-1) (4). TGF-β1 exerts its effects via binding to the membrane-bound TGF-β type II receptor (TβRII), causing the formation of heteromeric complexes. TβRII then transphosphorylates the type I receptor and activates the Smad signaling pathway to modulate gene transcription by phosphorylating Smad2/3. In addition, the accumulation of inflammatory cells and upregulated expression of proinflammatory mediators such as monocyte chemoattractant protein-1 (MCP-1) and intercellular adhesion molecule 1 (ICAM-1) contribute to the development of renal fibrosis in human and animal models of diabetic nephropathy (5).

Accumulated evidence indicates that calcium signaling cascades play a critical role in the functional activity of diverse tissues. Modification of the activity of Ca2+-activated K+ channels (KCa) leads to changes in the cellular and ultrastructural membrane potentials required for various cellular processes (6). KCa3.1 (also known as IK1, SK4, or KCNN4) belongs to the potassium intermediate/small conductance calcium-activated channel family. The intermediate-conductance KCa3.1 channel was first described by Gardos in erythrocytes in 1958 (7) and was subsequently cloned from pancreas, placenta, and lymphoid tissue in 1997 (8–10). KCa3.1 proteins are distributed in the membranes of both cytoplasm and mitochondria. KCa3.1 regulates K+ efflux, increasing the driving force for Ca2+ entry through hyperpolarization of the plasma membrane (11). KCa3.1-mediated Ca2+ influx is associated with inflammation, atherogenesis, and proliferation of endothelial cells, T lymphocytes, macrophages, and fibroblasts (12–16). KCa3.1 is a potential molecular target for pharmacological intervention in vascular restenosis, urinary incontinence, prostate cancer, and autoimmune disease (17–19). Recently, Grgic et al. have reported that the highly selective inhibitor of KCa3.1, TRAM34 (20,21), can reduce renal fibrosis in animal models of obstructive uropathy (22) and prevent acute kidney transplant rejection in rats if given in combination with a Kv1.3 blocker (23). However, the role of KCa3.1 in diabetic nephropathy has not been studied.

In this study, we investigated the therapeutic potential of KCa3.1 in diabetic nephropathy using two streptozotocin (STZ)-induced diabetic mouse models. Our results demonstrate that blockade of KCa3.1 was able to ameliorate albuminuria and minimize renal damage induced by diabetes. Furthermore, our results describe an additional mechanism for KCa3.1-mediated protection through the negative regulation of the TGF-β1 and Smad pathway.

RESEARCH DESIGN AND METHODS

Materials. Recombinant human TGF-β1 and the selective KCa3.1 blocker TRAM34 (1-[(2-chlorophenyl) diphenylmethyl-1H-pyrazole) were purchased from R&D Systems (Minneapolis, MN) and Sigma-Aldrich (St. Louis, MO), respectively. Anti-KCa3.1 was purchased from Abnova (Taipei City, Taiwan).
Anti-PAI-1, –TGF-β1, and –TβRII were purchased from BD Biosciences (Franklin Lakes, NJ), LifeSPAN (Seattle, WA), and Upstate (Billerica, MA). Anti-type III collagen (COL3) and –type IV collagen (COL4) were obtained from Abcam (Cambridge, MA). Anti-β-tubulin antibody was from Sigma-Aldrich. Anti-phospho-Smad2, –phospho-Smad3, -Smad2/3 were purchased from Cell Signaling Technology (Danvers, MA). Anti-F4/80 was obtained from AbD Serotec (Oxford, U.K.).

Human kidney biopsies. Human kidney biopsy specimens from patients with diabetic nephropathy were provided by the Department of Anatomical Pathology of the Royal North Shore Hospital. Kidneys removed from patients, generally due to peripheral tumor but without known kidney disease, served as controls. This study was approved by the Human Research Ethics Committee of the Royal North Shore Hospital.

Animal studies. KCa3.1+/+ mice were provided by Dr. James Melvin (National Institute of Dental and Craniofacial Research, Bethesda, MD). Eight-week-old male KCa3.1+/+ (C57B6), KCa3.1−/−, and eNOS−/− mice (Jackson Laboratory, Bar Harbor, ME) weighing ~20~25 g were assigned to receive either 55 mg/kg STZ (Sigma-Aldrich) diluted in 0.1 mol/L citrate buffer, pH 4.5, or citrate buffer alone by intraperitoneal injection as described previously (24). A group of KCa3.1−/− (n = 8) and eNOS−/− mice (n = 6) that received citrate buffer alone served as nondiabetic controls. eNOS−/− diabetic mice were then randomized into two groups, those receiving treatment with TRAM34, 120 mg/kg/day intraperitoneally, or vehicle (DMSO) alone for 24 weeks. Treatment commenced in vivo 24 h of the last STZ injection. All animals were housed in the Kears Animal Facility of the Kolling Institute of Medical Research, with a stable environment maintained at 22 ± 1°C with a 12/12-h light-dark cycle.

