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Decreased TRPM7 alleviates high glucose-induced renal tubular epithelial cell injury by inhibiting the HMGB1/TLR4 signaling pathway

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ABSTRACT

Objective: To explore the regulatory mechanism of transient receptor potential melastatin-7 (TRPM7) in high glucose-induced renal tubular epithelial cell injury.

Methods: The expression of TRPM7 in the serum of diabetic nephropathy patients and high glucose-induced HK-2 cells was detected by RT-qPCR. Then, the TRPM7 interference vector was constructed, and the downstream high mobility group box 1 (HMGB1)/Toll-like receptor 4 (TLR4) signaling pathway proteins were detected. Next, in addition to interference with TRPM7 expression, overexpression of HMGB1 in high glucose-induced HK-2 cells was performed. Cell activity, apoptosis, oxidative stress levels, and inflammation levels were determined by CCK8, TUNEL, Western blotting, immunofluorescence and related kits.

Results: TRPM7 expression was upregulated in the serum of diabetic nephropathy patients and high glucose-induced HK-2 cells. Interference with TRPM7 reduced cell damage, epithelial-mesenchymal transition, oxidative stress, and inflammatory response in high glucose-induced HK-2 cells *via* inhibiting the HMGB1/TLR4 signaling pathway. However, the effects induced by TRPM7 silencing were abrogated by HMGB1 overexpression.

Conclusions: Decreased TRPM7 alleviates high glucose-induced renal tubular epithelial cell injury by inhibiting the HMGB1/TLR4 signaling pathway. Further animal experiments and clinical trials are warranted to verify its effect.

KEYWORDS: Diabetic nephropathy; TRPM7; HMGB1/TLR4; High glucose; Renal tubular epithelial cell

1. Introduction

Diabetes is a type of metabolic disease accompanied by hyperglycemia. The prolonged course of diabetes often brings about chronic damage to other organs[1,2]. Diabetic nephropathy (DN) is the most harmful inflammatory complication and also the main microvascular complication of diabetes[3,4]. In diabetic patients, renal complications account for about 25%, among which end-stage renal disease is acknowledged to be primarily caused by DN in developed countries[5]. Therefore, studying the pathogenesis of DN is of great significance for early intervention, as well as effective prevention and treatment of disease progression.

Transient receptor potential melastatin-7 (TRPM7) is an ion

Significance

Transient receptor potential melastatin-7 (TRPM7) is abnormally expressed in diabetic nephropathy, but the role of TRPM7 in diabetic nephropathy and its regulatory mechanism have not been reported. This study shows that decreased TRPM7 alleviates high glucose-induced renal tubular epithelial cell injury by inhibiting the HMGB1/TLR4 signaling pathway.

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channel protein intensively expressed in various tissues, organs, and cells in mammals and vertebrates[6]. TRPM7 has been found to mediate renal injury, endothelial hyperpermeability, and mortality during endotoxemia[7]. TRPM7 is upregulated in renal ischemia-reperfusion injury and is considered a potential biomarker of ischemia-reperfusion-induced acute kidney injury[8,9]. These results indicated that inhibition of TRPM7 had an important renal protective function. In addition, a previous study has found that TRPM7 also plays an important regulatory role in diabetes[10]. High glucose (HG) can upregulate the expression of TRPM7 in human monocytes[11]. Interference with TRPM7 inhibits HG-induced endoplasmic reticulum stress and cell damage in neurons[12]. However, no relevant studies on TRPM7 in DN have been reported so far. In addition, the current research on targeted therapy of diseases is a hot spot. A previous study has shown that the TRPM7 channel can be used as a new therapeutic target for pulmonary hypertension[13]. Moreover, targeted silencing of TRPM7 ion channel induces replicative senescence and produces enhanced cytotoxicity with gemcitabine in pancreatic adenocarcinoma[14]. Therefore, in DN disease, targeting TRPM7 to improve DN disease may be an effective treatment.

It was also found that interference with TRPM7 reduced the expression of high mobility group box 1 (HMGB1) in renal ischemia-reperfusion[8]. HMGB1, a greatly conserved nuclear non-histone protein, is accumulated in the kidney and other important organs. HMGB1 can also be implicated in the initiation and development of various inflammatory diseases by serving as a proinflammatory cytokine[15,16]. Moreover, blocking HMGB1 can ameliorate DN in mice[9] and inhibition of the HMGB1/TLR4 signaling pathway can reduce diabetic kidney injury[17]. Therefore, this study aimed to investigate the role of TRPM7 in HG-induced renal tubular epithelial cell injury and elucidate the underlying mechanisms.

2. Materials and methods

2.1. Samples from DN patients

Sera were obtained from randomly selected DN patients (n=15) who were admitted to the People's Hospital of Qijiang District (Chongqing, China) between 2021 and 2022. All patients have signed the informed consent, and the Ethics Committee of the People's Hospital of Qijiang District has approved the research protocols. The clinical data of patients are shown in Supplementary Table 1.

2.2. Cell culture

Human renal tubular epithelial HK-2 cells were cultured in Dulbecco's modified Eagle' s medium (Invitrogen) with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Invitrogen) at 37℃, 5% CO_2. There were three groups as follows: 5.5 mM glucose was given to the control group for 24 h; the mannitol group (MA) was given 5.5 mM glucose, together with 24.5 mM MA for 24 h, and the HG group was given 30 mM glucose for 24 h[18].

