Inhibition of TRIM32 Attenuates the Apoptosis, Oxidative Stress and Inflammatory Injury of Podocytes Induced by High Glucose by Affecting the Akt/GSK-3 β /Nrf2 Pathway

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Abstract

Hyperglycemia-induced oxidative stress of podocytes exerts a major role in the pathological process of diabetic nephropathy. Tripartite motif-containing protein 32 (TRIM32) has been reported as a key protein in the modulation of cellular apoptosis and oxidative stress under various pathological processes. However, whether TRIM32 participates in the regulation of high glucose (HG)-induced injury in podocytes has not been investigated. The aims of this work were to assess the possible role of TRIM32 in mediating HG-induced apoptosis, oxidative stress and inflammatory response in podocytes *in vitro*. Herein, our results showed a marked increase in TRIM32 expression in HG-exposed podocytes. Loss-of-function experiments showed that the knockdown of TRIM32 improved the viability of HG-stimulated podocytes, and suppressed HG-induced apoptosis, oxidative stress and inflammatory response in podocytes. Further investigation revealed that the inhibition of TRIM32 enhanced the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) signaling associated with modulation of the Akt/glycogen synthase kinase-3 β (GSK-3 β) axis in podocytes following HG exposure. However, the suppression of Akt abrogated the TRIM32-knockdown-mediated activation of Nrf2 in HG-exposed podocytes. In addition, the knockdown of Nrf2 markedly abolished the TRIM32-inhibition-induced protective effects in HG-exposed podocytes. In summary, the results of this work show that the inhibition of TRIM32 protects podocytes from HG-induced injury by potentiating Nrf2 signaling via the modulation of Akt/GSK-3 β signaling. This study indicates a potential role of TRIM32 in mediating podocyte injury during the progression of diabetic nephropathy.

Introduction

Diabetic nephropathy is a severe complication of diabetes and represents a major contributor to end-stage renal disease [1]. Diabetic nephropathy is characterized by glomerular injury and accompanied by proteinuria [2]. Unfortunately, up to 40% of diabetic patients will eventually develop diabetic nephropathy [3]. However, the treatment of diabetic nephropathy remains a challenge in current years. Glomerular podocytes, major components of the glomerular filtration barrier, plays a vital role in the progression of diabetic nephropathy [4]. Persistent exposure to hyperglycemia evokes podocyte injury via the induction of apoptosis, oxidative stress, and inflammatory response, which is related to the pathogenesis of diabetic nephropathy [5–7]. However, the molecular mechanisms underlying hyperglycemia-evoked podocyte injury remain elusive. Therefore, a better understanding of the molecular mechanisms responsible for hyperglycemia-evoked podocyte injury may offer a new opportunity for the exploitation of innovative therapeutic options for diabetic nephropathy.

Tripartite motif-containing protein 32 (TRIM32) is a member of the TRIM family, which is related to a number of physiological and pathological processes [8]. TRIM32 harbors the typical RING domain, conferring E3 ligase activity, and is capable of ubiquitinating various substrates [9–11]. TRIM32 is widely expressed in adult tissues, and plays a multi-faceted role in diverse processes [12]. The dysregulation of TRIM32 is implicated in numerous pathological conditions, including myopathy, cardiomyopathy, neurological disorder and cancers [13–20]. Moreover, TRIM32 participates in the regulation of diverse

cellular activities, including differentiation, proliferation, apoptosis, oxidative stress and inflammatory response [21–24].

Nuclear factor erythroid 2-related factor 2 (Nrf2) plays an essential role in organizing the cellular protection network under adverse stimulus [25]. Nrf2 translocates to the nucleus where it is capable of binding to an antioxidant-response-element (ARE) in gene promoters to induce the expression of cytoprotective target genes [26]. Nrf2 is involved in plenty of pathological conditions by affecting apoptosis, oxidative stress and inflammatory responses [27–30]. Notably, increasing evidence has shown that Nrf2 exerts a key role in the pathogenesis of diabetic nephropathy [31–33]. The activation of Nrf2 ameliorates high glucose (HG)-induced apoptosis, oxidative stress and the inflammation of podocytes, which is conducive to curing diabetic nephropathy [34–36]. The activation of Nrf2 is modulated via various factors, such as Keap1 and Akt/glycogen synthase kinase-3 β (GSK-3 β) [37]. However, the regulation of Nrf2 activation in diabetic nephropathy remains poorly understood.