Mice were weighed and their blood glucose levels were measured using the ACCU-CHEK glucometer (Roche Diagnostics) weekly, and only STZ-treated mice were used for subsequent experiments. Blood pressure was noninvasively measured using a volume pressure recorder (T耀眼, Tokyo, Japan). Water intake was monitored daily, and food intake was measured weekly.

Kidney/body weight (mg/g) and Body weight (g) were calculated according to the formulae:

\[
\text{Kidney/body weight (mg/g)} = \frac{\text{Kidney weight (mg)}}{\text{Body weight (g)}}
\]

\[
\text{Body weight (g)} = \frac{\text{Kidney weight (mg)}}{\text{Kidney/body weight (mg/g)}}
\]

Blood glucose level (mmol/L) was measured using an Accu-Chek glucometer (Roche Diagnostics).

TABLE 1
Nucleotide sequence of the primers used for qRT-PCR

<table>
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<tr>
<th>Species</th>
<th>Molecules</th>
<th>Forward primer (5’-3’)</th>
<th>Reverse primer (5’-3’)</th>
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<tr>
<td>Human</td>
<td>COL3</td>
<td>CTGGAGCCCCAGGTCTTC</td>
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**TABLE 2**
Metabolic and physiological parameters of KCa3.1+/+ and KCa3.1−/− mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>KCa3.1+/+ control</th>
<th>KCa3.1+/+ DM</th>
<th>KCa3.1−/− DM</th>
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</thead>
<tbody>
<tr>
<td>Blood glucose level (mmol/L)</td>
<td>8.554 ± 0.223</td>
<td>22.69 ± 0.647‡</td>
<td>23.17 ± 0.665‡</td>
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<tr>
<td>Body weight (g)</td>
<td>30.3 ± 0.693</td>
<td>23.97 ± 0.983‡</td>
<td>29.81 ± 0.549§</td>
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<td>Kidney/body weight (mg/g)</td>
<td>6.435 ± 0.194</td>
<td>9.344 ± 0.423‡</td>
<td>8.16 ± 0.279§</td>
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<td>Blood pressure (systolic, mmHg)</td>
<td>110.9 ± 2.424</td>
<td>109.5 ± 1.609</td>
<td>107.2 ± 1.890</td>
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<tr>
<td>Spot albumin-to-creatinine ratio (mg/mg)</td>
<td>1.293 ± 0.345</td>
<td>4.947 ± 0.970‡</td>
<td>2.29 ± 0.634§</td>
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<tr>
<td>Albumin-to-creatinine ratio (mg/mg/24 h)</td>
<td>1.096 ± 0.253</td>
<td>4.169 ± 0.252‡</td>
<td>2.298 ± 0.695§</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. DM, diabetes mellitus. ‡P < 0.05, vs. KCa3.1+/+ control. §P < 0.05, vs. KCa3.1+/+ DM.
within the interstitium was assessed using Masson’s trichrome stain (American
MasterTech, Lodi, CA). In brief, endogenous peroxidase activity was blocked
by incubation in 0.3% hydrogen peroxide. After preincubation with 10% protein
block (Dako, Carpinteria, CA) for 10 min at room temperature to block non-
specific binding of antibodies, the tissues were incubated overnight at 4°C with
primary antibodies against KCa3.1, F4/80, type III and IV collagen, TGF-β1,
TβRII, and P-Smad2/3. After incubation with the appropriate secondary anti-
bodies, sections were developed with 3,3-diaminobenzidine (Dako) to produce
a brown color and counterstained with hematoxylin. Positive signals in the
renal cortex regions were quantified using Image J software as previously
described (26). The number of cells positive for F4/80+, phospho-Smad2/3+
was counted in 10 high-power fields (40×) of the tubulointerstitium.

