



Peroxiredoxin 6 alleviates high glucose-induced inflammation and apoptosis in HK-2 cells by inhibiting TLR4/NF- κ B signaling

Hao Wu^{1#}, Rong Wu^{2#}, Tianxi Liu², Hongbin Ma¹, Guozhong Xue¹, Minglong Liu²

¹Department of Nephrology, Affiliated Hospital of Gansu University of Chinese Medicine, Lanzhou, China; ²Department of Nephrology, The First Hospital of Lanzhou University, Lanzhou, China

Contributions: (I) Conception and design: H Wu, R Wu; (II) Administrative support: R Wu, T Liu; (III) Provision of study materials or patients: H Ma; (IV) Collection and assembly of data: G Xue; (V) Data analysis and interpretation: H Wu, M Liu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Rong Wu. Department of Nephrology, The First Hospital of Lanzhou University, No. 1 Donggang West Road, Chengguan District, Lanzhou 730000, China. Email: rongwu664@163.com.

Background: This research sought to elucidate the effects of peroxiredoxin 6 (*PRDX6*) on the biological processes in diabetic nephropathy (DN) and to identify the underlying regulatory mechanism related to toll-like receptor 4 (TLR4)/nuclear factor-kappa B (NF- κ B) signaling.

Methods: To induce an *in vitro* DN cellular model, human kidney 2 (HK-2) cells were treated with high glucose (HG). The mitochondrial membrane potential, adenosine triphosphate level, reactive oxygen species generation, and oxidative stress of the cells were then evaluated. After the *PRDX6* level had been determined, the effects of its overexpression on the mitochondrial membrane potential, adenosine triphosphate level, reactive oxygen species generation, and oxidative stress of the cells were assessed. Next, cytochrome c expression, cell viability, cell apoptosis, the inflammatory level, and the TLR4/NF- κ B signaling-related factors were assessed. After the addition of the TLR4 activator CRX-527 or the NF- κ B activator phorbol 12-myristate 13-acetate (PMA), cell viability, cell apoptosis and the inflammatory level were evaluated again.

Results: The results revealed that HG exposure triggered mitochondrial dysfunction and oxidative stress and decreased *PRDX6* expression in the HK-2 cells. *PRDX6* elevation exacerbated cell viability while alleviating mitochondrial membrane potential loss, oxidative stress, apoptosis, and inflammation in the HG-treated HK-2 cells. Further, *PRDX6* inhibited HG-induced TLR4/NF- κ B activation. The administration of CRX-527 or PMA reversed the effects of *PRDX6* on the cell viability, apoptosis, and inflammation of the HG-exposed HK-2 cells.

Conclusions: To conclude, *PRDX6* appears to protect HG-exposed HK-2 cells against inflammation and apoptosis by inhibiting TLR4/NF- κ B signaling.

Keywords: Diabetic nephropathy (DN); peroxiredoxin 6 (*PRDX6*); inflammation; apoptosis; TLR4/NF- κ B signaling

Submitted Nov 07, 2022. Accepted for publication Dec 23, 2022. Published online Jan 09, 2023.

doi: 10.21037/atm-22-6063

View this article at: <https://dx.doi.org/10.21037/atm-22-6063>

Introduction

Diabetes is a complicated metabolic disease that is characterized by elevated levels of blood glucose and insulin resistance (1,2). The International Diabetes Federation estimated that there were >463 million cases of diabetes

in 2019, and predicted that this figure would increase to 570 million by 2030 (3). Diabetic nephropathy (DN) is the most prevalent microvascular complication of diabetes, and it imposes a great burden on around 20–40% of individuals with diabetes (4,5). DN has also been confirmed to be a

primary cause of end-stage renal disease responsible for diabetic mortality (6). DN has a complex and distinctive pathogenesis; however, it is well documented that glucose metabolism disturbance, abnormal lipid metabolism, the release of inflammatory cytokines, and oxidative stress are closely associated with the initiation and process of DN (7-9). Currently, the therapeutic strategies for DN include hypoglycemic, anti-hypertensive, and hypolipidemic therapies, but the treatment of DN remains challenging and troublesome, and no definitive therapies are currently available (10). A deeper understanding of the cause of DN is crucial to improving the efficacy of DN therapies.

Peroxiredoxins (PRDXs) are a highly-conserved selenium-independent peroxidase family of PRDXs that maintain oxidative homeostasis (11). Peroxiredoxin 6 (*PRDX6*), which belongs to the PRDX family, is a recently discovered PRDX ubiquitously expressed in the lung, brain, testis, kidney, and liver with glutathione peroxidase and phospholipase A₂ activities (12,13). *PRDX6* activities play critical roles in antioxidant defense and the maintenance of phospholipid metabolism (14). Extensive research has shown that *PRDX6* is involved in the incidence and progression of inflammatory and neurodegenerative diseases; for example, the loss of *PRDX6* alleviates acute and chronic dextran sodium sulfate-triggered colitis (15). *PRDX6* has also been shown to be involved in cerebral ischemia/reperfusion inflammatory injury (16). Additionally, research has shown that *PRDX6* plays a role in the pathogenesis of diabetes (17) and protects against type 1 diabetes (18). Notably, *PRDX6*

elevation suppresses oxidative stress and ferroptosis to ease podocyte injury in DN. However, further research needs to be conducted into the more specific effects of *PRDX6* on DN and the potential regulatory mechanism related to mitochondrial dysfunction, oxidative stress, inflammation and apoptosis as well as the potential signaling pathways.

Toll-like receptor 4 (TLR4)/nuclear factor-kappa B (NF-κB) signaling is a classic inflammatory signaling pathway in which the activation of TLR4 binds to its ligand (such as, lipopolysaccharide and heat shock protein 60) to stimulate the release of the downstream transcription factor NF-κB, thereby eliciting an inflammatory response (19). There is extensive evidence that TLR4/NF-κB signaling is involved in sepsis-induced myocardial dysfunction, endometritis, and osteoarthritis via the modulation of inflammation (20-22). Further, TLR4/NF-κB signaling has been shown to drive inflammation in DN (23). Notably, *PRDX6* exerts a radioprotective effect by interacting with TLR4/NF-κB signaling (24). However, it is unclear whether *PRDX6* similarly controls biological functions in DN by modulating TLR4/NF-κB signaling.

This study sought to determine the effects of *PRDX6* on DN processes and the relationship between *PRDX6* and TLR4/NF-κB signaling. We present the following article in accordance with the MDAR reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-6063/rc>).