To date, whether TRIM32 plays a role in diabetic nephropathy is unknown. In this study, we aimed to elucidate the potential role of TRIM32 in regulating podocyte injury induced by HG. Our results showed marked increases in TRIM32 expression in podocytes following HG exposure. Functional studies showed that the inhibition of TRIM32 improved the viability of HG-stimulated podocytes, but suppressed HG-induced apoptosis, oxidative stress and the inflammatory response in podocytes. Further investigation revealed a regulatory effect of TRIM32 on Nrf2 signaling in podocytes following HG exposure. However, the suppression of Akt abrogated the TRIM32-knockdown-mediated activation of Nrf2 in HG-exposed podocytes. In addition, the knockdown of Nrf2 markedly abolished TRIM32-inhibition-induced protective effects in HG-exposed podocytes. Taken together, these results show that the inhibition of TRIM32 protects podocytes from HG-induced injury by potentiating Nrf2 signaling via modulation of the Akt/GSK-3 β axis.

Materials And Methods

Cell Culture and HG Treatment. The conditionally immortalized mouse podocyte cell line MPC5 was provided by BeNa Biotechnology (Beijing, China). The mouse podocytes were maintained in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and 10 U/ml interferon- γ at 33°C for cell proliferation. To induce differentiation, the medium was replaced with fresh medium without interferon- γ and cells were cultivated at 37°C for 14 days. To induce the HG injury of podocytes, MPC5 cells were placed in medium containing 30 mM glucose and cultivated for 48 h. Cells maintained in medium containing normal glucose (NG, 5.5 mM) were utilized as a control.

Real-Time Quantitative PCR (RT-qPCR). Total RNAs in cultured podocytes were extracted and purified using an RNApure Tissue & Cell Kit (Cowin Biosciences, Beijing, China). Total RNAs were reversely transcribed into cDNA using a HiFi-Script cDNA Synthesis Kit (Cowin Biosciences). FastSYBR Mixture (Cowin Biosciences) was adopted to amplify cDNA by RT-qPCR with appropriate primers. The generated

data by RT-qPCR was assessed via $2^{-\Delta\Delta Ct}$ method, and relative gene expression was obtained using GAPDH for normalization.

Western Blotting. Cultured podocytes were collected and lysed into lysis buffer (Solarbio, Beijing, China) supplemented with phosphatase inhibitors, proteinase inhibitors and phenylmethanesulfonyl fluoride. After being lysed, the lysates were centrifuged and the supernatants were collected. Protein concentration in the supernatants was quantified via a BCA Protein Assay Kit (Tiangen Biotech, Beijing, China). The same amounts of proteins were placed into each lane of sodium dodecyl sulfate (SDS) polyacrylamide gels, followed by being separated via electrophoresis. Then, proteins were transferred to Polyvinylidene Fluoride membranes via an electro-transfer method using Bio-Rad Trans-Blot apparatus (Bio-Rad Laboratories, Hercules, CA, USA). Polyvinylidene fluoride membranes were immersed into 5% skim milk for blocking prior to incubation with primary antibodies. The membranes were hybridized with matched secondary antibody, followed by incubation with enhanced chemiluminescent reagents (Solarbio) for the visualization of protein bands. The primary antibodies against TRIM32 (Abcam, Cambridge, UK), Akt (Proteintech Group, Wuhan, China), phospho-Akt (Ser473) (Proteintech Group), GSK-3 β (Proteintech Group), phospho-GSK-3 β (Ser9) (Proteintech Group), Nrf2 (Proteintech Group), GAPDH (Proteintech Group) and Lamin B1 (Proteintech Group).