### TABLE 3

<table>
<thead>
<tr>
<th>Metabolic and physiological parameters of eNOS&lt;sup&gt;−/−&lt;/sup&gt; control and diabetic mice treated with TRAM34 or vehicle</th>
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</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>Blood glucose level (mmol/L)</td>
</tr>
<tr>
<td>Body weight (g)</td>
</tr>
<tr>
<td>Kidney/body weight (mg/g)</td>
</tr>
<tr>
<td>Blood pressure (systolic, mmHg)</td>
</tr>
<tr>
<td>Spot albumin-to-creatinine ratio (mg/mg)</td>
</tr>
<tr>
<td>Albumin-to-creatinine ratio (mg/mg/24 h)</td>
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</tbody>
</table>

Data are presented as mean ± SEM. DM, diabetes mellitus. *P < 0.05, vs. control. †P < 0.05, vs. DM+DMSO.

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FIG. 1. KCa3.1 expression was increased in kidneys of human and mice with diabetic nephropathy. (A) Immunohistochemical analysis demonstrated increased KCa3.1 expression in kidney biopsies from patients with diabetic nephropathy (diabetes mellitus [DM]) compared with non-diabetic control kidneys (non-DM control) (n = 8). (B) The quantitation of KCa3.1 expression in human biopsies. (C) Immunohistochemical analysis demonstrated that the expression of KCa3.1 was increased in kidneys of diabetic KCa3.1<sup>+/−</sup> mice compared with normal mice. There is no KCa3.1 expression in KCa3.1<sup>−/−</sup> mice (n = 8). (E) Immunohistochemical analysis demonstrated increased KCa3.1 expression in kidneys of diabetic eNOS<sup>−/−</sup> mice (DM+DMSO) compared with normal mice (non-DM control), and TRAM34 suppressed KCa3.1 expression in the kidneys of diabetic eNOS<sup>−/−</sup> mice (DM+TRAM34) (n = 6). (D and F) The quantitation of KCa3.1 expression in mouse kidney. Results are presented as mean ± SEM. *P < 0.05; **P < 0.01. Original magnification ×400.

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**Statistical analysis.** The results from at least four independent experiments were expressed as mean ± SEM. Statistical analysis of data from two groups was compared by two-tailed Student’s t test. Data from multiple groups were analyzed by one-way ANOVA, followed by Tukey post hoc test. Statistical significance was determined as *P* < 0.05.

**RESULTS**

Blockade of KCa3.1 attenuates the renal injury in two STZ-induced diabetic models. To examine the role of KCa3.1 in the development of diabetic nephropathy in vivo, we conducted two animal studies: wild-type KCa3.1+/+ and KCa3.1−/− mice and secondly eNOS−/− mice with or without administration of TRAM34. The diabetic eNOS−/− mice are endorsed by the Animal Models of Diabetic Complications Consortium (AMDCC) for studies in diabetic nephropathy (27). As shown in Table 2, diabetic wild-type (KCa3.1+/+) mice showed significantly increased blood glucose levels and reduced body weight compared...
with the control group. The mean kidney-to-body weight ratio in diabetic KCa3.1+/+ mice was significantly higher than that of control (P < 0.05), which was reduced in diabetic KCa3.12/2 mice (P < 0.05). There was no difference in the blood pressure observed between control and diabetic mice. To determine renal function, spot urine albumin-to-creatinine ratio and 24-h urine albumin-to-creatinine ratio were measured at the time the mice were killed. Renal function was impaired in diabetic KCa3.1+/+ mice, and this impairment was significantly attenuated in KCa3.12/2 mice (P < 0.05). Similar results were found with pharmacological inhibition of KCa3.1 in diabetic eNOS−/− mice by administrating the specific inhibitor TRAM34 (Table 3). TRAM34 did not affect glucose levels or weight gain. Treatment of diabetic animals with TRAM34 significantly alleviated all indices of renal injury in eNOS−/− mice. These results indicate that blockade of KCa3.1 attenuates renal injury caused by diabetes, implicating the essential role of KCa3.1 in STZ-induced diabetic nephropathy. 

**KCa3.1 expression is increased in kidney tissues of human and mice with diabetic nephropathy.** To investigate whether KCa3.1 expression is altered in diabetic
nephropathy, we first examined the expression of KCa3.1 in kidney biopsies from patients with diabetic nephropathy and nondiabetic controls using immunohistochemical analysis. As shown in Fig. 1A, considerable staining for the KCa3.1 channel protein was observed in kidney proximal tubular cells of diabetic kidneys, whereas only a low basal level of KCa3.1 was expressed in nondiabetic controls ($P < 0.01$) (Fig. 1B).

