NFAT2 mediates high glucose-induced glomerular podocyte apoptosis through increased Bax expression

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Abstract

Background: Hyperglycemia promotes podocyte apoptosis and plays a key role in the pathogenesis of diabetic nephropathy. However, the mechanisms that mediate hyperglycemia-induced podocyte apoptosis is still far from being fully understood. Recent studies reported that high glucose activates nuclear factor of activated T cells (NFAT) in vascular smooth muscle or pancreatic β-cells. Here, we sought to determine if hyperglycemia activates NFAT2 in cultured podocytes and whether this leads to podocyte apoptosis. Meanwhile, we also further explore the mechanisms of NFAT2 activation and NFAT2 mediates high glucose-induced podocyte apoptosis.

Methods: Immortalized mouse podocytes were cultured in media containing normal glucose (NG), or high glucose (HG) or HG plus cyclosporine A (a pharmacological inhibitor of calcineurin) or 11R-VIVIT (a specific inhibitor of NFAT2). The activation of NFAT2 in podocytes was detected by western blotting and immunofluorescence assay. The role of NFAT2 in hyperglycemia-induced podocyte apoptosis was further evaluated by observing the inhibition of NFAT2 activation by 11R-VIVIT using flow cytometer. Intracellular Ca2+ was monitored in HG-treated podocytes using Fluo-3/AM. The mRNA and protein expression of apoptosis gene Bax were measured by real time-qPCR and western blotting.

Results: HG stimulation activated NFAT2 in a time- and dose-dependent manner in cultured podocytes. Pretreatment with cyclosporine A (500 nM) or 11R-VIVIT (100 nM) completely blocked NFAT2 nuclear accumulation. Meanwhile, the apoptosis effects induced by HG were also abrogated by concomitant treatment with 11R-VIVIT in cultured podocytes. Further, we also found that HG increased [Ca2+]i, leading to activation of calcineurin and subsequent increased nuclear accumulation of NFAT2 and Bax expression in cultured podocytes.

Conclusion: Our results identify a new finding that HG-induced podocyte apoptosis is mediated by calcineurin/NFAT2/Bax signaling pathway, which may present a promising target for therapeutic intervention.

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Introduction

Diabetic nephropathy (DN) is an important and common complication of both type 1 and type 2 diabetes leading to endstage renal disease (ESRD) [1]. Although current strategies slow disease progression [2–4], approximately one third of patients with diabetes develop end-stage renal disease requiring renal replacement therapy [1]. As a result, much effort has been devoted to understanding the mechanisms that promote glomerular damage in diabetic kidney disease with the hope of identifying new therapeutic strategies.

Podocytes are terminally differentiated cells reside on the outer surface of the glomerular basement membrane (GBM) and play a key role in maintaining the structure and function of the glomerular filtration barrier. Accumulating evidence suggests that glomerular podocytes play a pivotal role in the pathogenesis of diabetic kidney disease [5,6]. Some studies in type 1 and type 2 diabetes have demonstrated that podocyte depletion and loss represented one of the earliest mechanisms in the pathogenesis of DN [7,8]. High glucose (HG) induced podocyte apoptosis in vitro and in vivo [9–11], and podocyte apoptosis contributed to podocyte loss and reduced podocyte number [11]. It has been suggested that high glucose increased reactive oxygen species (ROS) may be a trigger mechanism in podocyte apoptosis and loss in DN [11]. However, the concert mechanisms that mediate hyperglycemia-induced podocyte apoptosis is still far from being fully understood. Due to this reason, there are no current interventions for DN specifically preventing podocyte apoptosis.

The nuclear factor of activated T cells (NFAT), which are the substrate for calcineurin (CaN), represent a family of Ca$^{2+}$ dependent transcription factors. Four isoforms, NFAT1, NFAT2, NFAT3 and NFAT4, have been identified [12]. NFAT are expressed in most immune system cells and play a pivotal role in the transcription of cytokine genes and other genes critical for the immune response [12,13]. Although originally thought to be largely restricted to cells of the immune system, abundant evidence now indicates that NFAT family members are expressed in nonimmune cells with some family members expressed ubiquitously [12,14–16]. NFAT is of critical importance in regulating the survival, proliferation, and function of multiple cell types. NFAT has been shown to regulate heart valve development, skeletal muscle and smooth muscle cell differentiation, and vascular development [17–20]. NFAT has also been implicated in the pathogenesis of cardiac and skeletal muscle hypertrophy [21,22].