Methods

Cell culture and treatment

The human renal tubular epithelial [human kidney 2 (HK-2)] cells were supplied by BeNa Culture Collection and maintained in Roswell Park Memorial Institute Medium-1640 (RPMI-1640; Beijing Solarbio Science and Technology Co., Ltd.) with 10% fetal bovine serum (Hunan Auragene Biotechnology Co., Ltd.) and 1% penicillin-streptomycin solution at 37 °C with 5% carbon dioxide. The cells were treated with 5.5 mM of glucose, 5 mM of glucose plus 24.5 mM of mannitol (MA), or 30 mM of glucose and divided into the Control, MA, and HG groups, respectively. Additionally, the TLR4 activator CRX-527 (0.5 ng/mL) (25) and the NF-κB activator phorbol 12-myristate 13-acetate (PMA; 1 μg/mL) (26) were used to treat the HK-2 cells.

Cell transfection

PRDX6 overexpression was achieved in the HK-2 cells

Highlight box

Key findings

- *PRDX6* protects HG-induced HK-2 cells against inflammation and apoptosis by inhibiting TLR4/NF-κB signaling.

What is known and what is new?

- *PRDX6* is involved in the incidence and progression of inflammatory diseases. *PRDX6* plays an important role in the pathogenesis of diabetes and *PRDX6* elevation suppresses oxidative stress and ferroptosis to ease podocyte injury in DN.
- *PRDX6* alleviates HG-induced inflammation and apoptosis of human renal tubular epithelial cells by inhibiting TLR4/NF-κB signaling.

What is the implication, and what should change now?

- This study identifies a novel molecular mechanism underlying the *PRDX6*-mediated DN process and provides further evidence supporting the use of *PRDX6*-based targeted therapy in the treatment of DN.

by transfection with recombinant pcDNA3.1-*PRDX6* plasmids (Oe-*PRDX6*, YouBio, Chongqing, China), and those transfected with pcDNA3.1 empty plasmids were used as the negative controls (Oe-NC). The procedure was conducted with Lipofectamine 2000 (Thermo Fisher) in accordance with the manufacturer's instructions.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Complementary deoxyribonucleic acid was produced using ReverTra-Plus™ kit (Toyobo Life Science) in accordance with the manufacturer's instructions after ribonucleic acid (RNA) was isolated. The amplification was performed in the MX3000p PCR system (Agilent, Santa Clara, CA) using the SYBR Premix Ex Taq kit (Shanghai Biosteel Biotechnology) with β -actin for normalization. The $2^{-\Delta\Delta C_t}$ method was used to analyze the messenger RNA levels (27).

Western blot analysis

Following HG stimulation, the HK-2 cells were lysed in lysis buffer (BestBio) and centrifuged (12,000 \times g, 4 °C) for 20 min to acquire total proteins. The proteins were then transferred to polyvinylidene fluoride membranes after sodium dodecyl-sulfate polyacrylamide gel electrophoresis. The membranes were placed in 5% non-fat milk at room temperature for 1 h before being incubated with the corresponding primary antibodies overnight at 4 °C, and the goat anti-rabbit horseradish peroxidase secondary antibody (1/2,000; Abcam) at room temperature for 2 h. The signals were developed using the enhanced chemiluminescence reagent (Advansta, Inc.), and the results were measured using ImageJ 1.46 (National Institutes of Health, USA).

JC-1 staining

Mitochondrial membrane potential was analyzed using a tetraethyl benzimidazolyl carbocyanine iodide (JC-1) detection kit (Nanjing KeyGen Biotech Co., Ltd.). Following HG exposure and plasmid transfection, the HK-2 cells were collected from the RPMI-1640 and washed with 5 μ mol/L of JC-1 staining buffer at 37 °C for half an hour protected from light, and then rinsed in cold phosphate buffered solution twice. The results were photographed with a fluorescence microscope (Olympus Corporation; magnification \times 200).

Determination of ATP and ROS contents

The HK-2 cells were lysed in lysis buffer (BestBio) and centrifuged (12,000 \times g, 4 °C) for 20 min. Adenosine triphosphate (ATP) activity in the cell supernatant was detected using the ATP Bioluminescent Assay Kit (Promega Corporation) in accordance with the manufacturer's instructions. To examine intracellular reactive oxygen species (ROS), 10 μ M of dichloro-dihydro-fluorescein diacetate (Sigma-Aldrich) was used to treat the HK-2 cells at 37 °C for half an hour in the dark and the cells were then washed thrice with serum-starved RPMI-1640. The results were photographed with a fluorescence microscope (magnification \times 200).

Cell Counting Kit-8 (CCK-8) assays

Cell viability was determined using the CCK-8 solution (Nanjing Keygen Biotechnology Co. Ltd.) in strict accordance with the manufacturer's instructions. The HK-2 cells (5×10^3 cells/well) were incubated in 96-well plates overnight. CCK-8 solution (10 μ L) was added to each well at 37 °C for an extra 2 h incubation. Then, the absorbance was measured using a 450-nm microplate reader (Tecan infinite).

Determination of intracellular SOD and MDA

The corresponding kits from Nanjing Jiancheng Bioengineering Co. Ltd. were employed to determine the levels of malonaldehyde (MDA; cat. no. A003-1-2) and superoxide dismutase (SOD; cat. no. A001-1-2) in the cell supernatant in accordance with manufacturer's instruction. Absorbance was assessed using a microplate reader at 450 nm.

TUNEL

Cell apoptosis was appraised using a terminal-deoxynucleotidyl transferase mediated nick end labeling (TUNEL) Apoptosis kit (Nanjing Biobox Biotech Co., Ltd.). Briefly, 4% paraformaldehyde was used to fix the HK-2 cells, and 0.1% Triton X-100 was then added for permeabilization. Subsequently, the cells were cultivated with TUNEL reaction reagent for 1 h and the nuclei were labeled with 10 mg/mL 4',6-diamidino-2-phenylindole for 10 min. Finally, 5 random fields of view were selected to capture images using a fluorescence microscope

(magnification $\times 200$) and analyzed using ImageJ 1.46.

Statistical analysis

All the experiments were repeated 3 times. All the data were reported as the mean \pm standard deviation and analyzed with GraphPad Prism 8.0 software. A 1-way analysis of variance followed by Tukey's post-hoc test was used to compare differences among multiple groups. A P value < 0.05 was considered statistically significant.