We next determined the expression of KCa3.1 in two STZ-induced diabetic mice models as described above. As shown in Fig. 1C and D, a low basal level of KCa3.1 expression was observed in kidney proximal tubular cells of nondiabetic KCa3.1+/+ mice, which was significantly upregulated in most proximal tubular cells of diabetic KCa3.1+/+ mice ($P < 0.01$). As expected, there was no KCa3.1 expression in KCa3.1-deficient diabetic mice. Similarly, the administration of TRAM34 significantly reversed the upregulated KCa3.1 expression in proximal tubular cells of diabetic eNOS−/− mice compared with that in the vehicle-treated group ($P < 0.01$) (Fig. 1E and F). Collectively, these data provided evidence for substantial KCa3.1 upregulation in diabetic kidneys, indicating a potential pathophysiological involvement of the KCa3.1 channel in diabetic nephropathy. **Blockade of KCa3.1 prevents inflammatory responses in diabetic mice.** To characterize the role of KCa3.1 in the regulation of inflammation, we examined two proinflammatory cytokines, MCP-1 and ICAM1, and one macrophage marker, F4/80, in kidney tissues. RT-PCR analyses of kidney tissues demonstrated that the expressions of MCP-1, ICAM1, and F4/80 were increased by 2.9-, 1.7-, and 2.2-fold, respectively, in the diabetic KCa3.1+/+ group, which were reduced in the diabetic KCa3.1−/− group ($P < 0.05$) (Fig. 2A–C). Remarkably, histopathological analysis of renal cross-sections demonstrated a 52% reduction of F4/80.
expression in diabetic kidneys of KCa3.1-deficient animals as compared with diabetic KCa3.1+/+ controls (P < 0.01) (Fig. 2D and E). Consistent with this finding, we also observed a significant decrease in MCP-1, ICAM1, and F4/80 expression in the kidneys of diabetic eNOS−/− mice compared with control mice but reduced mRNA expression with TRAM34 (n = 6). (F) Immunohistochemical staining of TGF-β1, TβRII, and P-Smad2/3 in the renal cortex from control, diabetic, and diabetic mice treated with TRAM34 (n = 6). The quantitation of TGF-β1 (D and J), TβRII (E and K), and P-Smad2/3 (F and L) expression in mouse kidney. Results are presented as mean ± SEM. *P < 0.05; **P < 0.01. Original magnification x400. DM, diabetes mellitus.

expression in diabetic kidneys of KCa3.1-deficient animals as compared with diabetic KCa3.1+/+ controls (P < 0.01) (Fig. 2D and E). Consistent with this finding, we also observed a significant decrease in MCP-1, ICAM1, and F4/80 expression in the kidneys of diabetic eNOS−/− mice compared with control mice but reduced mRNA expression with TRAM34 (n = 6). (F) Immunohistochemical staining of TGF-β1, TβRII, and P-Smad2/3 in the renal cortex from control, diabetic, and diabetic mice treated with TRAM34 (n = 6). The quantitation of TGF-β1 (D and J), TβRII (E and K), and P-Smad2/3 (F and L) expression in mouse kidney. Results are presented as mean ± SEM. *P < 0.05; **P < 0.01. Original magnification x400. DM, diabetes mellitus.
was observed within the tubulointerstitium after induction of diabetes. KCa3.1 deficiency significantly reduced excess matrix deposition ($P < 0.01$) (Fig. 3D and E). Diabetes also resulted in increased expression of type III ($P < 0.05$) (Fig. 3D and F) and IV collagen ($P < 0.01$) (Fig. 3D and G), whereas KCa3.1 deficiency attenuated this response. In line with these observations, renal gene expression levels of established fibrotic markers (PAI-1 and type III and IV collagen) were considerably lower in diabetic kidneys from TRAM34-treated mice compared with vehicle-treated mice ($P < 0.05$) (Fig. 3H–J). Consistently, the administration of TRAM34 significantly reversed tubulointerstitial damage in diabetic kidneys as compared with vehicle-treated controls ($P < 0.01$) (Fig. 3K and L). Furthermore, the immunohistochemical staining also showed a substantially increased abundance of immunostainable type III ($P < 0.05$) (Fig. 3K and M) and IV collagen ($P < 0.05$) (Fig. 3K and N) localized in the interstitial areas of diabetic kidneys compared with controls, which was reversed after TRAM34 treatment. Collectively, these results indicate that blockade of KCa3.1 suppresses interstitial matrix production and reduces renal interstitial fibrosis in diabetic nephropathy.