The activities of NFAT proteins are tightly regulated by the Ca$^{2+}$/calmodulin-dependent CaN, which can be inhibited by cyclosporine A (CsA). CaN controls the translocation of NFAT proteins from the cytoplasm to the nucleus of activated cells. In the nucleus, the NFATc proteins either alone or in combination with other nuclear partners form NFAT transcription complexes to control the transcription of target genes. In unstimulated cells, NFAT transcription factors are located in the cytoplasm and are highly phosphorylated. Dephosphorylation of NFAT isoforms by CN causes their translocation to the nucleus and stimulation of gene transcription [12].

Recent data suggest that high glucose activate nuclear factor of activated T cells (NFAT) in vascular smooth muscle, pancreatic $\beta$-cells [16,23]. A body of evidence has accumulated to suggest that NFAT were implicated in several types of cell apoptosis [15,24,25]. Meanwhile, recent studies has also demonstrated that the activation of NFAT was involved in podocyte injury and glomerulosclerosis [26,27], but it was unknown whether hyperglycemia activates NFAT2 in cultured mouse podocyte and whether this leads to podocyte apoptosis. The aim of the present study was to investigate if HG activated NFAT2 and its function in hyperglycemia-induced podocyte apoptosis.

Materials and methods

Cell culture and treatment

The conditionally immortalized mouse podocyte cell line (MPC) was kindly provided by Dr. Peter Mundel (University of Miami, Miami, FL, USA) and cultured as described previously [28]. Cells were cultured at 33 °C in RPMI-1640 medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS, Gibco BRL, USA) and recombinant IFN-γ (growth permissive conditions; CYT-358, ProSpec,Tany Technogene Ltd, Israel). To induce differentiation, podocytes were reseeded and cultured at 37 °C in 100 cm$^2$ culture dish coated with 12 μg/ml type-I collagen (Shengyou Biotechnology, China) and in DMEM culture medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% FBS, deprived of IFN-γ (growth restrictive conditions) for 10 to 14 days. After differentiation of podocytes was confirmed by the identification of synaptopodin, a podocyte differentiation marker, 10$^6$ cells were synchronized into quiescence by growing cells in serum-free Dulbecco’s Modified Eagle’s medium (DMEM: Invitrogen, USA) for 24 h, and then treated with normal glucose (NG, 5.3 mM) or high glucose (HG, 20 mM) or NG (5.3 mM) plus mannitol (14.7 mM; osmolality control) for 12, 24 and 48 h respectively before experimentation. MPC cells between passage 12 and 18 were used for all experiments. For inhibition experiments, two inhibitors of Calcineurin/NFAT pathway were enrolled in this study: CsA (Sigma, USA), a pharmacological inhibitor of Calcineurin and 11R-vivit, a special inhibitor of NFAT [29]. CsA at concentration of 500 nM and 11R-vivit at concentration of 100 nM were added to cells treated with HG at a concentration of 20 mM for 48 h respectively. Each reaction was repeated in triplicates.

Annexin V and propidium iodide staining assay.

Apoptotic cells in different groups were determined using an Annexin V/PI apoptosis detection kit according to manufacturer’s protocol (Nanjing KeyGEN Biotech. Co., Ltd. Nanjing, China). Briefly, the cell pellet was resuspended in 1× binding buffer followed by incubation with 5 μl of Annexin V (conjugated with FITC) and 5 μl of PI in the dark for 10 min. Cell fluorescence was then analyzed using a Cell Lab Quanta™ SC Flow cytometer (Beckman Colter, Inc, USA). Cells positive for Annexin V-FITC and negative for PI were considered apoptotic.