Cell Transfection. The siRNAs targeting TRIM32 or Nrf2 were synthesized via Genepharma (Shanghai, China). The transfection of siRNAs into podocytes was implemented via using TransIntro EL Transfection Reagent (Transgen, Beijing, China) according to the protocol provided by the manufacturer. The down-regulation of target genes was confirmed by RT-qPCR or western blotting after 48 h transfection.

Cell Viability Assay. MPC5 podocytes were cultured in a 96-well plate and transfected with indicated siRNAs when they reached ~ 70% confluence. After transfection for 48 h, the medium was replaced with fresh medium harboring HG, and podocytes were cultivated for a further 48 h. Then, cell counting kit-8 (CCK-8) reagents (Solarbio, Beijing, China) were added to each well to determine the viability of podocytes. The absorbance of each well at 450 nm was measured via a microplate reader (BioTeke, Beijing, China).

Terminal Deoxynucleotidyl Transferase dUTP Nick end Labeling (TUNEL) Assay. TUNEL assay was carried out using the TransDetect In Situ Fluorescein TUNEL Cell Apoptosis Detection Kit (Transgen, Beijing, China) following the manufacturer's protocol. In brief, podocytes were fixed by formaldehyde fixing solution at the time of detection. Then, 0.1% Triton X-100 solution was adopted to permeabilize the podocytes. Afterwards, podocytes were incubated with TdT reagent and Labeling Solution at 37°C for 1 h, protected from light. After being stained with DAPI, cells were visualized via a fluorescence microscope.

Annexin V-FITC/PI Apoptosis Assay. Annexin V-FITC/PI apoptosis assay was performed via flow cytometry analysis using an Annexin V-FITC/PI Apoptosis Kit (Solarbio, Beijing, China). Briefly, cultured podocytes were dissociated by trypsin digestion and washed with ice-cold phosphate buffer saline (PBS). Podocytes were collected and re-suspended into Binding Buffer, followed by adding Annexin V-FITC/PI

Solution. After being cultivated for 15 min at room temperature protected from light, cells were assessed via the FACScan flow cytometry system.

Detection of ROS Generation. The intracellular levels of ROS was evaluated via 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) which can be oxidized into the fluorescent DCFH. Generally, at the time of detection, the old medium was discarded and fresh medium supplemented with 10 μ M DCFH-DA (Beyotime, Shanghai, China) was added to cells. After being cultivated for 30 min at 37°C, cells were washed with PBS and then analyzed via the FACScan flow cytometry system to quantify the fluorescence intensity.

Measurement of Malondialdehyde (MDA) and Superoxide Dismutase (SOD) Activity. The contents of MDA and SOD in podocytes were measured by Lipid Peroxidation MDA Assay Kit (Beyotime, Shanghai, China) and Total Superoxide Dismutase Assay Kit (Beyotime), respectively, according to the protocols of the manufacturer.

Enzyme-Linked Immuno-Sorbent Assay (ELISA). The levels of pro-inflammatory cytokine levels, including interleukin (IL)-6, the tumor necrosis factor- α (TNF- α), and IL-1 β in the supernatants of cultured podocytes were quantified using ELISA kits (R&D Systems, Minneapolis, MN, USA) following the manufacturer's protocol.

Luciferase Activity Assays. Luciferase reporter vector pARE (Beyotime, Shanghai, China), which contains an ARE binding site, was adopted to measure the transcriptional activity of Nrf2. The luciferase reporter vector pNF- κ B (Beyotime) was utilized to detect the transcriptional activity of NF- κ B. TRIM32 siRNAs and corresponding luciferase reporter vectors were co-transfected into MPC5 podocytes and cultivated for 48 h prior to HG stimulation. Then, cells were collected and lysed to measure luciferase activity using a Luciferase Reporter Gene Assay Kit (Beyotime).

Statistical Analysis. Experimental results were expressed as mean $\pm$ standard deviation. Statistical analysis and graphing were implemented using GraphPad Prism 8. Student's t test was adopted for the two-group comparison. When there were three or more groups, comparisons were performed using one-way analysis of variance (ANOVA). Differences were considered statistically significant when $p < 0.05$.

