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Protective effects of pyrroloquinoline quinone against oxidative stress-induced cellular senescence and inflammation in human renal tubular epithelial cells via Keap1/Nrf2 signaling pathway

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ABSTRACT

Oxidative stress-induced cellular senescence and inflammation are important biological events in diabetic nephropathy (DN). Our recent studies have found that pyrroloquinoline quinone (PQQ) has protective effects against HG-induced oxidative stress damage and apoptosis in HK-2 cells. Nevertheless, whether PQQ has the effect of anti-inflammation and anti-senescence in HK-2 cells remains unclear. Here, we showed that low-dose PQQ treatment (100 nM) downregulates the expression of P16, P21, IL-1β, TNF-α and NF-κB in HG cultured HK-2 cells. A low dose of PQQ also upregulated the protein expression of SOD2, CAT and inhibited the generation of ROS. We also indicated that PQQ affected the activity of Keap1/Nrf2 pathway, increased the nuclear accumulation of Nrf2 and the downstream pathway protein expression of Keap1/Nrf2 signaling pathway (HO-1, NQO-1, GST and GPx-3). When ML385 was added to inhibit the activity of Keap1/Nrf2 signaling pathway, the effects of PQQ on anti-oxidative stress, anti-inflammation and anti-senescence in HK-2 cells under HG condition were weakened. In conclusion, our results suggest that PQQ could modulate HG-induced inflammation and senescence in HK-2 cells via the inhibition of ROS generation and achieves the protective effects through Keap1/Nrf2 pathway and upregulating the expression of its target protein.

1. Introduction

Diabetic nephropathy (DN) is one of the most common microvascular complications of diabetes mellitus [1–3]. Various studies have illustrated that high glucose (HG) increases the production of intracellular ROS and aggravates oxidative stress, thus leads to apoptosis, cellular senescence, inflammation, fibrosis and other damages of renal tubular epithelial cells [4–7]. Especially, oxidative stress-induced cellular senescence and inflammation are important biological events of renal tubular cells in DN [8–11]. Therefore, early intervention and treatment of renal tubular cell senescence and inflammation may effectively delay the development of DN.

Pyrroloquinoline quinone (PQQ) is an organic molecule as the third redox coenzyme following nicotinamides and flavines. PQQ is water-soluble, and the chemical properties are similar to the combined attributes of ascorbic acid and vitamin B6 [12]. Previous researches have demonstrated that PQQ has multiple physiological functions, including neural and cardiovascular protection [13–15], the promotion of growth and reproduction [16–18], and as an antioxidant to protect the cells from oxidative stress-induced damages [19–21]. In recent years, PQQ has become increasingly studied with its role in inflammation and cellular senescence [22,23]. Our recent studies have found that PQQ has protective effects against HG-induced oxidative stress damage and apoptosis in vitro model of diabetic nephropathy. PQQ (10 nM–10,000 nM) significantly increased the cell viability at 48 h under HG environment, especially in 100 nM concentration [24]. However, whether PQQ has the effect of anti-inflammation and anti-senescence in HK-2 cells remains unclear.

Nuclear factor-E2-related factor 2 (Nrf2), a key modulator of redox balance and signaling, which plays an important role in defending against oxidative stress damage and regulating antioxidant genes [25]. Previous studies have suggested that Keap1/Nrf2/ARE signaling

Abbreviations: DN, Diabetic nephropathy; PQQ, pyrroloquinoline quinone; NG, normal glucose; HG, high glucose; NAD+, nicotinamide adenine dinucleotide; HO-1, heme oxygenase-1; NQO-1, NAD(P)H quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; ARE, antioxidant response element; SA-β-gal, senescence associated-beta-galactosidase; GST, glutathione s-transferase; GPx-3, glutathione peroxidase 3; SOD2, manganese superoxide dismutase; CAT, catalase; ROS, reactive oxygen species

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pathway plays a critical role in delaying the progression of DN. Evidence suggests that Nrf2 may also play an important role in the regulation of inflammation and senescence [26–29]. In addition, Nrf2 is reported to be involved in suppression of pro-inflammatory situation via regulation of NF-kB signaling pathways [29,30]. Does PQQ affect the activity of Keap1/Nrf2 pathway in HK-2 cells? Whether PQQ exerts anti-oxidative stress, anti-inflammation and anti-aging effects through the Keap1/Nrf2 pathway remains unclear.

In the present study, we investigated the anti-inflammation and anti-senescence effect of PQQ in HK-2 cells under HG environment for 48 h. The effect of PQQ on the activity of Keap1/Nrf2 pathway was also further studied. The results indicated that PQQ attenuated oxidative stress-induced cellular senescence and inflammation in HK-2 cells. The protective effect of PQQ was mediated by Keap1/Nrf2 pathway.

2. Methods and materials

2.1. Chemicals and reagents

FBS and DMEM were purchased from Life Technologies BRL (Gaithersburg, MD). D-glucose, penicillin, streptomycin and pyrroloquinoline quinone (PQQ) were obtained from Sigma (St. Louis, Mo, USA). Antibodies for P16, P21, Nrf2, HO-1, NQO1, CAT, GPx-3, GST, Keap1 and Histone H3 were obtained from Abcam (Cambridge, UK). Antibodies for β-actin and SOD2 were purchased from Proteintech (Chicago, USA). Reverse Transcription System, qPCR Master Mix were purchased from Vazyme (New Jersey, USA). The β-Galactosidase Staining kit and reactive oxygen species assay kit were obtained from Solarbio (Beijing, China). The Nrf2 inhibitor ML385 was obtained from MedChem Express (New Jersey, USA). TRIzol reagent, NE-PER nuclear and cytoplasmic extraction kit were purchased from Thermo Fisher (Carlsbad, USA).

2.2. Cell culture and treatment

The Human proximal tubular epithelial cells (HK−2) were obtained from American Type Culture Collection (Manassas, USA). The cells were grown in low-glucose DMEM (5.5 mM D-glucose) that was supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37 °C. The cells were stimulated with normal concentration D-glucose medium (NG, 5.5 mmol/L), high D-glucose medium (HG, 30 mmol/L). Cells were incubated with PQQ and ML385 for the indicated concentration, respectively.

