Original Research

Formyl peptide receptor 1 promotes podocyte injury through regulation of mitogen-activated protein kinase pathways

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Impact statement

Nephropathy remains a major cause of morbidity and a key determinant of mortality in patients with type 1 or type 2 diabetes mellitus. Podocyte injury contributes to glomerular injury and is implicated in the pathogenesis of diabetic nephropathy. Alleviation of podocyte injury facilitates for the amelioration of diabetic nephropathy. In the present study, the silencing of FPR1 ameliorated glomerular and podocyte injury in rats with diabetic nephropathy, and suppressed podocyte apoptosis through inactivation of MAPKs. Therefore, FPR1 interference might be a potential therapeutic strategy for the treatment of diabetic nephropathy.

Abstract

Podocyte injury contributes to glomerular injury and is implicated in the pathogenesis of diabetic nephropathy. Formyl peptide receptor (FPR) 1 is abundantly expressed in neutrophils and mediates intracellular transport of Ca^{2+}. Intracellular Ca^{2+} regulates pathological process in renal podocyte and plays a role in diabetic nephropathy. However, the role of formyl peptide receptor 1 in podocyte injury of diabetic nephropathy has not been reported yet. Firstly, a rat model with diabetic nephropathy was established by streptozotocin injection, and a cell model was established via high glucose treatment of mouse podocytes (MPC5). Formyl peptide receptor 1 was enhanced in streptozotocin-induced rats and high glucose-treated MPC5. Secondly, streptozotocin injection promoted the glomerular injury with decreased nephrin and podocin. However, tail injection with adenovirus containing shRNA for silencing of formyl peptide receptor 1 attenuated streptozotocin-induced glomerular injury and the decrease in nephrin and podocin. Moreover, silencing of formyl peptide receptor 1 repressed cell apoptosis of podocytes in diabetic rats and high glucose-treated MPC5. Lastly, protein expression levels of p-p38, p-ERK, and p-JNK protein were up-regulated in streptozotocin-induced rats and high glucose-treated MPC5. Silencing of formyl peptide receptor 1 attenuated high glucose-induced increase in p-p38, p-ERK, and p-JNK in MPC5, and overexpression of formyl peptide receptor 1 aggravated high glucose-induced increase in p-p38, p-ERK, and p-JNK. In conclusion, inhibition of formyl peptide receptor 1 preserved glomerular function and protected against podocyte dysfunction in diabetic nephropathy.

Keywords: FPR1, podocyte injury, diabetic nephropathy, MAPKs, p-JNK, p-ERK

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Introduction

Diabetic nephropathy involves serious diabetic microangiopathy in patients with type 1 and type 2 diabetes, and becomes the most important cause of end-stage renal disease.[1] Progression of microalbuminuria and glomerular hyperfiltration are the main characteristic manifestations of diabetic nephropathy.[2] Injury of glomerulus is considered as the initial case of the renal injury during development of diabetic nephropathy.[3] Hyperglycemic podocyte dysfunction, including differentiation, apoptosis, and angiogenesis, is shown to be associated with the glomerular injury.[4] In the early stage of diabetic nephropathy, the number of podocytes is significantly reduced, which results in impaired integrity of the glomerular filtration barrier.[5] Podocyte injury may play a role in the pathogenesis of diabetic nephropathy,[6] and improving of podocyte injury facilitates for the amelioration of diabetic nephropathy.[7] Therefore, research on the mechanism of podocyte injury

might provide a promising therapeutic strategy for the treatment of diabetic nephropathy.

Formyl peptide receptor 1 (FPR1), a member of the G-protein-coupled pattern recognition receptor family, is mainly expressed in phagocytic leukocytes, and considered as a critical regulator in host defense and innate immunity.[8] Family with sequence similarity 19 (chemokine (C–C motif)-like) member A4, that was increased in lipopolysaccharide-induced macrophages and monocytes, binds to FPR1 to promote the phagocytosis of macrophages.[9] Annexin A1 interacts with FPR1 to regulate immune responses.[10] FPR1 interacts with the ligands or senses the endogenous signals of dysfunctional cells to contribute to inflammation or tissue damage.[11] For example, FPR1 is implicated in the pathogenesis of acute respiratory distress syndrome,[12] acute lung injury,[13] pulmonary fibrosis,[14] and neuroblastoma tumorigenesis.[15] FPR1 functions as a critical regulator of inflammatory environment through mediation of neutrophils migration and function.[16] Silencing of FPR1 was shown to repress ventricular remodeling and cardiomyocyte apoptosis in ischemia/reperfusion-induced rats.[17] FPR1 was also shown to induce changes in intracellular-free calcium concentration of neutrophils,[18] and intracellular Ca^{2+} regulates pathological process in renal podocyte and plays a role in diabetic nephropathy.[19] Moreover, FPR1 is abundantly expressed in macrophages, and recruitment of macrophages is implicated in the development and progression of diabetic renal injury.[20] Since FPR1 was up-regulated in pancreatic islets of diabetic mice,[21] and high glucose diet leads to increase in formyl-methionyl-leucyl-phenylalanine, which functions as a ligand of FPR1 to promote the expression of FPR1 during obesity-induced glucose intolerance,[22] we hypothesized that FPR1 might be related to podocyte injury during progression of diabetic nephropathy.

Expression of FPR1 in diabetic rats and high glucose-induced podocytes were firstly determined, and the functional role of FPR1 in glomerular injury and podocyte apoptosis was then investigated. The pathway involved in FPR1-mediated diabetic nephropathy was assessed in this study, and these results might provide potential therapeutic strategy for the treatment of diabetic nephropathy.