Results

PRDX6 levels decrease upon HG exposure

To show that the DN cellular model had been successfully established, the mitochondrial membrane potential, the content of ATP produced from the mitochondria, and activities of oxidative stress factors were measured. As *Figure 1A* shows, the experimental results of JC-1 staining showed that relative to the control group, the cells in the HG group emitted green fluorescence with little red fluorescence, suggesting that mitochondrial membrane potential was reduced. ROS activity increased in the HK-2 cells upon HG stimulation (*Figure 1B*). In addition, HG treatment resulted in a decrease in ATP production (*Figure 1C*). Additionally, the oxidative stress marker SOD level showed a downward trend while the MDA level was increased in the HG group compared to the control group (*Figure 1D, 1E*). Notably, RT-qPCR and western blot revealed that the level of *PRDX6* of the HK-2 cells in the HG group was significantly reduced compared to that of the control group (*Figure 1F, 1G*). Thus, HG stimulation decreased mitochondrial membrane potential and *PRDX6* expression but exacerbated oxidative stress in the HK-2 cells, which suggests that *PRDX6* might be involved in the DN process.

Effects of PRDX6 on oxidative stress during HG exposure

To further examine the effects of *PRDX6* on the biological activities of the HG-stimulated HK-2 cells, *PRDX6* overexpression was induced. The RT-qPCR and western blot experimental results indicated that *PRDX6* expression was significantly increased after the transfection of the Oe-*PRDX6* plasmids (*Figure 2A, 2B*). Subsequently, JC-1 staining showed that *PRDX6* overexpression alleviated the decrease in the mitochondrial membrane potential caused

by HG (*Figure 2C*). Subsequently, in the Oe-*PRDX6* + HG group, it was discovered that the increased ROS content in the HG-stimulated HK-2 cells was suppressed (*Figure 2D*). Additionally, the HG-suppressed ATP level significantly increased after *PRDX6* was overexpressed (*Figure 2E*). As anticipated, the decreased SOD level and the increased MDA level in the HG-treated HK-2 cells were both reversed on *PRDX6* overexpression (*Figure 2F, 2G*). Additionally, HG stimulation decreased the mito-cytochrome c protein level but increased the cyto-cytochrome c protein level, while *PRDX6* increased the mito-Cytochrome c protein level and decreased the cyto-cytochrome c protein level (*Figure 2H*), suggesting that *PRDX6* impeded the release of cytochrome c from the mitochondria. Thus, *PRDX6* overexpression mitigated mitochondrial dysfunction and oxidative stress in the HG-exposed HK-2 cells.

Effects of PRDX6 on apoptosis and the inflammatory response during HG exposure

Further, the CCK-8 assays showed that the suppressed viability of the HG-exposed HK-2 cells was increased when *PRDX6* was upregulated (*Figure 3A*). Conversely, as *Figure 3B* shows, the TUNEL staining results revealed that HG-induced apoptosis in the HK-2 cells was obstructed by *PRDX6* elevation (*Figure 3B, 3C*). The western blot results showed that *PRDX6* increased downregulated B-cell lymphoma 2 (Bcl-2) expression and decreased upregulated Bax expression in the HK-2 cells following HG treatment (*Figure 3D*). An examination of the inflammatory factors by western blot revealed that tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-11) protein levels decreased due to *PRDX6* elevation (*Figure 3E*). Thus, *PRDX6* exerted inhibitory effects on the apoptosis and inflammation of the HG-treated HK-2 cells.

PRDX6 ameliorates apoptosis and the inflammatory response by TLR4/NF- κ B signaling

More interestingly, the Western blot results indicated that the level of TLR4/NF- κ B signaling-related factors [i.e., TLR4, phosphor (p)-p65, and p-inhibitor of kappa B α (I κ B α)] was more increased in the HG group than the control group. Their expression levels in the HG + Oe-*PRDX6* group were significantly reduced compared to those in the HG + Oe-NC group (*Figure 4*). To further examine the roles of TLR4/NF- κ B signaling, the TLR4