2.3. Senescence-associated β-galactosidase (SA-β-gal) staining

The HK-2 cells were treated as previously indicated in 6-well plates. Senescent cells were detected using an SA-β-gal staining kit as the manufacturer's instructions. In brief, cells were washed with PBS and fixed at room temperature for 15 min in 4% formaldehyde and 0.2% glutaraldehyde. Then the cells were incubated for 12 h at 37 °C (without CO_2) with freshly prepared SA-β-gal stain solution. Positive cells stained with SA-β-gal were observed under microscope.

2.4. Detection of intracellular ROS and mitochondrial ROS analysis

The reactive oxygen species assay kit (2′,7′-dichlorofluorescein diacetate, DCFH-DA, 10 μM) and MitoSOX red(5 μM) were used to detect the intracellular and mitochondrial ROS generation. Cells were cultured in a 6-well plate at the density of 10^6 cells/well and stimulated with different medium for 48 h and then with DCFH-DA or MitoSOX Red at 37 °C for 30 min, washed again in PBS. The intracellular ROS generation was examined under a fluorescence microscope and mitochondrial ROS was examined under laser scanning confocal microscopy.

2.5. Immunofluorescence analysis

The HK-2 cells were grown on coverslips, washed with PBS for three times, fixed in 4% paraformaldehyde for 15 min, permeabilized with 0.3% Triton X-100 and then blocked with goat serum for 30 min at room temperature. The cells were then incubated in primary antibody solution (Nrf2, 1:200) at 4 °C overnight. Next day, the cells were incubated with secondary antibodies (1:150) for 1 h at 37 °C and DAPI for 10 min at room temperature. Images were obtained with a fluorescence microscope.

2.6. Total and nuclear protein extraction

Cells were washed twice with PBS. The total cell protein was extracted using RIPA lysis buffer containing protease and phosphatase inhibitor. Nuclear protein was extracted using nuclear and cytoplasmic extraction kit according to the manufacturer's instructions. The protein concentration was determined by a BCA method. The protein samples were denatured in the SDS sample buffer at 100 °C for 7 min and then stored at −70 °C until further experimentation.

2.7. Western blot analysis

A total of 30 μg protein was separated by 10–12% SDS-PAGE and then transfered to PVDF membranes, and then blocked with 5% skim milk for 1 h at 37 °C. The membranes were incubated with a primary antibody overnight at 4 °C. Next day, they were washed with TBST (3*15 min) and incubated with secondary antibodies (1:1000). Bands were detected with ECL detection reagent by the Odyssey Fc System (LICOR, USA). The protein bands intensity were analyzed using Image J software.

2.8. RNA extraction and real time-PCR analysis

Total cellular RNA was isolated using TRIzol reagent. RNA samples (2 μg) were then reverse-transcribed into cDNA using cDNA synthesis kit. Quantitative real-time PCR was carried out using qPCR Master Mix on an Agilent Mx3000P QPCR Systems (Agilent, CA), following the manufacturer's instructions. β-actin was used as an internal control. The primers used in the study were as follows: Nrf2 (forward: 5′-TCAGCG ACGGAAAGAGTATGA-3′ and reverse: 5′-CCACTGGTTTCTGACTGGA TGT-3′), β-actin (forward: 5′-TGACGTGGACATCCGCAAAG-3′ and reverse: 5′-CTGGAAGGTGGACAGCGAGG-3′). The cycling conditions were as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. Data were analyzed using the $2^{-\Delta\Delta CT}$ method.

2.9. Treatment with a Keap1/Nrf2 pathway inhibitor

Previous studies have demonstrated the discovery and validation of ML385 as a novel and specific Nrf2 inhibitor. ML385 interacts with Nrf2 and affects the DNA binding activity of the Nrf2-Maf protein complex. The addition of ML385 decreases anisotropy in a dose-dependent manner. A dose-dependent reduction in the Nrf2 transcriptional activity is observed and the maximum inhibitory concentration is 5 μM [31]. According to the above research, ML385 was used to inhibit the Keap1/Nrf2 pathway in the present study. ML385 was dissolved in DMSO to prepare a stock solution and then diluted it into 5 μM with medium to be used.

2.10. Statistical analysis

Statistical analysis was performed using SPSS 22.0 software. Data results were shown as mean ± standard deviation (SD). The biochemical measurements were analyzed by one-way ANOVA, followed by a Student-Newman-Keuls test. $P < 0.05$ was considered statistically significant.

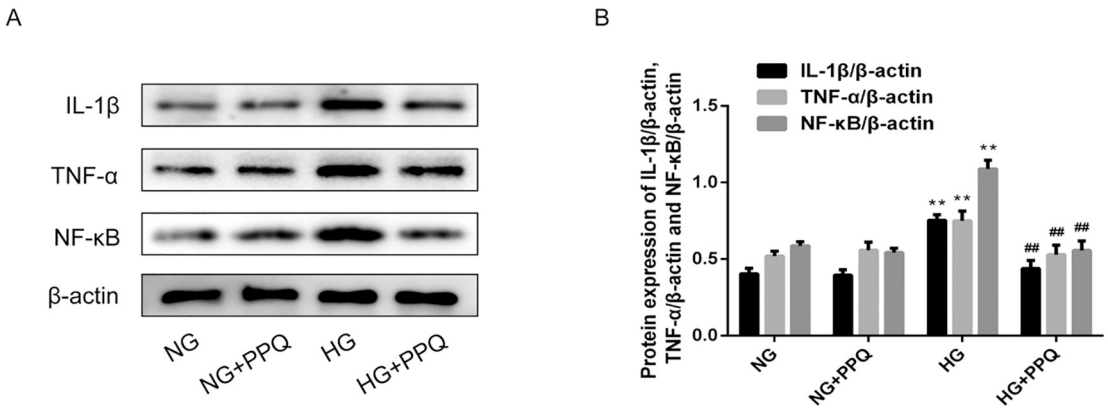

Fig. 1. PQQ inhibited HG-induced inflammation in HK-2 cells. HK-2 cells were treated with PQQ (100 nM) under NG or HG for 48 h. The protein levels of IL-1β, TNF-α and NF-κB were analyzed by Western blot ($n = 3$) (A, B). NG: normal glucose, 5.5 mM D-glucose; HG: high glucose, 30 mM D-glucose. NG + PQQ: PQQ (100 nM) plus 5.5 mM D-glucose. HG + PQQ: PQQ (100 nM) plus 30 mM D-glucose. $^{**}P < 0.05$ vs. NG; $^{\#\#}P < 0.05$ vs. HG.

