Role of the USF1 transcription factor in diabetic kidney disease

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Sanchez AP, Zhao J, You Y, Declèves A-E, Diamond-Stanic M, Sharma K. Role of the USF1 transcription factor in diabetic kidney disease. Am J Physiol Renal Physiol 301: F271–F279, 2011. First published May 4, 2011; doi:10.1152/ajprenal.00221.2011.—The predominant transcription factors regulating key genes in diabetic kidney disease have not been established. The transcription factor upstream stimulatory factor 1 (USF1) is an important regulator of glucose-mediated transforming growth factor (TGF)-β1 expression in mesangial cells; however, its role in the development of diabetic kidney disease has not been evaluated. In the present study, wild-type (WT; USF1+/+), heterozygous (USF1+/-), and homozygous (USF1-/-) knockout mice were intercrossed with Akita mice (Ins2/Akita) to induce type 1 diabetes. Mice were studied up to 36 wk of age. The degree of hyperglycemia and kidney hypertrophy were similar in all groups of diabetic mice; however, the USF1-/- diabetic mice had significantly less albuminuria and mesangial matrix expansion than the WT diabetic mice. TGF-β1 and renin gene expression and protein were substantially increased in the WT diabetic mice but not in USF1-/- diabetic mice. The underlying pathway by which USF1 is regulated by high glucose was investigated in mesangial cell culture. High glucose inhibited AMP-activated protein kinase (AMPK) activity and increased USF1 nuclear translocation. Activation of AMPK with AICAR stimulated AMPK activity and reduced nuclear accumulation of USF1. We thus conclude that USF1 is a critical transcription factor regulating diabetic kidney disease and plays a critical role in albuminuria, mesangial matrix accumulation, and TGF-β1 and renin stimulation in diabetic kidney disease. AMPK activity may play a key role in high glucose-induced regulation of USF1.

TGF-β1; renin; diabetic nephropathy; Akita; AMPK

THE DEVELOPMENT OF DIABETIC kidney disease is thought to be a complex interaction between metabolic and hemodynamic factors that depend on genetic determinants with inciting environmental cues (31). The hallmarks of diabetic nephropathy are albuminuria and extracellular matrix accumulation within the glomeruli of the kidney. Two of the most prominent pathways considered to drive diabetic kidney disease are the renin-angiotensin system (RAS) and the transforming growth factor-β1 (TGF-β1) system. The RAS is considered to play a major role in the hemodynamic alterations associated with diabetic kidney disease as well as regulating albuminuria, possibly via affecting podocyte function. TGF-β is considered to be the primary driver of both mesangial matrix accumulation and progressive tubulointerstitial fibrosis. Although both the RAS and TGF-β pathways have been consistently found to be upregulated in experimental models of diabetes and in the human condition, the specific transcription factors stimulating these pathways in diabetic kidney disease have not been clarified. Several transcription factors have been implicated in regulating renin and TGF-β1 gene transcription, including CREB, AP-1, Sp1, NF-κB, and the family of upstream stimulatory factors (USF1 and USF2) (22). Of these, there is an accumulating body of evidence supporting the roles of USF1 and USF2 (18, 22).

USF1 and USF2 are ubiquitously expressed transcription factors that bind the glucose-responsive element, an E-box motif (CANNTG or CACGTG), in the promoter region of the TGF-β1 gene (22, 38). USF1 and USF2 have also been shown to be involved in transcriptional regulation of thrombospondin 1 (THBS1), and osteopontin (SPP1) in response to high glucose, and binding sites have been reported in the promoters for plasminogen activator inhibitor 1 (SERpineI) and renin (REN) (2, 12, 18, 32, 34, 38). USF1 is a basic helix-loop-helix leucine zipper transcription factor that binds the promoter region as a USF1 homodimer, or as a USF1/USF2 heterodimer (1, 6). USF2 appears to only bind to promoter elements as a heterodimer (3). USF1 and USF2 have been linked to a variety of glucose-regulated genes in mesangial cells, hepatocytes, epithelial cells, and smooth muscle cells, and the relative amounts of each protein vary among cell types (29, 30, 38). In streptozotocin-induced diabetic rats, only USF1, and not USF2, is increased in glomeruli after 2 wk of diabetes (38). The USF1-/- mice are phenotypically normal (29, 30) but have not been previously studied with diabetes. In response to transient hyperglycemia (via a starvation-high carbohydrate refeeding protocol), WT and USF2-/- mice exhibit increased renal TGF-β1; however, USF1-/- mice do not (38). Overexpression of USF1 in transgenic mice influences metabolic traits such as obesity, lipid profiles, and glucose/insulin ratio (36), and USF1 SNPs have been identified in human linkage analysis with diabetic nephropathy (5). The USF2-/- mice have a short life-span and thus cannot be studied with chronic diabetes. Overexpression of USF2 in transgenic mice leads to enhanced albuminuria in streptozotocin-induced diabetic mice by 6 mo of age (14) and is accompanied by enhanced thrombospondin, TGF-β1, and renin gene expression.

