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Quercetin Alleviates Hyperglycemic-Generated Endoplasmic Reticulum Stress-Contacted Apoptosis of Rat Nucleus Pulposus Cells

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Background: Previous research has shown that apoptosis of nucleus pulposus (NP) cells contributes to intervertebral disc degeneration (IDD) progression. Endoplasmic reticulum (ER) stress is a reaction to diverse stimuli in eukaryotes and is tightly contacted with apoptosis. Quercetin, a naturally occurring flavonoid, exerts protective effects against degenerative diseases via ER stress. However, the effect of quercetin on NP cell apoptosis remains unclear.

Aims: To investigate the influences of quercetin on apoptosis and ER stress in a high-glucose-generated primary NP cell model.

Study Design: In vivo animal experimental study.

Methods: To investigate the influence of quercetin in a high-glucose-generated NP cell apoptosis model, control, glucose, and glucose + quercetin groups adopted with Sprague-Dawley rats primary NP cells. In the glucose group, cell apoptosis was generated by 200 mm high glucose. In the glucose + quercetin group, 60 μm quercetin was pretreated with NP cells for 2 h before glucose administration. In this research, we examined the change effect of quercetin on NP cell apoptosis, ER stress, and the protein kinase R-like ER kinase (PERK)-eukaryotic translation initiation element 2α (eIF2α)-activating transcription element 4 (ATF4).

Results: High glucose decreased the viability and induced ER stress-related apoptosis in NP cells. Quercetin modulated ER stress through the PERK-eIF2α-ATF4 pathway, thereby alleviating the apoptosis rank in NP cells.

Conclusion: Quercetin exerts antiapoptotic effects on NP cells, probably through ER stress, thereby showcasing potential as a therapeutic method for treating IDD.

INTRODUCTION

Intervertebral disc degeneration (IDD) is closely associated with lower back pain (LBP), which frequently results in poor quality of life and enormous clinical and socioeconomic burden.[1,2] As the principal tissue of the IVD, the nucleus pulposus (NP) plays an executive role in buffering mechanical stress in the joints. Therefore, increased NP cell death, predominantly through apoptosis, is the most prominent cause of IDD.[3,4] Several studies have argued that hyperglycemia is important in IDD etiology and that a high glucose concentration can cause NP cell apoptosis.[5,6] Based on this physiological correlation, relieving the NP cell death generated by high glucose ranks has become the key approach.[6-10]

Endoplasmic reticulum (ER) stress is a type of apoptosis initiation way that occurs in diverse diseases.[11] Once eukaryotic cells are displayed in a series of extracellular stimuli, including hypoxia, inflammation, and nutrient and energy metabolism disorders, ER stress balances such turbulence.[12-15] The 78-kDa glucose-regulated protein (GRP78) is regarded as an ER stress-activation marker.[13,16] Nevertheless, once ER stress is excessive or activated over a prolonged period, apoptosis can occur through the lower signal C/EBPα-homologous protein (CHOP) and caspase,[12] which is the ER-specific caspase.[12,17,18] Meanwhile, the impact of PKR-like endoplasmic reticulum kinase (PERK)-eukaryotic translation initiation element 2α (eIF2α)-ATF4 integrated stress response (ISR) cannot be ignored, which is a conservation principle wherein eukaryotic cells employ gene expression recombination to fit both endogenous and exogenous stress, a rapid, reversible process.

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The phosphorylate-eIF2α is an important ISR inducer under stress conditions.[19] Increasing numbers of studies have revealed that ER stress-related cell death is important in IDD.[20-22]

Quercetin is an approved drug that displays anti-senescence, anti-inflammatory, antioxidative, and anti-apoptosis properties by adjusting senescence, oxidative stress, autophagy, apoptosis, and ER stress in diverse chronic and degenerative diseases.[23-28] Evidence supports that quercetin attenuates apoptosis by inhibiting ER stress in chondrocytes.[29,30] Quercetin has also been reported to protect NP cells from interfering with the IDD process.[31,32] However, the molecular principles through which quercetin affects NP cells remain obscure. Considering the comprehensive and effective application of quercetin, we hypothesized that it exerts therapeutic potential against apoptosis and ER stress in NP cells.

Although some studies have verified that HG-generated apoptosis is an important degeneration modulator,[8,33] it remains elusive whether ER stress can induce HG-generated apoptosis in NP cells. However, the effects of quercetin on NP cells remain unexplored. Therefore, in the present study, we examined whether ER stress participates in the apoptotic phenomenon generated by high glucose levels and investigated the defense principle of quercetin against apoptosis in NP cells.

MATERIALS AND METHODS

Separation and fostering of primary cells

A total of 48 Sprague-Dawley (SD) rats (male, 200-230 g, 6-8 weeks old) were obtained from the Laboratory Animal Center and proceeded with the permission and guidance of the Ethics Committee of Beijing Jishuitan Hospital Guizhou Hospital Subcommittee of Ethics (approval number: KT2024090501, date: 05.09.2024). After the rats were euthanized through a pentobarbital sodium overdose, their thoracic, lumbar, and caudal discs were obtained under a microscope and sliced into 1 mm^3 fragments under aseptic conditions. The gel-like NP organizations were extracted with 0.1% type 2 collagenase (Solarbio, Beijing, China) for 6 h and centrifuged at 1000 rpm for 3 min; the precipitate was then cultivated in Dulbecco's Modified Eagle solution: F12 (DMEM/F-12, Hyclone, LA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Carlsbad, CA, USA) and 1% antibiotics (100 u/ml penicillin and 100 u/ml streptomycin) (Gibco) and grown in a humidified incubator (37 °C, 21% O_2 and 5% CO_2). The solution was replaced every 3 days, and NP cells were cultivated at a 1:3 ratio once they reached 75% confluence. The therapeutic drug quercetin was purchased from MedChemExpress LLC (MCE, New Jersey, USA).