Materials and methods

Animal model

A total of sixty six to eight weeks old Sprague Dawley rats (200–220 g weight) were acquired from The Jackson Laboratory (Bar Harbor, ME, USA) and housed in cages with free access to water and food under 21 ± 2°C. The experiments were approved by Second Affiliated Hospital of University of South China and in accordance the National Institutes of Health Laboratory Animal Care and Use Guidelines. Rats were randomly divided into control ($n = 30$) and streptozotocin (STZ, $n = 30$) groups. Following feed with high-fat diet (40% of calories from fat) for four weeks, rats in the STZ group were intraperitoneally injected with 30 mg/kg STZ dissolved in 0.1 mol/L citric acid buffer (pH 4.3; Sigma-Aldrich, St. Louis, MO, USA). Rats in the control group were fed with normal diet (4% calories from fat) for four weeks and then intraperitoneally injected with the same dose of 0.1 mol/L citric acid buffer (pH 4.3). Three days later, blood glucose of rats in tail-vein blood was measured by Accu-Chek Plasma blood glucose analyzer (Roche Diagnostics, Mannheim, Germany). Rats in the STZ group with glucose level over 16.7 mmol/L were regarded as diabetic rats. Twenty-four rats were successfully established as diabetic rats.

Adenovirus-containing shRNA for silence of FPR1 (Ad-shFPR1) and the negative control (Ad-shNC) were synthesized by Shanghai GenePharma (Shanghai, China). The control group was divided into three subgroups with 10 rats for each group: control, control with Ad-shNC, and control with Ad-shFPR1. The STZ group was also divided into three subgroups with eight rats for each group: STZ, STZ with Ad-shNC, and STZ with Ad-shFPR1. For Ad-shNC and Ad-shFPR1 administration, 1×10^9 PFU/mL Ad-shNC and Ad-shFPR1 in 0.5 mL of saline were injected into rats via tail vein injection. The weight, blood glucose, and urinary output of rats were measured every 20 days. Sixty days later, rats were killed by carbon dioxide inhalation, and the kidney tissues were excised and frozen in liquid nitrogen for other analysis.

Hematoxylin-eosin and periodic acid-Schiff stainings

Formalin-fixed and paraffin-embedded kidney tissues were sectioned into pieces with 4 μm thickness. Following deparaffinization in xylene and rehydration with graded series of ethanol, the sections were stained with hematoxylin and eosin (H&E) (Solarbio, Wuhan, China) or periodic acid-Schiff (PAS) (Sigma-Aldrich). The sections were measured under optical microscope (Carl Zeiss Vision, Munich, Germany).

Immunofluorescent staining

The sections of renal tissues were blocked in 5% BSA and incubated overnight with both FPR1 antibody and nephrin antibody (Abcam, Cambridge, MA, USA) at 4°C, respectively. Slides were then correspondingly incubated with fluorescein-5-isothiocyanate (FITC)- and tetramethyl rhodamine isothiocyanate (TRITC)-labeled secondary antibody, respectively. DAPI was used to stain the nuclei. The stained sections were viewed by fluorescence confocal microscopy (Carl Zeiss Vision).

Immunohistochemistry

The kidney tissues were fixed in formalin and embedded in paraffin, and then sectioned into pieces with 4 μm thickness. The sections were dewaxed in xylene and rehydrated with graded series of ethanol, and then incubated with EDTA solution. Following block in 3% bovine serum albumin, the sections were incubated with specific primary antibodies against nephrin (1:200, Abcam) or podocin (1:100, Abcam). Following incubation with horseradish peroxidase-labeled secondary antibody, the sections were performed with diaminobenzidine incubation and hematoxylin counterstaining before observe under microscope.

TUNEL assay

The kidney sections were incubated with 20 mg/mL proteinase K and then with 3% hydrogen peroxide. Following incubation with 0.1% Triton X-100 in 0.01% sodium citrate, the sections were incubated with TUNEL reaction mixture (Roche Diagnostics). The sections were counterstained with DAPI (BestBio, Shanghai, China) before observed under microscope.

Cell culture and treatment

Mouse podocytes (MPC5) were purchased from ATCC (Manassas, VA, USA) and cultured in collagen I-coated dishes (BD Biosciences, Bedford, MA, USA) with RPMI 1640 medium (Gibco BRL, Gaithersburg, MD, USA) and 10% fetal bovine serum (Gibco BRL) at 33°C. Recombinant murine IFN-γ (50 IU/mL; Sigma-Aldrich) was added into the medium. Cells were transferred to RPMI 1640 medium containing 5% fetal bovine serum without the murine IFN-γ at 37°C for two weeks to induce differentiation. For the treatment, the cultured medium of MPC5 was replaced by DMEM medium (Gibco BRL) containing normal glucose (5.5 mmol/L) or high glucose (30 mmol/L) for two days before other functional assays.

Cell transfection

MPC5 under high glucose treatment was infected with Ad-shNC or Ad-shFPR1 by Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA). pcDNA-mediated overexpression of FPR1 was also transfected into high glucose-induced MPC5. pcDNA-infected MPC5 was also incubated with 100 μM resveratrol (Sigma-Aldrich).

Table 1. Primer.

ID	Sequence(5′–3′)
GAPDH F	TGCACCACCAACTGCTTAGC
GAPDH R	GGCATGGACTGTGGTCATGAG
FPR1 F	TGGGAGGACATTGGCCTTTC
FPR1 R	GGATGCAGGACGCAAACAC

Flow cytometry

Trypsin digestion was used to harvest MPC5 with indicated transfection and treatment. Cells, resuspended with 100 μL binding buffer (Beyotime, Beijing China), were stained with Annexin V-fluorescein isothiocyanate apoptosis detection kit (Beyotime), and then analyzed by flow cytometer (Attune, Life Technologies, Darmstadt, Germany).

qRT-PCR

RNAs were isolated from kidney tissues by Trizol (Thermo Fisher Scientific) and then reverse-transcribed into cDNAs. SYBR Green Master (Roche) was applied to evaluate mRNA expression of FPR1 with GAPDH as the endogenous control. The primer sequences are shown in Table 1.