2.3. Quantitative reverse transcription-PCR (RT-qPCR)

Trizol reagent (Applied Biosystems) was used to accomplish the isolation of total RNA. cDNA was generated from 1 μg of total RNA using the Bio-rad iScript™ reverse-transcription system. Then, amplification of the cDNA was performed on a Bio-Rad CFX96 Touch™ Real-Time PCR Detection System using the iQ SYBR® Green Supermix protocol. Relative mRNA levels were measured using the $2^{-\Delta\Delta Ct}$ method[19] and normalized by *GAPDH*. Primer sequences were as follows: *TRPM7* forward: 5'-GGAGGAGTTGGTCGCACAAT-3', reverse: 5'-TCCGCCCCATACTTTCCAAC-3'; *HMGB1* forward: 5'-CATCTCAGGGCCAAACCGAT-3', reverse: 5'-CCTCTTGGGTGCATTGGGAT-3'; *GAPDH* forward: 5'-AATGGGCAGCCGTTAGGAAA-3', reverse: 5'-GCGCCCAATACGACCAAATC-3'.

2.4. Cell Counting Kit-8 (CCK-8)

After HG induction or indicated plasmid transfection, a CCK-8 kit (Beyotime) was adopted to detect cell viability[20]. Briefly, the CCK-8 solution was transferred to the medium at 37℃ for 4 h in a humid atmosphere with 5% CO_2. A microplate reader provided by Bio-Rad Laboratories Inc. was used to record A_{450}.

2.5. Western blotting assay

Isolation of protein was performed before centrifugation at 12000 ×g for 10 min at 4℃. A bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc.) was used to detect the protein concentrations. In brief, 30 μg of proteins on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were transferred onto PVDF membranes before 5% bovine serum albumin impeding. Then the membranes were incubated with primary antibodies including TRPM7 (1:1000, ab245408, Abcam), HMGB1 (1:1000, ab18256, Abcam), TLR4 (1:1000, ab13556, Abcam), NF-κB (1:1000, ab32360, Abcam), p-NF-κB (1:1000, ab194729, Abcam), Bcl-2 (1:1000, ab182858, Abcam), Bax (1:1000, ab32503, Abcam), cleaved-caspase-3 (1:1000, ab32042, Abcam), caspase-3 (1:1000, ab32351, Abcam), cleaved-caspase-9 (1:1000, # 20750S, Cell Signaling Technology), caspase-9 (1:1000, ab32539, Abcam), E-cadherin (1:1000, ab40772, Abcam), vimentin (1:1000, ab92547, Abcam), alpha-smooth muscle actin (α-SMA)

(1∶1 000, ab5694, Abcam), and β-actin (1∶1 000, ab8227, Abcam) overnight at 4 ℃. Horseradish peroxidase conjugated anti-rabbit or anti-mouse IgG was added for 1 h at room temperature on the second day. Lastly, the bands on the membrane were made visually using the super ECL reagent (Applygen Technologies Inc., Beijing, China) and analyzed with ImageJ (Version146, National Institutes of Health). β-actin was used as the loading control[21].

2.6. Cell transfection

The used siRNAs against TRPM7 in the current study target the mRNA sequence coding for the 170-188th N-terminal region of TRPM7. In brief, 1×10^6 HK-2 cells were transfected with 2 μg si-TRPM7#1/2 or si-NC (corresponding to a final concentration of 1.5 μM) employing Lipofectamine 2000 procured from Invitrogen. TRPM7 siRNA were as follows: Forward 5′-GUCUUGCCAUGAAAUACUCUU-3′ and reverse 5′-GAGUAUUUCAUGGCAAGACUU-3′ (si-TRPM7 #1), and forward 5′-AGGAGAAGAUGCAAUUAAATT-3′ and reverse 5′-UUUAAUUGCAUCUUCUCCUAG-3′ (si-TRPM7 #2). Si-NC was as follows: Forward 5′- UUCUCCGAACGUGUCACG UTT-3′ and reverse 5′-ACGUGACACGUUCGGAGA ATT-3′.

The synthetic HMGB1 overexpression plasmid (oe-HMGB1) and oe-NC were purchased from Zoman (Beijing, China). Oe-HMGB1 and oe-NC were transfected into HK-2 cells by Lipofectamine 3000 (Thermo Fisher Scientific). At 48 h after transfection, subsequent experiments were conducted.

2.7. Terminal deoxynucleotidyl transferase dUTP nick–end labeling (TUNEL) assay

After HG induction and indicated plasmid transfection, the cells received immobilization after the addition of 4% paraformaldehyde. TUNEL signals were examined by In Situ Cell Death Detection kit (Roche Molecular Biochemicals)[22].

2.8. Immunofluorescence

Treated HK-2 cells received immobilization with 4% paraformaldehyde for half an hour. Before cultivation with specific α-SMA antibody at 4 ℃ overnight, cells were permeabilized by Triton X-100 for 20 min. After the addition of Alexa Fluor 555 donkey anti-rabbit IgG, α-SMA protein was visualized with a single-photon excitation scanning confocal microscope system (Nikon, Tokyo, Japan) after DAPI staining for 5 min[23].