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(26). However, the role of USF1 has not been demonstrated in chronic diabetic kidney disease. In the present study, we developed a USF1−/− mouse in the B6 background and studied the effect of type 1 diabetes, using the Akita/Ins2 mouse, to investigate the role of USF1 on development and progression of diabetic kidney disease. We hypothesized that diabetic mice deficient in USF1 would be protected from TGF-β-induced extracellular matrix accumulation and that USF1 may also regulate the RAS.

MATERIALS AND METHODS

Animal studies. USF1−/− mice were originally obtained from Dr. Michele Sawadogo at the University of Texas MD Anderson Cancer Center (27). The USF1−/− mice were backcrossed at the University of California San Diego onto a C57Bl/6 (Jackson Labs) background for more than seven generations. Female USF1+/− and USF1−/− mice were then intercrossed with male Akita mice (Ins2/Akita, Jackson Labs), and male offspring carrying the Akita mutation and heterozygous for the USF1 allele, were then crossed again with USF1+/− mice carrying the Akita mutation. Mice were weaned, weighed, and their tails were clipped for genotyping at 4 wk of age. All male nondiabetic and diabetic offspring with the following USF1 genotypes were included in the study: wild type (+/+), +/−, and −/−. At weaning (4 wk), mice were weighed and a random, nonfasting blood glucose (Bayer glucometer) was obtained from tail vein sampling. Mice were deemed diabetic if random blood glucose was >300 mg/dl. At 6 and 20 wk of age, mice were weighed, blood was drawn via saphenous vein collection, and they were placed individually in Nalgene metabolic cages for 24-h urine collection. After each collection, urine was centrifuged at 3,500 g for 10 min to remove particulate matter. Twenty-four-hour urine collections were analyzed by ELISA for albumin using a mouse Albewell kit (Exocell, Philadelphia, PA). All assays were performed in triplicate. Mice were euthanized between 28 and 36 wk of age under isoflurane anesthesia. Kidneys were isolated, harvested, blotted, and weighed. Kidneys were sectioned into quarters, and the renal cortex was isolated and frozen for mRNA and protein studies, and sagittal sections were preserved in OCT for immunostaining and 4% paraformaldehyde for periodic acid-Schiff (PAS) staining. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of California San Diego.

Glomerular histology. To obtain light microscopic histology, the left kidney was fixed in buffered formalin and then embedded in paraffin. The fixed, embedded kidneys were cut into 3-μm sections and stained with PAS reagent. All slides were coded, and tissue evaluation was performed in a blinded manner. Twenty-five randomly selected glomeruli in the outer cortex of each kidney section were evaluated. Morphometry was performed by point-counting techniques (20). Briefly, the microscopic image (×50 magnification) of the kidney was overlaid with grids. The intersections of grids were counted and grouped as follows: capillary lumen, PAS-positive area, or nucleus. The number of all the grid intersections was calculated for glomerular size.

RNA isolation and quantitative real-time PCR analysis. Total RNA was isolated from the kidney cortex using TRizol reagent as previously described (38). Real-time PCR was performed as previously described (38). The primers for USF1, USF2, TGF-β1, osteopontin, αS(I) collagen, renin, angiotensinogen, nephrin, podocin, and synaptopodin have been previously described (10, 17, 28, 38) and are available upon request. To control for variations in cDNA, the levels of gene expression were normalized to 18S.

Immunohistochemistry. Kidney tissue was flash-frozen in liquid nitrogen after cortical tissue was placed in cassettes with OCT. Immunostaining of frozen mouse kidney sections was performed as described previously (16). OCT-embedded frozen kidneys were cut at 4-μm thickness and fixed in cold acetone for 3 min. Frozen sections were mounted on glass slides, fixed with paraformaldehyde, horse serum buffer, and primary antibody (podocin, TGF-β1/2/3, Santa Cruz Biotechnology; renin antibody, dilution 1:60, Anaspec). 4,6-Diamidino-2-phenylindole was used for nuclear staining. Images were obtained using a Zeiss confocal microscope at ×63.