Treatments of the NP cells

Three groups were created to demonstrate the influence of quercetin in the high glucose-generated NP cell apoptosis model, including the control, glucose, and glucose + quercetin groups. The third passage of primary NP cells of 3 SD rats was fostered in a 6 cm culture dish and used for each group. Before the experimental procedure, glucose was dissolved in DMEM/F-12 as 1 M mother liquid, and quercetin was pre-dissolved in dimethyl sulfoxide (DMSO, MCE) up to 1 mm and then diluted in DMEM/F-12 as 100 μm mother liquid. Then, 6 cm cell culture dishes were picked, corresponding to the 4 ml volume of solution. In the control group, NP cells were cultivated in DMEM/F-12. In the glucose group, the cells were generated by 200 mm high glucose for 2 h for apoptosis. In the glucose + quercetin group, 60 μm quercetin was pretreated with NP cells for 2 h and then cleaned before the administration of 200 mm glucose.

Hematoxylin-eosin and toluidine blue dyeing

First, the coverslip was cleansed with PBS when the cells converged to approximately 80% confluence. Next, the cells were fixed with 4% paraformaldehyde for 20 min and rinsed thrice with PBS. The nuclei were stained with the hematoxylin solution (Solarbio) for 8 min and rinsed under running water. The stained cells were then differentiated using a 1% acid-alcohol solution for 30 s and rinsed again under running water. The cells were stained in a saturated Li2CO3 solution for 30 s and rinsed under running water. Finally, the cells were stained with the eosin solution for 1 min and rinsed under running water.

Toluidine blue staining was performed when the cells reached approximately 80% confluence. The cells were immobilized with 95% alcohol for 15 s and rinsed thrice with phosphate-buffered saline (PBS, Thermo, Waltham, MA, USA). The cells were then stained with toluidine blue solution (Solarbio) for 5 min, and equal volumes of distilled water were incorporated for the next 15 min. Subsequently, the cells were rinsed under running water and dried by airing a glycerol-sealed piece. The cells stained with hematoxylin-eosin and toluidine blue were then observed using a reversed microscope (Leica Microsystems CMS GmbH, DFC450 C, Wetzlar, Germany) to observe the cell morphology. Hematoxylin-eosin and toluidine blue staining were performed to observe the cellular morphology under a reversed microscope (Leica Microsystems CMS).

Cell viability studies

To investigate the influences of diverse quality of high sugar and quercetin on the activity of rat NP cells, the Cell Counting Kit-8 (CCK-8) assay was used. Approximately 2000 cells were inoculated in the well of a 96-well plate and then incubated in a humidified atmosphere (37 °C, 21% O_2, and 5% CO_2) for 24 h. The adherent NP cells were treated with diverse quality of high-glucose or quercetin for 6 or 24 h. The wells were then rinsed three times with PBS. The CCK-8 assay reagent (Dojindo, Kumamoto, Japan) was incorporated into each solution of the disparate groups and then incubated for 1 h in a standard incubator. The absorbance was measured at 450 nm by using a microplate reader. Each assay was performed in triplicates.

Detection of the nuclear morphology

Hoechst 33258 (Beyotime Institute of Biotechnology, Shanghai, China) was used to examine the nuclear morphology by using a fluorescence microscope. Cured cells were fixed with 4% paraformaldehyde for 10 min at room temperature and rinsed twice with PBS. The cultured cells were stained with Hoechst 33258 for 5 min and rinsed twice with PBS, followed by observation of the cells under a fluorescence microscope (Leica Microsystems CMS GmbH) at a stimulated wavelength of 350 nm.

Western blotting

The cells were fostered in M-PER® Mammalian Protein Extraction Reagent (Thermo) containing protease and phosphatase inhibitor (Thermo) on an ice bath for 3 min and centrifuged at 4 °C at 12000 × g for 15 min to extract the supernatant. Sample proteins were electrophoresed by SDS-PAGE (Invitrogen, Mississauga, ON, Canada) and transferred onto PVDF membranes (EMD Millipore, Billerica, MA, USA, IPFL00010, USA). After sealing with 5% skim milk sealing film for 1 h, the membrane was treated with the following primary antibodies at 4 °C overnight: collagen II (1:1000), GRP78 (1:1000), PERK (1:1000), eIF2α (1:1000), ATF4 (1:1000), CHOP (1:1000), BAX (1:1000), Bcl-2 (1:1000) (Proteintech, Wuhan, China), caspase-12 (1:1000) (ABclonal, Wuhan, China), β-actin (1:5000), cleaved-caspase 3 (1:500), phospho-PERK (1:500), and phospho-eIF2α (1:1000) (CST, Danvers, MA, USA). The membranes were then rinsed with Tris-buffered saline-tween20 (TBST, Thermo) and treated with HRP-conjugated secondary antibody (1:2000) (CST) at 37 °C for 1 h. Subsequently, the protein bands on the PVDF membranes were visualized using the Western Chemiluminescent HRP Substrate (Millipore) and the CLINX Imaging System (CLINX Science Instruments, Shanghai, China). Protein expression was assessed using ImageJ software (National Institutes of Health, USA) and normalized to β-actin.

