RESEARCH ARTICLE

Podocyte-specific knockin of PTEN protects kidney from hyperglycemia

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Hyperglycemia in vivo remains unknown. The inducible podocyte-specific knockin of PTEN protected the kidney against hyperglycemia in vivo and motility and the inhibition of apoptosis. Our results showed that hyperglycemia in vivo was partly attributed with an improvement in autophagy and the face ment of podocyte foot processes, and incrassation of glomerular basement membrane (GFB) leads to extracellular matrix protein deposition, mesangial matrix expansion, and glomerular basement membrane (GBM) thickening, consequently developing into irreversible glomerulosclerosis and renal dysfunction. Limited treatment has proven to be effective to prevent the initiation and progression of DKD, although multiple studies have focused on its molecular mechanism. A new and effective therapeutic strategy is needed urgently.

Podocyte-specific knockin of PTEN knocksin the presence of high-glucose (HG), and knockout of PTEN in podocytes aggravated the progression of diabetic kidney disease (DKD). However, whether podocyte-specific knockin of PTEN protects the kidney against hyperglycemia in vivo remains unknown. The inducible podocyte-specific PTEN knockin (PPKI) mice were generated by crossing newly created transgenic loxP-stop-loxP-PTEN mice with podocin-iCreERT2 mice. Diabetes mellitus was inducted in mice by intraperitoneal injection of streptozotocin at a dose of 150 mg/kg. In vitro, small interfering RNA and adenovirus interference were used to observe the role of PTEN in HG-treated podocytes. Our data demonstrated that HG-induced PTEN was markedly reduced in the podocytes of patients with DKD and focal segmental glomerulosclerosis, as well as in those of db/db mice. Interestingly, podocyte-specific knockin of PTEN significantly alleviated albuminuria, mesangial matrix expansion, effacement of podocyte foot processes, and incrassation of glomerular basement membrane in diabetic PPKI mice compared with wild-type diabetic mice, whereas no alteration was observed in the level of blood glucose. The potential renal protection of overexpressed PTEN in podocytes was partly attributed with an improvement in autophagy and motility and the inhibition of apoptosis. Our results showed that podocyte-specific knockin of PTEN protected the kidney against hyperglycemia in vivo, suggesting that targeting PTEN might be a novel and promising therapeutic strategy against DKD.

Welcoming evidence indicated that PTEN was downregulated in glomeruli from humans and animals with DKD (21). PTEN deficiency aggravated podocyte injury under hyperglycemia in multiple ways in vivo or in vitro, including podocyte cytoskeletal rearrangement, apoptosis, autophagy, and epithelial-mesenchymal transition (21, 35, 43). Podocyte-specific deletion of PTEN resulted in increased urinary albumin excretion, moderate glomerulosclerosis, and the development of DKD in the presence of a high-fat diet (21). Although the effects and underlying mechanisms of PTEN insufficiency on podocytes challenged with high glucose (HG) have been studied, the impact of overexpression of PTEN in podocytes on the kidney suffering from hyperglycemia in vivo remains unknown. In the present study, the expression of PTEN was observed in glomeruli from humans with glomerular diseases, as well as diabetic mice. The effect of overexpression of PTEN in podocytes on glomerular function and progression of DKD was examined in a newly generated podocyte-specific-inducible PTEN knockin (PPKI) mouse model in the presence of diabetic kidney disease; hyperglycemia; podocyte; PTEN

INTRODUCTION

Diabetic kidney disease (DKD), a common and severe complication of diabetes mellitus (DM), is believed to be the major cause of end-stage renal disease globally (38). It has been projected that 592 million individuals will be diagnosed with diabetes worldwide by 2035 (11). Up to 40% of patients with Type 2 diabetes mellitus develop DKD, contributing to increased morbidity and mortality, as well as the increased economic cost (1, 11). Recently, podocyte injury, including effacement of podocyte foot processes, detachment, and apoptosis, has proven to be a vital hallmark of triggering DKD under hyperglycemia, with the initial manifestation of albuminuria (31). The destruction of glomerular filtration barrier (GBF) leads to extracellular matrix protein deposition, mesangial matrix expansion, and glomerular basement membrane (GBM) thickening, consequently developing into irreversible glomerulosclerosis and renal dysfunction. Limited treatment has proven to be effective to prevent the initiation and progression of DKD, although multiple studies have focused on its molecular mechanism. A new and effective therapeutic strategy is needed urgently.