Cell culture studies. A murine mesangial cell line was used in cell culture studies as previously described (35). Murine mesangial cells were maintained at 37°C in a humidified incubator with 5% CO2-95% air and propagated in DMEM (GIBCO BRL, Gaithersburg, MD) containing 10 mM d-glucose, 10% FCS, 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM supplemental glutamine. After near confluence, cells were rested in serum-free media overnight and then modulated with d-glucose at a concentration of 5.5 or 25 mM for 24 h. Mannitol was used as an osmotic control for 25 mM d-glucose. Cells were treated with 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR, Toronto Chemical) at 1 mM 30 min before glucose modulation. Total cell protein, cytosolic, and nuclear fractions of mesangial cells were isolated as previously described (21, 38).

Immunoblotting. The proteins extracted from total kidney were run on 4–12% SDS-PAGE gels, and separated proteins were blotted to nitrocellulose membranes. Immunoblotting was performed as described previously. Renin was detected with a polyclonal rabbit antibody (dilution 1:400, Anaspec). For verifying equal loading, an antibody to β-actin was used.

Mesangial cells were cultured as described above. Total protein from cells was solubilized in lysis buffer containing 1% Triton X-100, Protease Inhibitor Cocktail (Mini Complete Protease Inhibitor Cocktail, Roche, Germany), PMSF, and phosphatase inhibitors. Protein was resolved on SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA) as previously described (24). After the membrane was blocked by incubating with a blocking solution (PBST) for 1 h at room temperature, the membrane was hybridized in blocking buffer overnight at 4°C with a primary antibody. Primary antibodies included rabbit USF1 polyclonal antibody (Santa Cruz Biotechnology), p-AMP-activated protein kinase (AMPK)-α, polyclonal antibody, and rabbit anti-AMPK-α monoclonal antibody (Cell Signaling Technology). The filter was then washed four times with PBST and incubated with secondary antibody diluted 1:1,000 for 60 min at room temperature. Specific signals were detected by the enhanced chemiluminescence method. For verifying equal loading, an antibody to AMPK-α was used for the cytosol fraction and to histone for the nuclear fraction.

Statistical analyses. Data are presented as arithmetic means ± SE. A P value <0.05 was considered significant. Differences between data groups were evaluated for significance using an independent t-test of data or one-way ANOVA and Neuman-Keuls post hoc tests (GraphPad Prism Ver. 5, GraphPad Software, La Jolla, CA).

RESULTS

Hyperglycemia and diabetic kidney hypertrophy are not affected by USF1 allele status. The degree of hyperglycemia in the diabetic mice and the overall body weights were similar in all the diabetic groups (Table 1) at 6 and 36 wk of age. There was a tendency for the USF1−/− diabetic mice to gain less body weight than the WT or heterozygous mice, but this did not attain statistical significance. At the end of the study, kidney weights were similarly increased in all the diabetic groups (Table 1) at 6 and 36 wk of age. There was no difference between any of the USF1 genotypes at any of the measured time points. However, Akita mice and Akita mice heterozygous for the
USF1 allele had a significantly increased 24-h UAE at 6 wk of age (after ~2 wk of diabetes) and at 20 wk of age (~4 mo of diabetes). Diabetic USF1 −/− mice had a stable level of UAE throughout the course of diabetes, and it was not significantly elevated from nondiabetic WT mice at any of the time points (Fig. 1A).

Mesangial matrix accumulation and glomerular area were increased in the WT Akita diabetic mice. However, in the diabetic USF1 −/− mice, there was a significant reduction in both glomerular matrix and glomerular volume compared with WT diabetic mice (Fig. 1, B–D).

USF1 and USF2 mRNA expression in Akita mice. Expression of USF1 and USF2 genes was analyzed in all groups by real-time PCR. USF1 and USF2 mRNA were elevated in the kidney cortex of WT Akita mice (Fig. 2, A and B). The USF1 −/− mice did not have a compensatory increase in USF2 under nondiabetic conditions and had a modest but not significant increase in USF2 with diabetes. The heterozygous diabetic USF1 +/− mice had no increase in USF2 with diabetes but did have increased USF2 mRNA with diabetes. These results suggest that USF1 may play a regulatory role on USF2 gene expression under conditions of diabetes.