Western blot

Kidney tissues or MPC5 cells were lysed in RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific), and the concentration was determined by acid protein kit (Thermo Fisher Scientific). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gels, and electrotransferred onto PVDF membrane (Millipore, Bedford, MA, USA). Membranes were blocked with 5% BSA and then incubated overnight with primary antibodies: anti-FPR1 (1:2000, Abcam), anti-p38 and anti-p-p38 (1:2500, Abcam), anti-ERK, anti-p-ERK, anti-JNK and anti-p-JNK (1:3000, Abcam), anti-nephrin and anti-podocin (1:3500, Abcam), anti-Bcl-2 and anti-Bax (1:4000, Abcam), anti-cleaved caspase-3, and anti-GAPDH (1:4500, Abcam) overnight. Horseradish peroxidase-labeled secondary antibody (1:5000; Abcam) was applied to probe the membranes, and enhanced chemiluminescence (KeyGen, Nanjin, China) was used to detect the immunoreactivities.

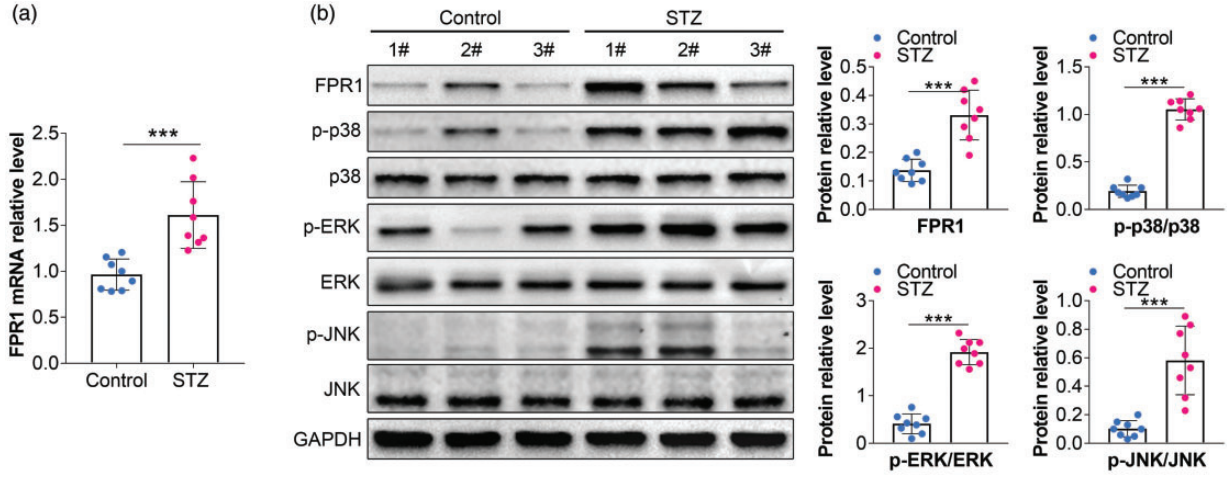

Figure 1. Enhanced level of FPR1 in rats with diabetic nephropathy. (a) mRNA expression of FPR1 was increased in the kidney tissues isolated from the diabetic rats. (b) Proteins expression of FPR1, p-p38, p-ERK and p-JNK, were enhanced in diabetic rats. $N = 10$. ***$p < 0.001$. (A color version of this figure is available in the online journal.)

Statistical analysis

Data with at least three repeats were expressed as mean ± SEM, and performed with one-way analysis of variance or Student's t test in GraphPad Prism software. Independent t test was used for comparison between the two groups. ANOVA was used for comparison among more than two groups, and LSD-t test was used for pair comparison between groups. The p value < 0.05 was considered as statistically significant.

Results

Enhanced level of FPR1 in rats with diabetic nephropathy

To assess the effects of FPR1 on diabetic nephropathy, a rat model was established through streptozotocin injection. Data from qRT-PCR showed that FPR1 were increased in the kidney tissues isolated from the diabetic rats (Figure 1 (a)). Protein expression of FPR1 was also up-regulated