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a member of protein tyrosine phosphatase superfamily located in 10q23.3., is a dual-specificity lipid and protein phosphatase, degrading phosphatidylinositol (3,4,5)-triphosphate (PIP3) into PIP2 as an antagonist of phosphatidylinositol 3 kinase/protein kinase B (PI3K/Akt) pathway (17, 23). Since its identification, PTEN has been studied extensively in the field of oncology as a unique tumor suppressor with phosphatase activity (18). Recently, its role in cell growth, metabolism, architecture, and motility as an intramolecular and regulatory mediator has gained widespread research interest as applied to nontumorous diseases, such as myocardial infarction, hepatic fibrosis, endocrine diseases, and DKD (3, 4, 22, 44, 45). Accumulating evidence indicated that PTEN was downregulated in glomeruli from humans and animals with DKD (21). PTEN deficiency aggravated podocyte injury under hyperglycemia in multiple ways in vivo or in vitro, including podocyte cytoskeletal rearrangement, apoptosis, autophagy, and epithelial-mesenchymal transition (21, 35, 43). Podocyte-specific deletion of PTEN resulted in increased urinary albumin excretion, moderate glomerulosclerosis, and the development of DKD in the presence of a high-fat diet (21). Although the effects and underlying mechanisms of PTEN insufficiency on podocytes challenged with high glucose (HG) have been studied, the impact of overexpression of PTEN in podocytes on the kidney suffering from hyperglycemia in vivo remains unknown. In the present study, the expression of PTEN was observed in glomeruli from humans with glomerular diseases as well as diabetic mice. The effect of overexpression of PTEN in podocytes on glomerular function and progression of DKD was examined in a newly generated podocyte-specific-inducible PTEN knockin (PPKI) mouse model in the presence of diabetes. Finally, the potential mechanism underlying the protective role of PTEN in HG-treated podocytes in vitro was further explored.

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MATERIALS AND METHODS

Cell culture and siRNA adenovirus infection. Conditionally immortalized mouse podocytes were cultured as described in a previous study (42). Briefly, the podocytes were cultured in 10% RPMI 1640 (containing 1:10,000 mouse recombinant IFN-γ) at 33°C for proliferation. Podocytes were maintained in 5% DMEM on BD BioCoat collagen I plates (BD Biosciences) at 37°C without IFN-γ for 8 to 12 days to induce differentiation. PTEN small interfering RNA (siRNA) was transfected into differentiated podocytes after 24 h of serum starvation. Subsequently, HG (30 mM) or normal glucose (5 mM, served as control) was added to the transfected podocytes for 48 h.

Apoptosis assay. Cell apoptosis was determined using the Annexin V-FITC/PI apoptosis detection kit (Bestbio). In brief, the cells were incubated with 800 μl of binding buffer, 10 μl of Annexin V-FITC, and 20 μl of PI for 10 min in the dark. Apoptotic cells were quantified as the number of dual-positive cells in a 20 x 20 horizon (average for six views) using a fluorescence microscope.

Immunofluorescence analysis. The expression of PTEN in the kidney of humans or animals was detected using immunofluorescence, as described in a previous study (20). Concisely, frozen slides were incubated with anti-PTEN antibody (1:100; Abcam) and anti-synaptopodin antibody (1:100; Life Technologies) followed by corresponding Alexa Fluor secondary antibodies (1:200 dilution; Life Technologies) before imaging and quantifying using a confocal microscope (LSM710; Zeiss). All human studies were performed with the approval of the Ethics Committee of Guangdong General Hospital, and informed consent was obtained from the patients. All animal studies were performed with the approval of the animal care and use committee of Guangdong General Hospital.

