Increased podocyte Sirtuin-1 function attenuates diabetic kidney injury

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Podocyte injury and loss contribute to the progression of glomerular diseases, including diabetic kidney disease. We previously found that the glomerular expression of Sirtuin-1 (SIRT1) is reduced in human diabetic glomeruli and that the podocyte-specific loss of SIRT1 aggravated albuminuria and worsened kidney disease progression in diabetic mice. SIRT1 encodes an NAD-dependent deacetylase that modifies the activity of key transcriptional regulators affected in diabetic kidneys, including NF-κB, STAT3, p53, FOXO4, and PGC1-α. However, whether the increased glomerular SIRT1 activity is sufficient to ameliorate the pathogenesis of diabetic kidney disease has not been explored. We addressed this by inducible podocyte-specific SIRT1 overexpression in diabetic OVE26 mice. The induction of SIRT1 overexpression in podocytes for six weeks in OVE26 mice with established albuminuria attenuated the progression of diabetic glomerulopathy. To further validate the therapeutic potential of increased SIRT1 activity against diabetic kidney disease, we developed a new, potent and selective SIRT1 agonist, BF175. In cultured podocytes BF175 increased SIRT1-mediated activation of PGC1-α and protected against high glucose-mediated mitochondrial injury. *In vivo*, administration of BF175 for six weeks in OVE26 mice resulted in a marked reduction in albuminuria and in glomerular injury in a manner similar to podocyte-specific SIRT1 overexpression. Both podocyte-specific SIRT1 overexpression and BT175 treatment attenuated diabetes-induced podocyte loss and reduced oxidative stress in glomeruli of OVE26 mice. Thus, increased SIRT1 activity protects against diabetes-induced podocyte injury and effectively mitigates the progression of diabetic kidney disease.

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Diabetic kidney disease (DKD) is the leading cause of end-stage renal disease in the United States.[1] The current therapy for DKD remains limited to the renin-angiotensin system blockade, which only provides partial renoprotection. Thus, many patients on angiotensin converting enzyme inhibitors or angiotensin receptor blockades continue to progress to end stage renal disease.[2] Therefore there is a large unmet need to develop more potent and safer therapies for patients with DKD.

The sirtuin family of nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylases plays an important role in aging,[3] metabolism,[4] cancer, and inflammation.[5] Sirtuin-1 (SIRT1) in renal tubular cells has been shown to protect renal tubular cells from cellular stresses associated with aging, cisplatin, and hypoxia.[6–8] Our previous studies have demonstrated that SIRT1 protein expression is reduced in podocytes and in glomerular cells of human diabetic kidneys, which was consistent with reduced SIRT1 mRNA expression in microdissected glomeruli of diabetic patients.[9,10] We have further shown that the global reduction of SIRT1 accelerated DKD progression in *db/db* mice[10] and that podocyte-specific knockout of *Sirt1* similarly accelerated DKD in streptozotocin-induced diabetic mice.[10,11] More recently, renal tubular SIRT1 expression was reported to mitigate diabetic glomerular injury.[12] However, whether the increased glomerular SIRT1 expression, particularly in the podocytes, is sufficient to attenuate DKD has not been addressed previously.

On the cellular level, SIRT1 has been shown to regulate autophagy,[13] energetic homeostasis,[14] mitochondrial biogenesis,[15] and apoptosis.[16] SIRT1 exerts these biological effects through deacetylation of transcription factors and consequently regulating their activities.[17] Among these substrates of SIRT1 are key transcription factors that are implicated in kidney disease progression, such as NF-κB p65 (RelA), STAT3, p53, FOXO, and PGC-1α.[18] Systems analysis has

revealed that JAK-STAT and NF-κB are key pathways activated in diabetic kidneys,[19,20] and we have recently shown that SIRT1 deacetylates STAT3 and NF-κB p65 to protect kidney from inflammation-induced kidney injury.[10] We also demonstrated that the attenuation of proteinuria and podocyte injury in diabetic *db/db* mice by pyridoxamine treatment was associated with restored SIRT1 expression and reduced NF-κB p65 and STAT3 acetylation and activation.[21] A large body of evidence also suggests that p53 mediates apoptosis of podocytes and tubular epithelial cells in DKD.[22–24] SIRT1 has been shown to promote cell survival by suppressing p53-dependent apoptosis in response to DNA damage and oxidative stress,[16] and recent data suggests that the interplay of SIRT1-p53 pathway controls cellular senescence.[25] Furthermore, SIRT1 was also shown to modulate PGC-1α activity and to attenuate aldosterone-induced mitochondrial damage and podocyte injury.[26]

Thus, together with the observation that SIRT1 expression is significantly reduced in glomeruli of mouse and human diabetic kidneys and that SIRT1 is a regulator of the previously mentioned transcription factors whose roles are implicated in DKD progression, we posited that the increased glomerular expression and/or activity of SIRT1 would confer therapeutic benefit against the disease progression. We have tested this hypothesis *in vivo* by employing both genetic and pharmacological approaches with the use of the inducible podocyte-specific SIRT1 overexpression mice and a novel SIRT1 agonist, respectively, in OVE26 type 1 diabetic mice.

