Epigenetic regulation of the thioredoxin-interacting protein (TXNIP) gene by hyperglycemia in kidney

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Diabetic kidney disease is the leading cause of end-stage renal disease. Genetic factors have been suggested to contribute to its susceptibility. However, results from genetic studies are disappointing possibly because the role of glucose in diabetic kidney disease predisposed by epigenetic mechanisms has not been taken into account. Since thioredoxin-interacting protein (TXNIP) has been shown to play an important role in the pathogenesis of diabetic kidney disease, we tested whether glucose could induce expression of *TXNIP* in the kidney by epigenetic mechanisms. In kidneys from diabetic Sur1-E1506K$^{+/+}$ mice, hyperglycemia-induced *Txnip* expression was associated with stimulation of activating histone marks H3K9ac, H3K4me3, and H3K4me1, as well as decrease in the repressive histone mark H3K27me3 at the promoter region of the gene. Glucose also coordinated changes in histone marks and *TXNIP* gene expression in mouse SV40 MES13 mesangial cells and the normal human mesangial cell line NHMC. The involvement of histone acetylation in glucose-stimulated *TXNIP* expression was confirmed by reversing or enhancing acetylation using the histone acetyltransferase p300 inhibitor C646 or the histone deacetylase inhibitor trichostatin A. Thus, glucose is a potent inducer of histone modifications, which could drive expression of proinflammatory genes and thereby predispose to diabetic kidney disease.

Kidney International (2016) **89,** 342–353; http://dx.doi.org/10.1016/j.kint.2015.12.018

KEYWORDS: diabetic kidney disease; histone acetyltransferase (HAT); histone deacetylase (HDAC); histone modification; hyperglycemia; thioredoxin-interacting protein (TXNIP)

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Received 28 January 2015; revised 10 October 2015; accepted 15 October 2015

Diabetic kidney disease (DKD) is one of the most devastating diabetic complications, affecting up to 30% of patients, and is the leading cause of renal failure worldwide.[1] While clustering in families implicates a genetic component for DKD,[2] it has been surprisingly difficult in genome-wide association studies to identify genetic variants contributing to DKD.[3] One possible reason for these pitfalls could be that genome-wide association studies have not taken into account the role of elevated glucose in the pathogenesis of DKD via, for instance, epigenetic mechanisms.[4–8] Michael Brownlee coined the term "metabolic memory" to describe the long-term effects of initial good or poor metabolic control for protection of or susceptibility to diabetic complications.[9–12] He also demonstrated that glucose could induce specific histone modifications in endothelial cells and thereby expression of nuclear factor-κB could be memorized by the cells up to 6 days.[13] These epigenetic changes induced by short-term changes in glycemia could thereby provide an explanation for the "metabolic memory."

Epigenetic regulation of gene expression is mediated by posttranslational modifications of the chromatin structure, including acetylation, methylation, phosphorylation, and ubiquitylation of the histone tails.[14] These modifications result in relaxation of chromatin and initiation of transcription of the gene.[15] Among such modifications, histone lysine acetylation was first correlated with gene activity,[16] which is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs add acetyl groups to the conserved lysine amino acids, and this process can be reversed by removal of acetyl groups by HDACs. A dynamic balance is maintained between the activity of HATs and HDACs, and gene transcription can be activated or repressed by hyperacetylation or hypoacetylation of the lysine residues.[17,18]

The mechanism by which high glucose increases expression of proinflammatory genes in kidney and thereby development of DKD is poorly understood but could involve epigenetic mechanisms similar to those described for nuclear factor-κB in blood vessels.[13] To address these questions, we investigated the effect of glucose on expression and histone modifications of a proinflammatory gene, thioredoxin-interacting protein

(TXNIP), which has been suggested to play a role in streptozotocin-induced diabetes nephropathy in mice.[19] Induced by glucose in most tissues including kidney, TXNIP is an endogenous inhibitor of the antioxidant protein thioredoxin.[20–22] Knockdown of TXNIP has been shown to limit glucose-induced ^{3}H-proline incorporation (a marker of collagen production) and oxidative stress in mesangial and proximal tubular kidney cells.[23] Here we demonstrated that *Txnip* gene expression was upregulated in kidneys from a diabetic mouse model, as well as mesangial cells exposed to high glucose, changes of which were associated with specific histone modifications. Furthermore, glucose-induced histone acetylation changes in mesangial cells could be reversed by a HAT inhibitor, which in turn prevented glucose-induced overexpression of the *TXNIP* gene. We hereby provide insights into the epigenetic mechanisms by which elevated glucose can induce gene expression in kidney and thereby potentially predispose to DKD. Importantly, our novel finding that glucose-induced gene expression of deleterious effect can be reversed by targeting HAT suggests a new therapeutic approach for improving DKD conditions.

