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Luteolin attenuates high glucose-induced podocyte injury via suppressing NLRP3 inflammasome pathway

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ABSTRACT

Aims: Diabetic nephropathy is a growing health concern, which is reported to be associated with inflammation. Luteolin has been explored for the treatment of some diabetic complications. Although several studies have verified the effect of luteolin on diabetic nephropathy, the mechanism by which the therapeutic effects of luteolin on diabetic nephropathy has not been established. Therefore, we aimed to investigate the effect of luteolin on diabetic nephropathy and its underlying mechanism.
Main methods: We used western blot, Real-time PCR, immunofluorescence and flow cytometry to analyze the effects of luteolin on podocyte injury and NOD-like receptor family and pyrin domain-containing protein 3 (NLRP3) inflammasome activation in high glucose (HG) condition. Reactive oxygen species (ROS) generation was measured by flow cytometry and malondialdehyde (MDA) level. To investigate the potential mechanism, we examined cell apoptosis upon transfection of siNLRP3.
Key findings: We showed that luteolin treatment could protect podocyte against HG-induced cell apoptotic and mitochondrial membrane potential collapse. In addition, luteolin significantly reduced NLRP3 inflammasome formation and subsequent interleukin-1β (IL-1β) secretion in HG-induced MPC-5 cells. Interestingly, siNLRP3 abolished the effect of luteolin on cell apoptosis, suggesting that the anti-apoptotic effect was found to be mostly related to NLRP3 inflammasome.
Significance: In summary, our data demonstrated the abilities of luteolin to inhibit podocyte injury and NLRP3 inflammasome activation, which could be used in the treatment of diabetic nephropathy.

1. Background

Diabetic nephropathy is one of the most serious diabetic complications and leads to the end-stage renal disease [1]. The albuminuria is reported to become the main risk marker of diabetic nephropathy [2]. Podocyte is a kind of terminally differentiated tubular epithelial cells, which can form glomerular filtration barrier [3]. Recent studies suggested that podocyte injury played a key role in diabetic nephropathy, and could be a typical characteristic of diabetic nephropathy [4]. However, the pathogenesis of podocyte dysfunction remains unclear. Therefore, it is important to explore a therapy which targets at podocyte to cure diabetic nephropathy in the early stages.

High glucose (HG) condition can promote reactive oxygen species (ROS) released in podocyte, in some cases, initiate the podocyte depletion and albumin excretion [5,6]. Several reports have shown that podocyte apoptosis could be induced by high concentration of glucose, which leads to the renal dysfunction [5,7]. Inflammation is also acted as a key mediator in diabetic nephropathy. Excessive inflammation which was triggered by inflammasome could lead to diabetic nephropathy [8]. Several kinds of inflammasome have been found, and NOD-like receptor family and pyrin domain-containing protein 3 (NLRP3) inflammasome has been reported to play a crucial role in diabetic nephropathy [9]. NLRP3 is identified as a NOD-like receptor, which can form a NLRP3 inflammasome after recruiting the adapter molecule ASC [10]. NLRP3 inflammasome can be activated by a variety of stimuli including extracellular ATP, viral DNA, bacterial infection and tissue damage [11]. Upon stimulation, NLRP3 can form a multi-protein complex which composed with ASC and the Interleukin-1 converting

Abbreviations: NLRP3, NOD-like receptor family and pyrin domain-containing protein 3; ASC, apoptosis-associated speck-like protein which contains a CARD; IL-1β, interleukin-1β; IL-18, interleukin-18; HG, high glucose; ROS, reactive oxygen species; FBS, fetal bovine serum; MDA, malondialdehyde; PBS, phosphate buffer saline; BSA, bovine serum albumin; MPC-5, mouse podocyte cell-5; NAC, *N*-acetyl-L-cysteine

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enzyme, Caspase-1, and subsequently leads to the activation of Caspase-1. Activation of the Caspase-1 can cause the maturation and secretion of some inflammatory cytokines including IL-1β and IL-18 [12]. Recent finding has shown that activation of NLRP3 inflammasome could promote diabetic nephropathy progression in non-myeloid-derived cells [13]. In addition, NLRP3 inflammasome mediates the effects of TLR4 on podocyte injury in HG condition [14].

Luteolin is a kind of natural flavonoid which functions as the active ingredient in a variety of fruits and vegetables [15]. Luteolin has a wide spectrum of biological and pharmacological actions including anti-apoptotic, anti-inflammatory, anti-oxidant and anti-tumor effects. It is widely known that the anti-apoptotic [16,17] and the anti-inflammatory effects [18] are the major pharmacological mechanism of luteolin in clinical treatments. However, there is little available information about the protective effects of luteolin in diabetic nephropathy. In this study, we investigated the involvement of NLRP3 inflammasome in protective effects by luteolin in diabetic nephropathy and reported an anti-apoptotic effect for luteolin mediated by inflammasome pathway.