Immunofluorescence staining

The experimental cells were inoculated on coverslips in a 24-well plate and incubated overnight. The post-healed cells attached to the coverslips were fixed with 4% paraformaldehyde for 20 min at room temperature. The coverslips were then rinsed with PBST and permeabilized with 0.2% Triton X-100 (Thermo). These cells were then sealed with 2% bovine serum albumin (BSA, Solarbio) for 1 h at 37 °C after washing thrice with PBST. Sequentially, the cells were treated with primary antibodies overnight at 4 °C and then with immunofluorescence secondary antibodies for 1 h at room temperature. The nuclei were stained with an antifade mounting solution with 4',6-diamidino-2-phenylindole (DAPI, Invitrogen) for 5 min and photographed under a fluorescence microscope. The primary antibodies used included collagen II (1:50, 28459-1-AP, Proteintech), GRP78 (1:50, 66574-1-lg, Proteintech), CHOP (1:50, 66741-1-lg, Proteintech), caspase-12 (1:50, A0217, ABclonal), and phospho-eIF2α (1:1000, #3398, CST). Immunofluorescence secondary antibodies included anti-mouse IgG (1:500, #4410, CST) and anti-rabbit IgG (1:500, #4413, CST).

Statistical analysis

SPSS V.24.0 (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analyses. The Kolmogorov-Smirnov test was performed to determine the normality of the distribution of continuous numerical variables. Depending on whether the variables were normally distributed, the variables were exhibited as averages with standard deviation or as medians with interquartile ranges. Statistical distinctions between two or multiple group averages were measured with Student's t-test or One-Way ANOVA for comparison, and the Bonferroni test was used for the post-hoc test of one-way ANOVA. $P < 0.05$ was considered to indicate statistical significance.

RESULTS

1. Identification of primary rat NP cells

Immunofluorescent staining for collagen II (green fluorescence), which mostly exists in the cytoplasm, was conducted (Figure 1a). Remarkably, the green fluorescence intensity increased near the nucleus (blue fluorescence). HE staining demonstrated that the rat NP cells appeared polygonal, spindle-shaped, or irregular (Figure 1b). In addition, toluidine blue stained the gathered proteoglycans enriched in the NP cells (Figure 1c).

2. Influences of diverse hyperglycemic quality on the viability, ER stress, and apoptosis of NP cells

We performed the CCK-8 assay to determine the influence of hyperglycemia on rat NP cell liveness. The liveness of NP cells decreased with increasing concentrations of high glucose. The percentage of living cells approached LC50 (50% lethal concentration) when the glucose concentration reached 300 mm, which was regarded as the half-lethal dose (Figure 2a). Western blotting was performed to detect the BAX, Bcl-2, and

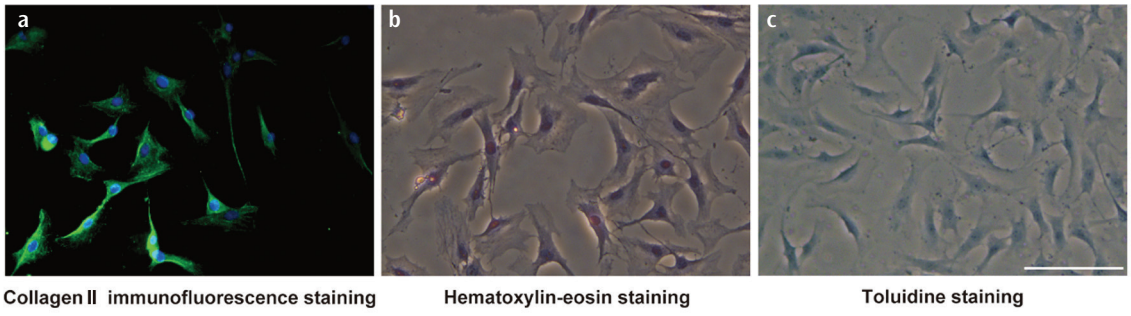

FIG. 1. Different staining assessment of rat NP cells. (a) Immunofluorescence staining: the nucleus depicts blue fluorescence stained by DAPI, while the high collagen II expression in cytoplasm is green-fluorescent in rat NP cells, (b) Hematoxylin-eosin staining: the nucleus appears round or oval, while NP cells polygonal, spindle, or irregular, (c) Toluidine blue staining: proteoglycans enriched in rat NP cells are stained strongly positive with toluidine blue. Scale bars 100 μm.

DAPI, 4',6-diamidino-2-phenylindole; NP, nucleus pulposus.

cleaved-caspase 3, the apoptotic executive protein, presenting the amount of apoptotic NP cells reached the peak at a high glucose concentration of 200 mM (Figure 2b). The Hoechst staining test demonstrated a brilliant, dense, and hyperchromatic morphology of apoptotic NP nuclei when the glucose concentration increased, which shared a similar trend with the results of Western blotting, suggesting that the hyperglycemic environment initiated apoptosis in NP cells (Figure 2c, d).