**Blockade of KCa3.1 inhibits the expression of TGF-β1, TβRII, and phosphorylation of Smad2/3 in diabetic mice.** Increased expression of TGF-β1 and/or TGF-β receptors is found in almost all forms of kidney diseases with interstitial fibrosis (28). To elucidate the mechanisms by which blockade of KCa3.1 inhibited renal fibrosis, we examined the effects of KCa3.1 on the expression of TGF-β1 and TβRII in diabetic kidneys using real-time PCR and immunohistochemical staining. At both mRNA and protein levels, expression of TGF-β1 and TβRII was significantly increased in diabetic KCa3.1−/− mice compared with control groups, and
KCa3.1 deficiency significantly reduced their levels ($P < 0.05$) (Fig. 4A–E). Phosphorylation of Smad2/3 and its subsequent nuclear translocation are critical steps in TGF-β1 signaling; therefore, the TGF-β1–induced Smad2/3 signaling pathway was examined. As shown in Fig. 4C, immunohistochemical staining showed that TGF-β1–Smad2/3 signaling was strongly activated in diabetic KCa3.1+/+ mice. However, the activation was inhibited in KCa3.1 deletion diabetic mice ($P < 0.01$) (Fig. 4F). Similarly, blockade of KCa3.1 with the administration of TRAM34 significantly decreased the expression of TGF-β1 and its receptor, TβRII, after diabetic injury ($P < 0.05$) (Fig. 4G–K) at both mRNA and protein levels. In addition, phosphorylation of Smad2/3 was suppressed in diabetic kidneys from TRAM34–treated mice compared with vehicle-treated mice ($P < 0.05$) (Fig. 4I and L). Taken together, these data indicate that KCa3.1 mediates expression of both TGF-β1 and its receptor, TβRII, through the Smad2/3 pathway in the diabetic kidneys.

Blockade of KCa3.1 inhibits TGF-β1–stimulated PAI-1 and type III and IV collagen expression via Smad2/3 pathway in human proximal tubular cells. To support the in vivo findings reported above, human proximal tubular cells (HK2 cells) exposed to TGF-β1 were concurrently exposed to KCa3.1 siRNA or TRAM34, respectively. As expected, exposure of HK2 cells to TGF-β1 resulted in significantly increased expression of PAI-1 ($P < 0.05$) (Fig. 5A and E), type III collagen ($P < 0.01$) (B and F), and type IV collagen ($P < 0.05$) (C and G). In addition, phosphorylation of Smad2/3 in HK2 cells that had been exposed to TGF-β1 was inhibited by KCa3.1 siRNA or TRAM34 ($P < 0.05$) (Fig. 5D and H). These data suggest that the antifibrotic effect of KCa3.1 is mediated by the TGF-β1/Smad signaling pathway.

FIG. 5. Blockade of KCa3.1 inhibited TGF-β1–stimulated PAI-1 and type III and IV collagen expression via the Smad2/3 pathway in human proximal tubular cells. Western blots demonstrate that TGF-β1 increased the expression of PAI-1 (A) and type III (B) and IV collagen (C) and activated Smad2/3 phosphorylation (D) in cultured human proximal tubular cells (HK2 cells) at 48 h, which were reversed in KCa3.1 siRNA–transfected HK2 cells. TRAM34 suppressed TGF-β1–induced PAI-1 (E) and type III (F) IV collagen (G) and phosphorylation of Smad2/3 (H) in HK2 cells. Results are presented as mean ± SEM. *$P < 0.05$; **$P < 0.01$. $n = 4$. 
DISCUSSION
This study was undertaken to address whether inhibition of the calcium-activated potassium channel KCa3.1 will ameliorate renal dysfunction and attenuate the renal fibrosis inherent in diabetic nephropathy, and to elucidate the possible underlying mechanisms. Our studies have first demonstrated that KCa3.1 expression is increased in both human and mice models with diabetic nephropathy. Subsequently, our findings demonstrate that KCa3.1 deficiency significantly attenuated inflammation, regulators of matrix production, and matrix protein expression and thus reduces renal fibrosis in the KCa3.1 knockout mouse model of diabetic nephropathy. In addition, with administration of the KCa3.1 inhibitor TRAM34, a highly selective inhibitor of KCa3.1, we provided evidence that pharmacological inhibition of KCa3.1 was similarly effective in mitigating the development of renal fibrosis after diabetic injury, which implies an important role for KCa3.1 in the pathogenesis of renal fibrotic disease. Furthermore, in vitro results supported that blockade of KCa3.1 inhibited TGF-β1–induced fibrotic responses through Smad2/3-dependent pathways.