2. Materials and methods

2.1. Chemicals and antibodies

Luteolin (> 99% pure) was purchased from DASF Bio-Tech Ltd. (Nanjing, China). Primary antibodies for NLRP3, WT-1, Nephrin, Desmin and Bcl-2 and β-actin were gained from Biogot Biotechnology Co., Ltd. (CA, USA); primary antibodies for cleavage caspase-3, cleavage caspase-6 and cleavage caspase-9 were purchased from Cell Signaling Technology (MA, USA); primary antibodies for ASC, Pro-Caspase-1, Caspase-1, Pro-IL-1β and IL-1β were obtained from Santa Cruz Biotechnology (CA, USA). NLRP3 siRNA and control siRNA were purchased from Shanghai Sango Biotechnology (Shanghai, China). SYBR Green Master Mix was obtained from Vazyme Biotech Co., Ltd. (Nanjing, China). *N*-acetyl-L-cysteine (NAC) was purchased from Beyotime, (Nanjing, China).

2.2. Cell culture

The mouse podocyte cell-5 (MPC-5) was obtained from Cell Bank of the Chinese Academic of Sciences (Shanghai, China) and cultured with dulbecco's modified eagle medium supplemented with 10% fetal bovine serum (FBS), under 95% humidified air with 5% CO2 at 37 °C. Cells were propagated at 33 °C and treated with interferon (10 U IFN-γ/ml). Then cells were harvested and incubated without IFN-γ at 37 °C for two weeks to allow their differentiation.

2.3. Measurement of MDA

Level of malondialdehyde (MDA) was measured by the malondialdehyde assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instruction.

2.4. Assay of cell apoptosis

Cells were treated with different stimulations. After that, cells were washed with phosphate buffer saline (PBS) and resuspended in binding buffer before staining by Annexin V-FITC/PI detection apoptosis kit (KeyGen Biotech, Nanjing, China) for 30 min. Accuri C6 flow cytometry (Becton Dickinson, CA, USA) was used to detect stained cells. The apoptosis rate was expressed with Annexin V+/PI− (early apoptosis) and Annexin V+/PI+ (late apoptosis).

2.5. Assay of mitochondrial membrane potential

Mitochondrial membrane potential was tested by JC-1 staining kit (KeyGen Biotech, Nanjing, China). MPC-5 cells were exposed with different stimulations and incubated with JC-1 for 30 min and washed. The fluorescence intensity of JC-1 was measured by Accuri C6 flow cytometry.

2.6. Measurement of ROS

Cell superoxide level was measured by DCFH-DA (Beyotime, Shanghai, China). MPC-5 cells were exposed with different stimulations and incubated with DCFH-DA for 20 min and washed. The fluorescence intensity of DCFH-DA was measured by Accuri C6 flow cytometry.

2.7. Western blot

Protein was separated by SDS-PAGE, followed by transferring to PVDF membranes (Millipore, MA, USA). After blocking in 3% bovine serum albumin (BSA), the membranes were incubated overnight with primary antibodies at 4 °C then incubated with secondary antibodies at 37 °C for 1.5 h. After that, the protein bands were identified by Tanon 5200 chemiluminescence imaging system (Shanghai, China).

2.8. Real-time PCR

TRIzol Reagent (Invitrogen, CA, USA) was used to extract total RNA from cells, following reversely transcribed into cDNA and prepared to test by real-time PCR using SYBR Green Master Mix. The results were analyzed by $2^{-\Delta\Delta CT}$ method. The primers were listed as follows: NLRP3: (forward) 5′-AAC ATT CGG AGA TTG TGG TTG GG-3′, (reverse) 5′-GTG CGT GAG ATT CTG ATT AGT GCT G-3′; caspase-1: (forward) 5′-TTA CAG ACA AGG GTG CTG AAC AA-3′, (reverse) 5′-TGA GGA GCT GGA AAG GAA GAA AG-3′; IL-1β: (forward) 5′-AGG CTG CTC TGG GAT TC-3′, (reverse) 5’-GCC ACA ACA ACT GAC GC-3′; GAPDH: (forward) 5′-AAG GTC GGA GTC AAC GGA TTT-3′ (reverse) 5′-AGA TGA TGA CCC TTT TGG CTC-3′.

2.9. Immunofluorescence assay

Cells were washed with PBS, fixed with paraformaldehyde and permeabilized with 0.2% Triton X-100. Cells were then blocked with 5% BSA for 1 h followed by incubation with primary antibody overnight. After washing with PBS and incubating with anti-rabbit FITC-conjugated secondary antibody, DAPI (Beyotime, Shanghai, China) was used to stain nuclei. Images were acquired by fluorescence microscope (Nikon, Tokyo, Japan).

2.10. Small interfering RNA transfection

NLRP3 siRNA was transformed by Lipofectamine 6000 reagent (Beyotime, Shanghai, China) according to the manufacturer's protocol. The sequence of NLRP3 siRNA was 5′-GGTGTTGGAATTAGACAAC -3′.

2.11. Statistical analysis

All experiments were performed three times independently. The data were considered statistically significant if $P < 0.05$ and were presented as mean ± SD. Statistical significance of differences was determined by student's two-tailed *t*-test or one-way analysis of variance using SPSS 22.0 software.