Intracellular Ca$^{2+}$ level assays

The differentiated podocytes were divided the following four groups, one treated with the normal glucose (5.3 mM) that
served as the negative control; one treated with normal glucose (5.3 mM)+Mannitol (14.7 mM) that served as a osmosality control; one treated with high glucose (20 mM); another treated with normal glucose (5.3 mM) plus lonomycin that served as the positive control. The intracellular Ca\(^{2+}\) was monitored using the Ca\(^{2+}\)-sensitive dye, Fluor-3/AM. The intracellular Ca\(^{2+}\) were labeled with Fluor-3/AM at a concentration of 5 μmol/L for 30 min at 37 °C, then abandoned the medium with Fluor-3/AM, washed with medium twice. The dye-loading glass coverslips were transferred to an observation chamber mounted on the stage of an inverse microscope equipped with laser confocal scanning microscopy (LCSM, Zeiss KS 400, Postfach, Germany). The level of intracellular Ca\(^{2+}\) at the resting stage was measured first. Following this, 20 mM HG was added to the observation chamber, and the change in the Ca\(^{2+}\) concentration was monitored. Confocal fluorescence images of 512 x 512 pixels were recorded every 5 s. Ca\(^{2+}\) labeling with fluo-3/AM was excited at 488 nm krypton/argon laser. The emitted light was detected at 505–530 nm. The numerical aperture of the objective was 1.0 and that of the ocular was 3.0.

**Nuclear localization of NFAT2**

NFAT2 localization in podocyte nuclei was measured using a commercial assay (Santa Cruz, USA) and Nuclear and Cytoplasmic Protein Extraction kit purchased from the Beyotime Institute of Biotechnology(China). In this assay we also used a mouse monoclonal anti-histone 3 (Abcam, Cambridge, MA). In separate experiments on NFAT localization, podocytes were planted on cover slides in six-well plates. After subjected to various treatments, differentiated podocytes were fixed with 4% paraformaldehyde at room temperature for 15 min. After pre-treatment with 0.2% Triton X-100 for 20 min at 37 °C, cells were blocked with 5% bovine serum albumin for 20 min at room temperature, and incubated with rabbit anti-NFAT2 (Santa Cruz, USA) overnight at 4 °C. After three washes with PBS, cells were incubated with the goat anti-rabbit Alexa Fluor 546 (Santa Cruz, USA) and Nuclear and Cytoplasmic Protein Extraction kit (Beyotime Institute of Biotechnology, China). In this assay we also used a mouse monoclonal anti-histone 3 (Abcam, Cambridge, MA). The suspension was vortexed for 30 min at 4 °C and then transferred to polyvinylidene fluoride membranes (LCSM, Zeiss KS 400, Postfach, Germany). All images were analyzed by two investigators blinded to the identity of the samples.

**Real-time quantitative RT-PCR**

Total RNA from cultured podocytes was extracted with TriZol Reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Then, Complementary DNA was synthesized from 2 μg of total RNA using the PrimeScriptTM RT regent Kit following the instructions provided by the manufacturer (Takara Biotechnology, China). Subsequently, the cDNA was subject to quantitative RT-PCR(qRT-PCR) using Power SYBR Green PCR Master Mix (Takara Biotechnology, China). Each real-time PCR reaction consisted of 2 μl diluted RT product, 10 μl SYBR Green PCR Master Mix (2 x) and 250 nM forward and reverse primers in a total volume of 20 μl. Reactions were carried out on 7500 qRT-PCR System (Applied Biosystems) for 40 cycles (95 °C for 15 s, 60 °C for 45 s) after an initial 10 min incubation at 95 °C. The primers used for real-time PCR were as follows: Bax, forward 5'-TGGCAACGTGACATGTTCTGTGAC-3', reverse 5'-CGTCCCAAC-CAGGGTCT-3', giving 195 bp PCR product. GAPDH, forward 5'GAAGTCAACGGATTTGGGTAT-3', reverse 5'GCCCTTCTCC-ATGGTGTGAAAGAC-3', giving 306 bp PCR product. In order to confirm amplification specificity, the PCR products from each primer pair were subjected to a melting curve analysis and subsequent agarose gel electrophoresis. A control without cDNA was run in parallel with each assay. Each reaction was amplified in triplicate and the fold change in expression of each gene was calculated using the ΔΔCt method, using GAPDH mRNA as an internal control.