2.9. Measurement of reactive oxygen species (ROS) production

HK-2 cells were seeded (1×10^6 cells/mL) into 6-well plates and the intracellular ROS level was measured using the oxidative conversion of cell permeable 2′,7′-dichlorofluorescein diacetate (DCFH□DA, Beyotime) to fluorescent dichlorofluorescein (DCF) after indicated treatment. DCFH□DA was added for half an hour at 37 ℃, then ROS level was examined[24].

2.10. ELISA

Interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1beta (IL-1β) levels in the supernatant of cells were determined by ELISA. The levels of malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) were detected by the corresponding kits (Beyotime) according to the protocol.

2.11. Statistical analysis

The data were expressed as mean±standard deviation (SD). A one-way ANOVA or Student's *t*-test was conducted using GraphPad Prism 6.0 software (La Jolla, CA, USA). P-value < 0.05 was considered significantly different.

3. Results

3.1. TRPM7 displays increased expression in DN patients and HG–induced HK–2 cells

RT-qPCR results showed that the expression of *TRPM7* in the DN group was markedly enhanced compared with the normal group (Figure 1A). After HK-2 cells were induced by HG, the expression of TRPM7 in the cells was detected by RT-qPCR and Western blotting analyses. The results showed that there was no significant difference in TRPM7 expression between the MA group and the control group. However, compared with the MA group, the expression of TRPM7 in the HG group was obviously augmented (Figure 1B and C).

3.2. Interference with TRPM7 inhibits the HMGB1/TLR4 signaling pathway in HG–induced HK–2 cells

After transfection of TRPM7 silencing plasmids, the results of RT-qPCR and Western blotting assays showed that the si-TRPM7#2 group had a more significant knockdown efficacy, so si-TRPM7#2 was chosen for subsequent experiments (Figure 2A and B). To measure the effect of TRPM7 on the HMGB1/TLR4 signaling, Western blotting was performed. HG administration remarkably enhanced HMGB1, TLR4, and p-NF-κB expression compared with the MA group. However, TRPM7 silencing decreased HMGB1, TLR4, and p-NF-κB expression under HG conditions (Figure 2C).

3.3. Interference with TRPM7 mitigates HG-induced HK-2 cell damage by inhibiting the HMGB1/TLR4 signaling pathway

To deeply uncover the mechanism, HMGB1 was overexpressed in HK-2 cells. RT-qPCR and Western blotting results demonstrated that the expression of HMGB1 in the oe-HMGB1 group was significantly upregulated compared with that in the oe-NC group (Figure 3A and B), indicating that cell transfection was successful. Therefore, cells were divided into the control, MA, HG, HG + si-NC, HG + si-TRPM7#2, HG + si-TRPM7#2 + oe-NC, and HG + si-TRPM7#2 + oe-HMGB1 groups. CCK-8 results corroborated that HG treatment prominently inhibited cell viability in comparison with the MA group. Compared with the HG + si-NC group, TRPM7 silencing increased cell viability. Compared with the HG + si-TRPM7#2 + oe-NC group, the cell viability in the HG + si-TRPM7#2 + oe-HMGB1 group was decreased (Figure 3C). According to TUNEL assay, HG administration promoted cell apoptosis, accompanied with lessened Bcl-2, and enhanced Bax, cleaved-caspase-3, and cleaved-caspase-9 expression compared with the MA group. In HG-treated HK-2 cells, apoptosis was mitigated by TRPM7 depletion, accompanied by elevated Bcl-2 and downregulated Bax, cleaved-caspase-3, and cleaved-caspase-9 expression. On this basis, apoptosis was significantly increased after the transfection of HMGB1 overexpression plasmid, accompanied by lessened Bcl-2 and augmented Bax, cleaved-caspase-3, and cleaved-caspase-9 expression (Figure 3D, E, and F).

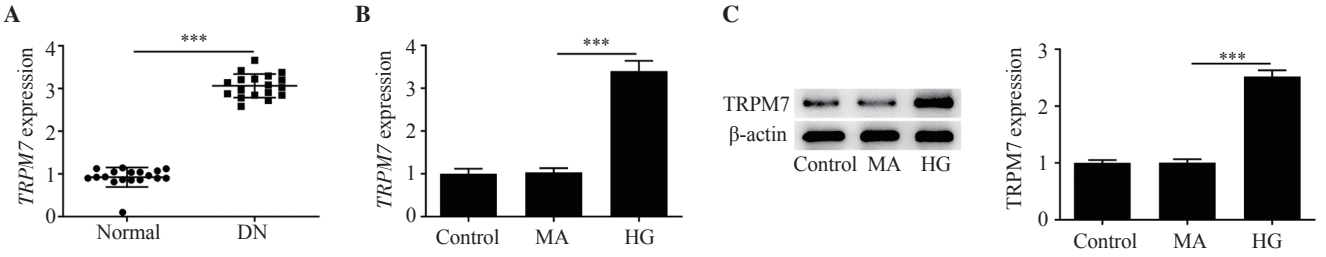

Figure 1. TRPM7 expression is increased in diabetic nephropathy (DN) patients and high glucose (HG)-induced HK-2 cells. The mRNA expression of *TRPM7* was detected (A) in the serum of DN patients and (B) in HG-induced HK-2 cells by RT-qPCR. (C) The protein expression of TRPM7 was detected in HG-induced HK-2 cells by Western blotting. The data are expressed as mean±standard deviation (SD) and analyzed by Student's *t*-test or one-way ANOVA. $^{***}P<0.001$. MA: mannitol.