3. Results

3.1. High-glucose condition activated NLRP3 inflammasome and induced podocyte injury in human podocyte

We first established podocyte injury model by treating with 30 mM of glucose for 24 h in MPC-5 cells. Podocyte will change the

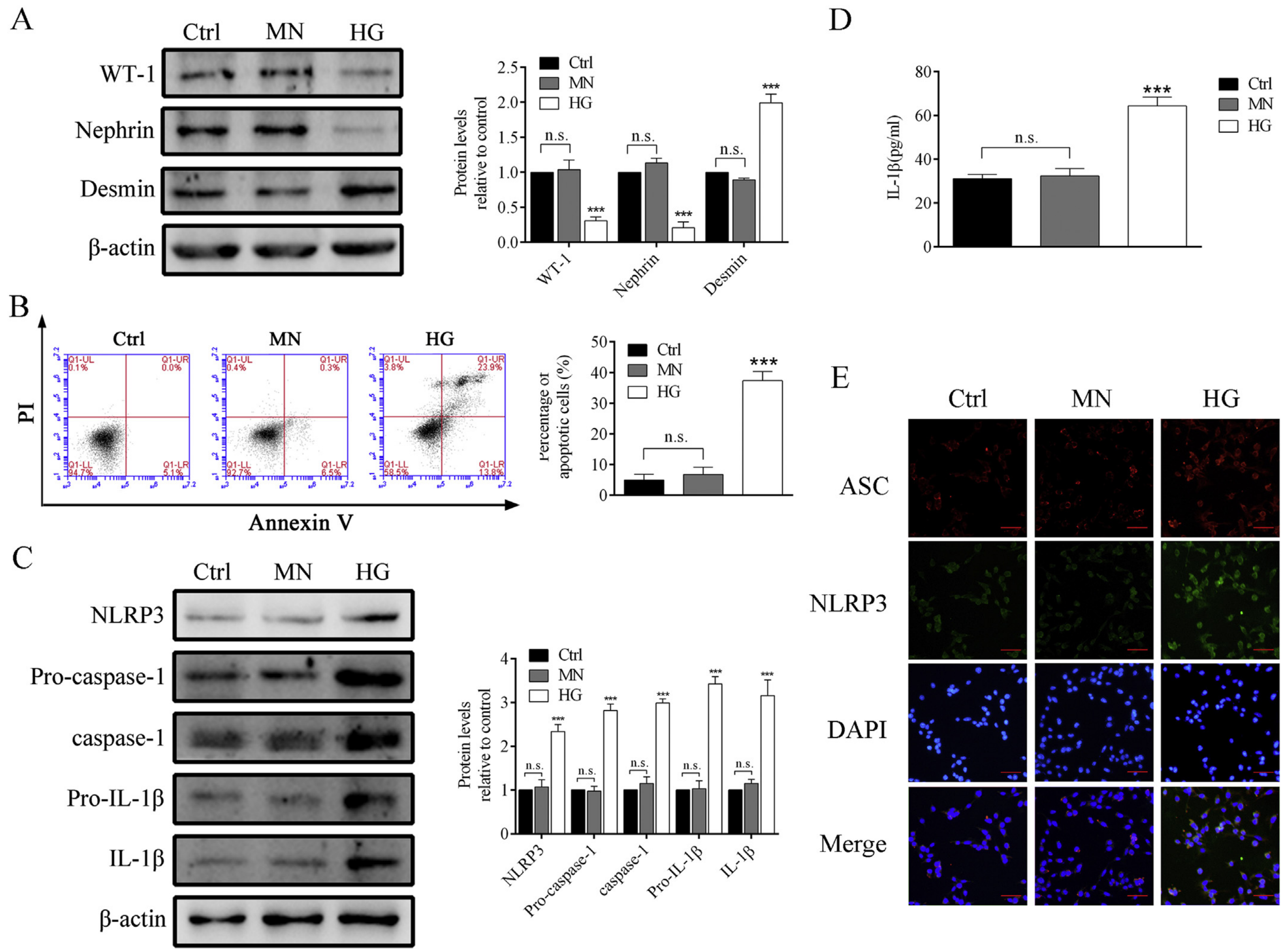

Fig. 1. HG condition induced NLRP3 inflammasome formation and podocyte apoptosis. MPC-5 cells were treated with mannitol and 30 mM glucose for 24 h. The expression of WT-1, Nephrin and Desmin were determined by western blot (A). The number of apoptotic cells was detected by flow cytometry (B). The expression of NLRP3, Pro-IL-1β, IL-1β, Pro-caspase-1 and caspase-1 were determined by western blot (C). The IL-1β secreted in supernatant was detected by ELISA (D). Immunofluorescence was used to detect the NLRP3 inflammasome formation by confocal NLRP3 and ASC after stimulation (scale bar, 50 μm) (E). The data (mean ± SD) are representative of three independent experiments. Ctrl: control group; MN: mannitol group; HG: high glucose group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control group.

morphological structure and increase their motility after exposing to HG, which can lead to proteinuria by impairing the integrity of the glomerular filtration barrier [19]. The expression of podocyte specific markers including WT-1, Nephrin and Desmin were determined by western blot. Compared with the normal glucose group (5.5 mM glucose) and mannitol control group (5.5 mM glucose + 24.5 mM mannitol), 30 mM glucose was sufficient to downregulate the expressions of WT-1 and Nephrin and upregulate the expression of Desmin (Fig. 1A), which suggested the successful establishment of podocyte injury model in MPC-5 cells. In addition, cell apoptosis is one of the signs of podocyte injury [20]. Therefore, we tested the number of apoptotic cells after exposing MPC-5 cells to HG. As shown in Fig. 1B, treatment with 30 mM glucose significantly increased the number of apoptotic cells in MPC-5 cells.