Calcium regulates a wide range of vital cell functions, including enzyme activities, attachment, motility, morphology, metabolic processes, cell-cycle progression, signal transduction, replication, gene expression, and electrochemical responses. It is well known that pathophysiological inflammation is implicated in the progression of diabetic nephropathy. KCa3.1-mediated elevation of intracellular calcium is necessary for the production of inflammatory chemokines and cytokines by T cells, macrophages, and mast cells (29,30). Activation of KCa3.1 is believed to contribute to migration, activation, and proliferation of immunologically active cells. KCa3.1 expression is upregulated in activated naive and central memory T cells and IgD+ B cells (31,32), and the channel has therefore been proposed as a target for the treatment of autoimmune diseases and transplant rejection (33). Recently, the combination of TRAM34 with the Kv1.3 blocking peptide ShK was further shown to reduce T-cell and macrophage infiltration in the early stages of...
chronic kidney transplant rejection in rats (23), suggesting that KCa3.1 blockers may represent a novel alternative therapy for prevention of kidney allograft rejection. In this study, we observed that blockade of KCa3.1 inhibited infiltration of macrophage F4/80 and suppressed the expression of MCP-1 and ICAM1 in the diabetic kidneys. These results suggest that inhibition of inflammatory responses may be a key mechanism by which KCa3.1 attenuates renal fibrosis.

Inhibition of KCa3.1 may elicit an antifibrotic effect by multiple mechanisms. As TGF-β1 signaling has a central role in a variety of fibrogenic processes, such as ECM protein accumulation in diabetic nephropathy and tubulointerstitial fibrosis (31,34), we examined the effect of KCa3.1 on the expression of TGF-β1 and TβRII in the kidneys of mice with diabetic nephropathy. Our results clearly indicated that expression levels of TGF-β1 and TβRII mRNA were upregulated in the diabetic kidney, and inhibition of KCa3.1 suppressed their expression (Fig. 4). Thus, we suggest that KCa3.1-targeted inhibition of renal fibrosis is likely mediated by antagonizing TGF-β1 signaling through suppression of TGF-β1 and TβRII expression. To further elucidate the mechanism by which blockade of KCa3.1 inhibits inflammatory cytokine and matrix protein expression, we examined whether blockade of KCa3.1 inhibited TGF-β1–mediated Smad2/3 activity. TGF-β receptor, a transmembrane Ser/Thr kinase receptor, phosphorylates receptor-regulated Smads, such as Smad2/3. Phosphorylated Smads enter the nucleus, where they activate the expression of target genes, including PAI-1 and matrix proteins, and subsequently contribute to tubulointerstitial fibrosis (35). In this study, we found that blockade of KCa3.1 successfully inhibited TGF-β1–stimulated target gene expression, including PAI-1, matrix proteins, and inflammatory cytokine through the Smad2/3 pathway. This study showed that the antifibrotic effects of KCa3.1 are at least partly mediated by the suppression of TGF-β1 signaling.

In summary, the current study is the first to report that blockade of KCa3.1 can inhibit excessive deposition of ECM and transcriptional expression of TGF-β1, TβRII, and several proinflammatory cytokines that are associated with diabetic nephropathy through Smad2/3 pathways. Therefore, inhibition of the KCa3.1 signaling pathway may provide a novel approach to prevent the development of diabetic nephropathy and attenuate the progression of renal fibrosis.

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No potential conflicts of interest relevant to this article were reported.

C.H. conceived and designed the research, performed and interpreted the results of experiments, analyzed data, prepared figures, and drafted and revised the manuscript. S.S., Q.M., J.C., and A.G. performed and interpreted partial experiments and revised the manuscript. C.A.P. and X.-M.C. conceived and designed the research, interpreted results of experiments, and revised the manuscript. All authors approved the final version of the manuscript. C.A.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The KCa3.1−/− mice were kindly provided by Dr. James Melvin, National Institute of Dental and Craniofacial Research, Bethesda, Maryland.

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