Results

TRIM32 Expression was Elevated in Podocytes Exposed to HG

To determine the possible relevance of TRIM32 in mediating HG-induced podocyte injury, we first analyzed the expression change of TRIM32 in MPC5 cells following exposure to HG. Our results showed that TRIM32 mRNA levels were obviously elevated in podocytes after HG treatment (Fig. 1A). Moreover, data for western blotting demonstrated significant increases in TRIM32 protein in HG-treated podocytes (Fig. 1B and C). The data indicate that TRIM32 is induced by HG in podocytes.

The Inhibition of TRIM32 Repressed HG-Induced Apoptosis of Podocytes

To understand the biological role of TRIM32 in mediating HG-induced podocyte injury, the loss-of-function experiment of TRIM32 was carried out. Transfecting TRIM32 siRNA into MPC5 cells markedly depleted TRIM32 expression with or without HG treatment (Fig. 2A-C). The viability assay showed that HG stimulation significantly reduced the viability of podocytes, which was markedly reversed by TRIM32 knockdown (Fig. 2D). The TUNEL assay showed that HG-induced apoptosis in MPC5 cells was significantly attenuated by TRIM32 knockdown (Fig. 2E and F). Moreover, the inhibitory effect of TRIM32 knockdown on HG-induced podocyte apoptosis was further confirmed by the Annexin V-FITC/PI apoptosis assay (Fig. 2G and H). Collectively, these data suggest that the inhibition of TRIM32 alleviates HG-induced podocyte apoptosis.

The Inhibition of TRIM32 Relieved HG-Induced Oxidative Stress in Podocytes

To further explore the role of TRIM32 in mediating HG-induced podocyte injury, we investigated the effect of TRIM32 inhibition on HG-induced oxidative stress in MPC5 cells. HG exposure caused high generation of ROS in podocytes, which was markedly decreased by TRIM32 knockdown (Fig. 3A and B). Moreover, the HG-induced elevation in MDA contents was also reduced by TRIM32 inhibition in podocytes (Fig. 3C). In addition, SOD activity, which was inhibited by HG, was significantly increased by TRIM32 inhibition in podocytes (Fig. 3D). Altogether, these data indicate that TRIM32 inhibition relieves HG-induced oxidative stress in podocytes.

The Inhibition of TRIM32 Repressed the HG-Induced Inflammatory Response in Podocytes

We next investigated the role of TRIM32 inhibition on the HG-induced inflammatory response in podocytes. We found that the knockdown of TRIM32 prominently decreased the release of pro-inflammatory cytokines, including IL-6, TNF- α and IL-1 β (Fig. 4A-C). Moreover, the knockdown of TRIM32 markedly suppressed the activation of NF- κ B in HG-exposed podocytes (Fig. 4D-F). Overall, these data suggest that the inhibition of TRIM32 decreases the HG-induced inflammatory response in podocytes.

The Inhibition of TRIM32 Potentiated Nrf2 Signaling in HG-Exposed Podocytes

To determine the molecular mechanism underlying TRIM32 inhibition-mediated effects in HG-exposed podocytes, we investigated the role of TRIM32 inhibition on Nrf2 signaling that contributes to the mediation of HG-induced apoptosis, oxidative stress and the inflammatory response in podocytes. The results showed that HG treatment decreased the level of nuclear Nrf2, which could be significantly up-

regulated by TRIM32 knockdown (Fig. 5A and B). Furthermore, the knockdown of TRIM32 markedly increased the transcriptional activity of Nrf2 (Fig. 5C). In addition, the knockdown of TRIM32 enhanced the expression of Nrf2 target genes, including HO-1 and NQO-1 (Fig. 5D). In short, these data imply that the inhibition of TRIM32 potentiates Nrf2 signaling in HG-exposed podocytes.