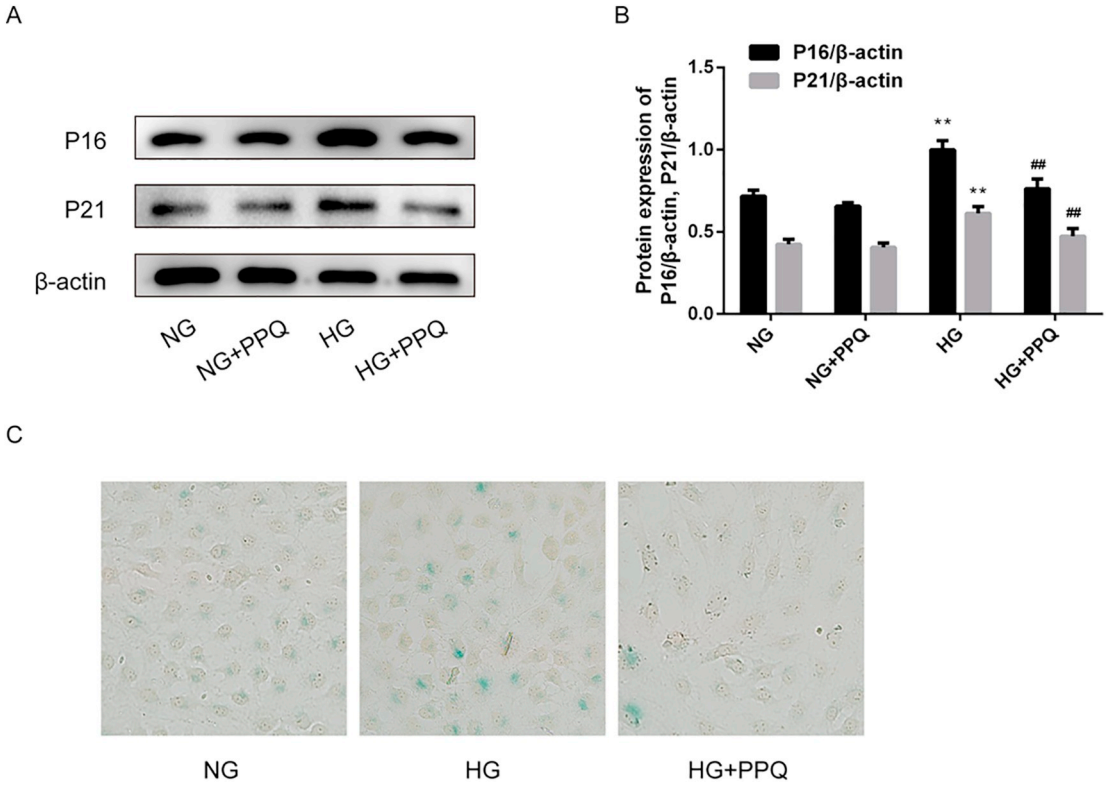

Fig. 2. PQQ inhibited HG-induced cellular senescence in HK-2 cells. HK-2 cells were treated with PQQ (100 nM) under NG or HG for 48 h. The protein levels of P16, P21 were analyzed by Western blot (n = 3) (A, B). Representative image of SA-β-gal staining in each group was captured with microscope. Blue staining means positive cells(C). Data are expressed as mean ± SD. ** $P < 0.05$ vs. NG; ## $P < 0.05$ vs. HG. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. PQQ inhibited HG-induced inflammation in HK-2 cells

In order to study whether PQQ could inhibit HG-induced inflammation in HK-2 cells, we tested the protein expression of IL-1β, TNF-α and NF-κB by western blot. As illustrated in Fig. 1A-B, the protein expression of IL-1β, TNF-α and NF-κB were significantly increased after HG stimulation for 48 h. However, PQQ treatment inhibited HG-induced secretion of these inflammatory cytokines ($P < 0.05$). These findings indicated that PQQ inhibited HG-induced inflammation in HK-2 cells.

3.2. PQQ inhibited HG-induced cellular senescence in HK-2 cells

Previous studies have shown that HG could induce cellular senescence in renal tubular epithelial cells [32]. In order to investigate the effect of PQQ on the cellular senescence in HG-induced HK-2 cells, the markers of cellular senescence including P16, P21 and cytoplasmic SA-β-gal activity were measured. The protein expression of P16 and P21 were dramatically increased at 48 h in HK-2 cells under HG condition ($P < 0.05$). Compared with HG group, the significantly increased of P16 and P21 could be reduced respectively in PQQ plus HG group ($P < 0.05$, Fig. 2A and B). Compared with NG group, more SA-β-gal positive cells were observed in HG-induced HK-2 cells. However, PQQ