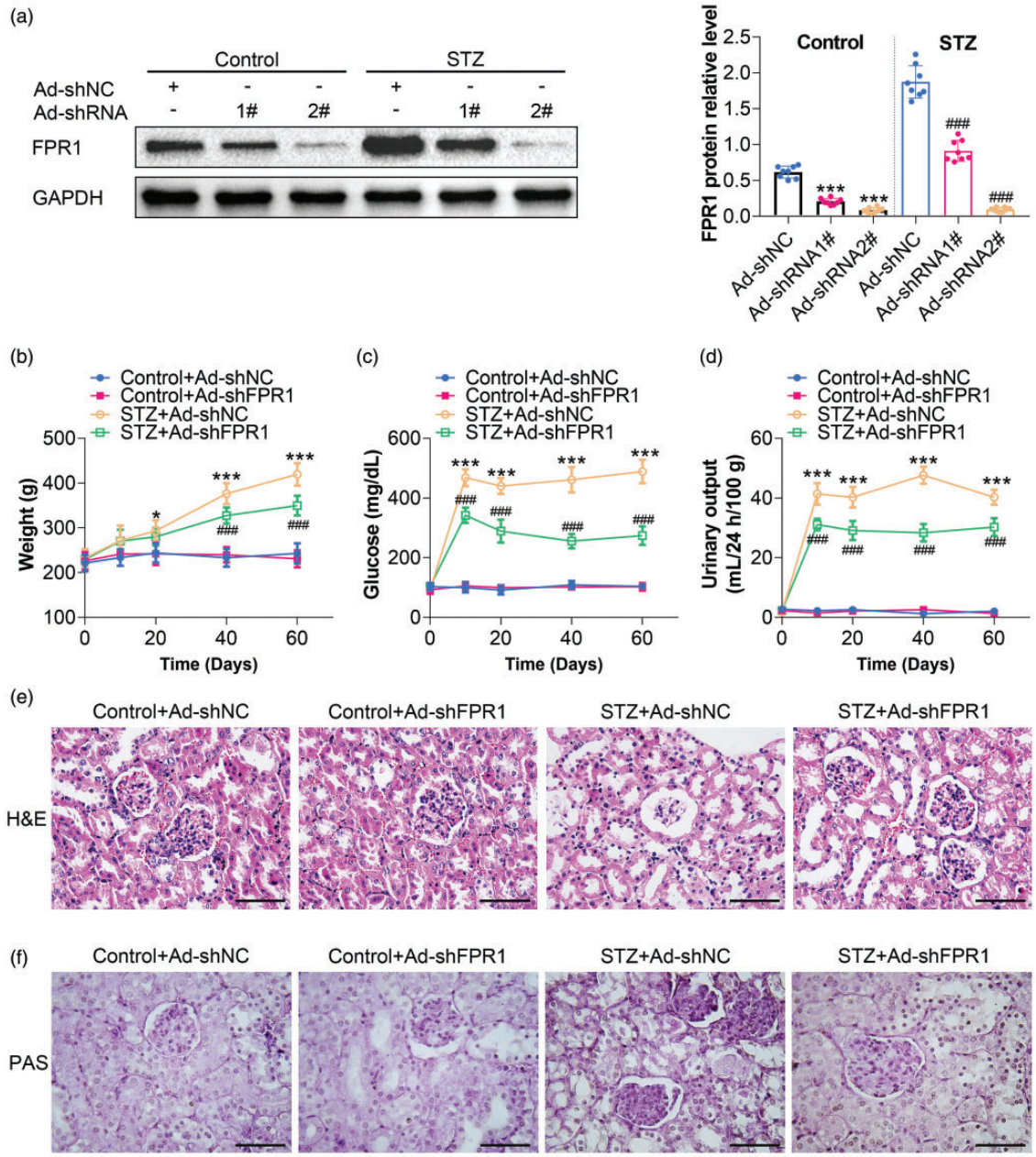

Figure 2. Silence of FPR1 ameliorated glomerular injury in rats with diabetic nephropathy. (a) Injection with Ad-shFPR1 decreased protein expression of FPR1 in diabetic rats. (b) Injection with Ad-shFPR1 attenuated streptozotocin injection-induced increase of body weight. (c) Injection with Ad-shFPR1 attenuated streptozotocin injection-induced increase of blood glucose. (d) Injection with Ad-shFPR1 attenuated streptozotocin injection-induced increase of urinary output. (e) H&E staining showed that injection with Ad-shFPR1 ameliorated streptozotocin-induced renal damage. (f) PAS staining showed that injection with Ad-shFPR1 ameliorated the glomerular injury through suppression of expanded glomerular mesangium and collapsed capillary in the glomerulus. $N = 10$. ***, ### $p < 0.001$. Scale bars: 100 μm. (A color version of this figure is available in the online journal.)

in rats post streptozotocin injection (Figure 1(b)). Immunofluorescent double staining of FPR1 and nephrin showed that FPR1 was located in both cytoplasm and nucleus of the podocytes with more in the cytoplasm (Supplemental Figure S1). Streptozotocin injection up-regulated the level of FPR1 (Supplemental Figure S1) and the ligand of FPR1, fMLF (Supplemental Figure S2(a)). Moreover, proteins involved in MAPK pathway, p-p38, p-ERK, and p-JNK, were enhanced in diabetic rats (Figure 1(b)), suggesting that FPR1 might participated in diabetic nephropathy, which might be regulated through MAPK pathway.

Silencing of FPR1 ameliorated glomerular injury in rats with diabetic nephropathy

Adenovirus-containing shFPR1 was injected into the diabetic rats through tail injection to silence FPR1 in diabetic rats. Western blot analysis demonstrated lower expression of FPR1 in diabetic rats injected with Ad-shFPR1 than that in rats injected with Ad-shNC (Figure 2(a)). Streptozotocin injection induced a significant increase in body weight (Figure 2(b)), blood glucose (Figure 2(c)), and urinary output (Figure 2(d)), while Ad-shFPR1 attenuated the increase in body weight (Figure 2(b)), blood glucose (Figure 2(c)), and urinary output (Figure 2(d)). Histological analysis of the renal morphology through H&E (Figure 2(e)) and PAS (Figure 2(f)) staining showed an apparent glomerulosclerosis in diabetic rats with expanded glomerular mesangium and collapsed capillary in the glomerulus. Injection with Ad-shFPR1 ameliorated the glomerular injury (Figure 2(e) and (f)), suggesting a protective effect on rats with diabetic nephropathy.

Silencing FPR1 ameliorated podocyte injury in rats with diabetic nephropathy

Immunohistochemical analysis of podocyte markers was used to evaluate podocyte injury. Result showed that streptozotocin injection induced less staining of nephrin and podocin than the control (Figure 3(a)), while injection with Ad-shFPR1 promoted the staining of nephrin and podocin (Figure 3(a)). Moreover, injection with Ad-shFPR1 attenuated streptozotocin-induced decrease in nephrin and podocin (Figure 3(b)), suggested that silencing of FPR1 ameliorated podocyte injury in rats with diabetic nephropathy.

Silencing of FPR1 suppressed podocyte apoptosis in rats with diabetic nephropathy

TUNEL staining was then applied to investigate role of FPR1 in podocyte apoptosis. Result showed that streptozotocin injection induced more TUNEL staining than the control (Figure 4(a)), while injection with Ad-shFPR1 decreased the TUNEL staining (Figure 4(a)). Moreover, injection with Ad-shFPR1 attenuated streptozotocin-induced decrease in Bcl-2 and increase in Bax and cleaved caspase-3 (Figure 4(b)), suggesting that silencing of FPR1