TGF-β1 is elevated in WT diabetic mice, but not in diabetic USF1 −/− mice. TGF-β1 mRNA expression was evaluated in all groups by real-time PCR. As there was no significant difference in gene expression of TGF-β1 among the three genotypes in nondiabetic conditions, these three groups were combined for ease of presentation and categorized as nondiabetic. With diabetes there was a significant increase in the kidney cortex of WT Akita diabetic mice, but not in the diabetic USF1 +/− or −/− mice (Fig. 2C). These data demonstrated that USF1 stimulation is necessary for diabetes-induced renal TGF-β1 gene upregulation. Immunostaining of kidneys demonstrated increased TGF-β staining in tubules and glomeruli in the diabetic mice, whereas it was reduced in diabetic USF1 −/− mice (Fig. 3, A and B).

Genes that contribute to matrix accumulation in diabetes and are potentially downstream of TGF-β were also evaluated. Osteopontin and type I collagen (α1(I)) were found to be increased in the diabetic WT mice but not in USF1 −/− diabetic mice (Fig. 4, A and B).

Diabetic USF1 −/− mice are protected from diabetes-induced stimulation of the RAS. Based on prior studies indicating that both renin and angiotensinogen promoter elements bind to USFs, the regulation of both renin and angiotensinogen was evaluated in WT and −/− groups. There was a marked 15-fold stimulation of angiotensinogen in the diabetic kidneys, which was significantly attenuated in the USF1 −/− diabetic mice (Fig. 5A). Renin gene expression was significantly increased in the diabetic WT mice but completely prevented in the USF1 −/− diabetic mice (Fig. 5B). Protein levels for renin were also evaluated by immunoblotting and immunostaining. Overall renal renin was increased with diabetes in WT but not in USF1 −/− mice (Fig. 5, C and D). Renin distribution in the juxtaglomerular apparatus was increased in the WT Akita diabetic mice but not in the USF1 −/− diabetic mice (Fig. 6, A and B). In addition, there was a marked upregulation of renin in the medullary tubular cells which was also not seen in USF1 −/− diabetic mice. (Fig. 6, C and D).

Nephrin is decreased in Akita mice, but not in diabetic USF1 knockout mice. As USF1 −/− diabetic mice had no increase in urine albumin during the course of diabetes, gene expression of podocyte-specific proteins was evaluated in WT and −/− groups. Nephrin was found to be significantly reduced with diabetes in WT but not USF1 −/− mice (Fig. 7A). Podocin and synaptopodin mRNA were not significantly altered with diabetes in WT or USF1 −/− mice (Fig. 7, B and C).

USF1 nuclear translocation is regulated by AMPK. To examine the underlying signaling pathway that may be important in regulating diabetes-induced stimulation of USF1, mesangial cells were examined in normal and high glucose. Similar to prior studies in murine podocytes, murine mesangial cells had a reduction in AMPK activity in response to high glucose (Fig. 8A) (there was no effect of the mannitol control, data not shown). USF1 was present in both the cytosolic and nuclear fractions of mesangial cells under normal glucose conditions; however, in high glucose USF1 was reduced in the cytosolic fraction and increased in the nuclear fraction (Fig. 8, B and C). Stimulation of AMPK with 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside was able to prevent the nuclear accumulation of USF1 with high glucose (Fig. 8, C and D).

**DISCUSSION**

In the present study, we demonstrate that USF1-deficient mice are protected against the major cardinal features of diabetic kidney disease, including albuminuria and mesangial matrix accumulation. The major mediators driving albuminuria and matrix accumulation include the RAS and the TGF-β system, and both systems are almost completely suppressed at the gene and protein levels in the USF1 −/− diabetic mice, thus demonstrating a dominant role for USF1. Our studies also demonstrate that a key pathway that mediates USF1 nuclear translocation with high glucose is the AMPK pathway.
The regulation of TGF-β1 promoter activity by high glucose in cell culture has been convincingly demonstrated to involve the USF family of transcription factors (7, 32, 38). This study is the first demonstration that USF1 is necessary for TGF-β1 gene stimulation in chronic diabetic kidney disease. Whether USF1 is sufficient by itself to stimulate TGF-β1 in the diabetic kidney remains unknown, although it is likely that there is contribution from other transcription factors, including USF2. USF2 appears to be a major driver of thrombospondin stimulation in diabetic kidney disease (14) and thrombospondin may enhance TGF-β activation and augment stimulation of TGF-β1 gene expression via a positive feedback loop (32). The lack of TGF-β1 gene stimulation in the USF1 −/− diabetic kidney likely contributes to the reduction of matrix gene expression of
Type 1 collagen and osteopontin and overall reduction of glomerular matrix accumulation.