**Whole, cytoplasmic, and nuclear protein extraction**

For isolation of total extract under different experimental conditions, 10\(^6\) cells were lysed with RIPA lysis buffer (50 mM Tris with pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS and 0.05 mM EDTA). The samples were centrifuged at 12,000g for 15 min at 4 °C and the supernatant was collected as total cell extracts. Cytoplasmic and nuclear extracts of cultured podocytes under different experimental conditions were prepared using the Nuclear and Cytoplasmic Protein Extraction kit (Beyotime Institute of Biotechnology, China) according to the manufacturer’s instructions. In brief, cells were washed with PBS, detached and resuspended in 200 ul of ice-cold cell lysis buffer A (10 mM HEPES with pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.4% Igepal CA-630, 5 μM leupeptin, 2 μM pepstatin A, 1 μM aprotinin and 1 mM phenylmethylsulfonyl fluoride) for 15 min. After centrifugation (12,000g for 5 min), supernatants (corresponding to cytoplasmic extracts) were collected and stocked at −70 °C. The nuclear pellets were washed in 200 μl of cell lysis buffer A and resuspended in 50 μl of nuclear extraction buffer B (20 mM HEPES with pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride). The suspension was vortexed for 30 min at 4 °C and centrifuged at 12,000g for 5 min. The supernatant fraction containing nuclear proteins was collected and stocked at −70 °C. Protein concentration was quantified by the Bradford Assay kit (Beyotime Institute of Biotechnology, China).

**Western blotting**

An aliquot of cell lysates containing 30 μg of protein was separated on 10% sodium dodecyl sulfate–polyacrylamide gels, and then transferred to polyvinylidene fluoride membranes (Amersham Biosciences) by electroblotting. The electroblotted membranes were immersed in a blocking solution that contained 5% nonfat dry milk and TBS-T [0.05% Tween 20, 20 mmol/l Tris-HCl, and 150 mmol/l NaCl (pH 7.6)]. Membranes were then incubated overnight at 4 °C with the following primary antibodies: (1) Rabbit polyclonal antibodies to NFAT2(Santa Cruz, USA,1:400); (2) a mouse monoclonal antibody to histone 3 (Abcam, Cambridge, MA,1:250); (3) mouse polyclonal antibodies to Bax (Cell Signaling Technology, USA, 1:1000) and (4) Anti-GAPDH Monoclonal antibodies (Santa Cruz, USA, 1:2000). Negative controls were performed without primary antibody. After washing, the horseradish peroxidase conjugated goat anti-rabbit IgG or goat anti-mouse IgG (Jackson Immuno Research, USA, 1: 5000) was added and incubated for 1 h at room temperature. The immunoblots were washed 3 times with TBS-T, immersed in ECL.
Plus Western Blotting Detection Reagents (Pluslight, Forevergen, China), and then exposed to X-ray film (Kodak, USA). The bands of the resulting autoradiographs were quantified densitometrically using Bandscan software. Protein expression was quantified as the ratio of specific band to histone 3 (nuclear fractions).

Statistical analysis

Data are presented as mean ± standard deviation (SD). For comparison of continuous variables between two groups, statistical significance was assessed by a Student’s t test using the InStat computer program (GraphPad Software, Inc., San Diego, CA). For comparisons between more than two groups, statistical analysis was performed using an ANOVA followed by a Bonferroni multiple comparisons posttest using the InStat program. P-values less than 0.05 were considered significant.

Results

High glucose induced podocyte apoptosis

To determine the effect of high glucose on podocyte apoptosis, podocytes were cultured in DMEM medium containing 5.3 mM glucose (NG group), 5.3 mM glucose plus 14.7 mM mannitol (M group, as an osmotic control) or 20 mM glucose (HG group) for

![Fig. 1 - Effects of HG on podocyte apoptosis. Podocytes were incubated with normal glucose (NG, 5.3 mM), NG+14.7 mM mannitol (MA) or high glucose (HG, 20 mM) for 12, 24 and 48 h respectively. (A) Podocytes were stained with Annexin V/PI for flow cytometry analysis (n = 6). Cells positive for Annexin V-FITC and negative for PI were considered apoptotic. (B) The results were expressed as apoptosis rate (%) and shown using a histogram. (C) The mRNA level of Bax was examined using real-time quantitative RT-PCR analysis and GAPDH mRNA was used as an internal control. Quantitative data was calculated by 2^{-ΔΔCT}. (D) The protein level of Bax was analyzed using western blot analysis (n = 4). (E) Densitometric analysis of three repetitions of the experiment shown in D. Note NG, Normal glucose (5.3 mM) group; HG, High glucose group (20 mM); MA, Normal glucose (5.3 mM)+Mannitol (14.7 mM) group, as an osmolality control; HG+V, High glucose (20 mM)+11R-VIVIT (100 nM, an inhibitor of NFAT2) group. All values are expressed as the mean ± SD. *p < 0.01, HG vs.NG, HG+V, respectively.](image-url)
High glucose induces NFAT2 nuclear accumulation in cultured podocyte.

