

## KCa3.1 as a novel therapeutic target for diabetic nephropathy

Chunling Huang\*, Carol A. Pollock and Xin-Ming Chen

Renal department, Level 9, Kolling Building, Sydney Medical School-Northern, University of Sydney, Royal North Shore Hospital, St Leonards, NSW, Australia

Accepted on August 21, 2017

Diabetic nephropathy (DN), a devastating complication of diabetes mellitus, is a worldwide health, societal and economic problem. Tubulointerstitial fibrosis has been shown to be the final common pathological consequence of almost all forms of chronic progressive renal disease, including diabetic nephropathy [1]. Numerous pathways have been identified in the pathogenesis of this disease, but clinical strategies based on these pathways for the management of diabetic nephropathy remain unsatisfactory, as the number of diabetic patients with nephropathy continues to increase [2-4]. New treatment modalities or strategies are needed to prevent or slow the progression of diabetic nephropathy.

KCa3.1 (also known as IK1, SK4 or KCNN4) is a member of the calcium-activated potassium channel (KCa) family. KCa3.1 has been suggested as a potential therapeutic target for diseases such as sickle cell anemia, autoimmunity and atherosclerosis [5,6]. Recent reports suggest that the highly selective inhibitor of KCa3.1, TRAM34, can reduce unilateral ureteral obstruction (UUO)-induced renal fibrosis [7] and prevent acute kidney transplant rejection in rats when combined with a KV1.3 blocker [8]. However, the role of the KCa3.1 channel in mediating diabetic nephropathy is still not clear. Therefore, our group has done a series of studies to explore the role of KCa3.1 in diabetic nephropathy. Two *in vivo* streptozotocin (STZ)-induced diabetic mouse models were studied: a genetic knockout of KCa3.1 and pharmacological inhibition of KCa3.1 with TRAM34 in eNOS<sup>-/-</sup> mice, to determine the therapeutic effect of KCa3.1 in diabetic nephropathy. Since proximal tubular cells are the predominant cell in the tubulointerstitium and fibroblasts are the main source for extracellular matrix (ECM), proximal tubular cells and fibroblasts were used as *in vitro* models to further determine the signaling pathways involved under transforming growth factor beta 1 (TGF- $\beta$ 1) or high glucose condition. Our studies have provided multiple lines of evidence that KCa3.1 channel is implicated in the pathogenesis of diabetic nephropathy and elucidated its possible underlying mechanisms and downstream signaling pathways [9-14].

Firstly, we demonstrated that KCa3.1 expression was increased in kidneys of both human and mouse models with DN. Blockade of KCa3.1 ameliorated the renal injury in two STZ-induced diabetic models [9]. In addition, blockade of KCa3.1 attenuated inflammation, regulators of matrix production and matrix protein expression and thus reduced

renal fibrosis in diabetic mice [9]. Furthermore, blocking KCa3.1 channel in both animal models led to the reduction of TGF- $\beta$ 1, T $\beta$ RII, and phosphorylation of Smad2/3, indicating that one mechanism by which KCa3.1 mediates renal fibrosis in DN is through TGF- $\beta$ 1/Smad signaling pathway [9].