We have discussed the influence of the diverse high glucose concentrations on ER stress. The GRP78, cleaved-caspase 12, and CHOP levels increased sharply when NP cells were placed in a hyperglycemic environment. Interestingly, we found that the expression of the above three proteins coincided with the peak glucose concentration of 200 mm, indicating that ER stress contributed to the apoptosis generated by high glucose and that the ER stress level was in accordance with the apoptosis level (Figure 3a-d).

3. Quercetin alleviates high glucose-generated ER stress and ER stress induced-apoptosis of NP cells

To determine quercetin safety and efficiency in NP cells, a CCK-8 assay was performed to examine cell viability. No significant distinction was detected in cell viability post-24 h of the treatment at a concentration ≤ 60 μm. Meanwhile, quercetin approximately reached LC50 at 100 μm. Consequently, we selected 60 μm as the quercetin treatment concentration to ensure optimal drug efficiency (Figure 4a).

To determine whether ER stress was contacted with the protective influence of quercetin, we examine the ER stress-associated proteins GRP78 and CHOP by Western blotting. As expected, a remarkable

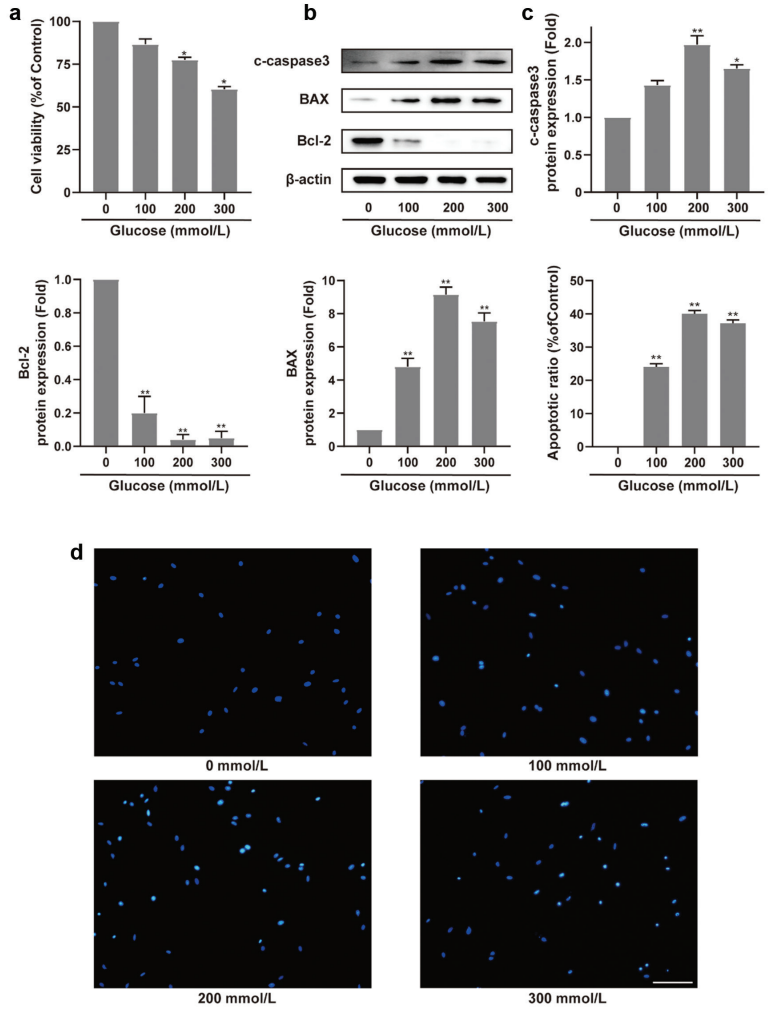

FIG. 2. Effects of glucose on rat NP cells viability and apoptosis. (a) Effect of diverse concentration of glucose on viability of rat NP cells detected by CCK-8, (b) Representative western blotting assay and quantitation of cleaved-caspase 3, BAX and Bcl-2; β-actin expression is used as the protein loading control, (c, d) Hoechst staining and the quantitation to detect NP cells apoptosis by evaluating apoptotic nuclei under high glucose. Scale bars 100 μm (data are shown as mean ± SD, $*p < 0.05$, $**p < 0.01$ vs. control group).

NP, nucleus pulposus; CCK-8, Cell Counting Kit-8; SD, standard deviation.

distinction was noted in the descending protein expression between GRP78 and CHOP in quercetin-treated cells in contrast with that in glucose-treated cells (Figure 4b, c). Moreover, immunofluorescence dyeing for GRP78 and CHOP was conducted in NP cells fixed with hyperglycemic for 6 h in the presence or absence of quercetin, which verified that quercetin could overtly lack ER stress caused by high glucose in NP cells. After the NP cells were exposed to hyperglycemic conditions, GRP78 demonstrated red fluorescence in the cytoplasm, whereas CHOP demonstrated green fluorescence in the nucleus. However, in contrast to their expression in the pretreated groups, the fluorescence intensities of GRP78 and CHOP were significantly weaker (Figure 4d-g).