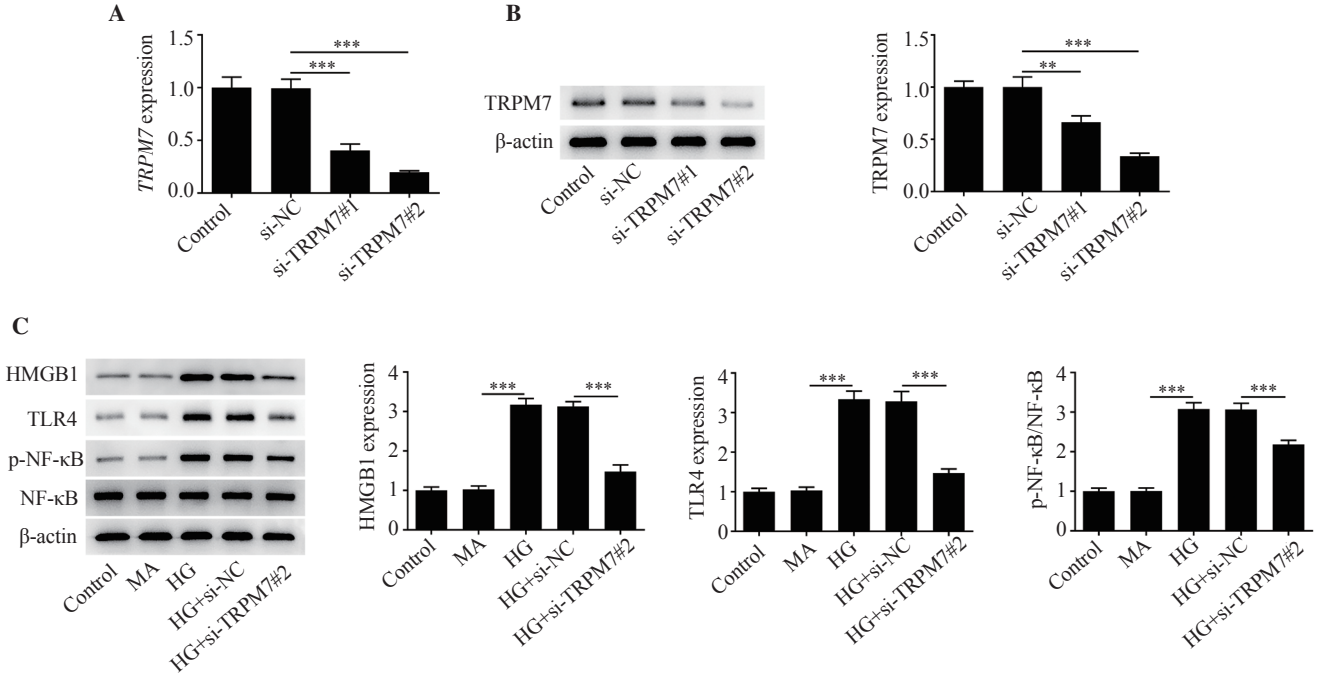

Figure 2. Interference with TRPM7 inhibits the HMGB1/TLR4 signaling pathway in HG-induced HK-2 cells. (A-B) The mRNA and protein expression of TRPM7 was detected by RT-qPCR and Western blotting assays in HG-induced HK-2 cells after TRPM7 silencing. (C) The expression of proteins related to the HMGB1/TLR4 pathway was analyzed in HG-treated HK-2 cells by Western blotting. The data are expressed as mean±SD and analyzed by one-way ANOVA. $^{**}P<0.01$, $^{***}P<0.001$.