Previous studies showed that HG could lead to inflammation [21]. In addition, diabetic nephropathy is widely considered as a kind of inflammatory disease. So we detected the expression of NLRP3 inflammasome components in MPC-5 cells after treating with high glucose. As shown in Fig. 1C, HG condition could increase the expressions of NLRP3, Pro-caspase-1, caspase-1, Pro-IL-1β and IL-1β. HG also induced a concentration-dependent increase of IL-1β secretion in MPC-5 cells (Fig. 1D). In addition, ASC speck formation is another hallmark of inflammasome activation. ASC can assemble this large protein complex, which is necessary for caspase-1 activation [22]. Our data showed that the formation of ASC speck was remarkably increased after HG treatment, as proved by confocal analysis of NLRP3/ASC (Fig. 1E). Taken together, these data demonstrated that HG could induce podocyte injury and NLRP3 inflammasome formation in MPC-5 cells.

Luteolin inhibited the activation of NLRP3 inflammasome in HG condition.

We had previously shown that HG condition could activate NLRP3 inflammasome, and increase the production of IL-1β. Thus, we further explored if luteolin could inhibit NLRP3 inflammasome activation in MPC-5 cells. Cells were treated with various concentrations of luteolin for 24 h after glucose challenge. The results of western blot (Fig. 2A) and ELISA (Fig. 2B) showed that luteolin was sufficient to inhibit NLRP3 expression, caspase-1 cleavage and mature IL-1β secretion in a concentration-dependent manner. Consistent with these results, mRNA levels of NLRP3, caspase-1 and IL-1β were also distinctly reduced in HG-induced MPC-5 cells after the addition of luteolin (Fig. 2C). Furthermore, stimulating with high concentration of glucose induced ASC speck formation, whereas luteolin treatment could reduce ASC speck formation in HG condition, as proved by confocal analysis of NLRP3/ASC (Fig. 2D). Data above collectively suggested that luteolin