Immunoblot and immunohistochemistry analysis. Association of fibrosis protein was assessed using Western blot analysis, as described previously (41). The antibodies antifibronectin (FN; 1:1,000), anti-collagen IV (Col IV; 1:2,000), anti-connective tissue growth factor (CTGF; 1:1,000), anti-PTEN (1:5,000), and antinephrin (1:1,000) were bought from Abcam. Anti-beclin-1 (1:1,000), anti-Bcl2 (1:1,000), and anti-LC3-B (1:1,000) were bought from Cell Signaling Technology. For immunohistochemistry, the sections were incubated with primary antibody to WT-1 (1:50; Santa Cruz Biotechnology) and then with horseradish peroxidase-labeled secondary antibody (Beyotime). Podocyte number was determined by WT-1-positive cells per glomerulus, counted by two renal pathologists in a blinded method.

Podocyte-specific PTEN knockin mice. Podocin-iCreERT2 mice (C57BL/6J background) were kindly provided by Professor Farhad R. Danesh (MD Anderson Cancer Research Center). A transcriptional stop cassette flanked by loxP recombination sites before the PTEN coding region was introduced to create transgenic loxP-stop-loxP-PTEN mice, which silence PTEN until the introduction of Cre recombinase. In addition, a neocassette driven by the podocyte-specific podocin promoter during timed exposure to tamoxifen was used to create transgenic mice (20). The PCR primers were as follows: Rosa-Pten: primer 1: 5'-AAA GTC GCT CTG AGT TAT-3', primer 2: 5'-GGC AAG AGT TTG TCC TCA ACC-3', and primer 3: 5'-GGC GCG GGA AAA ATG ATG ATG-3', yielding a 300-bp PCR product; iCre-forward: 5'-TCA ACA TGC TAC GGA GAT-3' and iCre-reverse: 5'-ACC ATA GAT CAG GGC GTG GGT-3', yielding a 500-bp PCR product. PCR reactions were carried out with an initial denaturation of 94°C for 4 min followed by 30 cycles of reaction (94°C, 30 s; 57°C, 30 s; 72°C, 1 min), with a final extension of 72°C for 10 min (Takara, RR001A). Then, 10–50 ng of genomic DNA was used as a template per reaction.

Diabetic model. Eight-week-old PPKI mice and their control littermates (sex ratio 1:1 with body weights ranging from 15 to 25 g) were intraperitoneally injected with tamoxifen at 2 mg/day for 10 days to induce the expression of PTEN. After a 2-wk break, all mice were randomly treated with either streptozotocin (STZ) or citric acid solution at a dose of 150 mg/kg, as previously reported (40–42). Diabetic mice, whose random blood glucose levels measured weekly after STZ injection were higher than 16.7 mmol/l for 2 wk without increased urinary albumin excretion, were enrolled in the following experiment. All experimental mice were fed a normal chow diet and housed in an environment with a 12:12-h light-dark cycle and 22°C room temperature.

Biochemical analysis. At the age of 36 wk, the mice were anesthetized with 2% pentobarbital sodium (40 mg/kg ip). Blood and kidney samples were harvested for further analysis. Urinary albumin (mouse albumin ELISA kit; Bethyl Laboratories), urine creatinine (creatinine urinary colorimetric assay kit; Cayman Chemicals), serum creatinine (creatinine serum colorimetric assay kit; Cayman Chemicals), serum cholesterol (cholesterol fluorometric assay kit; Cayman Chemicals), and serum triglyceride levels (triglyceride colorimetric assay kit; Cayman Chemicals) were determined according to the manufacturer’s protocols.

Periodic acid Schiff staining. Kidney samples were immersed in 4% paraformaldehyde and then embedded in paraffin for Periodic acid Schiff (PAS) staining, according to the manufacturer’s guideline (Sigma-Aldrich). Glomerular and mesangial matrix areas of glomerular cross sections from light microscopic views were quantified using Image-Pro Plus by two individuals blinded to group allocation. The mesangial matrix index was calculated as the ratio of mesangial matrix area to the glomerular area (% area) (41).

Oil Red O staining and lipid quantification. Frozen sections were stained with Oil Red O, as described previously (13). Total cholesterol (normalized by protein concentration) in the renal cortex was extracted and determined, according to the instruction on the commercial kit (E1015; Applygen).