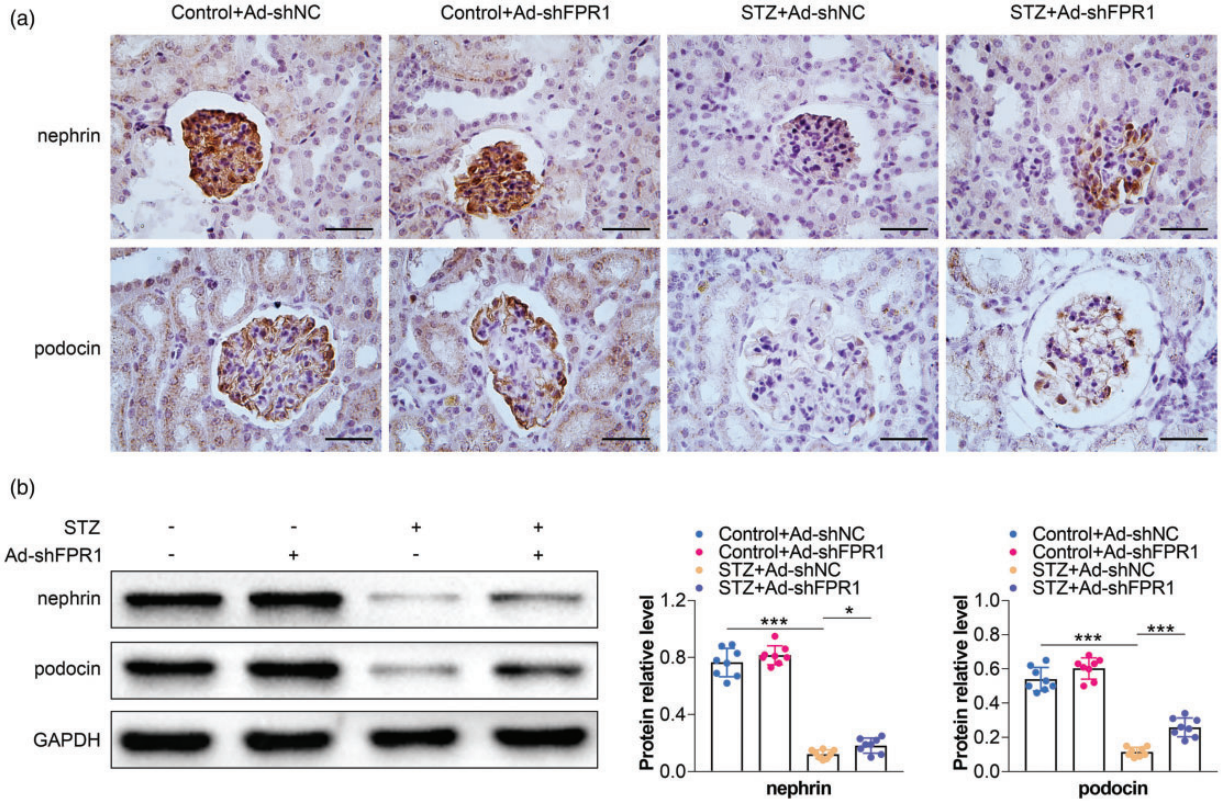

Figure 3. Silence of FPR1 ameliorated podocyte injury in rats with diabetic nephropathy. (a) Immunohistochemical analysis showed that injection with Ad-shFPR1 promoted the staining of nephrin and podocin. (b) Injection with Ad-shFPR1 attenuated streptozotocin-induced decrease of nephrin and podocin. $N = 10$. $^{*}p < 0.05$, $^{***}p < 0.001$. Scale bars: 50 μm. (A color version of this figure is available in the online journal.)