RESULTS

The mouse model Sur1-E1506K$^{+/+}$ develops diabetes and early signs of DKD

The Sur1-E1506K$^{+/+}$ mouse, which has a knock-in mutation equivalent to the human mutation (E1506K) in the SUR1 (*ABCC8*) gene, causes a reduction, but not complete loss, of K_{ATP} channel activity.[24] The mouse model was developed to recapitulate the human mutation, which is associated with neonatal hyperinsulinism and hypoglycemia, while patients later in life develop diabetes due to reduced functional β-cell mass.[25] We compared homozygous Sur1-E1506K$^{+/+}$ mice with wild-type littermates; the former showed hyperglycemia already at 8 weeks of age, which persisted after 16, 24, and 32 weeks (Figure 1a). Glucose tolerance was assessed by i.p. glucose tolerance test in mice fasted for 6 hours. Significant differences in plasma glucose during i.p. glucose tolerance test were observed between homozygous Sur1-E1506K$^{+/+}$ mice and wild-type littermates at 15 minutes after the glucose bolus at 8, 24, and 32 weeks (Supplementary Figure S1 online). It has been shown that hyperglycemia is predominantly caused by decreased insulin secretion and functional β-cell mass in the Sur1-E1506K$^{+/+}$ mice compared to wild-type littermates;[24] this was confirmed in the current study. The Sur1-E1506K$^{+/+}$ is thus a diabetic mouse model associated with both decreased insulin and elevated glucose concentrations, making it a good *in vivo* model to determine whether hyperglycemia increases expression of the *Txnip* gene via histone modifications.

To obtain insight into the pathology of the kidneys of Sur1-E1506K$^{+/+}$ mice, we performed Alcian blue/periodic acid–Schiff (ABPAS) staining, which showed a gradual increase of mesangial matrix and mesangial cells in kidneys at 8, 16, 24, and 32 weeks. Histologic differences were well established between diabetic and control tissue at 32 weeks (Figure 1b). However, there was no sign of nodular sclerosis or Kimmelstiel–Wilson lesions, suggesting that the glomerular changes observed reflected early stages of DKD that had not yet reached the sclerotic phase. The degree of tubular atrophy was negligible, and there were no signs of arteriosclerosis or vascular hyalinosis. Neither was there any sign of tubulointerstitial nephritis. Quantification of mesangial expansion, measured as percentage of the glomerular ABPAS-positive mesangial matrix, confirmed a robust increase in mesangial matrix in mutant kidney (Figure 1c). Kidney function was assessed by measuring albumin-to-creatinine ratio in a 2- to 3-hour urine sample collection in both mutant and wild-type mice ranging from 14 to 86 weeks of age. No differences in albumin-to-creatinine ratio were detected between genotypes (Figure 1d). Altogether the data indicate that Sur1-E1506K$^{+/+}$ mice are characterized by signs of early DKD.[26]

Txnip mRNA expression increased by time in kidneys of diabetic Sur1-E1506K$^{+/+}$ mouse

We next tested whether *Txnip* gene expression is altered in kidneys of Sur1-E1506K$^{+/+}$ mutant and wild-type littermates. *Txnip* mRNA expression increased progressively at 8, 16, 24, and 32 weeks in the Sur1-E1506K$^{+/+}$ mutant compared to wild-type littermates. In the wild-type mice, there was a slight age-related increase in *Txnip* expression from 24 weeks onward. In the mutant mice, increased *Txnip* expression was already observed at 16 weeks (Figure 2a). These observations suggest that *Txnip* expression in the Sur1-E1506K$^{+/+}$ mouse kidney was increased by age, which was further accelerated by hyperglycemia.

Effect of age and hyperglycemia on activating and inactivating histone marks in diabetic Sur1-E1506K$^{+/+}$ mouse kidney

We then determined the involvement of histone modifications in increased *Txnip* gene expression using chromatin immunoprecipitation (ChIP) with antibodies binding to various histone modification loci. Precipitated chromatin DNA was quantified using primers flanking the promoter region of the *Txnip* gene (Figure 2b). In line with the age-related upregulation of *Txnip* gene expression, we observed a modest but significant age-dependent increase in histone marks H3K9ac (Figure 2c), H3K4me1 (Figure 2d), and H3K4me3 (Figure 2e) and a decrease in H3K27me3 (Figure 2f) from week 24 onward in both mutant diabetic Sur1-E1506K$^{+/+}$ mice and wild-type littermates. Furthermore, increased H3K9ac, H3K4me1, and HeK4me3 (Figure 2c–e) and decreased H3K27me3 (Figure 2f) were much more pronounced in the mutant mice already at 16 weeks. These data suggest that age- and glucose-related increases in *Txnip* expression are temporally associated with changes in activating and inactivating histone marks that could contribute to the increased *Txnip* expression. Furthermore, we observed strong positive correlations between *Txnip* expression and levels of H3K9ac (Figure 3a), H3K4me1 (Figure 3b), and H3K4me3 (Figure 3c) and negative correlation with H3K27me3 (Figure 3d).