Transmission electron microscopy. Ultrastructural observation for the podocyte foot process and GBM thickness was performed using a transmission electron microscope as before (42). Podocyte foot process effacement was quantified by foot process width. Briefly, the total number of podocyte foot processes along the GBM from each photograph was counted, divided by the total length of GBM measured in each picture, and multiplied by 1/4. Calculations were performed on six animals per group (39).

Statistical analyses. All data were presented as means ± SE. Quantitative comparisons of four groups of mice and cultured podocytes were performed by one-way ANOVA followed by Bonferroni multiple-comparison test, whereas the Western blot of db/db mice was analyzed by an independent samples t-test with Graph-Pad Prism 6.0 (GraphPad). A P value <0.05 was considered statistically significant.

RESULTS

Expression of PTEN in human glomerular diseases and mice DKD model. To investigate the role of PTEN in glomerular diseases, we first determined the expression of PTEN in the glomeruli of patients with diverse chronic kidney diseases and...
healthy controls. Our data demonstrated that PTEN was expressed predominantly in podocytes of healthy donor individuals. In contrast, PTEN was significantly decreased in patients with DKD and focal segmental glomerulosclerosis, compared with healthy controls (Fig. 1, A and B). Consistent with a previous study (33), our data indicated that PTEN contributed to the glomerular diseases evidenced by downregulated expression in podocytes.

Twenty four-week-old db/db mice with albuminuria and certain pathologic changes related to DKD were used to investigate the expression of PTEN in the kidney of diabetic mice, age-matched wild-type (db/m) mice as controls. Consistent with the finding in podocytes of humans with DKD, both immunofluorescence and Western blot analyses showed that PTEN was downregulated in podocytes in mice with DKD compared with control mice (Fig. 1, C–F). Interestingly, we found that PTEN was upregulated in heart and spleen but downregulated in liver and kidney in the presence of diabetes. These data suggested that the expression level of PTEN changed variably in every specific organ of db/db mice compared with db/m mice.

**Generation of podocyte-specific PTEN knockin mice.** The strategy for generating loxP-stop-loxP-PTEN mice by targeting Pten into Rosa 26 loci through homologous recombination was illustrated in Fig. 2A. The Rosa-Pten plasmid shown in Fig. 2B, was authenticated by restriction enzyme and sequencing shown in the electrophoresis map of digested plasmid (Fig. 2B). Sequentially, loxP-stop-loxP-PTEN mice were crossed with podocin-iCreERT2 mice to generate double-copy, Cre-activated, PPKI mice and PTEN lox/lox mice (referring to control mice). No significant abnormalities in appearance and size were found between the age-matched PPKI and control mice (Fig. 2C). After an intraperitoneal injection of tamoxifen, the mice were euthanized for authenticating the recombination of Pten and the expression of PTEN protein in the kidney. The PCR analysis showed that Rosa-pter (300 bp) was detected in

![Fig. 1. Expression of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) in podocytes of humans with glomerular diseases and db/db mice. A: representative PTEN-Synpo bifluorescence of human glomeruli. PTEN, red; Synpo, green; Merge, yellow. Scale bar: 50 μm. B: quantification of PTEN mean fluorescence intensity (MFI) in the glomerular area (each group from three human subjects). C: representative Western blot of PTEN in the kidney from db/m and db/db mice. D: densitometric analysis of PTEN in Western blot via three independent experiments (independent-sample t test). E: representative PTEN-Synpo bifluorescence of glomeruli from db/m and db/db mice. PTEN, red; Synpo, green; Merge, yellow. Scale bar: 10 μm. F: quantification of PTEN MFI in mice (each group from three mice). DKD, diabetic kidney disease; FSGS, focal segmental glomerulosclerosis; Synpo, synaptopodin.](https://www.ajprenal.org/content/doi/10.1152/ajprenal.00575.2017/fig1)
PPKI and control mice except for wild-type (WT) mice (Fig. 2D1); iCre (500 bp) was examined in PPKI mice but not in control and WT mice (Fig. 2D2). The Western blot analysis displayed that the expression of PTEN protein in kidney in PPKI mice was remarkably higher than that in control mice (Fig. 2E). No abnormalities in serology, morphology, and histology were observed in PPKI mice compared with their littermates. After identification, PPKI and control mice were grouped, and diabetes was established as shown in Fig. 3.