Severe or prolonged ER stress causes excessive protein depletion and apoptosis. To determine the relationship between NP cell apoptosis and ER stress, we determined the protein level of cleaved-caspase 3 and caspase 12 by Western blotting. The results indicated that quercetin resisted the cleaved-caspase 3 and caspase 12 rank, which were both upregulated by the high glucose concentration of 200 mm (Figure 5a, b). Moreover, as per the immunofluorescence data, caspase-12 was evidently upregulated in the high-glucose group, while quercetin administration alleviated this response (Figure 5c, d).

4. Quercetin inhibited PERK-eIF2α-ATF4 ISR activation under high-glucose conditions in NP cells

To further determine the effect of quercetin in high glucose-generated ER stress, we investigated whether quercetin could modulate PERK-eIF2α-ATF4 ISR activation in NP cells. Interestingly, Western blotting analysis displayed a clear increase in the protein expression of the ISR-related genes phosphorylated-PERK, phosphorylated-eIF2α, and ATF4 in the high-glucose group of NP cells, while quercetin exerted a reverse effect (Figure 6a-d). We also noted the intensity of phosphorylated-eIF2α downregulation by quercetin in contrast with that in the high-glucose group under the immunofluorescence scope (Figure 6e, f). These results suggested that the PERK-eIF2α-ATF4 pathway of ISR is activated under hyperglycemic-generated ER stress in NP cells and that quercetin decreases the progress to some extent.

DISCUSSION

IDD is the principal reason for LBP. Currently, surgical operations such as discectomy are conducted to treat IDD patients when the conservative treatment fail.[34] However, diverse surgical risks are associated with surgery.[35] Therefore, it seems crucial for drug therapy to be equipped with the potential to ameliorate IDD progression from a molecular perspective. Over the years, natural herbal extracts

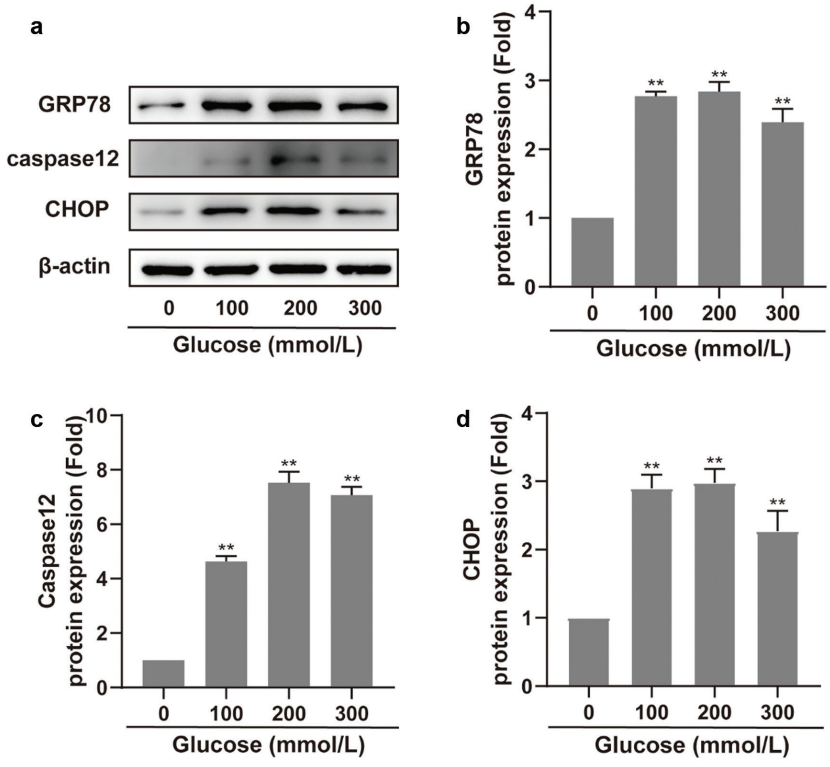

FIG. 3. Effects of glucose on ER stress and ER stress-related apoptosis of rat NP cells. NP cells are exposed to diverse concentrations of glucose for 6 h, (a-d) Representative western blotting assay and quantitation of ER stress and related apoptosis molecules GRP78, caspase 12, and CHOP; β-actin expression is used as the protein loading control (data are shown as mean ± SD, *$p < 0.05$, **$p < 0.01$ vs. control group).

ER, endoplasmic reticulum; NP, nucleus pulposus; GRP78, glucose-regulated protein 78; CHOP, C/EBPα-homologous protein; SD, standard deviation.

have been reported to heal clinical illness owing to their diverse molecular modulatory functions and the few side-effects.[36-38] Here, we have demonstrated that treatment with quercetin, a natural medicine, modulates apoptosis in NP cells.

NP cells are a special type of chondrocyte that share similar characteristics. Generally, there is no specific identification of NP cells, albeit researchers are inclined to identify chondrocyte features for substitution.[39] Collagen II and proteoglycans are the primary molecules that maintain the gelatinous nature of NP tissues.[3] Herein, we identified primary NP cells based on the characteristics of collagen II and proteoglycans.