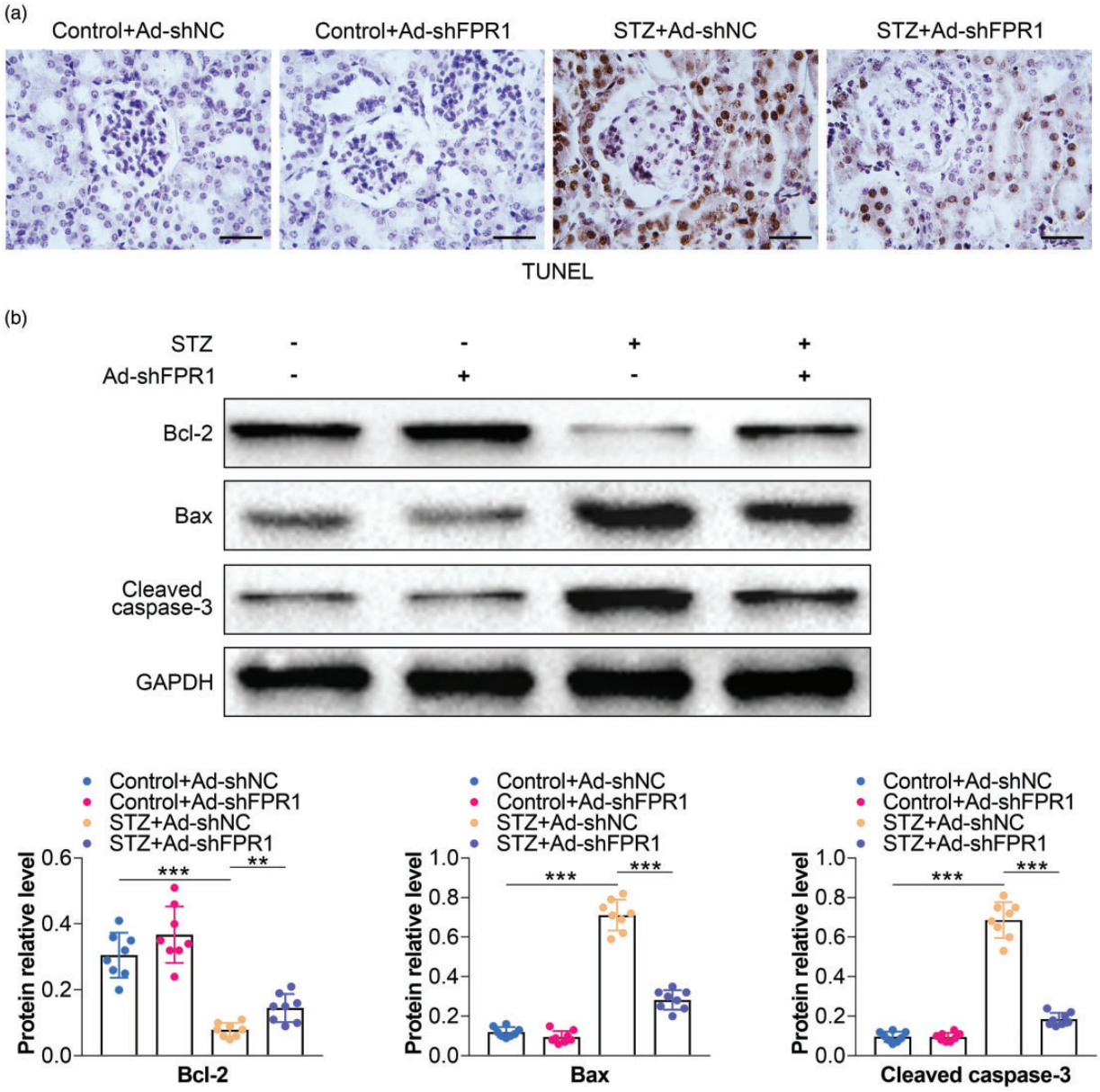

Figure 4. Silence of FPR1 suppressed podocyte apoptosis in rats with diabetic nephropathy. (a) TUNEL staining showed that injection with Ad-shFPR1 decreased the TUNEL staining. (b) Injection with Ad-shFPR1 attenuated streptozotocin-induced decrease of Bcl-2 and increase of Bax and cleaved caspase-3. $N = 10$. $^{**}p < 0.01$, $^{***}p < 0.001$. Scale bars: 50 μm. (A color version of this figure is available in the online journal.)

suppressed podocyte apoptosis in rats with diabetic nephropathy suggesting that silence of FPR1.

Silencing of FPR1 attenuated high glucose-induced increase of podocyte apoptosis

In vitro high glucose-induced mouse podocyte (MPC5) model was established to investigate role of FPR1 in renal podocyte damage. Treatment with high glucose demonstrated higher protein expression of FPR1 (Figure 5(a)) and fMLF (Supplemental Figure S2B) than that of normal glucose. Ad-shFPR1 transfection decreased the protein expression of FPR1 (Figure 5(a)). Cell apoptosis of MPC5 was promoted by high glucose treatment (Figure 5(b)), accompanied by decrease in Bcl-2 and increase in Bax and cleaved caspase-3 (Figure 5(c)). However, knockdown of FPR1 attenuated high glucose-induced increase in podocyte apoptosis (Figure 5(b)), and the decrease in Bcl-2 and increase in Bax and cleaved caspase-3 induced by high glucose were also reversed by silencing of FPR1 (Figure 5(c)). Treatment with FPR1 agonist, fMLF, promoted the cell apoptosis of high glucose-induced MPC5 (Supplemental Figure S2(c)). However, FPR1 inhibitor, BOC-MLF, suppressed the cell apoptosis of high glucose-induced MPC5 (Supplemental Figure S2(c)). These results showed that silencing of FPR1 demonstrated anti-apoptotic role against high glucose treatment in podocytes.