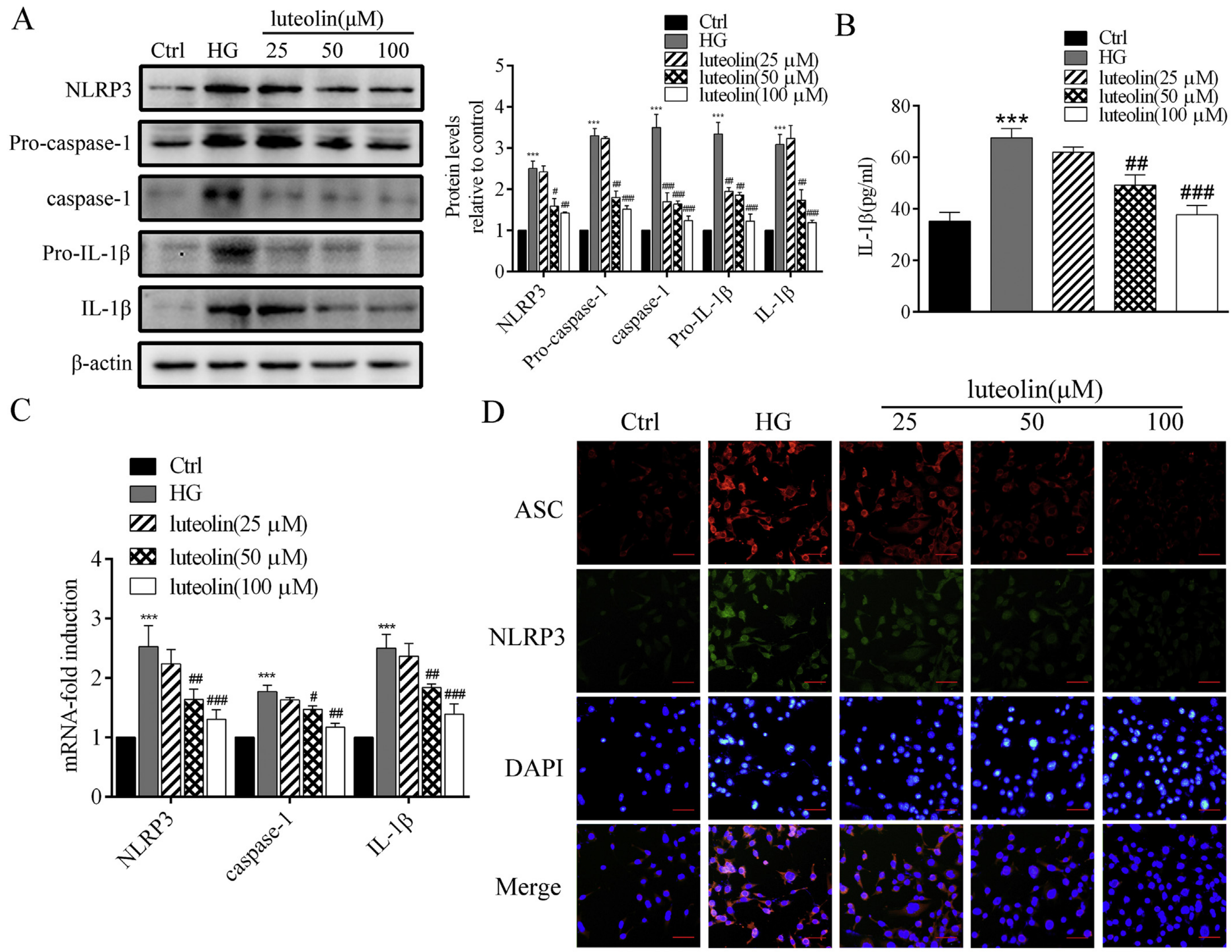

Fig. 2. The effect of luteolin on NLRP3 inflammasome activation. HG-pretreated MPC-5 cells were treated with luteolin for 24 h, then NLRP3, Pro-IL-1β, IL-1β, Pro-caspase-1 and caspase-1 were detected by western blot (A) and real-time RT-PCR (C). The IL-1β secreted in supernatant was detected by ELISA (B). Immunostaining of endogenous NLRP3/ASC was performed (scale bar, 50 μm) (D). The data (mean ± SD) are representative of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with HG group.

significantly inhibited the NLRP3 inflammasome complex formation which was induced by HG condition.

3.2. Luteolin suppressed HG-induced ROS generation in podocyte

Next, we investigated the potential mechanism of the inhibitory effect of luteolin on NLRP3 inflammasome activation. It is widely known that ROS plays a key role in diabetic nephropathy. In this study, we found a significant increase in ROS level in HG-treated MPC-5 cells, and luteolin inhibited HG-induced ROS generation in a concentration-dependent way (Fig. 3A and B). In addition, HG-treated MPC-5 cells showed an increase in MDA level, and luteolin treatment concentration-dependently reversed it (Fig. 3C). Previous studies showed that ROS could promote the deubiquitination of NLRP3, which was associated with NLPR3 inflammasome activation and IL-1β secretion. Accordingly, we found that when MPC-5 cells were pretreated with NAC, a ROS scavenger, to degrade cytosolic ROS, luteolin had not inhibitory effect on IL-1β generation (Fig. 3D). Altogether, these results demonstrated that luteolin suppressed NLRP3 inflammasome activation via preventing the ROS generation.

3.3. Luteolin prevented apoptosis induced by HG in MPC-5

To determine the effect of luteolin on podocyte apoptosis in HG condition, flow cytometry was employed. As shown in Fig. 4A, luteolin significantly decreased the apoptotic cells in HG-induced MPC-5 cells. In addition, excessive ROS was reported to be associated with mitochondrial membrane permeability [23,24]. Our data showed that HG treatment caused mitochondrial membrane potential collapse. However, luteolin concentration-dependently restored the mitochondria membrane potential and rescued mitochondria damage (Fig. 4B).

Based on the observation that luteolin could regulate apoptosis in MPC-5 cells, we detected the apoptotic markers including Bcl-2, cleavage caspase-3, cleavage caspase-6 and cleavage caspase-9. In HG-induced MPC-5 cells, luteolin treatment significantly upregulated the expression of Bcl-2, while downregulated the expressions of cleavage caspase-3, cleavage caspase-6 and cleavage caspase-9 (Fig. 4C). Taken together, these data suggested that luteolin could reverse the apoptosis induced by HG condition.

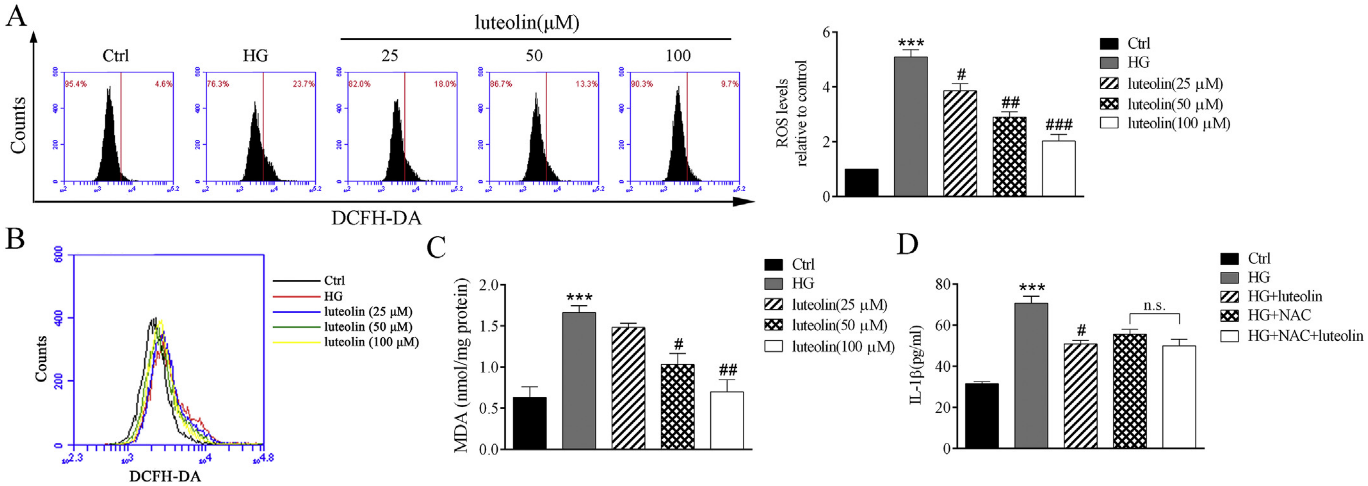

Fig. 3. Luteolin inhibited the generation of ROS in podocyte. MPC-5 cells were treated with HG, followed by incubation of luteolin and then stained with DCFH-DA (A and B). The MDA levels were detected by MDA assay (C). The IL-1β secreted in supernatant was detected by ELISA (D). The data (mean ± SD) are representative of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with HG group.