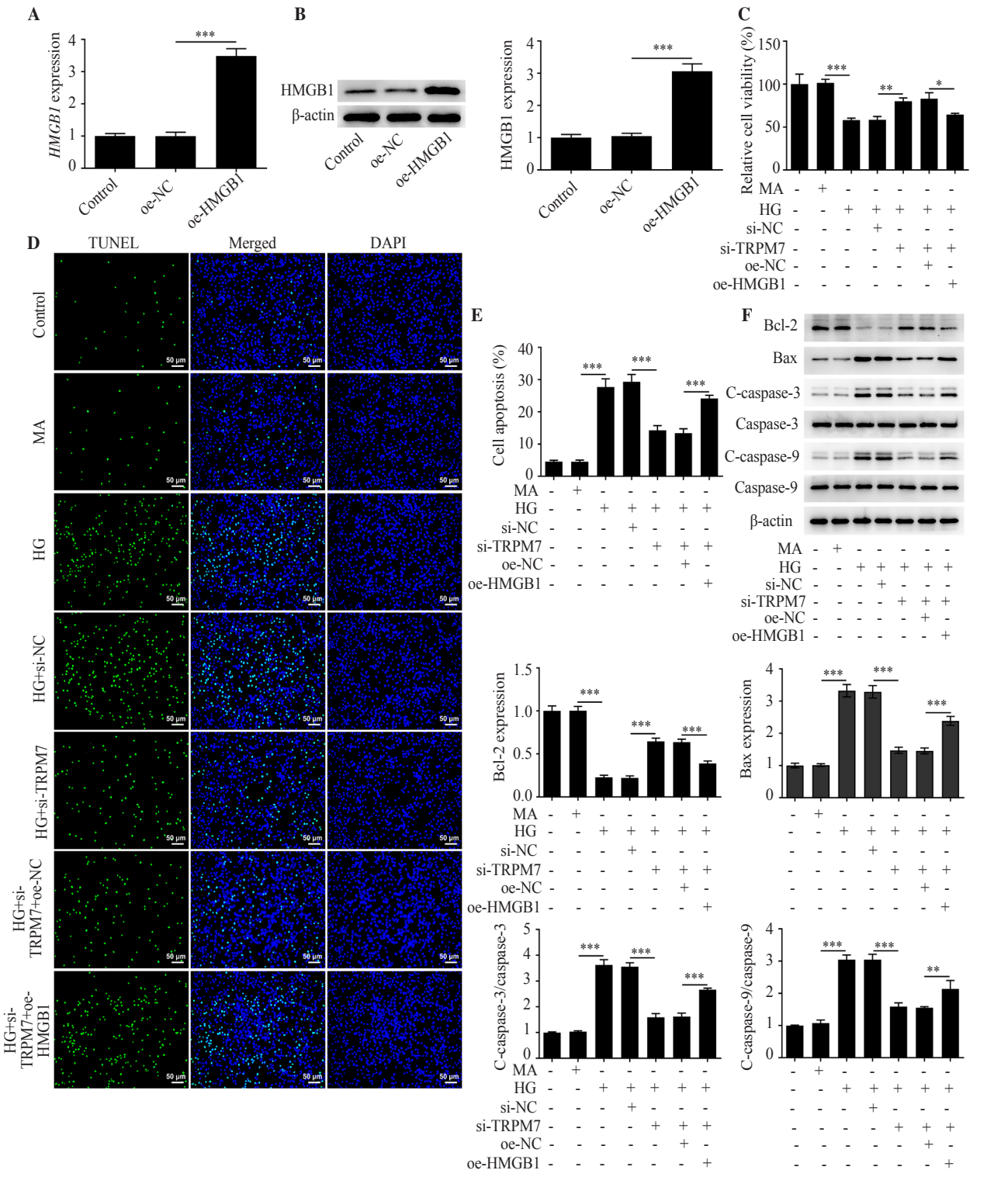

Figure 3. Interference with TRPM7 mitigates HG-induced HK-2 cell damage by inhibiting the HMGB1/TLR4 signaling pathway. (A-B) The mRNA and protein expression of HMGB1 was detected in HG-induced HK-2 cells by RT-qPCR and Western blotting assays. (C) The viability of HG-induced HK-2 cells was determined by CCK-8 assay. (D) The apoptosis of HG-treated HK-2 cells was detected by TUNEL assay (magnification: ×200). (E) The percentage of cell apoptosis. (F) The expression of apoptosis-related proteins was determined in HG-induced HK-2 cells by Western blotting. The data are expressed as mean±SD and analyzed by one-way ANOVA. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. C-caspase-3: cleaved-caspase-3; C-caspase-9: cleaved-caspase-9.