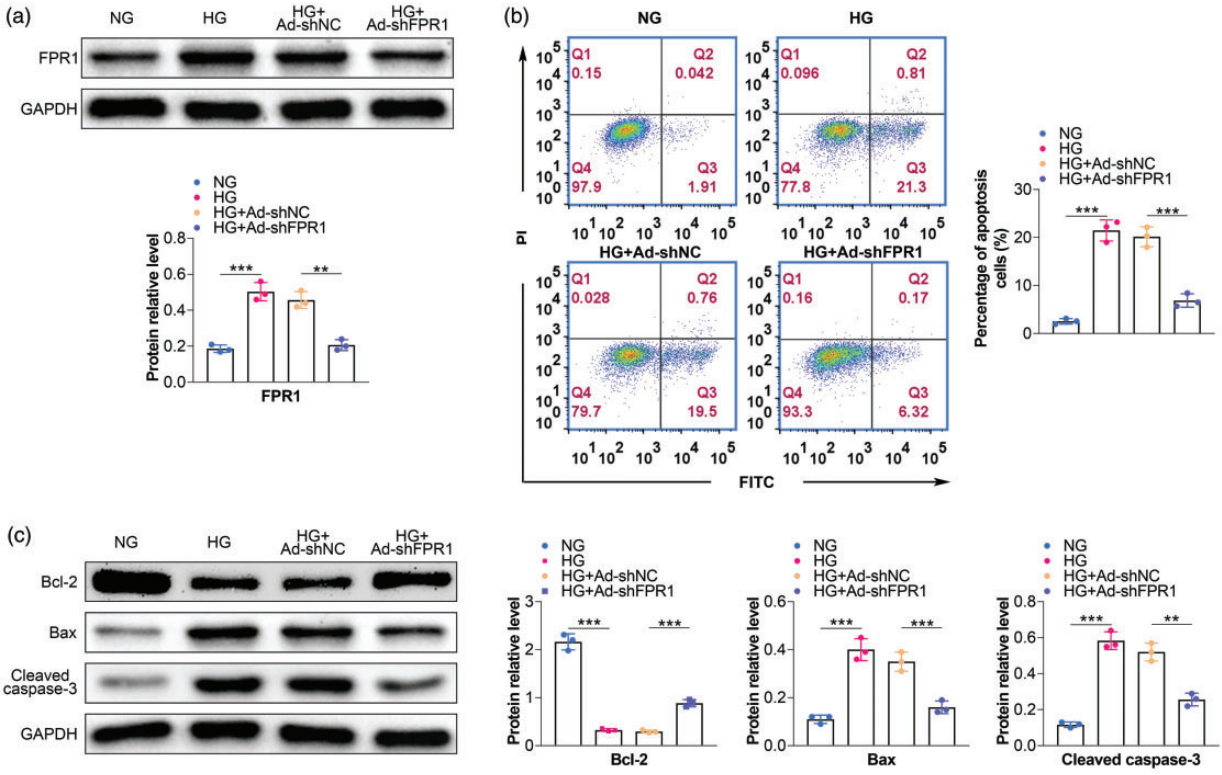

Figure 5. Silence of FPR1 attenuated high glucose-induced increase of podocyte apoptosis. (a) Ad-shFPR1 injection attenuated high glucose-induced increase of FPR1 in MPC5. (b) Ad-shFPR1 injection attenuated high glucose-induced increase of MPC5 apoptosis. (c) Injection with Ad-shFPR1 attenuated high glucose-induced decrease of Bcl-2 and increase of Bax and cleaved caspase-3 in MPC5. $N = 3$. $^{**}p < 0.01$, $^{***}p < 0.001$. (A color version of this figure is available in the online journal.)

FPR1 promoted podocyte injury through activation of MAPKs

To unravel the underlying mechanism involved in FPR1-mediated podocyte injury, Western blot analysis of MAPKs was applied. Results showed that high glucose induced activation of MAPKs through up-regulation of p-p38, p-ERK, and p-JNK (Figure 6(a)). Knockdown of FPR1 decreased protein expression of p-p38, p-ERK, and p-JNK (Figure 6(a)) to suppress the activation of MAPKs. Moreover, over-expression of FPR1 aggravated high glucose-induced up-regulation of p-p38, p-ERK, and p-JNK in MPC5 (Figure 6(b)), while inhibition of MAPKs (resveratrol) reversed the promotive effect of FPR1 on MAPKs activation (Figure 6(b)). Similarly, over-expression of FPR1 promoted high glucose-induced increase in podocyte apoptosis (Figure 6(c)), and the cell apoptosis was attenuated by resveratrol treatment (Figure 6(c)). Therefore, FPR1 promoted podocyte injury through activation of MAPKs.

Discussion

Podocytes are terminally differentiated visceral epithelial cells that reside along the glomerular basement membrane.[23] Podocytes connect to the endothelial and mesangial cells in the glomerulus across the basement membrane,[24] and keep the integrity of the glomerulus through formation of the glomerular filtration barrier.[25] Injurious stimuli, such as high glucose and the metabolites in diabetes, that contribute to podocyte dedifferentiation could promote mesenchymal features and retard the podocyte-specific properties, thus leading to podocyte dysfunction and glomerular filtration barrier damage.[26] Glomerulus is considered as the primary site of diabetic kidney injury, and the crosstalk between podocytes and glomerulus suggests that podocyte injury is closely correlated with severity of diabetic nephropathy.[24] Previous study has shown that FPR1 was up-regulated in pancreatic islets of diabetic mice,[21] and pharmacological inhibition of FPR1 ameliorated glucose tolerance through increased insulin levels.[22] The role and mechanism of FPR1 in podocyte injury during the development of diabetic nephropathy were then investigated in this study.

FPR2 was enhanced in the adipose tissues of diabetic mice, and the silencing of FPR2 reduced body weight gain and contributed to the alleviation of diet-induced insulin resistance.[27] Inhibitor and antagonist of FPR demonstrated anti-inflammatory and anti-angiogenic capacities for proliferative diabetic retinopathy.[28] In this study, we firstly established a rat model with diabetic nephropathy through low dose (30 mg/kg) streptozotocin injection according to American Diabetes Complications Consortium (http://www.amdcc.org).[7] *In vitro* cell model of diabetic nephropathy was also established through treatment with high glucose in podocyte.[29] Our results indicated that FPR1 was up-regulated in the kidney tissues of diabetic rats and high-glucose-induced podocyte. These results highlighted the significance of FPR1 in the podocyte pathophysiology of diabetic nephropathy.