The Inhibition of TRIM32 Enhanced Nrf2 Signaling via Modulation of the Akt/GSK-3 β Axis in HG-Exposed Podocytes

TRIM32 plays a vital role in mediating the activation of Akt/GSK-3 β axis. Considering that the Akt/GSK-3 β axis also regulates the activation of Nrf2 signaling, we sought to determine whether TRIM32 modulates Nrf2 signaling via the Akt/GSK-3 β axis in HG-exposed podocytes. Interestingly, we found that TRIM32 inhibition increased the phosphorylation of Akt and GSK-3 β in HG-exposed podocytes (Fig. 6A-C). Treatment with Akt inhibitors markedly decreased the phosphorylation of Akt and GSK-3 β induced by TRIM32 inhibition in HG-exposed podocytes (Fig. 6A-C). Notably, TRIM32 inhibition-induced Nrf2 activation in HG-exposed podocytes was markedly abolished by Akt inhibition (Fig. 6D-F). In summary, these findings confirm that the inhibition of TRIM32 enhances Nrf2 signaling via modulation of the Akt/GSK-3 β axis.

The Knockdown of Nrf2 Reversed TRIM32-Inhibition-Mediated Protective Effects in HG-Exposed Podocytes

To confirm whether TRIM32 inhibition protects podocytes from HG injury via Nrf2 signaling, we detected the effect of Nrf2 knockdown on TRIM32-inhibition-mediated effects in HG-exposed podocytes. The transfection of Nrf2 signaling markedly decreased the activation of Nrf2 induced by TRIM32 inhibition in HG-exposed podocytes (Fig. 7A-C). As expected, the knockdown of Nrf2 significantly abolished TRIM32-inhibition-induced suppressive effects on HG-induced apoptosis (Fig. 7D and E) and ROS generation (Fig. 7F and G). In addition, the inhibitory effects of TRIM32 inhibition on the HG-induced release of IL-6, TNF- α and IL-1 β were also partially reversed by Nrf2 knockdown (Fig. 7H-J). To summarize, these data confirm that TRIM32 inhibition protects podocytes from HG injury via Nrf2 signaling.

Discussion

In the present work, we have determined the pivotal role of TRIM32 in regulating HG-evoked injury of podocytes. TRIM32 expression was significantly elevated in podocytes stimulated by HG. The inhibition of TRIM32 by siRNA-mediated gene silencing markedly suppressed HG-evoked podocyte apoptosis, oxidative stress and inflammatory response. Moreover, we further identified that the inhibition of TRIM32 conferred anti-HG injury in podocytes by potentiating Nrf2 signaling via regulation of the Akt/GSK-3 β axis (Fig. 8). Overall, our work indicates a vital role of the TRIM32/Akt/GSK-3 β /Nrf2 axis in mediating HG-induced podocyte injury, highlighting the possible relevance of TRIM32 in diabetic nephropathy.

TRIM32 exerts a key role in regulating the survival and apoptosis of various cell types. TRIM32 can inhibit the apoptosis of cancer cells and enhances cell survival under exposure to chemotherapeutics [38–40]. The overexpression of TRIM32 protects keratinocytes from apoptosis induced by ultraviolet b and tumor necrosis factor- α [23]. Despite the anti-apoptotic function of TRIM32 having been documented, the pro-apoptotic role of TRIM32 has been also reported in certain contexts. In TRIM32-knockout mice, traumatic brain injury-induced cell apoptosis in cortex is markedly reduced compared that in wild-type mice [41]. Moreover, the knockdown of TRIM32 alleviates oxygen-glucose deprivation-induced apoptosis of hippocampal neurons [42]. The silencing of TRIM32 decreases the apoptosis of nucleus pulposus cells induced by IL-1 β or TNF- α [43]. Notably, TRIM32 expression is induced by hydrogen peroxide or rotenone in human embryonic kidney cells, and TRIM32 up-regulation enhances human embryonic kidney cells to hydrogen peroxide- or rotenone-induced cell death [23]. Therefore, these studies indicate that the inhibition of TRIM32 is conducive to survival under noxious stimuli. However, whether TRIM32 is involved in regulating podocyte apoptosis induced by HG is unknown. Herein we found that HG stimulation markedly up-regulated TRIM32 expression in the mouse podocyte MPC5 *in vitro*. Notably, the inhibition of TRIM32 improved the viability of HG-injured podocytes, and attenuated HG-induced podocyte apoptosis. Collectively, our work confirms that the inhibition of TRIM32 exerts an anti-apoptotic role in mediating HG-induced podocyte injury.