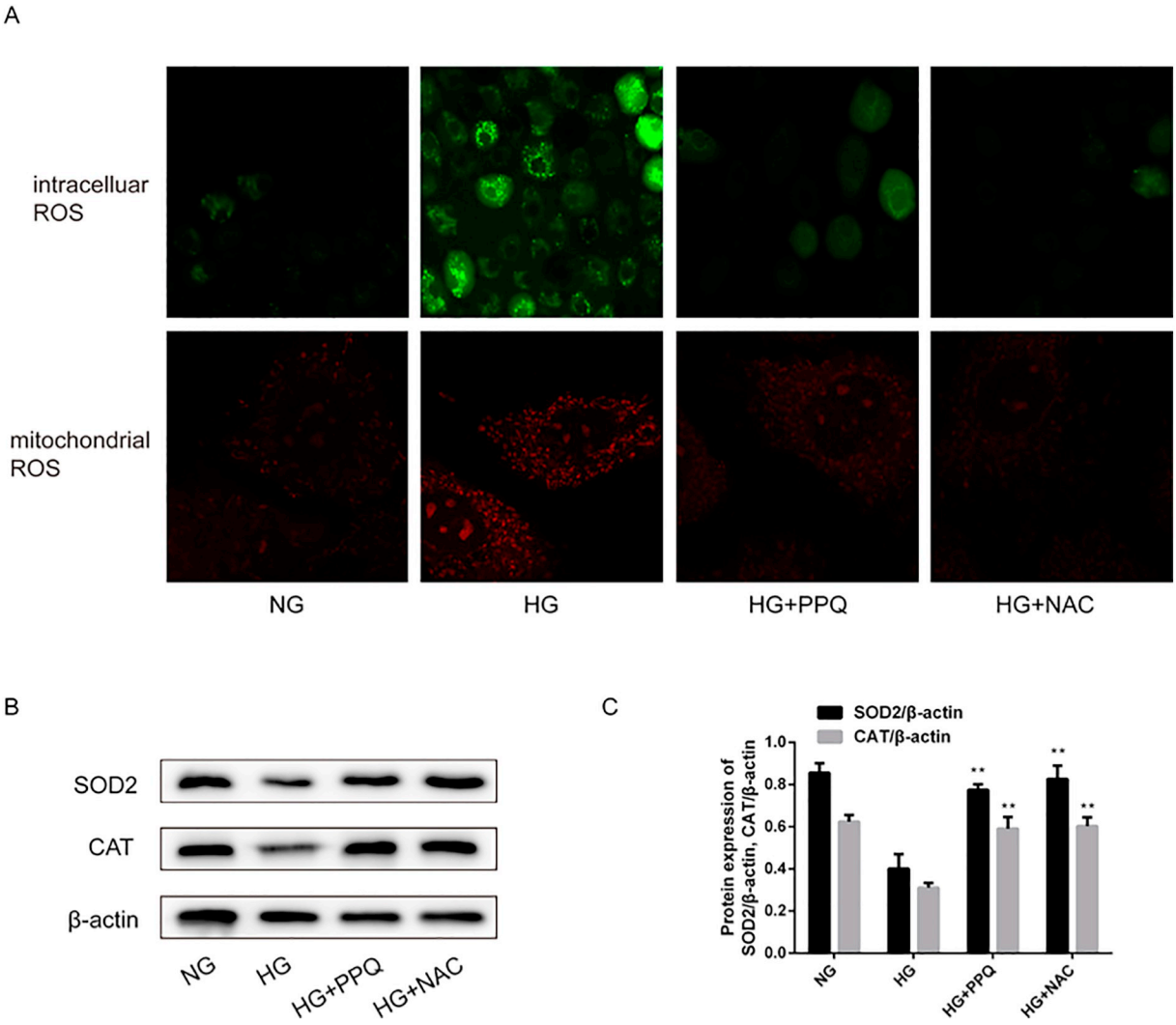

Fig. 3. Anti-oxidative stress effect of PQQ during HG-induced inflammation and cellular senescence. ROS accumulation in Intracellular and mitochondrial were detected by a fluorescence microscope in each groups (A). The protein levels of SOD2 and CAT were analyzed by Western blot (n = 3) (B, C). HG + NAC: *N*-acetylcysteine (5 mM) plus 30 mM D-glucose. Data are expressed as mean ± SD. $^{**}P < 0.05$ vs. NG; $^{\#\#}P < 0.05$ vs. HG.

treatment resulted in less SA-β-gal positive cells in comparison with the HG group (Fig. 2C).

3.3. Anti-oxidative stress effect of PQQ during HG-induced inflammation and cellular senescence

It has been demonstrated that HG-induced inflammation and cellular senescence are associated with increased reactive oxygen species (ROS) production [30,33]. Therefore, we hypothesized that PQQ could modulate HG-induced inflammation and senescence in HK-2 cells via the inhibition of ROS generation. To determine the effect of PQQ on reducing HG-induced ROS generation, we detected the mitochondrial ROS and intracellular ROS levels separately. As shown in Fig. 3A, compared with NG group, a remarkable increase of intracellular ROS and mitochondrial ROS were observed in HK-2 cells under HG conditions. However, co-treatment with PQQ significantly inhibited the generation of intracellular ROS and mitochondrial ROS under HG-induced conditions. Indeed, treatment with the known anti-oxidant NAC further reduced the level of intracellular ROS and mitochondrial ROS in the HG condition. This data suggested that PQQ could possess strong anti-oxidant properties.

We also tested the expression of SOD2 and CAT as the anti-oxidant protein. We found that the protein levels of SOD2 and CAT were reduced in HK-2 cells after exposed to HG for 48 h ($P < 0.05$, Fig. 3B, C). However, compared with HG group, the levels of anti-oxidant protein were restored significantly by PQQ ($P < 0.05$). This data clearly demonstrated that SOD2 and CAT played a central role in PQQ-mediated anti-oxidant effects.

3.4. PQQ regulated the activity of Keap1/Nrf2 pathway in HK-2 cells

Keap1/Nrf2 pathway is an important signaling pathway which is involved in oxidative stress, inflammation and cellular senescence [26,29]. GPx-3, GST, HO-1 and NQO-1 are activated by Nrf2. Therefore, we attempted to determine whether PQQ mediated the activity of Keap1/Nrf2 pathway and affected the expression of downstream pathway proteins. As shown in Fig. 4 A–C, E and F, the levels of protein Nrf2, GPx-3, GST, HO-1 and NQO-1 were reduced in HK-2 cells exposed to HG for 48 h ($P < 0.05$). However, the expression of Nrf2 and downstream pathway proteins were increased post PQQ stimulation. In addition, we found that HG significantly increased the protein level of Keap1. However, the protein expression of Keap1 was inhibited by PQQ. PQQ treatment also enhanced HG-induced mRNA expression levels of Nrf2 (Fig. 4D). The results indicated that Keap1/Nrf2 pathway was activated by PQQ in HG-induced HK-2 cells.