Emerging evidence suggests that a hyperglycemic environment may have harmful influences on IVD cells through signaling pathways, which eventually induce morphological and molecular changes in the IVD.[40,41] Several studies have verified that a high-glucose environment is a stable model for IVD cells with degenerative

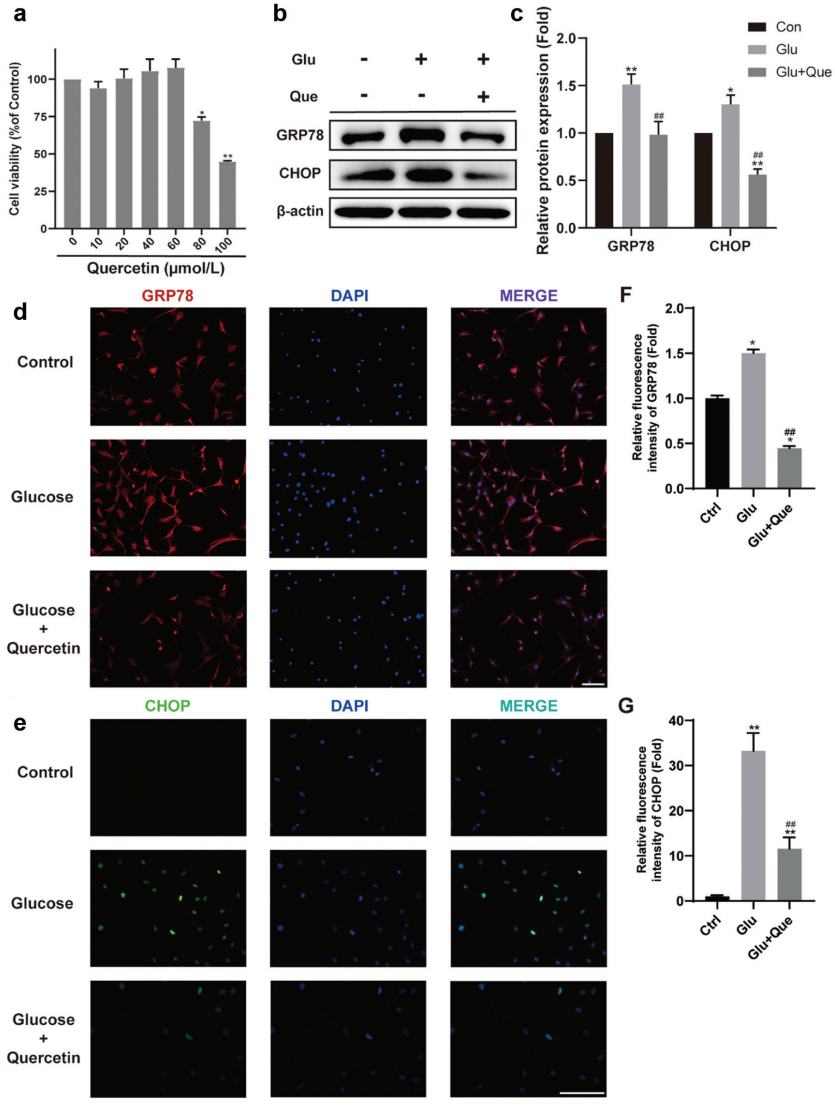

FIG. 4. Quercetin downregulates ER stress triggered by high glucose in rat NP cells. (a) Effect of diverse concentration of quercetin on viability of rat NP cells detected by CCK8. NP cells are exposed to glucose (200 mm) for 6 h except for control group. Quercetin (60 μm) is pre-treated for 2 h in the corresponding group. (b, c) Representative western blotting assay and quantitation of ER stress related molecules GRP78 and CHOP; β-actin expression is used as the protein loading control. (d, e) Immunofluorescence staining is used to detect the expression and localization of GRP78 and CHOP in rat NP cells. The GRP78 depicts red fluorescence predominantly in cytoplasm while the CHOP depicts green in nucleus, and the nucleus depicts blue stained by DAPI. Scale bars 100 μm. (f, g) Quantitative analysis of immunofluorescence staining of GRP78 and CHOP results. Scale bars 100 μm (data are shown as mean ± SD, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. glucose treatment).

ER, endoplasmic reticulum; NP, nucleus pulposus; CCK-8, Cell Counting Kit-8; GRP78, glucose-regulated protein 78; CHOP, C/EBPα-homologous protein; SD, standard deviation; DAPI, 4′,6-diamidino-2-phenylindole.

changes in vitro.[10,42-45] Herein, we assessed changes in the nuclear morphology of NP cells, the gene expression of the proapoptotic protein cleaved-caspase 3, as well as BAX and Bcl-2, to determine NP cell apoptosis. Our results demonstrated that hyperglycemic conditions promoted nuclear apoptosis in contrast with the baseline culture. Our results verify the effects of the 200 mm concentration of glucose on NP cell apoptosis as also suggested in previous studies.[10,43-45] The intervertebral disc is a tissue lacking blood vessels; when it degenerates, it releases an intense stimulus in the local microenvironment, which explains why high-concentration glucose could be used in vitro.[10,43-45]

The ER pathway is one of the classic apoptotic modes reported in recent years, and ER stress is an important intrinsic apoptotic element.[17] It is widely acknowledged that overwhelming ER stress can evolve into cell apoptosis, which includes the transcriptional initiation of GRP78-CHOP, caspase family, and PERK-eIF2α-ATF4 ISR activation.[19,46] Further research on ER stress-related apoptosis in IDD remains limited, regardless of its impact on several different diseases and corresponding tissues, including IVD. Herein, we demonstrated that the activation of a series of ER stress-associated genes may be involved in the apoptosis of NP cells, including GRP78, CHOP, caspase 12, and the PERK-eIF2α-ATF4 pathway. These results indicated severe ER stress and ER stress-related apoptosis in NP cells under a high glucose environment.