Inflammation has been proposed to be important in the pathogenesis of DN with increased expression of chemokines. Monocyte chemoattractant protein-1 (MCP-1) is an important chemokine, playing a key role in monocyte/macrophage recruitment and contributing to inflammation in DN [14]. To investigate the role of KCa3.1 in the regulation of TGF- $\beta$ 1 induced MCP-1 expression and the underlying signal transduction pathway, we combined whole-cell electrophysiology together with biochemical and molecular techniques in HK2 cells, a proximal tubular cell line [10]. Our data showed that TGF- $\beta$ 1 not only increased the expression of KCa3.1 but also the channel activity, indicating a direct link between TGF- $\beta$ 1 treatment and induction of this channel [10]. Consistent with the *in vivo* results, both gene knockdown and pharmacological inhibition of KCa3.1 suppressed TGF- $\beta$ 1-induced MCP-1 expression in HK2 cells [10]. Using the specific inhibitor, we further demonstrated that the inhibitory effect of KCa3.1 was dependent on the activation of Smad3, p38 and ERK1/2 signaling pathways [10]. Chemokine (C-Cmotif) ligand 20 (CCL20) has also been shown to be involved in the pathogenesis of DN. As demonstrated previously by our group CCL20 was significantly increased by high glucose in renal proximal tubule cells as well as in the kidneys of diabetic rats [16]. To determine the effect of KCa3.1 on CCL20, we exposed HK2 cells to high glucose for 6 days to mimic diabetic milieu [13]. *In vitro* results showed that blockade of KCa3.1 with TRAM34 was able to attenuate high glucose-induced upregulation of CCL20 expression in HK2 cells, which was further confirmed by *in vivo* mice models [13]. The anti-inflammatory effect of KCa3.1 inhibition on DN was shown to be closely associated with suppression of NF- $\kappa$ B activation, which is an important transcription factor in the regulation of inflammation [13]. Together, these results further demonstrated that inhibition of inflammatory responses may be another key mechanism by which KCa3.1 attenuates diabetic nephropathy.

Previously KCa3.1 has been reported to be involved in renal fibroblast proliferation [7]. However, the role of KCa3.1 in the activation of fibroblasts in DN was required

to be delineated. We investigated the effects of KCa3.1 on fibroblast activation, matrix synthesis and degradation induced by TGF- $\beta$ 1 in human primary renal interstitial fibroblasts and explored the underlying mechanisms in two mouse models of DN [12]. Our results demonstrated that blockade of KCa3.1 suppressed TGF- $\beta$ 1-induced activation of fibroblasts and ECM production, which occurs through Smad2/3 or ERK1/2, but independent of P38 or JNK pathways [12]. Furthermore, blockade of KCa3.1 normalized regulators of matrix production and matrix protein expression and thus reduced renal fibrosis in both animal models of DN [12]. Hence, the results suggest that targeting the signaling pathways that mediate the activation of fibroblasts may be the third mechanism whereby KCa3.1 inhibition suppresses the pathological consequences of DN.

Recently, autophagy has drawn increasing attention as dysregulated autophagy has been implicated in the pathogenesis of diabetic nephropathy [17]. The role of autophagy in diabetic nephropathy has not been fully elucidated [18]. A study has been undertaken to identify the role of KCa3.1 in dysfunctional tubular autophagy in diabetic nephropathy [19]. Our data clearly demonstrated dysfunctional tubular autophagy in the presence of TGF- $\beta$ 1 *in vitro* as well as in a mouse model of diabetic nephropathy [19]. In addition, our results suggested that TGF- $\beta$ 1-induced overexpression of KCa3.1 in tubular cells contributed to the impairment of autophagic flux [19]. Furthermore, TGF- $\beta$ 1-induced KCa3.1 activation was likely to mediate dysregulation of tubular autophagy through PI3K/Akt/mTOR signaling pathways [19]. Therefore, our data demonstrated that blockade of KCa3.1 attenuated diabetes induced dysregulation of tubular autophagy, indicating a further mechanism by which KCa3.1 mediates diabetic renal fibrosis is through dysregulation of autophagy.

In summary, given the evidence developed in the studies, we propose that the KCa3.1 channel is a promising therapeutic target for DN based on its expression and demonstrated role in fibrosis and inflammation in tubular epithelia cells and fibroblasts in *in vitro* and *in vivo* models of diabetic nephropathy.