In order to further determine whether PQQ could affect the

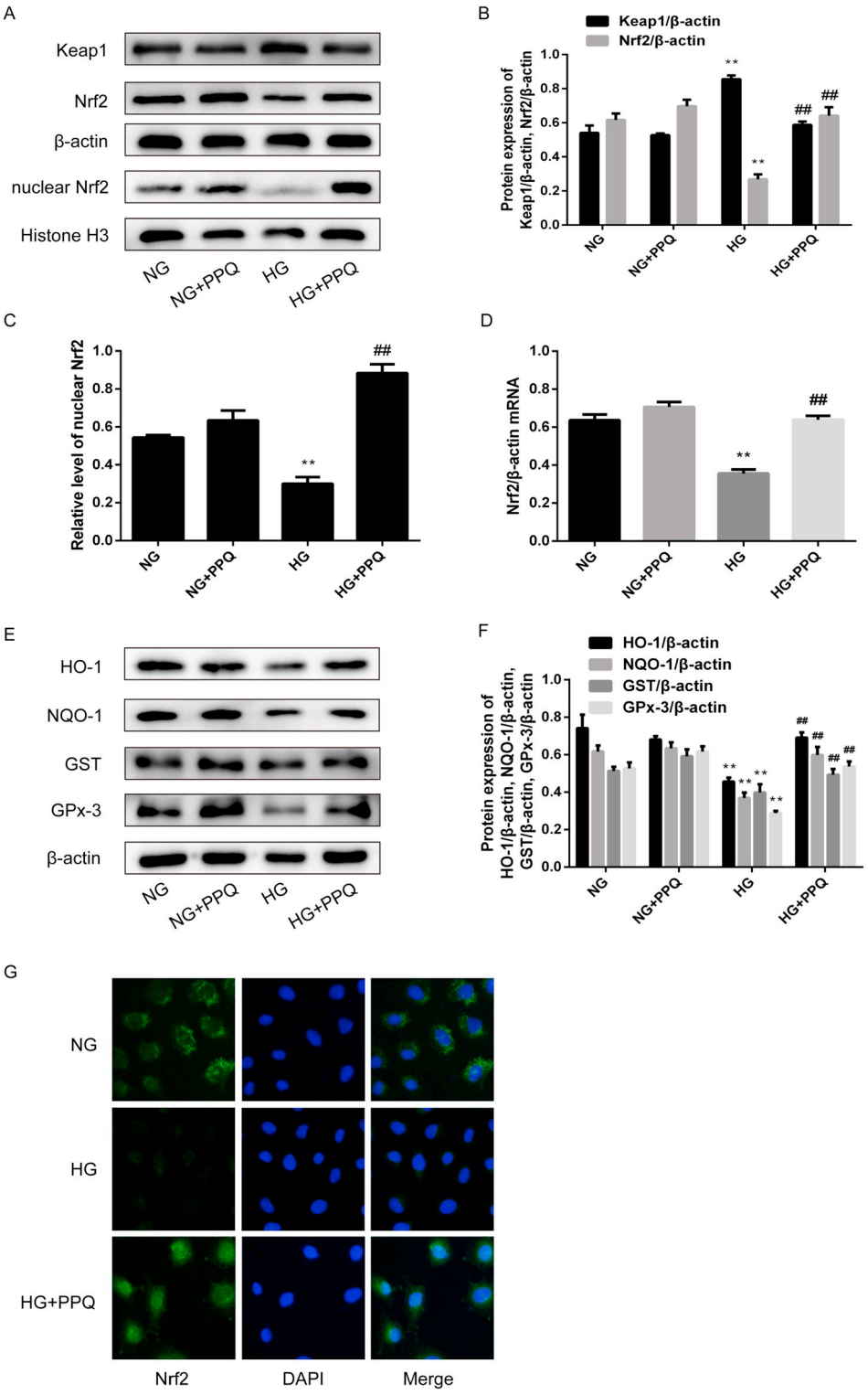

Fig. 4. PQQ regulated the activity of Keap1/Nrf2 pathway in HK-2 cells. Western blot analysis of total Nrf2, Keap1, HO-1, NQO-1, GST, GPx-3 and nuclear Nrf2 protein expression in HK-2 cells were cultured in different groups (A–C, E, F). Quantification of Nrf2 mRNA expression levels in different conditions-treated HK-2 cells by realtime-PCR (D). Immunofluorescence microscopy was used to visualize the nuclear translocation of Nrf2 (Green), Nuclei were visualized using DAPI counterstaining (Blue) (G). Data are expressed as mean ± SD. n = 3, $^{**}P < 0.05$ vs. NG; $^{\#\#}P < 0.05$ vs. HG. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

transcriptional activity of Nrf2, we monitored the nuclear translocation of Nrf2 by immunofluorescence analysis and the change of protein levels of nuclear Nrf2. As immunofluorescence staining showed that Nrf2 was mainly expressed in cytoplasm under NG conditions in HK-2 cells (Fig. 4E). There was no significantly Nrf2 signal in either the cytoplasm or nucleus in HK-2 cells under HG condition for 48 h. However, when the cells were co-treated with PQQ, the nuclear accumulation of Nrf2 was significantly increased. Compared with the NG control group, HG significantly reduced the protein levels of nuclear Nrf2 ($P < 0.05$, Fig. 4A and C). However, the protein expression of nuclear Nrf2 was increased when the cells were co-treated with PQQ. The result strongly supported that PQQ could affect the activation of Nrf2-dependent antioxidant genes and the nuclear translocation of Nrf2.