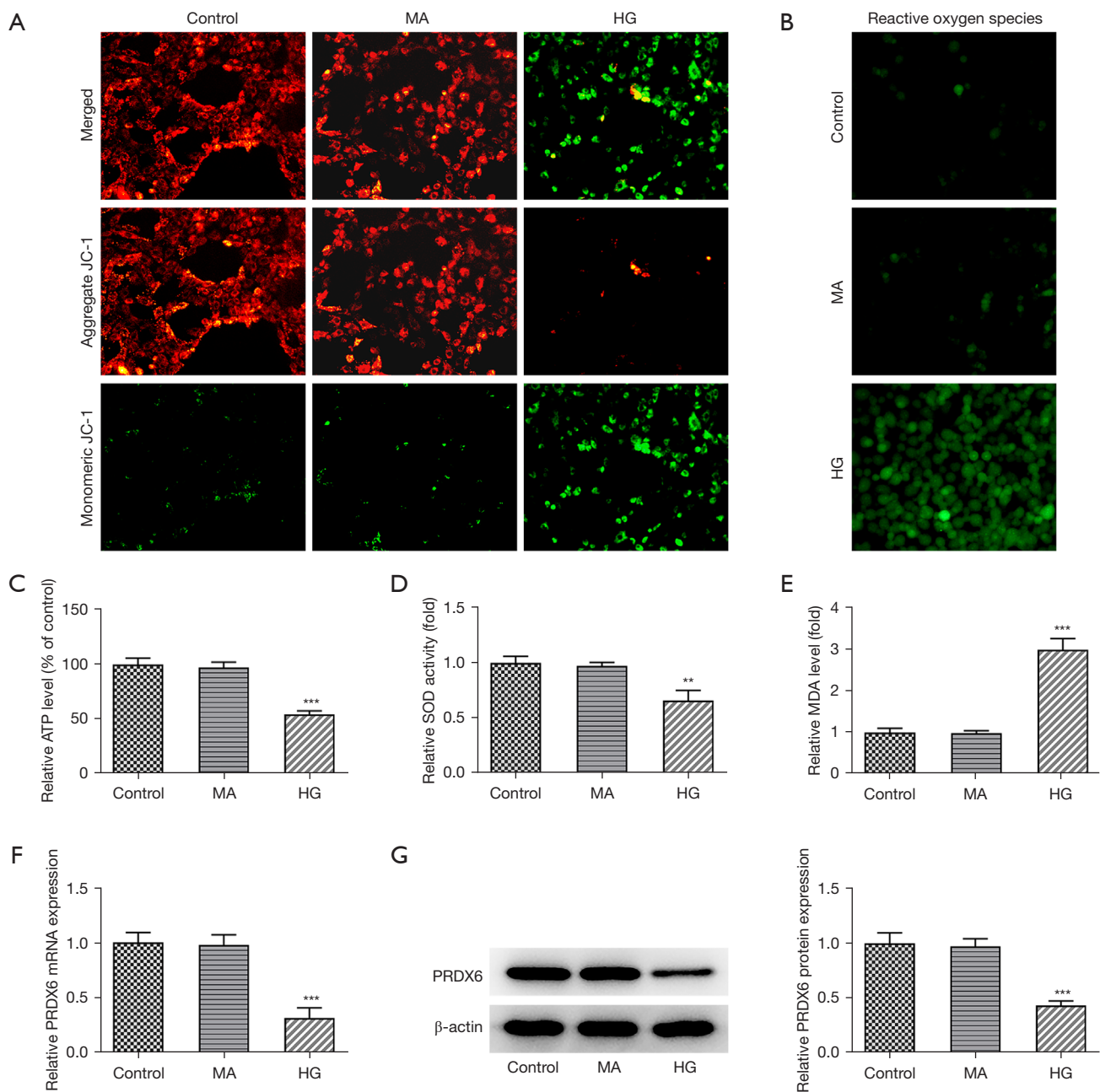


Figure 1 *PRDX6* levels decrease upon HG exposure. (A) JC-1 staining revealed mitochondrial membrane potential. Magnification, 200 \times . (B) ROS production and (C) ATP content were both tested using the corresponding assay kits. Magnification, 200 \times . Enzyme-linked immunoassay analysis results of (D) SOD and (E) MDA levels. (F) RT-qPCR and (G) Western blot were conducted to examine *PRDX6* expression in the HK-2 cells exposed to HG. ** $P < 0.01$, *** $P < 0.001$ vs. Control group. HG, high glucose; MA, mannitol; ROS, reactive oxygen species; ATP, adenosine triphosphate; SOD, superoxide dismutase; MDA, malonaldehyde; HK-2, human kidney 2; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

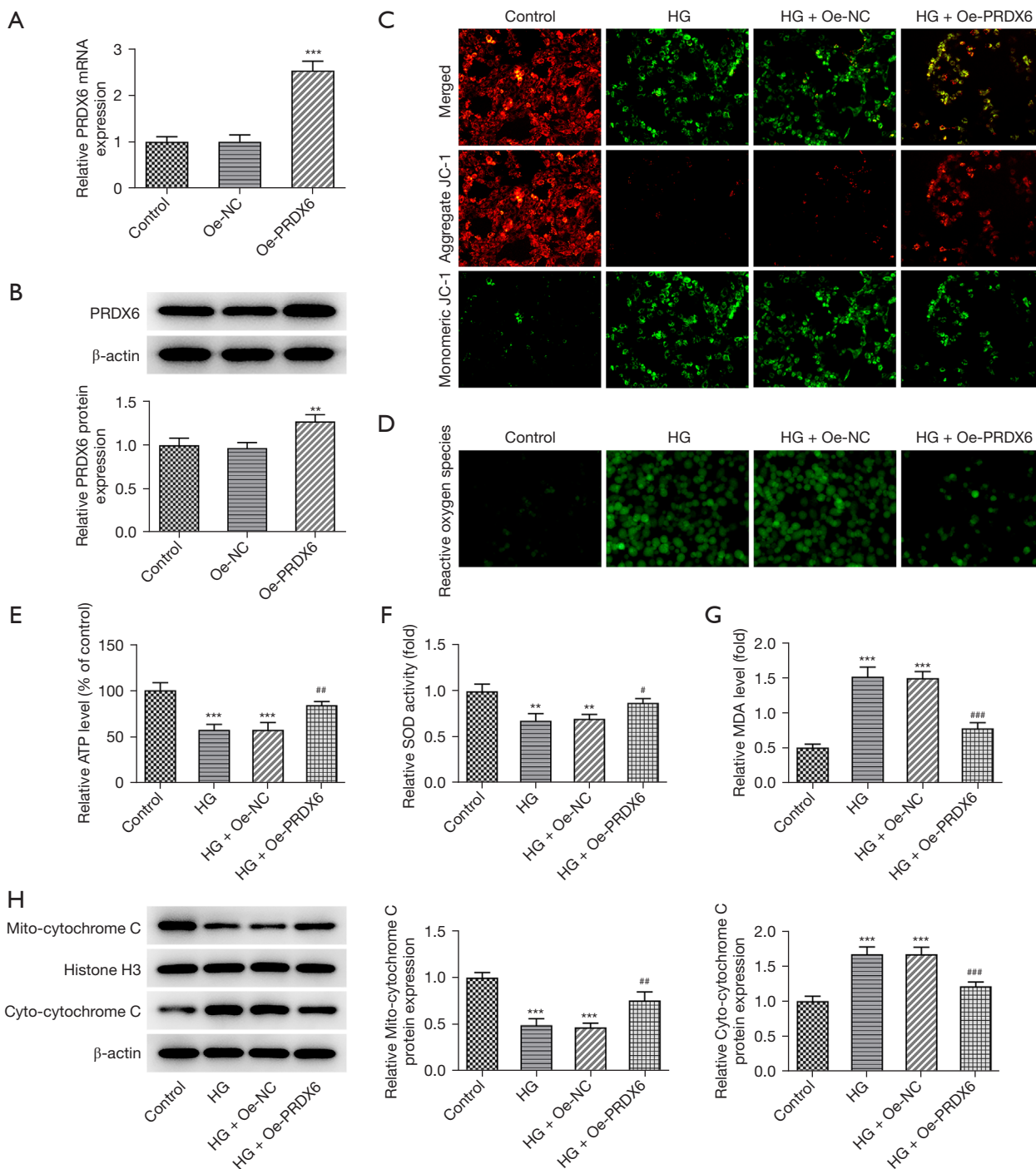


Figure 2 Effects of *PRDX6* on oxidative stress during HG exposure. Analysis of the overexpression efficacy of Oe-*PRDX6* plasmid by (A) RT-qPCR and (B) Western blot. (C) JC-1 staining appraised mitochondrial membrane potential. Magnification, 200 \times . (D) ROS production and (E) ATP contents were both tested using the corresponding assay kits. Enzyme-linked immunoassay analysis results of (F) SOD and (G) MDA levels. Magnification, 200 \times . Normal transfected MPC-5 cells and the HG treatment were used as the Controls. (H) Western blot analysis of mito-cytochrome c and cyto-cytochrome c expression. ** $P < 0.01$, *** $P < 0.001$ vs. Control group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. HG + Oe-NC group. HG, high glucose; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; ROS, reactive oxygen species; ATP, adenosine triphosphate; SOD, superoxide dismutase; MDA, malonaldehyde; Oe-NC, overexpression-negative control.