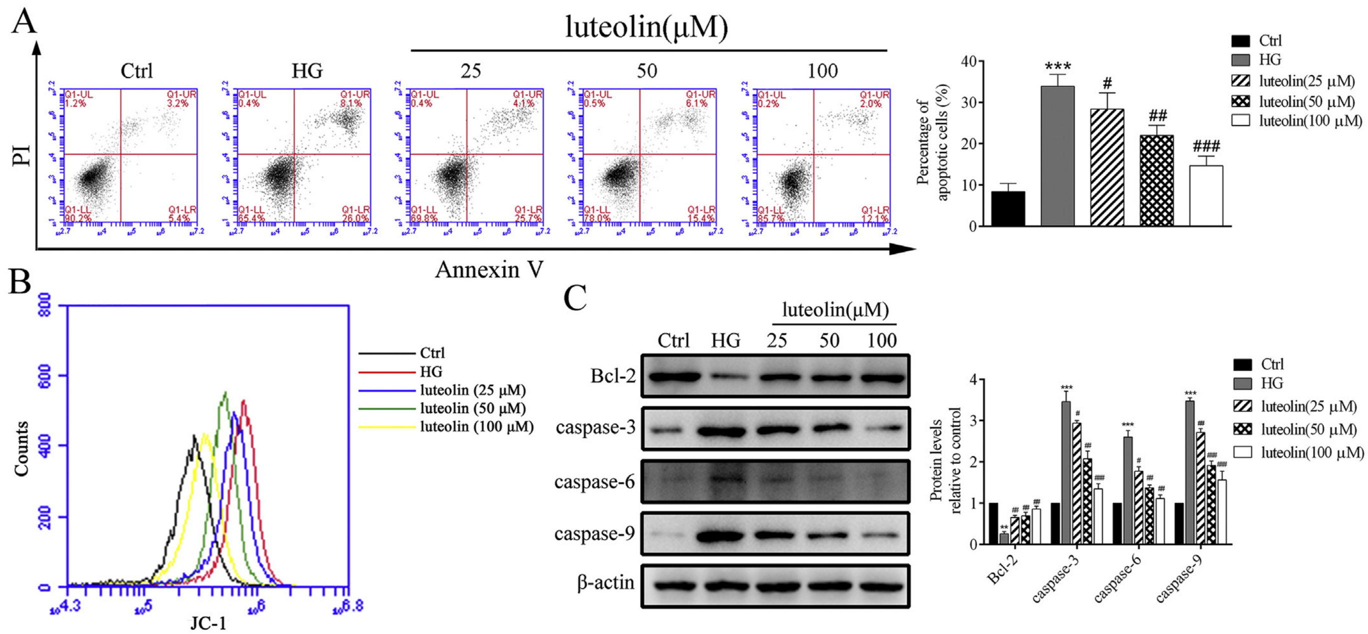

Fig. 4. Luteolin attenuated apoptosis induced by HG. MPC-5 cells were treated with HG, followed by incubation of luteolin. Apoptotic cells were detected by flow cytometry (A). Mitochondrial membrane potential was determined by JC-1 staining (B). The expression of Bcl-2, caspase-3, caspase-6 and caspase-9 were detected by western blot (C). The data (mean ± SD) are representative of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with HG group.

3.4. The anti-apoptotic effect of luteolin was dependent on NLRP3 inflammasome in MPC-5 cells

Prior works implicated that excessive activation of NLRP3 inflammasome was involved in cell apoptosis under oxidative stress [25–27]. To explore the mechanism by which luteolin regulated apoptosis, NLRP3 siRNA was used. Successful transfection was proved by western blotting (Fig. 5A), and the protein levels of caspase-1 and IL-1β were decreased in MPC-5 cells after siNLRP3 transfection, suggesting the inactivation of inflammasome. As shown in Fig. 5B, knockdown of NLRP3 significantly decreased HG-induced cell apoptosis, but the anti-apoptotic effect of luteolin was impaired when NLRP3 was absent in MPC-5 cells. Similarly , significant attenuation of JC-1 green was caused by NLRP3 silencing, indicating a rescue in mitochondrial membrane potential in HG condition. But fluorescence intensity of JC-1 green could not be further decreased by the addition of luteolin in siNLRP3-pretreated cells (Fig. 5C). In addition, knockdown of NLRP3 significantly increased the expression of Bcl-2 and decreased the expressions of cleavage caspase-3, cleavage caspase-6 and cleavage caspase-9 in HG-induced MPC-5 cells. However, luteolin treatment scarcely changed the expressions of these apoptotic markers in siNLRP3-pretreated cells, compared with the siNLRP3 alone treatment group (Fig. 5D). Therefore, these data demonstrated that luteolin exerted anti-apoptotic effect by inhibiting the NLRP3 inflammasome formation in MPC-5 cells.