Quercetin, extracted and biologically identified in 1936, is a natural metabolite belonging to the flavonoid group present in fruits,

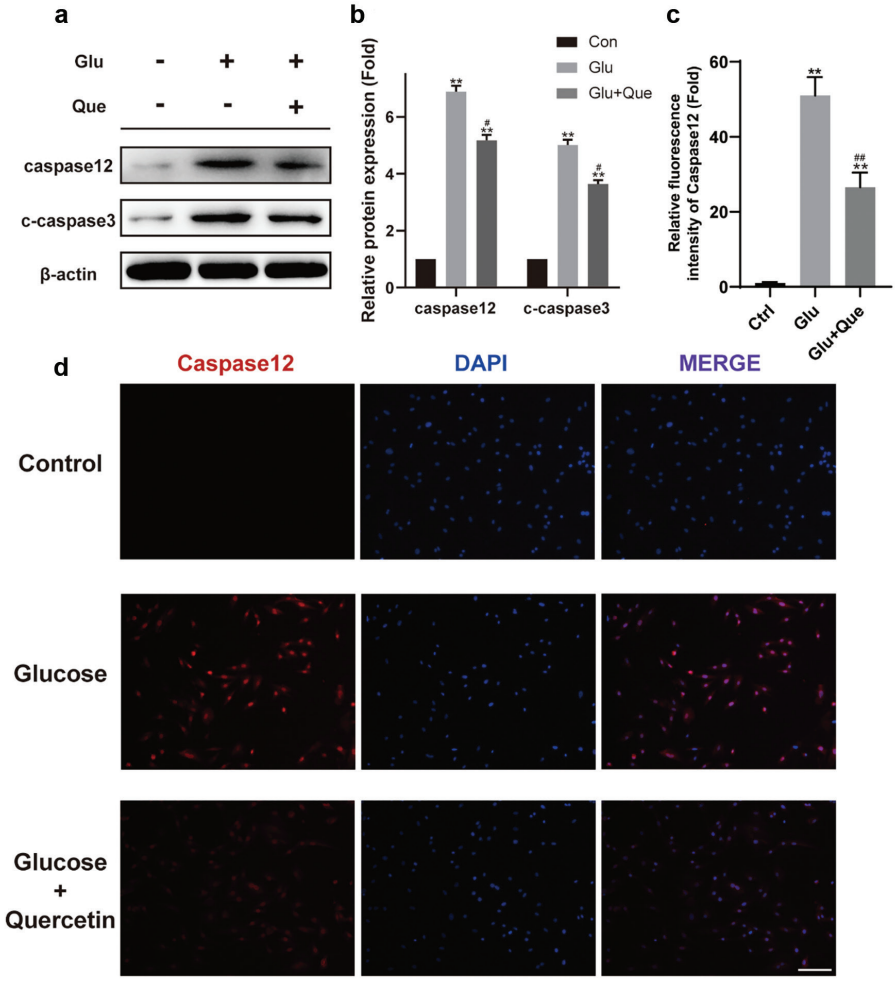

FIG. 5. Quercetin suppresses cleaved-caspase 3 and caspase 12 under high glucose stimulation. NP cells are exposed to glucose (200 mm) for 6h except in the control group. Quercetin (60 μm) is pre-treated for 2 h in the corresponding group. (a, b) Representative western blotting assay and quantitation of cleaved-caspase 3 and caspase 12; β-actin expression is used as the protein loading control. (c) Quantitative analysis of Immunofluorescence staining of caspase 12 results. (d) Immunofluorescence staining is used to detect caspase 12 expression, which depicts red fluorescence in rat NP cells. Nuclei are stained blue with DAPI. Scale bars 100 μm (data are shown as mean ± SD, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. glucose treatment).

NP, nucleus pulposus; DAPI, 4′,6-diamidino-2-phenylindole; SD, standard deviation.

vegetables, tea, and other edible plants.[25,47] Previous studies have demonstrated that quercetin regulates phenotypes, including inflammation, senescence, ECM descent, and autophagy in NP cells and IDD rat models.[31,32] Recent studies have demonstrated that quercetin has a custodial influence on ER stress-mediated apoptosis.[48] Quercetin also has a protective influence on chondrocytes through the inhibition of apoptosis and ER stress.[29,49] However, the potential protective impact of quercetin in regulating apoptosis and ER stress in patients with IDD is not well known. The present research is the first to determine the therapeutic effect of quercetin in ER stress-associated apoptosis of NP cells. Fortunately, our findings demonstrated that when ER stress-induced apoptosis peaked, quercetin mitigated the turbulent conditions in NP cells to some extent. As depicted by our results, the ER stress upstream initiator GRP78 and downstream CHOP both decreased when NP cells were treated with quercetin. Simultaneously, the ER stress-related apoptotic protein caspase 12, including the apoptotic executor cleaved-caspase 3, tended to decline under the influence