Podocyte-specific PTEN knockin ameliorated urinary albumin excretion in diabetic mice. The physical and biochemical parameters of all the mice were displayed in Fig. 4. No significant difference in body weight was observed between all of the groups during the experimental period (Fig. 4A). At 36 wk of age (26 wk after STZ administration), all diabetic mice exhibited a marked increase in blood glucose, serum cholesterol, serum triglyceride, and serum creatinine levels compared with nondiabetic controls. Interestingly, the serum creatinine level was significantly ameliorated in diabetic PPKI mice compared with diabetic controls (Fig. 4, C–F). Despite no difference in kidney weight/body weight (kidney index) between all of the groups (Fig. 4B), diabetic mice developed progressive albuminuria, and urinary albumin/creatinine ratio was significantly reduced at 28 and 36 wk in PPKI mice compared with control mice in the presence of diabetes (Fig. 4G). However, no significant difference in the blood glucose level was found between PPKI mice and their controls before or after STZ injection. These collective results revealed that the overexpression of PTEN in podocytes ameliorated renal function and destruction of GFB with the typical manifestation of albuminuria, which was independent of hyperglycemia.

Podocyte-specific PTEN knockin alleviated the progression of DKD. Double-staining immunofluorescence analysis showed that PTEN was obviously expressed in the podocytes of PPKI mice compared with control mice. Consistent with the findings in patients with DKD and db/db mice, PTEN in the podocytes markedly reduced in diabetic control mice compared with nondiabetic controls, confirming that PTEN was downregulated in response to hyperglycemia (Fig. 5, A and B).

Persistent hyperglycemia promotes the deposition of extracellular matrix protein in renal tissue and finally leads to glomeruli fibrosis and glomerulosclerosis (47). The present data showed that the expression of FN, Col IV, and CTGF in the renal cortex was markedly increased in diabetic mice compared with nondiabetic mice. However, the synthesis of FN, Col IV, and CTGF in diabetic PPKI mice was significantly
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PTEN regulated podocyte autophagy, apoptosis, and motility upon HG stimulation. To elucidate the mechanism of PTEN protection in podocytes against hyperglycemia, PTEN was knocked down and overexpressed in cultured podocytes before HG stimulation, using siRNA and adenovirus interference, respectively. As shown in Fig. 6, the expression levels of LC3-II, Beclin-1, Bcl-2, and nephrin were downregulated, and the expression level of Bax was upregulated in HG-treated podocytes compared with the control. Knockdown of PTEN in podocytes exacerbated the decrease of LC3-II, Beclin-1, Bcl-2, and nephrin seen with HG treatment and an increase of Bax. Conversely, forced expression of PTEN in podocytes blocked these effects of HG. Consistent with the result of Western blot analysis, Annexin V-FITC/PI dual-positive podocytes were aggrandized when treated with HG. Repressive PTEN further increased the number of apoptotic cells in the presence of HG, whereas enhanced PTEN exhibited an inverse effect (Fig. 7, A–D).

The scratch test was executed to better understand the effect of PTEN on podocyte motility (16, 34). After knockdown or forced expression of PTEN, as described previously, the podocytes were incubated with HG for 48 h after scratching. Figure 7, E–H clearly shows that PTEN regulated podocyte motility when exposed to HG; downregulation of PTEN disrupted the ability for migration while upregulation of PTEN restored the dynamics in podocytes.

Together, these data demonstrated a potential beneficial role of PTEN elevation in podocytes against HG-triggered podocyte damage through modulating autophagy, apoptosis, and motility response to HG.

DISCUSSION

The current study demonstrated that PTEN was downregulated in DKD in podocytes in both human subjects and diabetic mice. An innovative and important podocyte-specific inducible PTEN knockin mouse model was generated and in the presence of diabetes. Induction of PTEN in podocytes with tamoxifen protected against a number of classical features of DKD. Furthermore, the underlying renoprotection mechanism of PTEN in podocytes was partly associated with the improvement of autophagy and motility, and the inhibition of apoptosis in HG.