Stimulation of the RAS is a hallmark of diabetic kidney disease, and clinical studies have clearly demonstrated the benefits of inhibiting the RAS in diabetic nephropathy. Our study supports the accumulating evidence (13, 26, 37) that the key genes renin and angiotensinogen are likely the initial drivers of stimulating RAS activity in diabetic kidney disease. There was a >10-fold induction of angiotensinogen and a >4-fold induction of renin with diabetes. Both genes were close to control levels in the diabetic USF1 −/− mice. These data are similar to what was found in the USF2 transgenic mouse as there was increased renin gene stimulation (26). It is therefore likely that USF1 and USF2 both contribute to stimulation of renin and may well act synergistically. Of note, the USF2 transgenic mouse did not upregulate angiotensinogen whereas the USF1 −/− diabetic kidney had a marked inhibition of angiotensinogen, suggesting that some genes may have a preferential response only to USF1. A separate study with liver angiotensinogen also found preferential binding of USF1 to the angiotensinogen promoter although both USF1 and USF2 are able to modulate the promoter activity (4). As USF1 SNPs have been linked to diabetic nephropathy (5), it will be of interest to determine whether the response to RAS blockade may be linked to USF activity.

Fig. 2. USF1, USF2, and transforming growth factor (TGF)-β1 gene expression. A: USF1 mRNA were analyzed by real-time PCR from kidneys of WT and heterozygous mice. There is a significant increase in USF1 in the Akita diabetic WT group [A/a (+/−)] vs. nondiabetic groups. There was a significant reduction of USF1 mRNA in the diabetic heterozygous mouse [A/a (+/−)] vs. WT diabetic mice. B: USF2 mRNA was measured in all groups. USF2 was significantly increased in the WT and heterozygous diabetic groups but not in the homozygous USF1 −/− diabetic group. C: TGF-β1 mRNA expression in the kidney cortex was analyzed by real-time PCR from kidneys of all the nondiabetic groups and in the individual diabetic groups. TGF-β1 mRNA was only increased in the WT diabetic kidneys but not in the heterozygous or homozygous diabetic kidneys. Values are standardized by 18S and expressed as means ± SE from 6–8 mice/group. *P < 0.05 vs. (+/+) or corresponding nondiabetic group. **P < 0.05 vs. A(+/+).

Fig. 3. Immunofluorescence labeling of TGF-β in mouse kidneys. A: immunostaining of frozen kidney tissue was performed with anti-TGF-β antibodies, anti-podocin, and 4,6-diamidino-2-phenylindole (DAPI). Green = TGF-β; red = podocin; blue = DAPI nuclear stain. There is a marked stimulation of glomerular TGF-β in the A(+/+) diabetic kidneys but little increase in the USF1 −/− [A(−/−)] diabetic group. B: integrated densitometric values of TGF-β-positive staining demonstrates a significant increase only in the A(+/+) kidney cortex. The A(−/−) group had a significant reduction of cortical TGF-β compared with the A(+/+) group. Values are means ± SE. *P < 0.05 vs. (+/+), **P < 0.05 vs. A(+/+).

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Stimulation of the RAS is a hallmark of diabetic kidney disease, and clinical studies have clearly demonstrated the benefits of inhibiting the RAS in diabetic nephropathy. Our
The USF1−/− diabetic mouse not only had a reduced degree of mesangial matrix accumulation but also had a surprising protection against albuminuria in the early and sustained phases of diabetes. This result suggests that USF1 may be closely involved in regulating podocyte function and structure. Indeed, the nephrin promoter has been found to contain an E-box (15), and in our studies nephrin was significantly reduced only in the WT diabetic mice and not in the USF1−/− diabetic mice. Additional studies linking USFs to the nephrin promoter will be of major interest. Apart from nephrin, other podocyte-specific genes (podocin, synaptopodin) were not suppressed in the WT diabetic kidney.