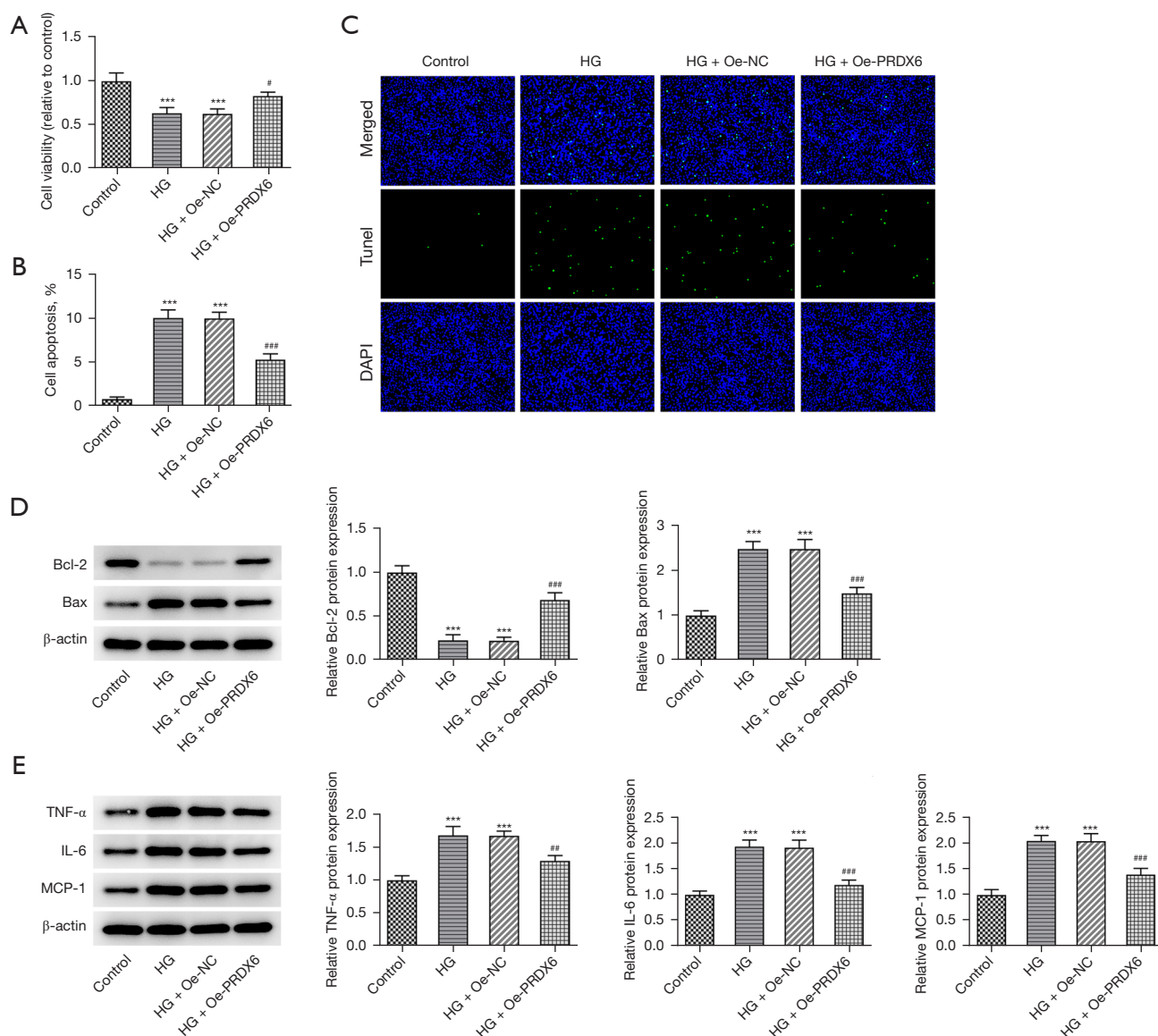


Figure 3 Effects of *PRDX6* on apoptosis and the inflammatory response during HG exposure. (A) The effects of *PRDX6* on the viability of the HG-treated HK-2 cells were examined using CCK-8 assays. (B,C) TUNEL assays were used to examine the effect of *PRDX6* on the apoptosis of the HG-stimulated HK-2 cells. Magnification, 200 \times . Western blot analysis of the expression of (D) apoptosis-related factors and (E) inflammatory factors. *** $P < 0.001$ vs. Control group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. HG + Oe-NC group. HG, high glucose; DAPI, 4',6-diamidino-2-phenylindole; HK-2, human kidney 2; CCK-8, Cell Counting Kit-8; TUNEL, terminal-deoxynucleotidyl transferase mediated nick end labeling; Oe-NC, overexpression-negative control.

activator CRX-527 or the NF- κ B activator PMA were used to treat the HK-2 cells. Based on the CCK-8 assay results, the protective role of *PRDX6* in HG-induced HK-2 cell viability injury was offset by the administration of CRX-527 or PMA (Figure 5A). Conversely, the decreased apoptosis of the HG-stimulated HK-2 cells induced by *PRDX6*

upregulation was again promoted by CRX-527 or PMA (Figure 5B,5C). Similarly, the increase in Bcl-2 expression and decrease in Bax expression in the HG-induced HK-2 cells caused by *PRDX6* were both counteracted by the addition of CRX-527 or PMA (Figure 5D). Thus, CRX-527 or PMA partially restored TNF- α , IL-6, and MCP-1