## References

1. Zeisberg M, Neilson EG. Mechanisms of tubulointerstitial fibrosis. *J Am Soc Nephrol.* 2010;21(11):1819-34.
2. Arora MK, Singh UK. Molecular mechanisms in the pathogenesis of diabetic nephropathy: An update. *Vascul Pharmacol.* 2013;58(4):259-71.
3. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: Challenges, progress and possibilities. *Clin J Am Soc Nephrol.* 2017.
4. Gnudi L, Coward RJ, Long DA. Diabetic nephropathy: Perspective on novel molecular mechanisms. *Trends Endocrinol Metab.* 2016;27(11):820-30.
5. Wulff H, Castle NA. Therapeutic potential of KCa3.1 blockers: Recent advances and promising trends. *Expert Rev Clin Pharmacol.* 2010;3(3):385-96.
6. Chou CC, Lunn CA, Murgolo NJ. KCa3.1: Target and marker for cancer, autoimmune disorder and vascular inflammation? *Expert Rev Mol Diagn.* 2008;8(2):179-87.
7. Grgic I, Kiss E, Kaistha BP, et al. Renal fibrosis is attenuated by targeted disruption of KCa3.1 potassium channels. *Proc Natl Acad Sci U S A.* 2009;106(34):14518-23.
8. Grgic I, Wulff H, Eichler I, et al. Blockade of T-lymphocyte KCa3.1 and Kv1.3 channels as novel immunosuppression strategy to prevent kidney allograft rejection. *Transplant Proc.* 2009;41(6):2601-6.
9. Huang C, Shen S, Ma Q, et al. Blockade of KCa3.1 ameliorates renal fibrosis through the TGF-beta1/Smad pathway in diabetic mice. *Diabetes.* 2013;62(8):2923-34.
10. Huang C, Day ML, Poronnik P, et al. Inhibition of KCa3.1 suppresses TGF-beta1 induced MCP-1 expression in human proximal tubular cells through Smad3, p38 and ERK1/2 signaling pathways. *Int J Biochem Cell Biol.* 2014;47:1-10.
11. Huang C, Lin MZ, Cheng D, et al. Thioredoxin-interacting protein mediates dysfunction of tubular autophagy in diabetic kidneys through inhibiting autophagic flux. *Lab Invest.* 2014;94(3):309-20.
12. Huang C, Shen S, Ma Q, et al. KCa3.1 mediates activation of fibroblasts in diabetic renal interstitial fibrosis. *Nephrol Dial Transplant.* 2014;29(2):313-24.
13. Huang C, Pollock CA, Chen XM. High Glucose Induces CCL20 in Proximal Tubular Cells via Activation of the KCa3.1 Channel. *PLoS One.* 2014;9(4):e95173.
14. Huang C, Pollock CA, Chen XM. The role of the potassium channel KCa3.1 in diabetic nephropathy. *Clin Sci (Lond).* 2014.
15. Giunti S, Barutta F, Perin PC, et al. Targeting the MCP-1/CCR2 System in diabetic kidney disease. *Curr Vasc Pharmacol.* 2010;8(6):849-60.
16. Qi W, Chen X, Zhang Y, et al. High glucose induces macrophage inflammatory protein-3 alpha in renal proximal tubule cells via a transforming growth factor-beta 1 dependent mechanism. *Nephrol Dial Transplant.* 2007;22(11):3147-53.
17. Yamahara K, Yasuda M, Kume S, et al. The role of autophagy in the pathogenesis of diabetic nephropathy. *J Diabetes Res.* 2013;2013:193757.
18. Kume S, Yamahara K, Yasuda M, et al. Autophagy: Emerging therapeutic target for diabetic nephropathy. *Semin Nephrol.* 2014;34(1):9-16.

19. Huang C, Lin MZ, Cheng D, et al. KCa3.1 mediates dysfunction of tubular autophagy in diabetic kidneys via PI3k/Akt/mTOR signaling pathways. *Sci Rep.* 2016;6:23884.

**\*Correspondence to:**

Dr. Chunling Huang  
Renal department,  
Level 9, Kolling Building  
Sydney Medical School-Northern  
University of Sydney  
Royal North Shore Hospital  
St Leonards, NSW  
AUSTRALIA 2065  
Phone: 61-2-9926 4784  
E-mail [chunling.huang@sydney.edu.au](mailto:chunling.huang@sydney.edu.au)