3.5. ML385, a specific Nrf2 inhibitor, suppressed the effect of PQQ on Keap1/Nrf2 pathway in HG-cultured HK-2 cells

In order to further confirm whether Keap1/Nrf2 signaling pathway

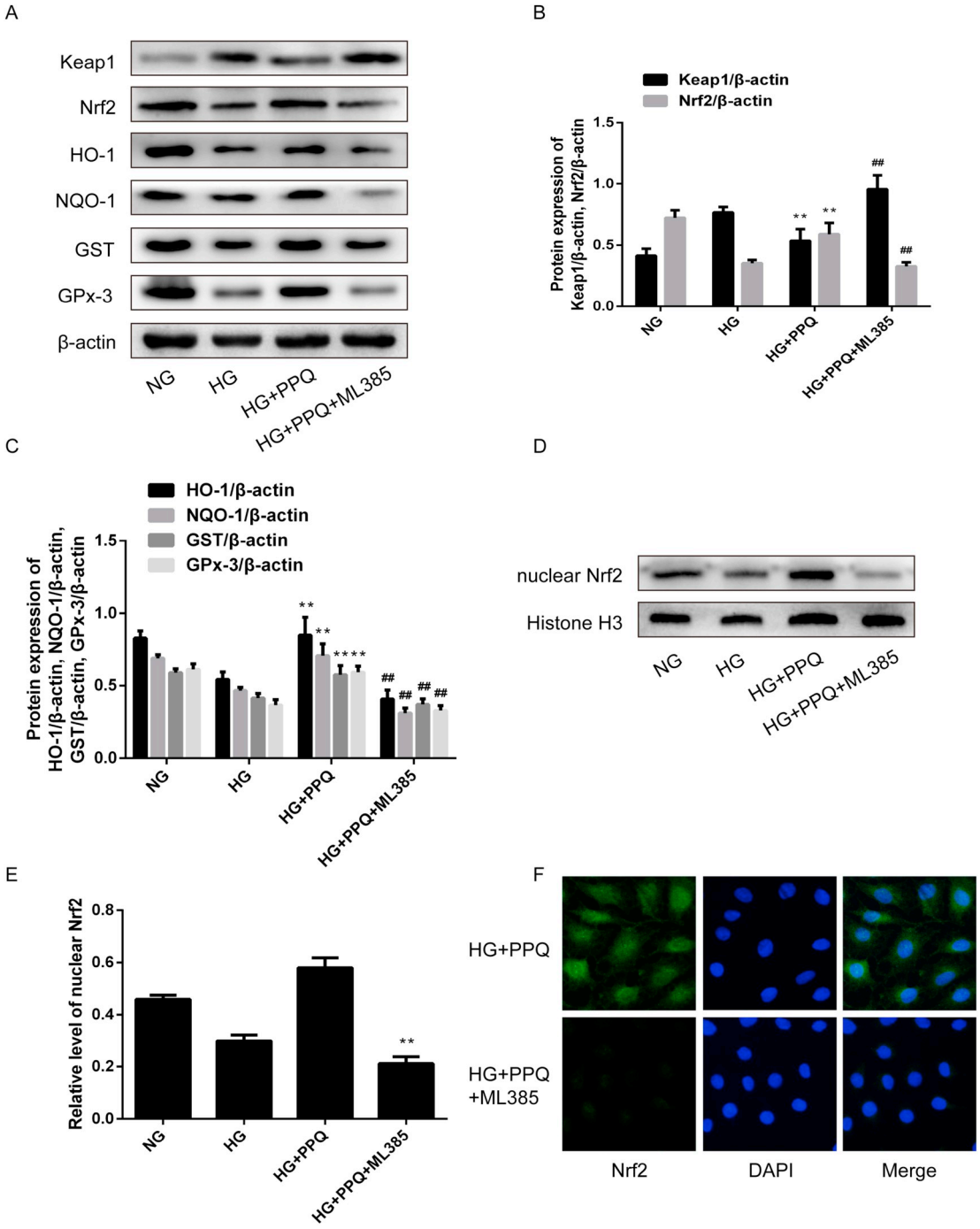

Fig. 5. ML385, a Nrf2 inhibitor, suppressed the effect of PQQ on Keap1/Nrf2 pathway in HG-challenged HK-2 cells. Total Nrf2, Keap1, HO-1, NQO-1, GST, GPx-3 and nuclear Nrf2 protein expression of HK-2 cells in different groups were analyzed by western blot (A–E). The nuclear translocation of Nrf2 was visualized by Immunofluorescence microscopy (F). Data are expressed as mean ± SD. n = 3, HG + PQQ + ML385: PQQ (100 nM), ML385 (5 μM) plus 30 mM D-glucose. **P < 0.05 vs. HG + PQQ.

was involved in the protective effects of PQQ, ML385 was used to inhibit the pathway activity. As shown in Fig. 5A-C, treatment with ML385 (5 μM), the expression of Nrf2 was markedly decreased in HK-2 cells under HG plus PQQ condition ($P < 0.05$). We also monitored the effect of ML385 on the nuclear translocation of Nrf2. After the addition of ML385, the nuclear transfer effect of PQQ on Nrf2 was weakened ($P < 0.05$, Fig. 5A, D and E). The pro-expression effect of PQQ on GPx-3, GST, HO-1 and NQO-1 were also inhibited ($P < 0.05$, Fig. 5A and C). This indicated that ML385 could block the excitatory effect of PQQ on Nrf2 in HK-2 cells under HG condition.

3.6. When ML385 was added to inhibit the activity of Keap1/Nrf2 signaling pathway, the effects of PQQ on anti-inflammation and anti-senescence in HK-2 cells under HG condition were weakened

Next, we explored the effect of PQQ on HG-induced HK-2 cell inflammation and cellular senescence after inhibiting Keap1/Nrf2 pathway. Compared with the HG + PQQ group, the protein expression of IL-1β, TNF-α, NF-κB, P16 and P21 were increased ($P < 0.05$, Figs. 6 and 7) after adding ML385. More SA-β-gal positive cells were observed in HK-2 cells. The results suggested that the protective effect of PQQ was weakened by exposure to ML385.

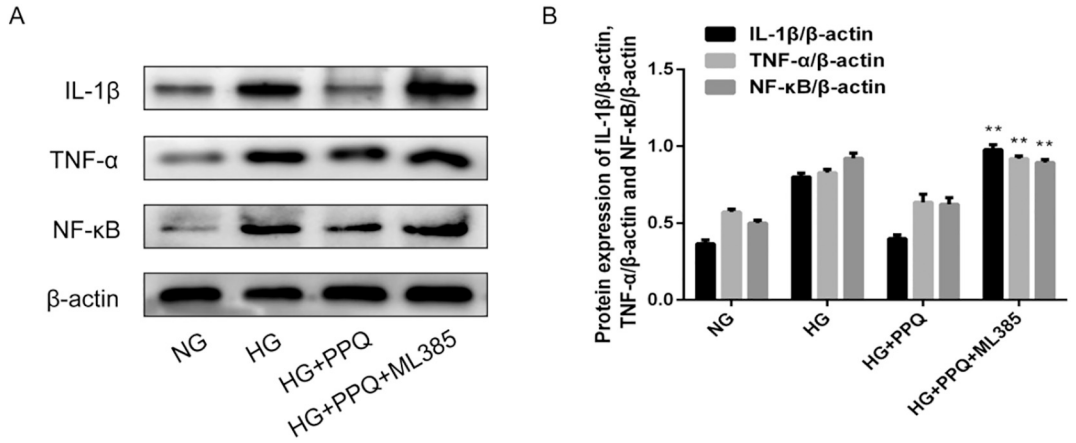

Fig. 6. Inhibition of Nrf2 suppressed the effect of PQQ on HG-induced inflammation in HK-2 cells. The protein levels of IL-1β, TNF-α and NF-κB were analyzed by Western blot (n = 3) (A, B). Data are expressed as mean ± SD. n = 3, **P < 0.05 vs. HG + PQQ.