TRIM32 is involved in modulating oxidative stress. The overexpression of TRIM32 enhances the production of ROS induced by hydrogen peroxide or rotenone [23]. The inhibition of TRIM32 decreases the generation of ROS induced by oxygen-glucose deprivation in hippocampal neurons, and up-regulates the contents of SOD [42]. In line with these findings, we found that the knockdown of TRIM32 inhibited the production of ROS and MDA, while increasing the contents of SOD in podocytes exposed to HG. Therefore, our work confirms the crucial role of TRIM32 in modulating oxidative stress.

TRIM32 plays a vital role in regulating inflammatory response. TRIM32-knock out mice have reduced production of pro-inflammatory cytokines and chemokines post *S. suis* infection [24]. The overexpression of TRIM32 promotes the release of pro-inflammatory cytokines in TNF- α -stimulated fibroblast-like synoviocytes [44]. Consistent with these studies, our work showed that the inhibition of TRIM32 markedly repressed the production of pro-inflammatory cytokines induced by HG in podocytes. Moreover, TRIM32 regulates the inflammatory response associated with the modulation of NF- κ B [44]. Herein, we demonstrated that the inhibition of TRIM32 impeded the nuclear translocation of NF- κ B p65, and reduced the transcriptional activity of NF- κ B. Therefore, our study indicates that the inhibition of TRIM32 attenuates HG-induced inflammation in podocytes associated with the down-regulation of NF- κ B activation.

Nrf2 signaling plays a vital role in modulating HG-induced apoptosis, oxidative stress and inflammation of podocytes [34–36]. Interestingly, our study reported that the inhibition of TRIM32 potentiated Nrf2 signaling in HG-exposed podocytes, which is consistent with a recent study demonstrating that TRIM32 plays a role in regulating Nrf2 activation in oxygen-glucose deprivation-induced neurons [42]. Moreover, we found that TRIM32 inhibition potentiated Nrf2 signaling via modulation of the Akt/GSK-3 β axis.

Indeed, the Akt/GSK-3 β axis contributes to modulation of Nrf2 activation [45–47]. In addition, TRIM32 also acts a vital regulator of the Akt/GSK-3 β axis. It is reported that the inhibition of TRIM32 enhances plakoglobin binding to PI3K, which leads to Akt activation [48]. Furthermore, the knockout of TRIM32 significantly increased the phosphorylation of Akt and GSK-3 β in cardiomyocytes under hypertrophic stresses [49]. Notably, our data demonstrated that the inhibition of Akt markedly reversed TRIM32-inhibition-mediated Nrf2 activation in HG-exposed podocytes. Therefore, our data confirm that TRIM32 inhibition potentiates Nrf2 signaling via modulation of the Akt/GSK-3 β axis.

Conclusion

Taken together, the findings of this study demonstrate that the inhibition of TRIM32 ameliorates HG-induced apoptosis, oxidative stress and inflammatory responses in podocytes by potentiating Nrf2 signaling via modulation of the Akt/GSK-3 β axis. These data suggest that the TRIM32/Akt/GSK-3 β /Nrf2 axis is a new mechanism in regulating the HG-induced injury of podocytes. This work indicates that TRIM32-mediated podocyte injury may play a vital role in the pathogenesis of diabetic nephropathy, and suggests TRIM32 as a potential target for podocyte protection. However, the precise role of TRIM32 in mediating diabetic nephropathy requires further investigation using animal models *in vivo*.

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not Applicable.

CONSENT FOR PUBLICATION

All authors have agreed for the publication of this paper.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available on request from the corresponding author.

COMPETING INTERESTS

The authors declare that they have no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Zhao Chen designed the study, performed the experiments and drafted the manuscript. Lifang Tian performed the experiments. Li Wang performed the experiments. Xiaotao Ma performed the experiments. Fuqian Lei collected and analyzed the data. Xianghui Chen collected and analyzed the data. Rongguo Fu designed the study and reviewed the manuscript. All authors have read and approved the final version of this manuscript.