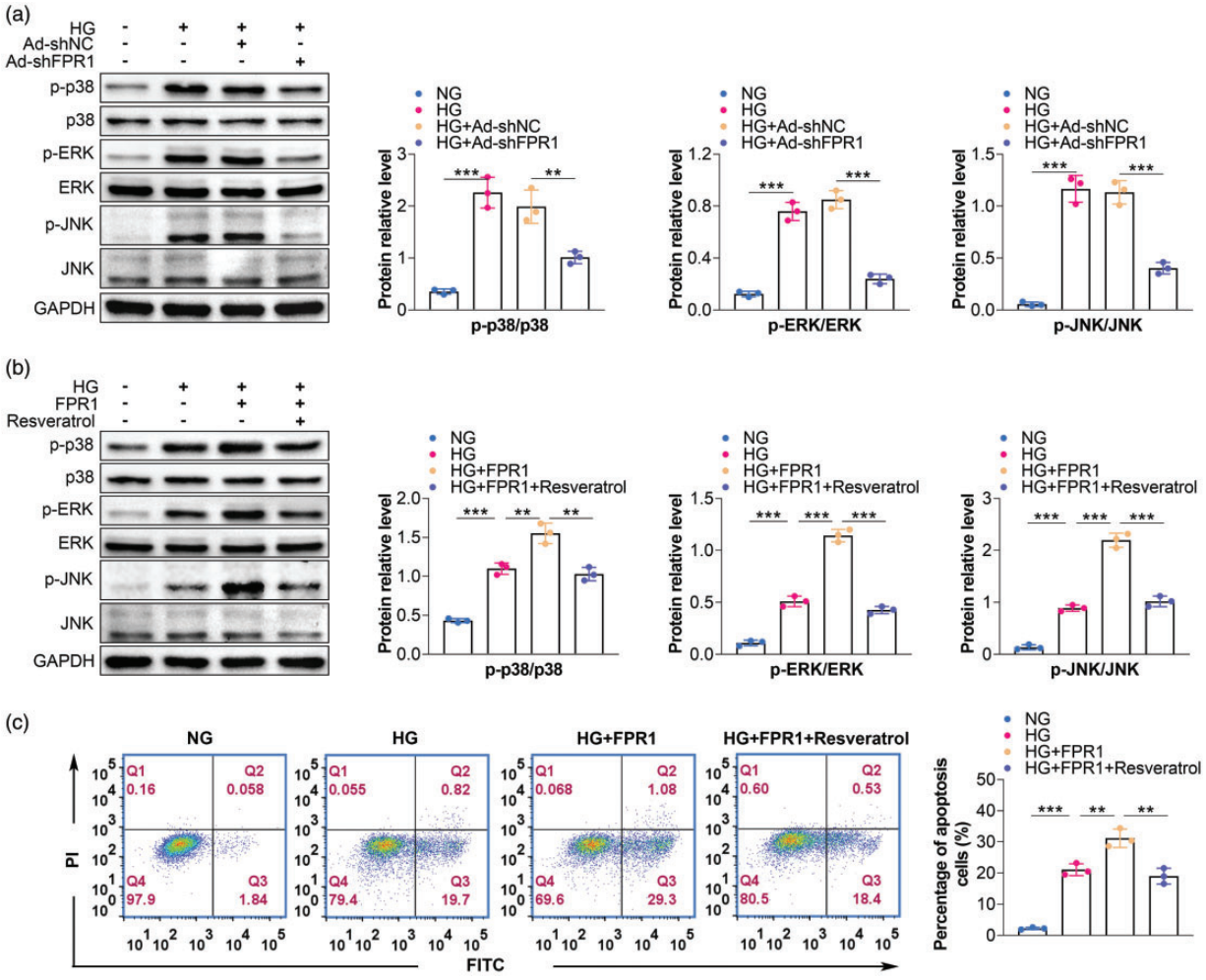

Figure 6. FPR1 promoted podocyte injury through activation of MAPKs. (a) Knockdown of FPR1 attenuated high glucose-induced increase of p-p38, p-ERK and p-JNK in MPC5. (b) Over-expression of FPR1 aggravated high glucose-induced up-regulation of p-p38, p-ERK and p-JNK in MPC5, while resveratrol reversed the promotive effect of FPR1 on MAPKs activation. (c) Over-expression of FPR1 promoted high glucose-induced increase of podocyte apoptosis, and the cell apoptosis was attenuated by resveratrol treatment. $N = 3$. $^{**}p < 0.01$, $^{***}p < 0.001$. (A color version of this figure is available in the online journal.)

The novel finding of this study was that Ad-shFPR1 injection ameliorated diabetic nephropathy, which was authenticated by several lines of evidence. Firstly, silencing of FPR1 mitigated glomerulosclerosis in diabetic rats through suppression of expanded glomerular mesangium and collapsed capillary in the glomerulus. Secondly, silencing of FPR1 promoted the expression of podocyte markers, nephrin, and podocin, to mitigate podocyte injury in diabetic rats. Thirdly, silencing of FPR1 suppressed podocyte apoptosis in diabetic rats and attenuated high glucose-induced cell apoptosis of podocyte. Apoptosis or necrosis of podocytes results in podocyte depletion and detachment from the basement membrane, thereby contributing to progression of diabetic nephropathy.[30] High glucose induces accumulation of reactive oxygen species to promote podocyte apoptosis during onset of diabetic nephropathy.[31] Suppression of podocyte apoptosis facilitated for the amelioration of diabetic nephropathy.[30] Therefore, results of this study suggested that silencing of FPR1 demonstrated anti-apoptotic effects against podocyte injury to ameliorate progression of diabetic nephropathy.

Multiple pathways, including transforming growth factor β, Notch, and p38 MAPKs, are involved in podocyte apoptosis.[30] p-ERK and p-p38MAPK-positive cells were found in glomeruli of patients with diabetic nephropathy, and the number of p-ERK-positive cells in glomeruli was associated with the progression of glomerular lesions, suggesting that MAPK phosphorylation contributed to progression of diabetic nephropathy.[32] Inhibition of MAPKs pathway by beraprost sodium demonstrated protective effect on diabetic nephropathy.[33] The present study showed that MAPK phosphorylation (p-p38, p-ERK, and p-JNK) was up-regulated in the kidney tissues of diabetic rats and high-glucose-induced podocyte. Over-expression of FPR1 aggravated high glucose-induced increase in MAPK phosphorylation. Silence of FPR1 was reported to repress cardiomyocyte apoptosis through inhibition of MAPKs pathway.[17] Our results also showed that Ad-shFPR1 injection decreased protein expression of p-p38, p-ERK, and p-JNK, and MAPK inhibitor attenuated FPR1-induced increase in MAPK phosphorylation. These results indicated that FPR1 interference suppressed

podocyte injury through inhibition of MAPKs pathway to retard progression of diabetic nephropathy.

Oxidative stress,[34] autophagy,[35] and inflammation[36] contributed to podocyte injury during the progression of diabetic nephropathy. FPR1 plays important role in acute inflammation[37] and oxidative stress.[18] Whether FPR1 was implicated in the inflammation, oxidative stress, or autophagy of podocyte during the development of diabetic nephropathy needs to be further investigated. The appropriate therapeutic strategies, including signal therapy of FPR1 silencing and combinational therapy of FPR1 silencing and MAPK inhibitor, warranted further exploration for the treatment of diabetic nephropathy.

In general, this study, for the first time, demonstrated that FPR1, via activation of MAPKs, aggravated podocyte injury, and silencing of FPR1 was effective for reversing podocyte injury in diabetic nephropathy. This study provides a potential therapeutic target for the treatment of diabetic nephropathy.

AUTHORS' CONTRIBUTIONS

All authors participated in the design, interpretation of the studies and analysis of the data and review of the article; JZ and TD designed the study, supervised the data collection, DXT analyzed the data, interpreted the data, JPW and PH prepare the article for publication and reviewed the draft of the article.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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