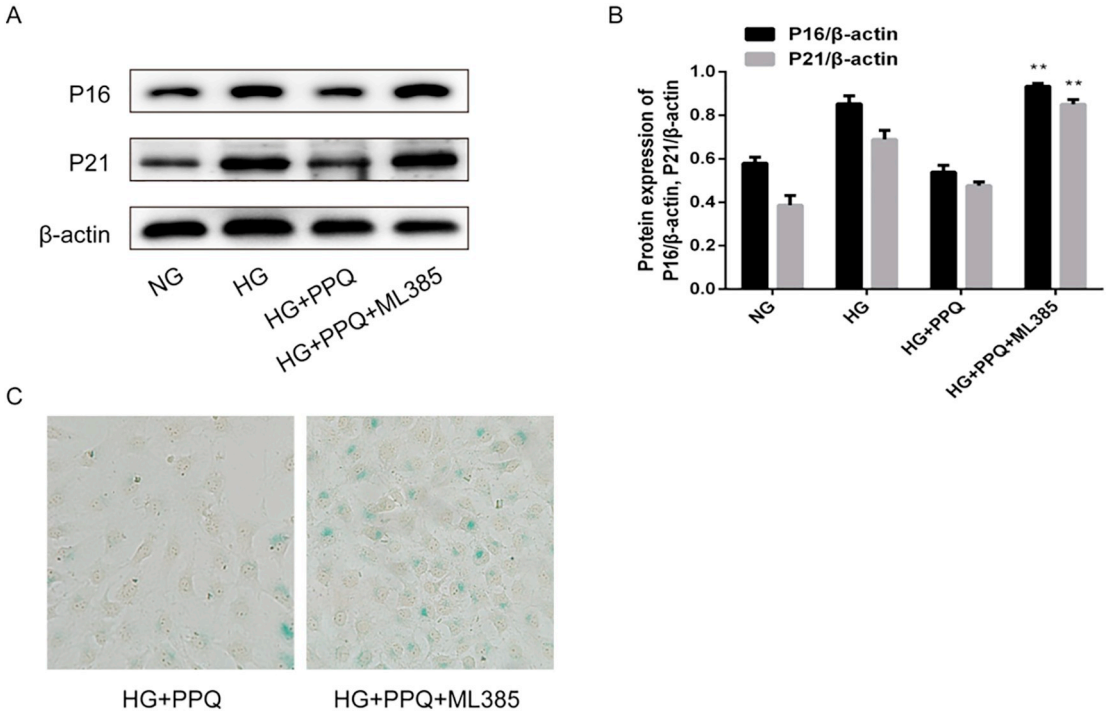

Fig. 7. Inhibition of Nrf2 suppressed the effect of PQQ on HG-induced cellular senescence in HK-2 cells. The protein levels of P16, P21 were analyzed by Western blot (n = 3) (A, B). Representative image of SA-β-gal staining in each group was captured with microscope (C). Data are expressed as mean ± SD. n = 3, **P < 0.05 vs. HG + PQQ.

4. Discussion

The results suggest that PQQ has protective effects against HG-induced inflammation and cellular senescence in HK-2 cells. The protective effects of PQQ are associated with inhibition of ROS generation and upregulating the level of antioxidants. In this study, we also illustrate that PQQ could affect the activity of Keap1/Nrf2 pathway, promote the translocation of Nrf2 to nucleus and upregulate the protein expression downstream pathway of Keap1/Nrf2 signaling pathway. PQQ achieves the anti-inflammation and anti-senescence effects through Keap1/Nrf2 pathway. When the activity of Keap1/Nrf2 pathway was inhibited with ML385, the effects of PQQ on anti-oxidative stress, anti-inflammation and anti-senescence in HK-2 cells under HG condition were weakened.

It has been demonstrated that various inflammatory and cellular senescence processes play an important role in the pathology of DN [32,34]. Therefore, it is significantly to search for effective components with anti-inflammatory and anti-senescence characteristics to delay HG-induced injury. PQQ has been reported to exert protective effects against oxidative stress in the heart, liver and brain through decreasing ROS and attenuating oxidative stress [35,36]. In a recent study, PQQ was reported to protect human dermal fbroblast from ultraviolet A irradiation induced senescence [23]. Our previous research also found that PQQ protects HK-2 cells against HG-induced oxidative stress and apoptosis through Sirt3 and PI3K/Akt/FoxO3a signaling pathway [24]. In the present study, we focused on the protective effects of PQQ on HG-induced inflammatory and cellular senescence in HK-2 cells. It was demonstrated that the protein expression of IL-1β, NF-κB, TNF-α, P16 and P21 could be reduced by PQQ, as well as SA-β-gal activity. These results suggested that PQQ has a protective effect on HG-induced HK-2 cells. In this research, the results showed that PQQ could increase the level of SOD2, CAT and down-regulate the generation of ROS in HK-2