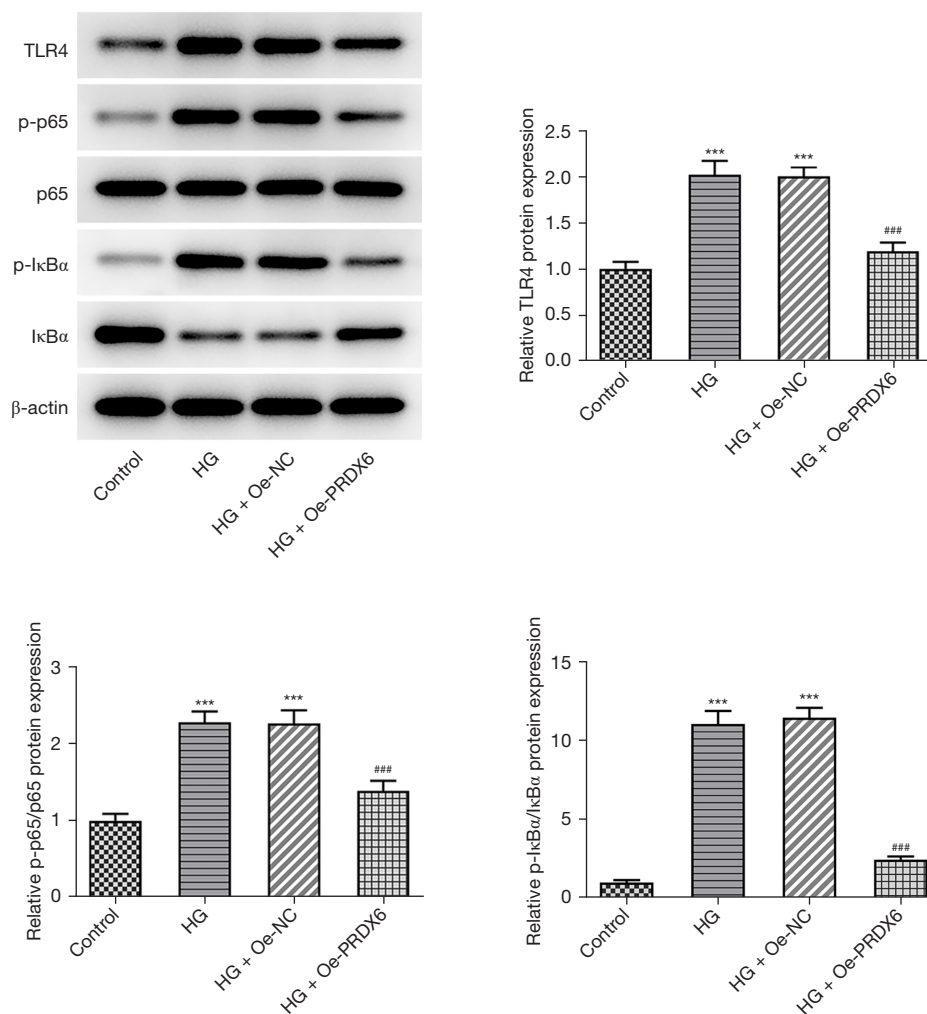


Figure 4 *PRDX6* blocks the TLR4/NF-κB signaling. Western blot analysis of the TLR4/NF-κB signaling-associated factors. *** $P < 0.001$ vs. Control group; ### $P < 0.001$ vs. HG + Oe-NC group. TLR4, toll-like receptor 4; NF-κB, nuclear factor-kappa B; HG, high glucose; Oe-NC, overexpression-negative control.

expression in HG-induced HK-2 cells with *PRDX6* overexpression (Figure 5E). Collectively, *PRDX6* inhibited TLR4/NF-κB signaling to ease apoptosis and inflammation in the HG-exposed HK-2 cells.

Discussion

DN refers to a series of pathological and physiological changes in diabetes resulting from the mutual effects of multitudinous factors, including environmental, genetic, epigenetic factors, oxidative stress, inflammation, and mitochondrial dysfunction (28,29). Long-term exposure to HG stress is a driving factor triggering DN, which

may stimulate oxidative stress by generating massive ROS through enzymatic or non-enzymatic reactions (30). Additionally, the excessive production and accumulation of ROS may lead to mitochondrial dysfunction, which may in turn further aggravate ROS production (31). Thus, mitochondrial function and oxidative stress under HG conditions were used to successfully establish an *in vitro* DN cell model. In this experiment, the HK-2 cells were stimulated under an HG environment. As anticipated, the results showed that HG treatment significantly damaged mitochondrial membrane potential and exacerbated ROS generation. ATP is thought to be a source of chemical energy in organisms, and ATP is frequently supplied by