The relative roles of USF1 and USF2 were examined by gene expression in the present studies. In the Akita WT diabetic kidney, there was a significant upregulation of both USF1 and USF2 gene expression. Interestingly, with USF1 deficiency (+/−, −/−) there is a reduction of USF2 stimulation in the diabetic kidney. These data suggest that USF1 is a positive stimulator of USF2 under the diabetic condition and in line with prior data demonstrating an E-box in the USF2 promoter that binds to USF1 (3). Several studies have found that an increase in USF1 stimulates USF2 as well (3, 11); however, upregulation of USF2 does not consistently increase

Fig. 4. Osteopontin (OPN) and type I collagen gene expression. There was a significant increase in renal osteopontin (A) and α(1) collagen (B) gene expression in the diabetic WT group but not in the USF1−/− diabetic group. The USF1 (+/−) diabetic group had intermediate values between A(+/+) and A(−/−). Values are standardized by 18S and expressed as means ± SE from 6–8 mice/group. *P < 0.05 vs. nondiabetic group.

Fig. 5. Renin-angiotensin system in diabetic WT and USF1−/− kidneys. A and B: angiotensinogen (AGT) and renin mRNA expression was analyzed by real-time PCR from kidneys of nondiabetic and diabetic WT and USF1−/− mice. Values are standardized by 18S and expressed as means ± SE from 6–8 mice/group. *P < 0.05 vs. nondiabetic. **P < 0.05 vs. A(+/+). C and D: Western blot analysis of renin and β-actin in the kidney cortex from each of the groups (C) and quantitative data from 3 separate experiments (D). Values are standardized by β-actin and expressed as means ± SE from 3 mice/group. **P < 0.01 vs. indicated group.
USF1. The regulation of USF1 at the gene level with diabetes remains largely unexplored, although of major importance. Interestingly, recent studies have identified that the E-box that closely regulates collagen and TGF-β1 production under diabetic conditions is regulated by specific microRNAs that function to reduce suppressors on the E-box (8, 9). Additional studies would be of interest to evaluate how microRNAs affect USF1/USF2 binding and activity of the specific E-boxes relevant to diabetic kidney disease. Previously, we found that elevation in glucose concentration, even in the high normal range, was sufficient to stimulate USF1 protein levels in the nucleus (38). In the present study we found that high glucose leads to reduced cytosolic levels of USF1, likely due to translocation to the nucleus.

The signaling pathways regulating USF1 under high-glucose conditions may involve the AMPK and the PKC pathways. Prior studies have identified a role for PKC in regulating both USF1 and USF2 in high-glucose conditions (32, 38). In the present report, we found that AMPK activity is reduced with high glucose and that AMPK activation blocks the nuclear

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Fig. 6. Renin distribution in WT and diabetic kidneys. A: representative photomicrographs of immunofluorescence labeling of renin (green) in the cortex of mouse kidneys. Antibody to podocin (red) was used to label glomeruli and identify juxtaglomerular structures. DAPI (blue) was used to label nuclei. The arrow shows positive specific juxtaglomerular (JG) renin immunofluorescence. B: there is an increase in JG renin staining in the WT diabetic [A(+/+)] kidneys but not in the USF1 A(+/−) JG area. C: representative photomicrograph of renin immunostaining in the medulla. D: there is an increase in renin immunoreactivity in the medulla of A(+/+) but not in USF1 −/− diabetic mice. Values are means ± SE. *P < 0.05 vs. nondiabetic groups. **P < 0.05 vs. A(+/+).
translocation of USF1 under high glucose conditions. High glucose inhibits AMPK activity, similar to what we have previously described in podocytes (23, 25). Thus reduced AMPK activity may be the initial energy-sensing pathway that mediates downstream effects on key transcription factors to regulate the mesangial cell response to high glucose. As the PKC pathway has been clearly linked to diabetic kidney disease, it will be of interest to demonstrate cross talk between the AMPK and the PKC pathways.

In summary, our studies demonstrate that USF1 is of major importance in mediating diabetes-induced gene stimulation of TGF-β1, renin, angiotensinogen, osteopontin, type I collagen, and nephrin. The lack of USF1 is able to protect the chronic diabetic kidney from accumulation of mesangial matrix and albuminuria. Furthermore, AMPK inhibition may be a critical early step in regulating USF1 activity in the diabetic milieu.
GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