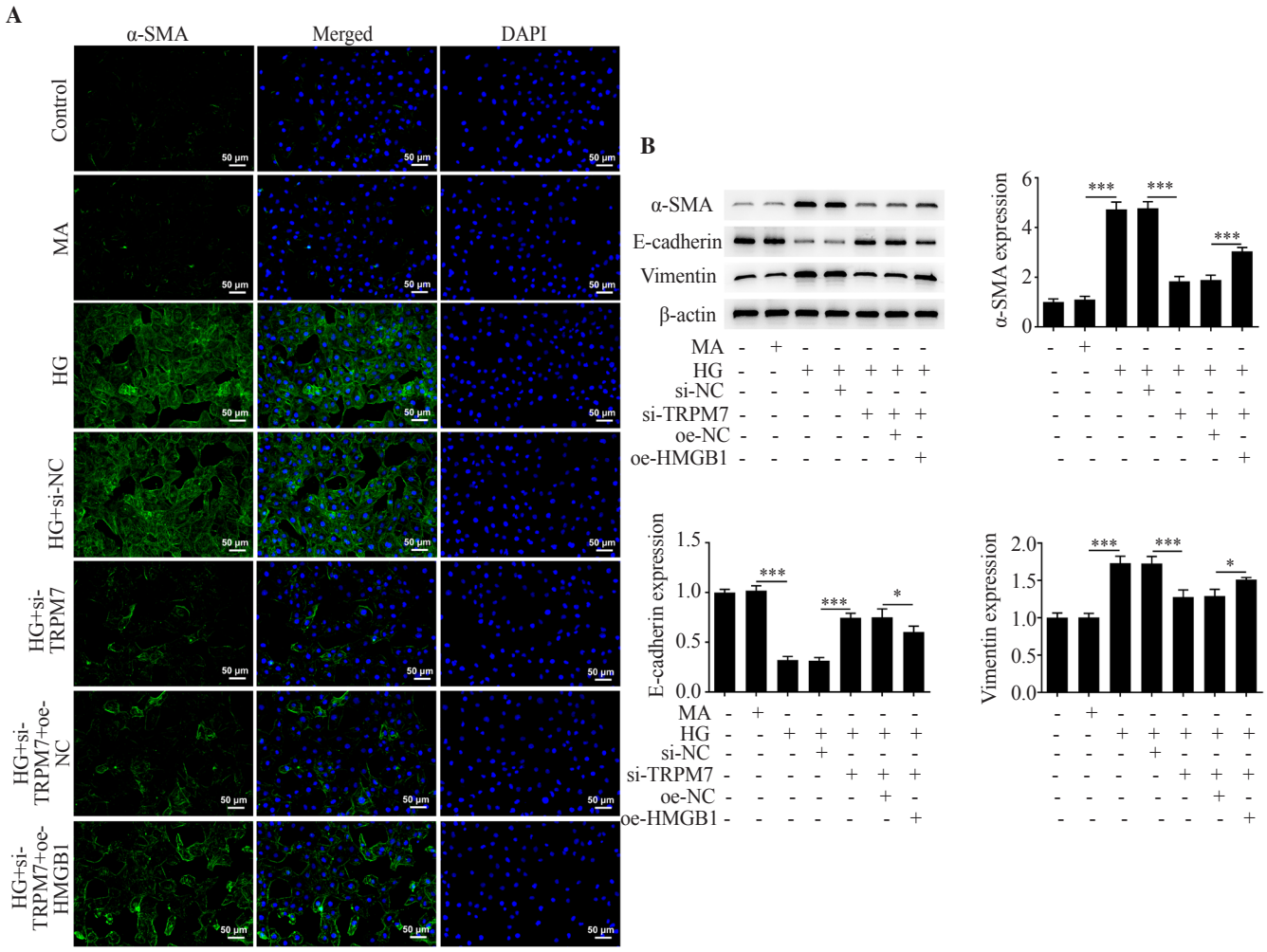

Figure 4. Interference with TRPM7 reduces epithelial-mesenchymal transition in HG-induced HK-2 cells by inhibiting the HMGB1/TLR4 signaling pathway. (A) α-SMA expression was examined by immunofluorescent assay (magnification: ×200). (B) The expression of epithelial-mesenchymal transition-related proteins was determined in HG-induced HK-2 cells by Western blotting. The data are expressed as mean±SD and analyzed by one-way ANOVA. $^{*}P<0.05$, $^{***}P<0.001$.

3.4. Interference with TRPM7 reduces epithelial–mesenchymal transition (EMT) in HG–induced HK–2 cells by inhibiting the HMGB1/TLR4 pathway

The results of immunofluorescent assay displayed that HG condition upregulated the expression of α-SMA in comparison with the MA group. α-SMA expression in the HG+si-TRPM7#2 group was significantly decreased compared with the HG + si-NC group. Compared with the HG + si-TRPM7#2 + oe-NC group, the expression of α-SMA in the HG + si-TRPM7#2 + oe-HMGB1 group was significantly upregulated (Figure 4A). These results were further confirmed by Western blotting. HG greatly upregulated the expression of α-SMA and vimentin while dramatically diminishing the expression of E-cadherin. TRPM7 silencing noticeably diminished the expression of α-SMA and vimentin and enhanced the expression of E-cadherin. Furthermore, HMGB1 overexpression reversed these effects induced by TRPM7 silencing (Figure 4B).

3.5. Interference with TRPM7 reduces oxidative stress and inflammatory response in HG–induced HK–2 cells by inhibiting the HMGB1/TLR4 pathway

ROS level was evidently increased after HG induction according to DCFH-DA fluorescence results. However, inhibition of TRPM7 expression in HG-induced HK-2 cells significantly reduced ROS level, which was reversed by HMGB1 elevation (Figure 5A). Moreover, the expression of MDA was obviously upregulated by HG stimulation, whereas the expression of SOD and CAT displayed an opposite trend. However, inhibition of TRPM7 expression in HG-induced HK-2 cells significantly reduced the expression of MDA, while fortifying SOD and CAT expression, which was abrogated by HMGB1 elevation (Figure 5B). HG also stimulated inflammatory response, as shown by elevated TNF-α, IL-6, and IL-1β. TRPM7 interference reduced TNF-α, IL-6, and IL-1β levels, which were increased by HMGB1 overexpression (Figure 6).