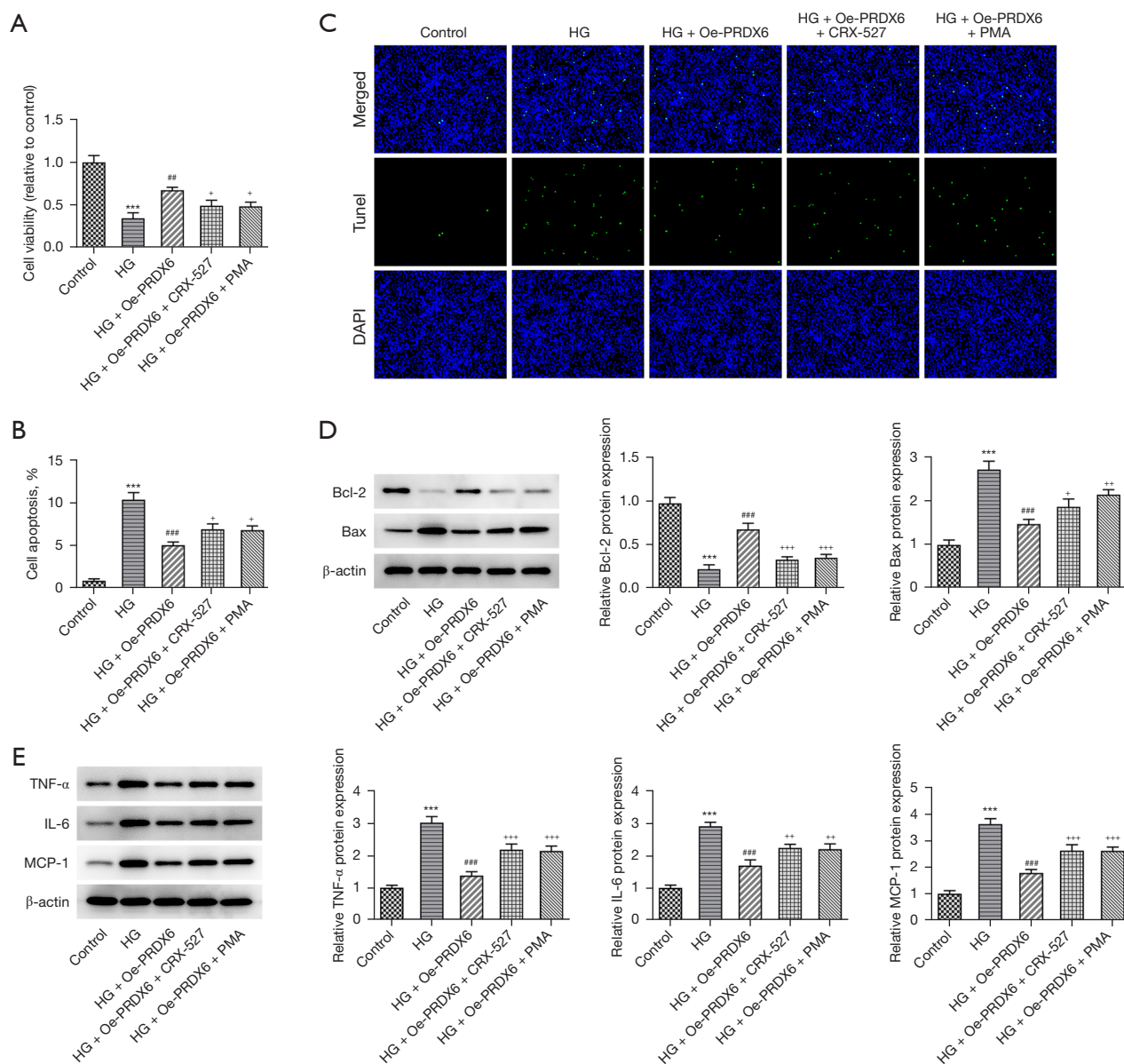


Figure 5 *PRDX6* ameliorates HG-triggered apoptosis and the inflammatory response via TLR4/NF- κ B signaling. (A) Effect of TLR4 activator CRX-527 or NF- κ B activator PMA on the viability of the *PRDX6*-overexpressing HK-2 cells in a HG environment were examined by CCK-8 assays. (B,C) TUNEL assays were used to examine the effect of CRX-527 or PMA on the apoptosis of *PRDX6*-overexpressing HK-2 cells in a HG environment. Magnification, 200 \times . Western blot analysis results of the expression of (D) apoptosis-related factors and (E) inflammatory factors. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Control group; *** $P < 0.001$ vs. HG group; ### $P < 0.01$, #### $P < 0.001$ vs. HG + Oe-*PRDX6* group. HG, high glucose; TLR4, toll-like receptor 4; NF- κ B, nuclear factor-kappa B; PMA, phorbol 12-myristate 13-acetate; HK-2, human kidney 2; CCK-8, Cell Counting Kit-8; TUNEL, terminal-deoxynucleotidyl transferase mediated nick end labeling.

the mitochondria (32). The experimental data in this study also confirmed that HG exposure resulted in ATP depletion. MDA forms as a decomposition product of lipid peroxidation where ROS attacks free unsaturated fatty

acids (33). SOD is a vital antioxidant for ROS elimination, which reflects antioxidant capacity (34). The results of this study showed that the SOD level decreased while the MDA level increased in the HK-2 cells under HG conditions.

These results imply that HG treatment contributes to mitochondrial dysfunction and oxidative stress.

It was recently conjectured that the activity regulation of *PRDX6* is closely related to the occurrence of a number of human diseases (11). In the PRDX family, *PRDX6* is capable of eliminating ROS to defend against oxidative stress-dependent on glutathione (GSH) peroxidase activity (35). Our experimental results first revealed that *PRDX6* expression was significantly reduced in the HG-exposed HK-2 cells. The functional experiments showed that in the HG-exposed HK-2 cells, *PRDX6* elevation significantly improved mitochondrial membrane potential, suppressed ROS generation, promoted ATP production, and halted oxidative stress. These findings highlighted that *PRDX6* alleviated mitochondrial dysfunction and oxidative stress injury in the HK-2 cells exposed to HG, which was in line with the findings of Zhang *et al.* (36) that Sp1-activated *PRDX6* relieves oxidative stress in podocytes under HG stimulation in DN.

Oxidative stress is a leading cause of apoptosis (37). In the process of DN, oxidative stress is implicated in the inflammatory response (38). The release of proinflammatory cytokines is also believed to deteriorate renal function (39). The present study showed that *PRDX6* hindered HG-triggered viability injury and apoptosis. Similarly, the lessened anti-apoptotic Bcl-2 expression and the augmented pro-apoptotic Bax expression caused by the HG treatment were both reversed by the overexpression of *PRDX6*. As a dominant inflammatory factor, TNF- α not only activates NF- κ B signaling but also stimulates cytotoxicity to induce renal injury in DN. IL-6 and MCP-1 are also prototypical proinflammatory chemokines (40). Similarly, *PRDX6* was discovered to decrease the upregulated expression of TNF- α , IL-6, and MCP-1 in the HK-2 cells in a HG environment.

TLR4 is a major receptor in the congenital immune and inflammatory responses (41). NF- κ B is the most significant intracellular nuclear transcription factor and is responsible for modulating multiple inflammatory genes and proteins (42). The binding of TLR4 to its ligands pushes the translocation of NF- κ B from the cytoplasm into the nucleus, and NF- κ B signaling participates in the inflammatory response (19). It's worth noting that TLR4/NF- κ B signaling has a stimulative effect on inflammation in DN (23). Notably, *PRDX6* has been shown to exert a radioprotective effect by interacting with TLR4/NF- κ B signaling (24). This study showed that HG exposure strengthened the expression of TLR4/NF- κ B signaling-

associated proteins TLR4, p-p65, and p-I κ B α . The subsequent administration of TLR4 activator CRX-527 or NF- κ B activator PMA partially counteracted the effects of *PRDX6* on the viability, apoptosis, and inflammation of the HG-exposed HK-2 cells. The present study provided experimental support for the use of *PRDX6* for the targeted therapy of DN, which laid foundation for the application of *PRDX6* in DN treatment.

Conclusions

To summarize, *PRDX6* inhibited apoptosis and inflammation in the HG-induced renal tubular epithelial cells in DN via TLR4/NF- κ B signaling inactivation. Thus, this study identified a novel molecular mechanism underlying the *PRDX6*-mediated DN process and provides further evidence supporting the use of *PRDX6*-based targeted therapy in the treatment of DN. However, only *in vitro* experiments were performed in this paper, and the lack of *in vivo* animal experiments is a limitation of this study, which will be conducted to support the conclusion of this paper our future research.

Acknowledgments

Funding: None.

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-6063/rc>

Data Sharing Statement: Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-6063/dss>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-6063/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons

Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

- Blair M. Diabetes Mellitus Review. *Urol Nurs* 2016;36:27-36.
- Harreiter J, Roden M. Diabetes mellitus-Definition, classification, diagnosis, screening and prevention (Update 2019). *Wien Klin Wochenschr* 2019;131:6-15.
- Saeedi P, Petersohn I, Salpea P, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. *Diabetes Res Clin Pract* 2019;157:107843.
- Schernthaner G, Schernthaner GH. Diabetic nephropathy: new approaches for improving glycemic control and reducing risk. *J Nephrol* 2013;26:975-85.
- Sagoo MK, Gnudi L. Diabetic Nephropathy: An Overview. *Methods Mol Biol* 2020;2067:3-7.
- Warren AM, Knudsen ST, Cooper ME. Diabetic nephropathy: an insight into molecular mechanisms and emerging therapies. *Expert Opin Ther Targets* 2019;23:579-91.
- Samsu N. Diabetic Nephropathy: Challenges in Pathogenesis, Diagnosis, and Treatment. *Biomed Res Int* 2021;2021:1497449.
- Stadler K, Goldberg IJ, Susztak K. The evolving understanding of the contribution of lipid metabolism to diabetic kidney disease. *Curr Diab Rep* 2015;15:40.
- Arora MK, Singh UK. Molecular mechanisms in the pathogenesis of diabetic nephropathy: an update. *Vascul Pharmacol* 2013;58:259-71.
- Lu Y, Liu D, Feng Q, et al. Diabetic Nephropathy: Perspective on Extracellular Vesicles. *Front Immunol* 2020;11:943.
- Lee YJ. Knockout Mouse Models for Peroxiredoxins. *Antioxidants (Basel)* 2020.
- Fisher AB. Peroxiredoxin 6: a bifunctional enzyme with glutathione peroxidase and phospholipase A₂ activities. *Antioxid Redox Signal* 2011;15:831-44.
- Yamada Y, Limmon GV, Zheng D, et al. Major shifts in the spatio-temporal distribution of lung antioxidant enzymes during influenza pneumonia. *PLoS One* 2012;7:e31494.
- Fisher AB. Peroxiredoxin 6 in the repair of peroxidized cell membranes and cell signaling. *Arch Biochem Biophys* 2017;617:68-83.
- Melhem H, Spalinger MR, Cosin-Roger J, et al. Prdx6 Deficiency Ameliorates DSS Colitis: Relevance of Compensatory Antioxidant Mechanisms. *J Crohns Colitis* 2017;11:871-84.
- Shanshan Y, Beibei J, Li T, et al. Phospholipase A2 of Peroxiredoxin 6 Plays a Critical Role in Cerebral Ischemia/Reperfusion Inflammatory Injury. *Front Cell Neurosci* 2017;11:99.
- Pacifici F, Arriga R, Sorice GP, et al. Peroxiredoxin 6, a novel player in the pathogenesis of diabetes. *Diabetes* 2014;63:3210-20.
- Novoselova EG, Glushkova OV, Lunin SM, et al. Peroxiredoxin 6 Attenuates Alloxan-Induced Type 1 Diabetes Mellitus in Mice and Cytokine-Induced Cytotoxicity in RIN-m5F Beta Cells. *J Diabetes Res* 2020;2020:7523892.
- Maher DP, Walia D, Heller NM. Morphine decreases the function of primary human natural killer cells by both TLR4 and opioid receptor signaling. *Brain Behav Immun* 2020;83:298-302.
- Luo M, Yan D, Sun Q, et al. Ginsenoside Rg1 attenuates cardiomyocyte apoptosis and inflammation via the TLR4/NF-κB/NLRP3 pathway. *J Cell Biochem* 2020;121:2994-3004.
- Zhang H, Wu ZM, Yang YP, et al. Catalpol ameliorates LPS-induced endometritis by inhibiting inflammation and TLR4/NF-κB signaling. *J Zhejiang Univ Sci B* 2019;20:816-27.
- Ding Y, Wang L, Zhao Q, et al. MicroRNA-93 inhibits chondrocyte apoptosis and inflammation in osteoarthritis by targeting the TLR4/NF-κB signaling pathway. *Int J Mol Med* 2019;43:779-90.
- Mudaliar H, Pollock C, Panchapakesan U. Role of Toll-like receptors in diabetic nephropathy. *Clin Sci (Lond)* 2014;126:685-94.
- Sharapov MG, Glushkova OV, Parfenyuk SB, et al. The role of TLR4/NF-κB signaling in the radioprotective effects of exogenous Prdx6. *Arch Biochem Biophys* 2021;702:108830.
- Legat A, Thomas S, Hermant P, et al. CD14-independent responses induced by a synthetic lipid A mimetic. *Eur J Immunol* 2010;40:797-802.
- Duan Y, Luo Q, Wang Y, et al. Adipose mesenchymal stem cell-derived extracellular vesicles containing microRNA-

- 26a-5p target TLR4 and protect against diabetic nephropathy. *J Biol Chem* 2020;295:12868-84.
27. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001;25:402-8.
 28. Lin TA, Wu VC, Wang CY. Autophagy in Chronic Kidney Diseases. *Cells* 2019;8:61.
 29. Papadopoulou-Marketou N, Paschou SA, Marketos N, et al. Diabetic nephropathy in type 1 diabetes. *Minerva Med* 2018;109:218-28.
 30. Aluksanasuwan S, Plumworasawat S, Malaitad T, et al. High glucose induces phosphorylation and oxidation of mitochondrial proteins in renal tubular cells: A proteomics approach. *Sci Rep* 2020;10:5843.
 31. Wei PZ, Szeto CC. Mitochondrial dysfunction in diabetic kidney disease. *Clin Chim Acta* 2019;496:108-16.
 32. Annesley SJ, Fisher PR. Mitochondria in Health and Disease. *Cells* 2019;8:680.
 33. Cooley JC, Lunte CE. Detection of malondialdehyde in vivo using microdialysis sampling with CE-fluorescence. *Electrophoresis* 2011;32:2994-9.
 34. Wang Y, Branicky R, Noë A, et al. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *J Cell Biol* 2018;217:1915-28.
 35. Manevich Y, Reddy KS, Shuvaeva T, et al. Structure and phospholipase function of peroxiredoxin 6: identification of the catalytic triad and its role in phospholipid substrate binding. *J Lipid Res* 2007;48:2306-18.
 36. Zhang Q, Hu Y, Hu JE, et al. Sp1-mediated upregulation of Prdx6 expression prevents podocyte injury in diabetic nephropathy via mitigation of oxidative stress and ferroptosis. *Life Sci* 2021;278:119529.
 37. Ogura S, Shimosawa T. Oxidative stress and organ damages. *Curr Hypertens Rep* 2014;16:452.
 38. Turkmen K. Inflammation, oxidative stress, apoptosis, and autophagy in diabetes mellitus and diabetic kidney disease: the Four Horsemen of the Apocalypse. *Int Urol Nephrol* 2017;49:837-44.
 39. Betjes MG. Immune cell dysfunction and inflammation in end-stage renal disease. *Nat Rev Nephrol* 2013;9:255-65.
 40. Panagi L, Poole L, Hackett RA, et al. Happiness and Inflammatory Responses to Acute Stress in People With Type 2 Diabetes. *Ann Behav Med* 2019;53:309-20.
 41. Flier S, Concepcion AN, Versteeg D, et al. Monocyte hyporesponsiveness and Toll-like receptor expression profiles in coronary artery bypass grafting and its clinical implications for postoperative inflammatory response and pneumonia: An observational cohort study. *Eur J Anaesthesiol* 2015;32:177-88.
 42. Mitchell JP, Carmody RJ. NF-κB and the Transcriptional Control of Inflammation. *Int Rev Cell Mol Biol* 2018;335:41-84.
- (English Language Editor: L. Huleatt)

Cite this article as: Wu H, Wu R, Liu T, Ma H, Xue G, Liu M. Peroxiredoxin 6 alleviates high glucose-induced inflammation and apoptosis in HK-2 cells by inhibiting TLR4/NF-κB signaling. *Ann Transl Med* 2023;11(2):41. doi: 10.21037/atm-22-6063