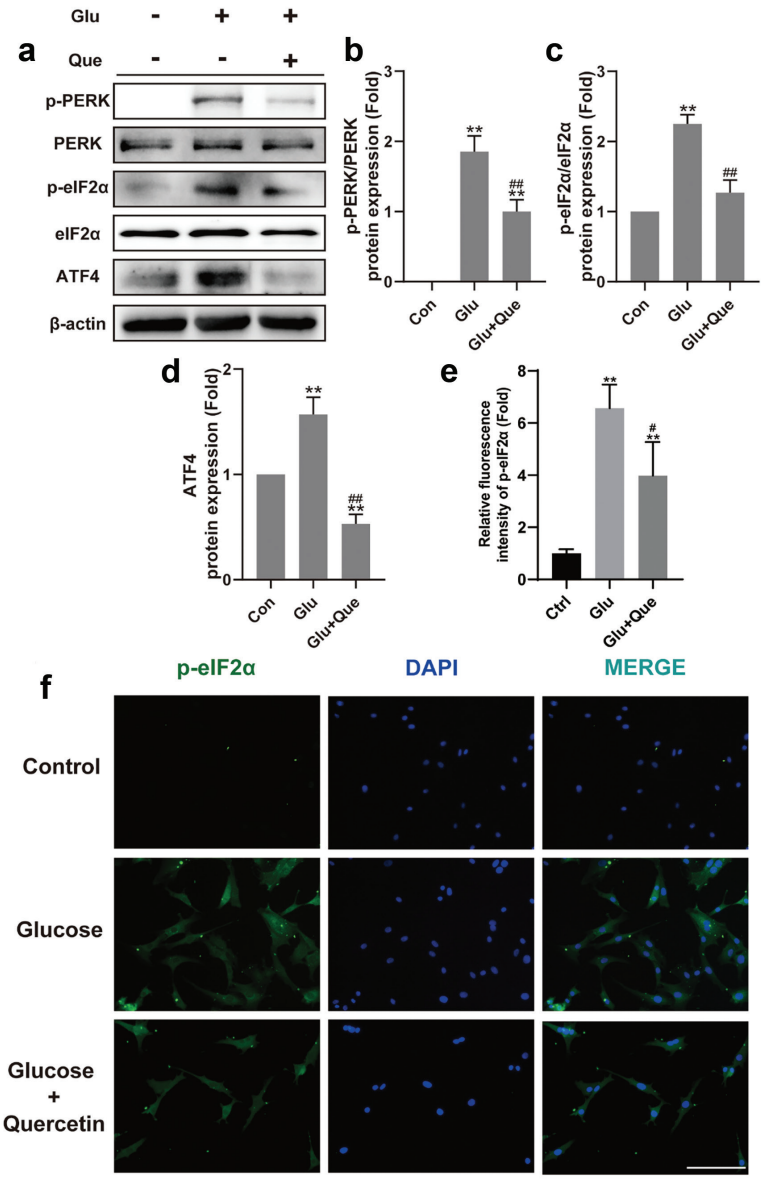

FIG. 6. Quercetin modulates ER stress through PERK-eIF2α-ATF4 pathway in NP cells. (a-d) Representative western blotting assay and quantitation of p-PERK, PERK, p-eIF2α, eIF2α, and ATF4; β-actin expression is used as the protein loading control. (e) Quantitative analysis of immunofluorescence staining of p-eIF2α results. (f) Immunofluorescence staining is used to detect p-eIF2α expression, which depicts green fluorescence in rat NP cells. The nucleus depicts blue is stained by DAPI. Scale bars 100 μm (data are shown as mean ± SD, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. glucose treatment).

ER, endoplasmic reticulum; PERK, protein kinase R-like endoplasmic reticulum kinase; eIF2α, eukaryotic translation initiation element 2α; ATF4, activating transcription element 4; NP, nucleus pulposus; DAPI, 4′,6-diamidino-2-phenylindole; SD, standard deviation.

of quercetin. Moreover, we found that the PERK-eIF2α-ATF4 pathway participated in all the processes under high-glucose stimulation and the quercetin rescue effect. Cumulatively, our results indicate that quercetin can not only relieve ER stress turbulence in NP cells but also the resultant apoptosis, probably through the PERK-eIF2α-ATF4 pathway.

Our findings thus demonstrate the protective effect of quercetin against NP cell apoptosis. We suggested that high glucose is a stable model for imitating IDD in vitro by inducing NP cell apoptosis, which is primarily mediated by ER stress. In addition, quercetin administration could prevent severe ER stress conditions and related apoptosis, probably by the PERK-eIF2α-ATF4 signaling pathway in NP cells. Unfortunately, we could not investigate the protective feature of quercetin against ER stress-generated apoptosis in vivo. However, quercetin administration, which interferes with human IDD progression, warrants further research. We plan to pursue these issues for future research.

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Ethics Committee Approval: This study was approved by the Ethics Committee of Beijing Jishuitan Hospital Guizhou Hospital Subcommittee Laboratory Animal Center.

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authorship Contributions: Concept- Z.Y., Xia.W., A.Z.; Design- Z.Y., Xia.W., X.X., A.Z.; Supervision- Y.W., A.Z.; Materials- Y.H., Xin.W.; Data Collection or Processing- Z.Y., Xia.W., Z.Z.; Analysis and/or Interpretation- Z.Y., Z.Z., Y.W.; Literature Review- Z.Y., Xia.W, Y.H., Xin.W.; Writing- Z.Y.; Critical Review- Y.W., A.Z.

Conflict of Interest: The authors declare that they have no conflict of interest.

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