As a tumor suppressor for neoplastic diseases, PTEN was found to be downregulated in clear cell renal cell carcinoma (ccRCC) and to induce apoptosis in KU19–20 and Caki-2 cells as an Akt inhibitor (12). PTEN reduced the incidence rate and delayed the onset of renal cysts and ccRCC by preventing deficiency of the product of the von Hippel–Lindau (or pVHL) (8). Systemic overexpression of PTEN exhibited miraculous cancer resistance and energy expenditure improvement (10, 26). The current data showed that no neoplastic diseases occurred in PPKI mice. Recently, a reduction in the expression of PTEN was also observed in diabetic nephropathy, ischemic renal injury, aristolochic acid nephropathy, and unilateral ureteral occlusion, suggesting that PTEN might be involved in maintaining renal homeostasis (33). Consistently, the present study confirmed a consistent reduction in PTEN in podocytes of patients with diverse glomerular diseases, confirming that PTEN is an essential biomarker in podocyte injury.

The present study, along with others (21, 43, 47), confirmed that PTEN was downregulated in podocytes of both human and mice DKD models. Podocyte-specific knockout of PTEN resulted in prominent albuminuria, moderate podocyte efface-
ment, glomerular obliteration of capillaries, and glomerular sclerosis, predisposing mice toward the development of DKD (21). In the present study, the variation in the expression of PTEN among organs in db/db mice implied that PTEN might work heterogeneously and specifically in different tissues (15). For instance, PTEN deficiency was found to enhance systemic insulin sensitivity and insulin-stimulated glucose uptake in liver-specific PTEN knockout mice, resulting in decreased blood glucose levels (10, 28). The tissue-dependent effects of PTEN have induced the advancement of the cell-specific model, to avoid systemic side effects. Thus, transgenic mice overexpressing PTEN specifically in podocytes (PPKI mice) were generated to examine the effect of this modification on the development of DKD. Our data demonstrated that PPKI genotype had no effect on blood glucose and serum lipid levels. However, serum creatinine and urinary albumin excretion normalized for creatinine excretion were lower in diabetic PPKI than in diabetic control mice. Histologically, the amount of FN, Col IV, CTGF, and mesangial matrix in glomeruli was reduced in PPKI than in control mice in the presence of diabetes. Similarly, the effacement of podocyte foot processes, as well as GBM thickness, was also ameliorated in diabetic PPKI mice. The present findings were the first to illustrate the relevance of podocyte-specific overexpression of PTEN to hyperglycemia in the kidney in vivo and suggested that the overexpression of PTEN in podocytes protected the kidney.

Fig. 5. Histological and morphological features related to DKD in the kidney of experimental mice. A: double-immunofluorescence of PTEN and Synpo in glomeruli. PTEN, red; Synpo, green; Merge, yellow. Scale bar: 10 μm. B: quantification of PTEN MFI in glomeruli (n = 6 mice in each group). C–G: renal extracellular matrix deposition in mice at the age of 36 wk. Representative Western blot of fibronectin, collagen IV, and CTGF in the renal cortex (C). Densitometric analysis of fibronectin (D), collagen IV (E), nephrin (F) and CTGF (G) using Western blot by three independent experiments. H: representative periodic acid Schiff (PAS) staining for glomerular mesangial matrix at the age of 36 wk. Scale bar: 30 μm. I: semiquantitative sclerosis analysis evaluated mesangial matrix expansion by mesangial matrix index (MMI) (n = 20 glomeruli in each group). J: representative Oil Red O staining for glomeruli at the age of 36 wk. Scale bar: 30 μm. K: quantification of total cholesterol (normalized by protein concentration) in the renal cortex (n = 6 mice in each group). L: representative immunohistochemical staining of WT-1 in mice at 36-wk-old. Scale bar: 20 μm. M: quantification of average number of WT-1-positive cells per glomerulus. Twenty glomeruli per kidney were counted. N: representative electron microscopy for observing podocyte foot process effacement and GBM thickness. Scale bar: 500 nm. O: quantification of podocyte foot process effacement by foot process width (FPW). P: quantification of GBM thickness. CTGF, connective tissue growth factor; GBM, glomerular basement membrane; Synpo, synaptopodin.
from hyperglycemia via rescuing central characteristics of DKD.