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Not Applicable.

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Figures

Figure 1

The effect of HG treatment on TRIM32 expression in MPC5 cells. MPC5 cells were cultured for 48 h in the presence of NG or HG. (A) RT-qPCR was utilized to determine the effect of HG on TRIM32 mRNA levels. (B, C) Western blotting was adopted to measure the effect of HG on TRIM32 protein levels. N=3, **p<0.01.

Figure 2

The effect of TRIM32 inhibition on HG-induced podocyte apoptosis. MPC5 cells were transfected with TRIM32 siRNA or control siRNA for 48 h and then subjected to HG exposure. The down-regulation of TRIM32 level by siRNA transfection was confirmed by (A) RT-qPCR and (B, C) western blotting (N=3). (D) The effect of TRIM32 inhibition on podocyte viability was assessed via CCK-8 assay (N=4). The effect of

TRIM32 inhibition on podocyte apoptosis was evaluated via (E, F) TUNEL assay and (G, H) Annexin V-FITC/PI assay (N=3). **p<0.01.

Figure 3

The effect of TRIM32 knockdown on HG-induced oxidative stress in podocytes. (A, B) The effect of TRIM32 inhibition on ROS generation was monitored via DCFH-DA staining method (N=3). (C) The effect of TRIM32 inhibition on MDA contents was assessed via MDA assay (N=4). (D) The effect of TRIM32 inhibition on SOD activity was evaluated via SOD assay (N=4). *p<0.05 and **p<0.01.

Figure 4

The effect of TRIM32 knockdown on the HG-induced inflammatory response in podocytes. The effect of TRIM32 inhibition on concentration levels of (A) IL-6, (B) TNF- α and (C) IL-1 β in the culture supernatants was measured via ELISA (N=4). (D, E) The effect of TRIM32 inhibition on the levels of nuclear NF- κ B p65 protein was determined via western blotting. Lamin B1 served as the loading control (N=3). (F) The effect of TRIM32 inhibition on the transcriptional activity of NF- κ B was measured via luciferase reporter assay (N=5). *p<0.05, **p<0.01 and ***p<0.001.

Figure 5

The effect of TRIM32 inhibition on Nrf2 signaling in HG-exposed podocytes. (A, B) The effect of TRIM32 inhibition on the level of nuclear Nrf2 was measured via western blotting (N=3). Lamin B1 served as the loading control. (C) The effect of TRIM32 inhibition on the transcriptional activity of Nrf2 was determined via luciferase reporter assay (N=5). (D) The effect of TRIM32 inhibition on the levels of HO-1 and NQO-1 was assessed via western blotting. GAPDH served as the loading control. *p<0.05 and **p<0.01.

Figure 6

The effect of Akt inhibition on TRIM32-mediated Nrf2 signaling. MPC5 cells were transfected with TRIM32 siRNA for 48 h in the presence or absence of Akt inhibitor, MK-2206 2HCl, prior to HG treatment. Levels of (A-C) phospho-Akt and phospho-GSK-3β, and (D, E) nuclear Nrf2 were determined via western blotting (N=3). (F) Nrf2 transcriptional activity was monitored via luciferase activity (N=5). *p<0.05 and **p<0.01.

Figure 7

The effect of Nrf2 inhibition on TRIM32-inhibition-mediated protective effects in HG-exposed podocytes. TRIM32 siRNA and Nrf2 siRNA were co-transfected into MPC5 cells followed by incubation for 48 h before HG exposure. (A-C) Levels of nuclear Nrf2 were examined via western blotting (N=3). (D, E) Podocytes apoptosis was measured via the Annexin V-FITC/PI assay (N=3). (F, G) ROS levels in podocytes were assessed via the DCFH-DA staining method (N=3). Concentration levels of (H) IL-6, (I)

TNF- α and (J) IL-1 β in culture supernatants of podocytes were monitored via ELISA (N=4). *p<0.05 and **p<0.01.

Figure 8

A proposed model for TRIM32 in mediating HG-induced podocyte injury.