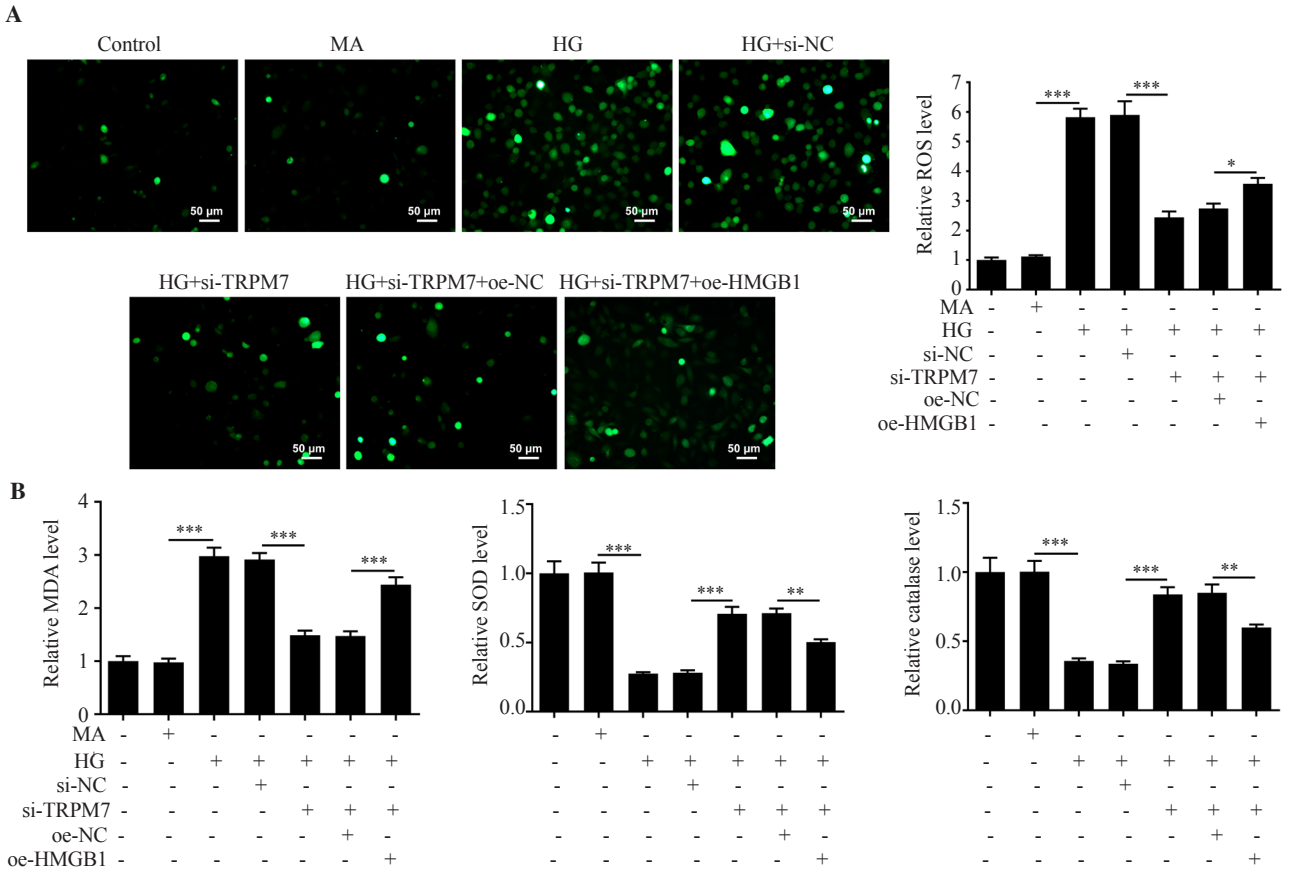

Figure 5. Interference with TRPM7 reduces oxidative stress in HG-induced HK-2 cells by inhibiting the HMGB1/TLR4 signaling pathway. (A) ROS levels were detected by DCFH-DA fluorescence (magnification: ×200). (B) The expression levels of oxidative stress-related factors were detected by the related kits. The data are expressed as mean±SD and analyzed by one-way ANOVA. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. ROS: reactive oxygen species; MDA: malondialdehyde; SOD: superoxide dismutase.

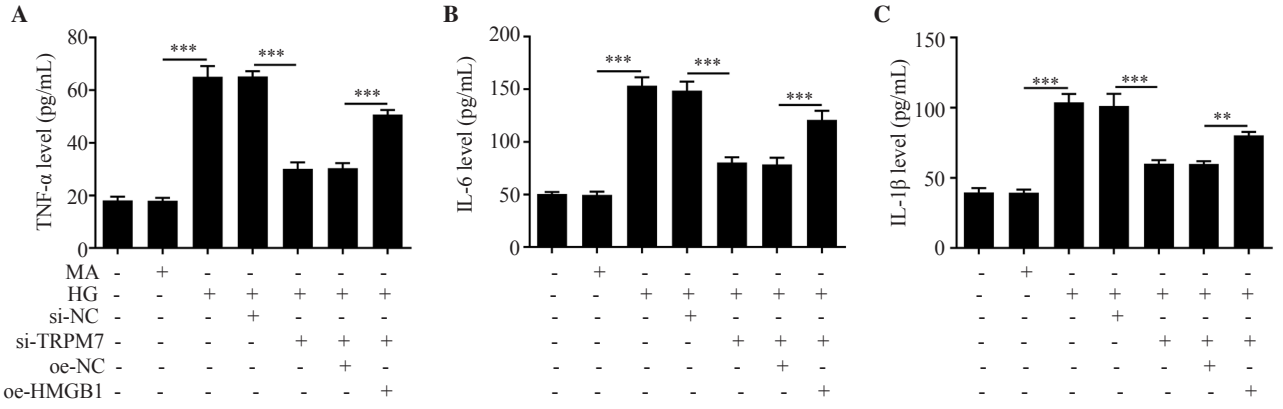

Figure 6. Interference with TRPM7 reduces inflammatory response of HG-induced HK-2 cells by inhibiting the HMGB1/TLR4 signaling pathway. The levels of (A) TNF-α, (B) IL-6, and (C) IL-1β were determined by ELISA kits. The data are expressed as mean±SD and analyzed by one-way ANOVA. $^{**}P<0.01$, $^{***}P<0.001$.