To determine whether high glucose induces nuclear translocation of NFAT2, we assessed subcellular localization of NFAT2 in cultured podocyte under different exposure by immunoblotting of nuclear fractions and immunofluorescence. Time- and dose-response experiments reveal significantly increased NFAT2 nuclear accumulation after 2-hours exposure to 20 mM HG (Fig. 2A and B). In dose-response experiment, a stronger band corresponding to NFAT2 was observed in the nuclear extracts of the podocyte exposure to 20, 30 and 40 mM glucose (Fig. 2A). Densitometric quantitation of the immunoblots is shown in Fig. 2C. In time-response experiment, a stronger band corresponding to NFAT2 was observed in the nuclear extracts of the podocyte after 2 h exposure to 20 mM HG (Fig. 2B). Densitometric quantitation of the immunoblots is shown in Fig. 2D. Using immunoblotting of nuclear fractions, we also observed that the effects of high glucose on nuclear localization of NFAT2 were also blocked by concurrent treatment with 100 nM 11R-VIVIT, an inhibitor of the NFAT2, and 500 nM CsA, an inhibitor of the phosphatase calcineurin (Fig. 2E,F). Similar results using confocal microscopy to examine localization of NFAT also show that high glucose induce nuclear accumulation in cultured podocyte, and this effect was blocked by concurrent treatment with CsA and 11R-VIVIT.

NFAT2 mediates high glucose-induced apoptosis in podocytes

To further study the role of NFAT2 in high glucose induced podocyte apoptosis, we employed 11R-VIVIT, an inhibitor of NFAT2, to assess the effect of NFAT2 in podocytes apoptosis result from HG stimulation. As shown in Fig. 3A, we observed that the apoptosis-inducing effect of high glucose was abolished by cotreatment with 11R-VIVIT (Fig. 3A). These results suggest that NFAT2 mediates high glucose-induced apoptosis and NFAT2 may potentially play a proapoptotic role in high glucose-induced podocyte apoptosis.

High glucose increase intracellular \( \text{Ca}^{2+} \) concentration in cultured podocytes

To measure changes in the intracellular \( \text{Ca}^{2+} \) concentration caused by high glucose treatment, the levels of \( \text{Ca}^{2+} \) labeled with Fluo-3/AM were determined by LCSM. The results showed that 20 mM high glucose treatment results in a significant increase of \( \text{Ca}^{2+} \) levels in cultured podocyte (NG, 1.00 ± 0.01 vs HG, 1.24 ± 0.10, \( p < 0.01 \), Fig. 4).

Discussion

There is mounting evidence demonstrating that podocytes have a central role in the progression of glomerular diseases \[6,32,33\]. Recently, much work has demonstrated that podocyte apoptosis is an early glomerular phenotype and plays an important role in the pathogenesis of proteinuria and progression of diabetic nephropathy \[9–11,34,35\]. Experimental and clinical studies have shown that a decrease in podocyte number due to apoptosis or detachment leads to proteinuria in diabetic nephropathy \[7,8,11,35\]. Therefore, preventing or inhibiting podocyte apoptosis should become an obvious and promising therapeutic target for treatment of diabetic nephropathy.
In this study, we demonstrated that high glucose (20 mM) could induce podocyte apoptosis in vitro, and along with the upregulation of mRNA and protein Bax. Moreover, we provide the first evidence that podocyte apoptosis under high glucose mediated through activation of NFAT2, which is a substrate for calcineurin (CaN). We also further found that this activation is probably due to the increased intracellular Ca\textsuperscript{2+} concentration induced by high glucose, following activation of calcineurin and NFAT2. These data suggest that CaN/NFAT2/Bax signal pathway may potentially play a proapoptotic role in high glucose-induced podocyte apoptosis.

The NFAT family of transcription factors is composed of four family members NFAT1, NFAT2, NFAT3 and NFAT4, and is best
NFAT proteins are dephosphorylated by CaN, and then translocated from the cytoplasm to the nucleus, and become transcriptionally active [12]. Indeed, we found that the level of NFAT2 expression in the nuclei was dramatically increased at 2 h of podocytes treatment with 20 mM high glucose, the nuclear translocation of NFAT2 is abolished by treatment with the calcineurin inhibitor CsA and the specific NFAT-inhibitor 11R-VIVIT in vitro. The present findings indicate that NFAT2 expressed in podocytes can be activated, i.e. from the cytoplasm into the nuclei by stimulation with high glucose.