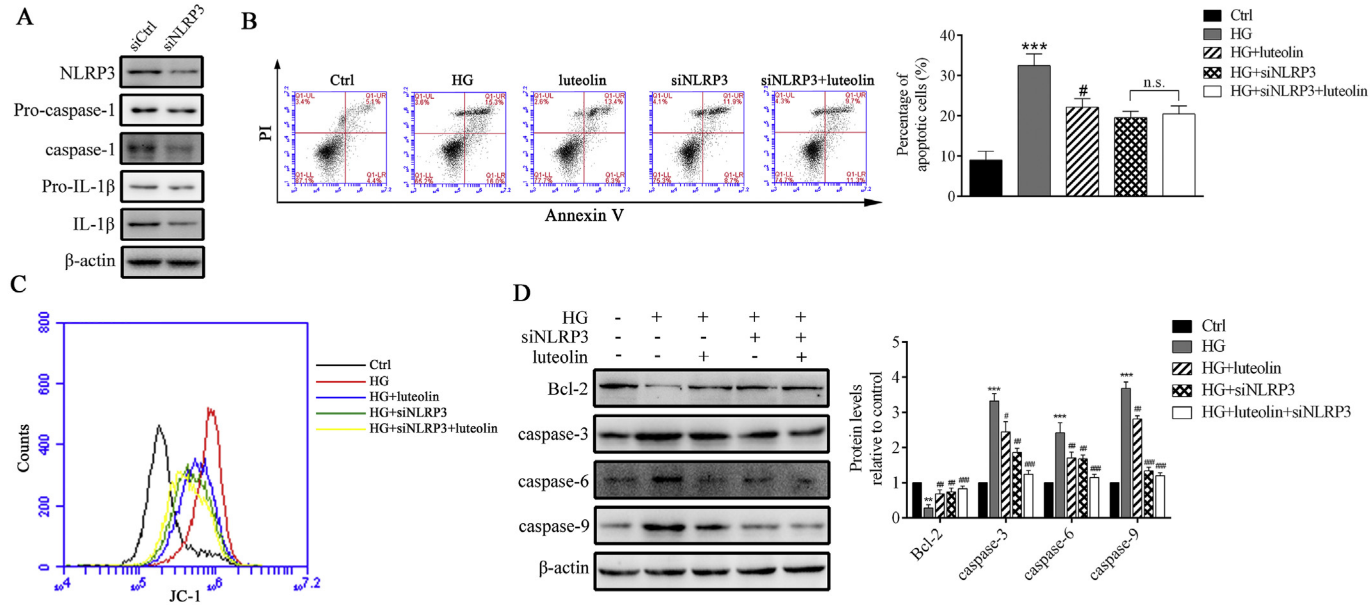

Fig. 5. Luteolin exerted its effect on apoptosis by forming NLRP3 inflammasome in MPC-5 cells. The transfection efficiency of siNLRP3 was determined by western blot (A). Cells were pretreated with siNLRP3 for 24 h before treating with luteolin in MPC-5 cells. Apoptotic cells were detected by flow cytometry (B). Mitochondrial membrane potential was determined by JC-1 staining (C). The expression of Bcl-2, caspase-3, caspase-6 and caspase-9 were detected by western blot (C). The data (mean ± SD) are representative of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with HG group.

4. Discussion

Despite the effects of luteolin on several diabetic complications such as diabetic cardiomyopathy, diabetic neuropathy, diabetic cataract and diabetic encephalopathy have been well recognized, the potential mechanism of it in diabetic nephropathy is not fully clarified. In this study, we showed that inhibition of NLRP3 inflammasome was essential in luteolin-mediated protective effects in diabetic nephropathy. Our data demonstrated that luteolin significantly suppressed NLRP3 inflammasome activation and ROS generation induced by HG condition in MPC-5 cells. In addition, we presented here an anti-apoptotic effect of luteolin in MPC-5 cells through the NLRP3 inflammasome pathway. Therefore, our data proved that luteolin ameliorated HG-induced podocyte injury via inhibiting NLRP3 inflammasome.

Podocyte is linked by slit diaphragm and covers the surface of glomerular basement membrane to modulate the permeability. It wraps the capillaries to form a filtration slit, which can maintain structural integrity of glomerular filtration membrane [28]. Podocyte loss is a crucial factor contributing to the progression of some kidney diseases including diabetic nephropathy. During diabetic nephropathy, podocyte undergoes detachment in hyperglycemia due to the loss of foot process [29]. WT-1 and nephrin are the vital molecules in the maintenance of cytoskeleton, which is downregulated during the podocyte injury [30]. In addition, desmin is another kind of cytoskeletal protein, which is upregulated during the podocyte injury [31]. In this study, we successfully established podocyte injury model using 30 mM glucose, as proved by the upregulation of desmin and downregulation of WT-1 and nephrin.