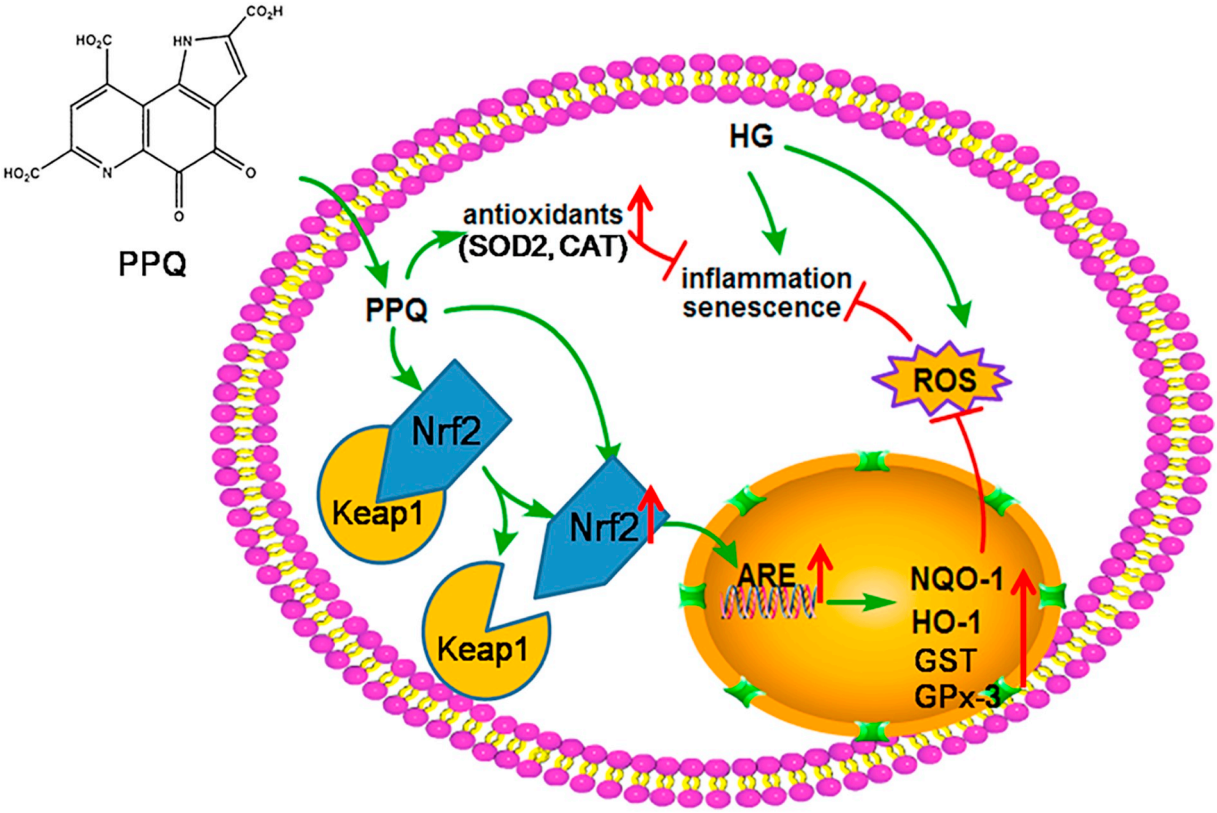

Fig. 8. Proposed mechanism of PQQ in the prevention of inflammation and cellular senescence. Pyrroloquinoline quinone (PQQ), a new B vitamin, has been demonstrated to be important in anti-inflammation and anti-senescence effects. PQQ may enter the cells and act directly as antioxidant, anti-inflammatory and anti-senescence molecules, promote the upregulation of Keap1/Nrf2 pathway. Nrf2 migrates to the nucleus and induces antioxidant enzymes and rises the cell antioxidant capacity. Then, along with reducing of the ROS generation and upregulating the expression of antioxidant protein, the oxidative stress-induced cellular senescence and inflammatory response are attenuated. PQQ, Pyrroloquinoline Quinone; Nrf2, nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; NQO-1, NAD(P)H dehydrogenase quinone 1; HO-1, heme oxygenase-1; ROS, reactive oxygen species; GST, Glutathione S-Transferase; GPx-3, Glutathione Peroxidase 3.

cells. We also found that the anti-oxidative stress effect of PQQ was similar to that of NAC. *N*-acetylcysteine (NAC), an antioxidant, attenuated inflammatory and premature senescence, and its effects were associated with its anti-oxidative stress effect [37,38]. Taken together, these findings suggest that the anti-inflammatory and anti-senescence effect of PQQ may be dependent on the clearance of ROS.

The remaining question is how PQQ protects HK-2 cells from HG-induced inflammatory and cellular senescence. Previous studies have revealed that Keap1/Nrf2 signaling pathway is a major regulatory system controlling the expression of antioxidant and detoxification enzymes, which has also played a role in alleviating inflammation and cellular senescence [39,40]. Nrf2 regulates the expression of some related downstream protein including NQO-1, HO-1, GST, GPx-3 and ferritin [41–43]. Treatment of mouse hepatoma epithelial-like cells with PQQ increases PGC-1 promoter activity, which induces NRF1 and NRF2 and Tfam. The downstream targets of PGC1 (NRF-1, NRF-2, and Tfam) are upregulated to drive mitochondrial biogenesis [44]. However, the mechanistic study of PQQ is in the early stage. To our knowledge, the activation of Keap1/Nrf2 pathway by PQQ in HG-induced HK-2 cells has not yet been reported. In this study, we measured the expression level of Nrf2, Keap1, HO-1, NQO-1, GST and GPx-3 to demonstrate that PQQ could increase the activity of Keap1/Nrf2 signaling pathway and the downstream targets of Nrf2 (HO-1, NQO-1, GST and GPx-3). The results also showed that PQQ treatment could promote the translocation of Nrf2 to nucleus. Furthermore, ML385 was noted to significantly inhibit the expression of Nrf2. When Nrf2 was inhibited, the protective effect of PQQ on oxidative stress, senescence and inflammation disappeared. Our findings indicated that the Keap1/Nrf2 pathway and its target gene played an important role in PQQ-regulated protective effect in HK-2 cells (Fig. 8).

In conclusion, our results illustrate that PQQ could protect HK-2 cells from HG-induced inflammation and senescence. The possible mechanism may be involved of its anti-oxidative stress effect, increasing the activity of Keap1/Nrf2 pathway, promoting the translocation of Nrf2 to nucleus and up-regulating its target gene.

Conflicts of interest

The authors declare no conflict of interest.

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