PTEN was also involved in lipid metabolism (9, 14, 46). In the liver, insulin resistance caused excess lipid deposition due to the stimulatory effect of insulin on lipogenesis, resulting in steatosis. In contrast, ameliorative insulin resistance lowered insulin levels and liver lipid content (6). Consistently, our data showed that diabetic mice had a higher level of cholesterol.
Fig. 6. PTEN regulated autophagy and apoptosis in HG-treated podocytes. A and B: inhibition of PTEN suppressed autophagy and aggravated apoptosis in cultured podocytes treated with HG. Representative Western blot of nephrin, Beclin-1, PTEN, Bcl-2, Bax and LC3-II (A). Densitometric analysis using Western blot via three independent experiments (B). (C and D) Elevation of PTEN restored autophagy and apoptosis damage caused by HG in cultured podocytes. Representative Western blot of nephrin, Beclin-1, PTEN, Bcl-2, Bax and LC3-II (C). Densitometric analysis using Western blot by three independent experiments (D).
Fig. 7. PTEN regulated apoptosis and motility in high glucose (HG)-treated podocytes. A: repressive Annexin V-FITC/PI dual fluorescence in cultured podocytes treated with si-PTEN. B: quantification of the number of apoptotic cells. C: repressive Annexin V-FITC/PI dual fluorescence in cultured podocytes treated with ad-PTEN. D: quantification of the number of apoptotic cells. E: scratch test in cultured podocytes treated with si-PTEN. F: quantification of the number of cells in the scraping zone. G: scratch test in cultured podocytes treated with ad-PTEN. H: quantification of the number of cells in the scraping zone.
accumulation in the kidney compared with nondiabetic mice. However, the podocyte-specific overexpression of PTEN reduced the level of total cholesterol in the kidney without disturbing serum cholesterol and serum triglyceride levels, in agreement with the findings of previous studies (29, 30). PTEN disruption resulted in the elevated amount of lipid droplets by upregulating PI3K/AKT/mTOR pathway and dysregulating the lipid-mediated checkpoint (5, 27, 46). Increased PTEN led to the inhibition of macrophage scavenger receptor A in macrophages, subsequently ameliorating acLDL uptake and foam-cell formation in apoE−/− mice (7, 19). Generally, PTEN alleviated the accumulation of lipid in the kidney, possibly by inhibiting lipid biogenesis. The difference between serum and tissue lipid levels might be explained by the localized accumulation of PTEN in the kidney without disturbing the main lipid metabolism organs, such as the liver.

The renal protective role of PTEN in podocytes under HG is attributed mainly to its capacity to regulate the PI3K/Akt signaling pathway. Nonetheless, PTEN acts beyond the PI3K/Akt pathway, such as PKR-eIF2α, p38 MAPK-MAPKAPK2-HSP27, and NR2B-tau phosphorylation pathways (2, 25, 36). For example, the dramatic upregulation of PTEN inhibited the activity of RhoA/Rac1/Cdc42 signaling in podocytes via degrading PIP3, thus suppressing cytoskeletal rearrangement and albumin permeability under a hyperglycemic condition (21). PTEN was also observed to upregulate the expression of podocalyxin and nephrin and downregulate the expression of desmin and α-smooth muscle actin, so as to prevent HG-induced phenotypic transition by silencing the PI3K/Akt pathway (43). PTEN antagonized HG-induced podocyte injuries, including increased oxidative stress and apoptosis, deficient cell viability and autophagy, downregulated expression of nephrin and synaptopodin, and restrained insulin-stimulated glucose uptake (35). Consistent with previous studies, the present data showed that knockdown of PTEN in cultured podocytes mimicked or exacerbated the expression of autophagy-related (LC3-II, Beclin-1), as well antiapoptotic and autophagy-related (LC3-II, Beclin-1), as well antiapoptotic (Bcl-2) proteins, and increased the expression of proapoptotic PTEN was confirmed by the expression of PTEN in human subjects and db/db mice, while WF, LTT, LI, WYH and LS were responsible for histological examination and evaluation. WWJ designed the study. WHZ and WWJ wrote the paper. All authors participated in drafting and revising the manuscript. All authors approved the final version.

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