4. Discussion

The complicated pathogenesis of DN involves renal interstitial damage, metabolism, hemodynamic changes, and many other factors[25]. Renal tubular epithelial cell injury occupies a critical position in the etiology of DN, as one of the markers of DN pathological injury, and is closely related to clinical indicators of renal impairment[26]. A previous study has shown that inhibiting renal tubular injury can effectively alleviate the progression of DN to end-stage renal disease[27,28]. In addition, it has been pointed out that apoptosis and oxidative damage of renal tubular cells can be observed in the early stage of DN[29]. In the present study, renal

tubular epithelial cells were damaged under HG conditions to form a DN cell model.

EMT of renal tubular epithelial cells is one of the most important promoters of early DN and a key factor of nephron loss in DN[30]. Mesenchymal phenotypic markers represented by α-SMA and vimentin and epithelial phenotypic markers represented by E-cadherin were abnormally expressed in renal tubular epithelial cells with EMT[31]. α-SMA and vimentin proteins are the markers of myofibroblasts, and myofibroblasts-secreted collagen is a crucial source of extracellular matrix. Increased α-SMA and vimentin expression during extracellular matrix leads to increased deposition of ECM proteins[32]. E-cadherin is strongly responsible for maintaining the morphology and adhesion of epithelial cells, and E-cadherin expression is in a downtrend during EMT, resulting in the adhesion loss of epithelial cells[33]. In this experiment, α-SMA, vimentin, and E-cadherin proteins were detected to explore the alternations in EMT levels. HG caused abnormal expression of these proteins.

In the present study, the expression of TRPM7 was discovered to be markedly increased in the serum of DN patients. TRPM7 is engaged in cellular events and gene expression to exert significant properties in cancers[34–36]. A recent study has shown that the expression of TRPM7 is significantly increased in endoplasmic reticulum stress and HG-induced neuron injury, and TRPM7 overexpression exacerbates HG-induced endoplasmic reticulum stress and cell injury[12]. In addition, it has been reported that specific inhibitors of TRPM7 can prevent renal atrophy in unilateral ureteral obstruction kidneys and preserve tubular formation[37]. Inhibition of TRPM7 can reduce kidney transplantation injury[38]. However, the role of TRPM7 in DN and the effect of TRPM7 on HG-induced renal tubular epithelial cell injury and EMT have not been reported so far. The present study showed that HG remarkably fortified the expression of TRPM7 in HK-2 cells. In addition, it was found that inhibition of TRPM7 expression could significantly inhibit the apoptosis, EMT, oxidative stress, and inflammation in HK-2 cells upon exposure to HG. Taken together, silencing TRPM7 expression could significantly reduce HG-induced HK-2 cell damage, thus mitigating the development of DN disease.

With the in-depth development of gene research, diseases have entered the era of targeted therapy[39]. Transient receptor potential (TRP) channels have been shown to make important contributions to the pathophysiology of a variety of cancers and can be used as targets for disease therapy[40]. In addition, TRPM7 is one of the important non-glutamate mechanisms in stroke. At present, some scholars have developed ischemic stroke drugs targeting the inhibition of TRPM7, providing evidence that TRPM7 is a promising target for stroke treatment[41]. Therefore, based on the findings of our study, TRPM7 can be used as a therapeutic target for DN diseases. Targeted inhibition of TRPM7 can alleviate cell apoptosis, inflammatory response, and oxidative stress in DN, thereby improving the effect on DN disease. However, whether TRPM7's targeted treatment of DN disease can be used as an alternative to existing therapies still needs further animal experiments and clinical trials.

It was reported that TRPM7 was positively correlated with HMGB1 in ischemia-reperfusion injury diseases[9]. It has shown that TRPM7 can regulate the expression of HMGB1 in renal injury models, thus modulating inflammation and apoptosis[8]. HG-induced release of HMGB1 can induce renal injury in diabetic rats[42]. Moreover, lipopolysaccharides triggered TRPM7-dependent Ca^{2+} elevations essential for TLR4 endocytosis and the subsequent activation of the transcription factor IRF3, which identified the TRPM7 as a central component of the TLR4 signaling[43]. TRPM7 mediates endotoxin regulation of the TLR4/NoX-2 /ROS/NF-κB signaling pathway to affect endothelial permeability and mortality in sepsis[7]. In addition, inhibition of the HMGB1-TLR4-NF-κB pathway could ameliorate streptozotocin-induced oxidative kidney injury[44]. However, the regulatory effects and mechanisms of TRPM7, HMGB1, and TLR4 in DN diseases have not been reported so far. The present study showed that HMGB1 expression was significantly increased in HG-induced HK-2 cells. The downstream mechanism of TRPM7 regulation on HG-induced HK-2 cells was further explored by simultaneously inhibiting the expression of TRPM7 and overexpressing HMGB1. It was noted that interference with TRPM7 inhibited the HMGB1/TLR4 signaling in HG-induced HK-2 cells, thus attenuating HK-2 cell damage, EMT, oxidative stress, and release of inflammatory cytokines.

However, there are some limitations in our article. The experiment was only carried out in HK-2 cells, without verification in other cell lines. Further experiments will be conducted with other cell lines. In addition, the role of TRPM7 in DN diseases was only verified in a cell model. Animal experiments and clinical trials should be carried out to further confirm its effect.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Authors' contributions

HC and FMG wrote the manuscript and analyzed the data. HC, FMG, and ZYC performed the experiments and supervised the study.

WF searched the literature and revised the manuscript for important intellectual content. HC confirmed the authenticity of all the raw data.

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