The increase in intracellular Ca\(^{2+}\) concentration is presumed to be required for NFAT dephosphorylation by the Ca\(^{2+}\)/CaM-

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**Fig. 3 – Effects of 11R-VIVIT on high glucose-induced podocyte apoptosis.** (A) Podocytes were incubated with normal glucose (NG, 5.3 mM), or high glucose (HG, 20 mM) or normal glucose (5.3 mM)-mannitol (14.7 mM) or HG plus 11R-VIVIT (HG+V) for 48 h respectively. The apoptosis rate was examined using flow cytometry. MA, normal glucose (5.3 mM)-mannitol (14.7 mM); HG+V, HG+11R-VIVIT. (B) The results were expressed as apoptosis cell (%), and shown using a histogram. Values are expressed as the mean±SD. The assays were done in triplicate. *p<0.05, HG vs. NG or HG-V vs. HG, respectively.
dependent protein CaN [12]. Therefore, we further investigated whether high glucose also increased intracellular Ca\(^{2+}\) concentration in cultured podocyte. As expected, in present study, we found there was increased Ca\(^{2+}\) concentration in podocytes following 20 mM high glucose treatment for 5 min. These results indicate that the effect of glucose on NFAT2 activation involves increased intracellular Ca\(^{2+}\) concentration, and subsequent activation of calcineurin and NFAT2.

The NFAT is expressed in many cell types and contributes to diverse cellular functions [12,14–20]. Therefore, it has become increasingly apparent that this pathway also plays an important role in the regulation of a wide variety of cellular responses. Recently a body of evidence has accumulated to suggest that NFAT were implicated in several types of cell apoptosis [15,24,25]. Especially, a role for NFAT in podocyte signaling was also implicated by more recently studies in cultured cells using a luminescent NFAT promoter [36,37]. Whether NFAT are involved in high glucose-mediated podocytes apoptosis? Based on a recent finding that podocyte-specific over-expression of NFAT2 leads to albuminuria and glomerulosclerosis [26]. Therefore, we investigated whether NFAT2 are involved in high glucose-mediated podocyte apoptosis. In present study, we found that nuclei paralleled the timing of the onset of apoptosis as detected by annexin-V labeling. Meanwhile, we also found that the effects of high glucose induced podocyte apoptosis is blocked by concomitant treatment with 11R-VIVIT. This further suggests that NFAT2 is implicated in high glucose-induced podocytes apoptosis.

To further explore the mechanisms of NFAT2 mediated high glucose-induced podocyte apoptosis, we next investigated the expression of Bax, which is a well recognized indicator of apoptosis [31,32]. The mRNA and protein expression levels of Bax were significantly increased in podocytes with HG stimulation, and this effect was abrogated cotreatment with CsA and 11R-VIVIT. Similar to the apoptosis results, mannitol had no effect on the mRNA and protein expression of Bax.

Taken together, here, for the first time, we investigated whether hyperglycemia activates NFAT2 in cultured mouse podocyte and whether this leads to podocyte apoptosis. We also further explore the mechanism of the activation of NFAT2 induced by high glucose in cultured podocytes. Our results indicate that high glucose induced apoptosis in a time-dependent manner in cultured podocytes. The apoptosis inducing effect of high glucose was abolished by cotreatment with 11R-VIVIT. High glucose also significantly increased NFAT2 nuclear accumulation, and this effect was completely blocked by 11R-VIVIT, and was also blocked by the calcinerin inhibitor CsA. In addition, we also found that high glucose increased intercellular Ca\(^{2+}\) concentration, and subsequent activation of calcineurin and NFAT2. These results suggest that CaN/NFAT2/Bax signal pathway may present a promising target for preventing podocyte apoptosis under hyperglycemia conditions.

**Authors’ contributions**

W. Shi and R.Z Li designed the study; R.Z Li and L. Zhang performed all the experiments, L. Zhang wrote the paper. All the authors read and approved the final manuscript.

**Conflict of interest statement**

None of the authors has any potential financial conflict of interest related to this manuscript.

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