RESULTS

Generation of diabetic mice with inducible podocyte-specific SIRT1 overexpression

To generate transgenic mice with tetracycline-inducible SIRT1 overexpression, human SIRT1 cDNA (Addgene, Cambridge, MA) was subcloned into pLP-TRE2 vector (Clontech Laboratories, Mountain View, CA), resulting in pLP-TRE2-SIRT1 expression construct as described in the Materials and Methods section. Doxycycline (Dox)-inducible expression of pLP-TRE2-SIRT1 construct was first confirmed *in vitro* in U2OS cell line (Supplementary Figure S1A) and used for microinjection to generate the TRE-SIRT1 transgenic mice in the FVB/N background (TRE-SIRT1^{OV}). Inducible SIRT1 overexpression *in vivo* was first tested in TRE-SIRT1^{OV} mice bred with transgenic mice with the universal expression of reverse tetracycline-controlled transactivator transgene under the cytomegalovirus early enhancer element and chicken beta-actin promoter (CAG-rtTA). Upon Dox supplementation (625 mg/kg in chow), there was a robust expression of SIRT1 in kidney cortices of CAGs-rtTA;TRE-SIRT1^{OV} mice (Supplementary Figure S1B). TRE-SIRT1^{OV} mice were subsequently crossed with podocin-rtTA transgenic mice to generate podocin-rtTA;TRE-SIRT1^{OV} (referred to hereafter as Pod-SIRT1^{OV}) for podocyte-specific SIRT1 expression. Dox-dependent induction of SIRT1 overexpression in podocytes was confirmed by Western blot analysis of primary podocytes isolated from Pod-SIRT1^{OV} mice with or without Dox supplementation (Supplementary Figure S1C). In order to ascertain whether the podocyte-specific overexpression of SIRT1 can mitigate the diabetic kidney injury, Pod-SIRT1^{OV} mice were crossed with type 1 diabetic OVE26 mice[27] to generate OVE26;Pod-SIRT1^{OV}. Their littermates without SIRT1 transgene (OVE26;WT) and age-matched healthy FVB mice were used as controls. Consistent with previous reports,[27,28] OVE26 mice exhibited significant albuminuria by 16 weeks of age. Thus, we next determined whether the overexpression of SIRT1 in podocytes in OVE26 mice starting at 16 weeks of age can curtail the progress of an established DKD.

Podocyte-specific SIRT1 overexpression attenuates DKD progression in diabetic OVE26 mice

Healthy nondiabetic littermate control, OVE26;WT, and OVE26;Pod-SIRT1^{OV} mice were given Dox-supplemented chow starting at 16 weeks of age for 6 weeks as outlined in Figure 1a. Blood glucose and urinary albumin levels were monitored starting at 8 weeks until 22 weeks of age when they were killed. OVE26 mice displayed pronounced hyperglycemia at 8 weeks of age that was sustained throughout the duration of the study (Figure 1b). Dox-induced SIRT1 overexpression in OVE26;Pod-SIRT1^{OV} mice did not affect blood glucose levels or overall body weight in comparison to OVE26;WT mice (Figure 1b and c). However, kidney-to-body weight ratio, which was found to be significantly increased in OVE26;WT mice compared with healthy controls, were suppressed in OVE26;Pod-SIRT1^{OV} mice (Figure 1d). Weekly spot collection of urine showed apparent albuminuria in both OVE26;WT and OVE26;Pod-SIRT1^{OV} mice at 8 weeks of age, which had further escalated by 22 weeks of age in OVE26;WT but was significantly curtailed in OVE26;Pod-SIRT1^{OV} mice (Figure 1e). The reduction in albuminuria by SIRT1 overexpression was further confirmed by 24-hour urine excretion at 22 weeks of age, which showed a marked reduction in OVE26;Pod-SIRT1^{OV} mice as compared with OVE26;WT (Figure 1f).

Histologically, although both diabetic groups displayed glomerular hypertrophy and mesangial matrix expansion in comparison with nondiabetic controls, they were both significantly attenuated in OVE26;Pod-SIRT1^{OV} compared with OVE26;WT (Figure 2a–c). Consistently, electron microscopic analysis showed significant reduction in podocyte foot process effacement and glomerular basement membrane (GBM) thickening in OVE26;Pod-SIRT1^{OV} mice compared with OVE26;WT mice (Figure 2d). Together, our data suggest that the overexpression of SIRT1 in podocytes in diabetic kidneys mitigates diabetes-induced podocyte injury and effectively blunts the progression of DKD in OVE26 mice.

BF175 is a new potent agonist of SIRT1

Given that podocyte-specific overexpression of SIRT1 for 6 weeks in OVE26 mice with established DKD was sufficient to