Epidemiologic studies demonstrated that inflammatory microenvironment was involved in the pathogenesis of diabetic nephropathy, which indicated that there was a close relationship between chronic inflammation and diabetic nephropathy [32]. Given the fact that diabetic nephropathy is considered as a metabolic disease due to the inflammation, the inflammasome may be a potential candidate which acts as an molecular switch to turn on the inflammatory response in podocyte [8,9]. In this study, we observed that the expressions of NLRP3, caspase-1 and IL-1β were significantly upregulated after stimulating with 30 mM glucose, indicating the activation of NLRP3 inflammasome. However, we observed reduction in ASC speck formation and the expressions of NLRP3, caspase-1 and IL-1β when MPC-5 cells were sequentially treated with luteolin. Actually, activation of the NLRP3 inflammasome needs a two-step process (priming and activation). The priming step, also named “signal 1”, which is NF-κB-dependent transcription of NLRP3. The activation step, also named “signal 2”, which is triggered by a variety of stimuli including K^{+} efflux, mitochondrial dysfunction or lysosomal rupture [12]. It is widely known that ROS can produce from many inflammatory stimuli, which is essential for priming and activation steps in NLRP3 inflammasome activation [33]. Initially, NLRP3 transcription step (priming) requires a ROS-sensitive pro-inflammatory signal, and it can be blocked by ROS scavenger [34]. Bauernfeind F and Liu X also demonstrated that blockade ROS by chemical antioxidants suppressed the priming of NLRP3 inflammasome [35,36]. Based on the well-defined anti-oxidative effect of luteolin, we hypothesized that luteolin suppressed the activation of NLRP3 inflammasome through a ROS-dependent manner. In this study, an increase of ROS was found in podocyte after treating with HG, and this increase was ameliorated by luteolin treatment. Additionally, luteolin scarcely changed the IL-1β secretion in NAC-pretreated cells, compared with the NAC alone treatment group. Thus, our results suggested that ROS served as the ligand of NLRP3 inflammasome in HG condition, and luteolin could function as a ROS scavenger to suppress the priming step of NLRP3 inflammasome in MPC-5 cells.

Recent studies have suggested that podocyte apoptosis played a key role in the pathogenesis of diabetic nephropathy [37,38]. Consistent with these studies, we found that HG condition induced apoptosis in MPC-5 cells. Cell apoptosis is reported to be associated with the caspase cascades and Bcl-2 protein family [39]. In this study, the expression of Bcl-2 was remarkably decreased during the process of HG-induced apoptosis, while the expressions of caspase-3, caspase-6 and caspase-9 were significantly increased in the same condition. We also showed that treating with luteolin attenuated the apoptosis induced by HG condition, which was indicated by the upregulation of Bcl-2 and the downregulation of the caspase-3, caspase-6 and caspase-9. Oxidative damage is reported to cause mutation in some mitochondrial genome and give way to mitochondrial dysfunction, which results in the increase of the production of ROS and mitochondria permeability [23,24]. In our study, an increase in ROS-induced damage was found in podocyte under HG condition, as shown by JC-1 staining. Furthermore, luteolin treatment concentration-dependently decreased the JC-1 accumulation in podocyte compared with the HG group.

NLRP3 inflammasome activation could be a cause of podocyte injury under HG conditions [9]. NLRP3 normally locates in the cytoplasm and stays in resting state in the basal circumstance. Once cells were stimulated with endogenous or exogenous stimuli, NLRP3 could recruit ASC and Pro-caspase-1 to form a NLRP3 inflammasome, thereby resulting in the secretion of inflammatory cytokines [12]. It was reported that pyroptosis is a kind of inflammatory cell death, which is induced by inflammasome formation and activation. This contrasts with apoptosis, a kind of non-inflammatory programmed cell death [40]. However, crosstalk between apoptosis and pyroptosis has been reported in many studies. It is widely known that the NLRP3 inflammasome can activate caspase-1, which subsequently leads to pyroptotic cell death [41]. But a study showed that NLRP3 inflammasome could also activate cell apoptosis. NLRP3 inflammasome are able to activate caspase-3 and initiate apoptosis, in addition to caspase-1-dependent pyroptosis. The balance between apoptosis and pyroptosis depends on the mount of DNA. Pyroptosis has a higher threshold for initiation and apoptosis predominated at lower DNA concentrations. Pyroptotic responses to inflammasome activation may be missed in many experimental settings, due to the low level of stimuli that are generally used [42]. It was reported that blockade of NLRP3 inflammasome could mitigate podocyte injury in HG condition [43]. In addition, recent study suggested that knockdown of NLRP3 or ASC could repress NLRP3 inflammasome formation, thereby attenuating HG-induced apoptosis [14]. Consistent with these studies, the novel finding in our study was that luteolin functioned dependently of the NLRP3 inflammasome in podocyte. Our results showed that inhibition of NLRP3 inflammasome pathway by siNLRP3 abolished the effect of luteolin on MPC-5 cells. Thus, NLRP3 inflammasome could act as a key mediator which linked luteolin and diabetic nephropathy.

In summary, our study demonstrated that luteolin could protect podocyte from HG-induced injury. In addition, luteolin alleviated podocyte apoptosis by inhibiting the activation of NLRP3 inflammasome, which might be used for the treatment of diabetic nephropathy.

Conflict of interests

The authors state no conflict of interest.

Acknowledgements

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