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Regular paper

Endoplasmic reticulum stress involves the high glucose-induced nucleus pulposus cell pyroptosis

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Objective: Diabetes has been identified as a risk factor for intervertebral disc degeneration (IDD). The aim of this study is to investigate the potential mechanism underlying diabetes-related pyroptosis in nucleus pulposus (NP) cells. Methods: We used a high-glucose environment to mimic diabetes *in vitro* and examined the endoplasmic reticulum stress (ERS) and pyroptotic response. Furthermore, we utilized activators and inducers of ERS to explore the role of ERS in high-glucose-induced pyroptosis in NP cells. We evaluated the ERS and pyroptosis levels using immunofluorescence (IF) or RT-PCR and measured the expression of collagen II, aggrecan, and MMPs. Additionally, we used ELISA to determine the levels of IL-1β and IL-18 in the culture medium, and CCK8 assay to test cell viability. Results: High-glucose conditions promoted the degeneration of NP cells and triggered ERS and pyroptosis. A high level of ERS aggravated pyroptosis, and partially suppressing ERS resisted high-glucose-induced pyroptosis and alleviated the degeneration of NP cells. Inhibiting caspase-1-based pyroptosis under high-glucose conditions helped relieve the degeneration of NP cells but did not affect ERS levels. Conclusions: High-glucose induces pyroptosis in NP cells via the mediation of ERS, and suppressing ERS or pyroptosis protects NP cells under high-glucose conditions.

Keywords: Nucleus pulposus cells, endoplasmic reticulum stress, high-glucose, pyroptosis

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Abbreviations: ANOVA, Analysis of variance; 2-DG, 2-deoxyglucose; 2-DG6P, 2-DG-6-phosphate; CCK-8, Cell counting kit-8; DMEM, Dulbecco's modified eagle medium; ECM, Extracellular matrix; ELISA, Enzyme-linked immunosorbent assay; ER, Endoplasmic reticulum; ERS, Endoplasmic reticulum stress; IDD, Intervertebral disc degeneration; IF, Immunofluorescence; NP, Nucleus pulposus; OD, Optical density; RT-PCR, Reverse transcription-polymerase chain reaction; SPSS, Statistical Product and Service Solutions

INTRODUCTION

Low back pain is a common clinical symptom, which may or may not be accompanied by radiating pain, numbness, and muscle strength changes in the lower limbs (Chou, 2014). Intervertebral disc degeneration (IDD) is one of the leading causes of low back pain, pathologically characterized by the dysfunction of nucleus pulposus (NP) cells, the degradation of extracellular matrix (ECM), and the progressive fibrosis of the intervertebral disc (Vadala *et al.*, 2015; Zhao *et al.*, 2007; Che *et al.*, 2020). In addition to age, mechanical damage, and other biomechanical risks, metabolic diseases, especially diabetes, have also been confirmed to have a strong correlation with IDD. The diffusion, distribution, and utilization of glucose play a vital role in the normal function of the intervertebral disc (Cannata *et al.*, 2020). Therefore, the imbalance in the maintenance of normal blood glucose levels in diabetic condition has become a risk factor for IDD (Agius *et al.*, 2016).

In a prospective study by Jhawar and others (Jhawar *et al.*, 2006), the relative risk factor of diabetes for lumbar disc herniation was found to be 1.52, which was higher than smoking, hypertension, and hyperlipidemia. Huang and others (Huang *et al.*, 2016) also found that diabetes duration of more than ten years is a high-risk factor for intervertebral disc herniation. Additionally, Robinson and others (Robinson *et al.*, 1998) discovered that diabetic patients have a lower content of aggrecan in their intervertebral discs compared to non-diabetic patients. Despite the strong correlation between diabetes and IDD that has been reported, the pathogenesis of IDD, especially the mechanism of high-glucose-induced IDD, has not been extensively studied.

Pyroptosis shares several morphological characteristics with apoptosis, such as nuclear condensation and chromosome DNA degradation, as well as a reliance on Caspase activation, making it easy to confuse the two (Shi *et al.*, 2017). However, they can be distinguished from one another based on their biological characteristics and molecular mechanisms. The most significant difference between pyroptosis and apoptosis is the series of inflammatory reactions that occur due to the destruction of cell membrane integrity (Fang *et al.*, 2020). Caspase-1/4/5/11 activation and the release of IL-1β and IL-18 accompany pyroptotic cells, leading to the recruitment of more inflammatory cells and the expansion of local and systemic inflammatory responses (Toldo *et al.*, 2018).

The endoplasmic reticulum (ER) is a critical organelle responsible for protein synthesis and folding in eukaryotic cells, making it very sensitive to various stimuli. Disorders in cellular glucose metabolism can cause protein misfolding and accumulation in the ER cavity, resulting in endoplasmic reticulum stress (ERS) (Wagner & Moore, 2011). Recent studies have shown that ERS is a common feature in subjects with the development of IDD and is closely related to the activation of pyroptosis-related NLRP3 inflammasomes (Han *et al.*, 2018). Since ERS plays a role in the processes of glucose me-

Table 1. Primer sequences for RT-PCR

Gene name	Forward (5′>3′)	Reverse (5′>3′)
Collagen II	TGGACGATCAGGCGAAACC	GCTGCGGATGCTCTCAATCT
Aggrecan	ACTCTGGGTTTTCGTGACTCT	ACACTCAGCGAGTTGTCATGG
MMP3	AGTCTTCCAATCCTACTGTTGCT	TCCCCGTCACCTCCAATCC
MMP-13	ACTGAGAGGCTCCGAGAAATG	GAACCCCGCATCTTGGCTT
Caspase-4	AAGAGAAGCAACGTATGGCAGGAC	GGACAAAGCTTGAGGGCATCTGTA
Caspase-11	GGACGCCTTGTGGGAGAATG	TCAATGACCTTACACTGACGC
GADD34	ATGATGGCATGTATGGTGAGC	AACCTTGCAGTGTCCTTATCAG
GRP78	CATCACGCCGTCCTATGTCG	CGTCAAAGACCGTGTTCTCG
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

RT-PCR, Reverse Transcription-Polymerase Chain Reaction

tabolism, pyroptosis, and IDD, we can experimentally explore the connection and mechanism of ERS in the high-glucose-induced pyroptosis of NP cells.

MATERIALS AND METHODS

Patient tissue collection and NP cells isolation

We recruited 9 patients (4 males, 5 females; every age: 42 years) who underwent lumbar fracture and needed discectomy. The inclusion criteria for patients is no history of the underlying disease and IDD. This research was approved by the Ethics Committee of the Zhoushan Hospital of Traditional Chinese Medicine. After cutting from patients, the disc tissue was conserved in a culture medium and shipped to the laboratory for NP cell isolation immediately.

The gelatinous NP tissue was removed from the disc, washed 3×PBS, and cut into small pieces. Then, the fragment was digested with type II collagenase (Sigma, St. Louis, MO, USA) for 6 h. After centrifuge, the cell pellets were resuspended in the DMEM low glucose medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Gibco, Rockville, MD, USA). NP cells were cultured at 37°C, 5% CO_2 concentration, and 95% relative humidity. The medium was changed every three days.

NP Cells isolation and treatments

NP cells were initially cultured in low glucose DMEM (1 mg/mL), and extra glucose (Beyotime, Shanghai, China) was added to the medium to achieve a final concentration of 3, 5, 10, and 15 mg/mL. After assessing cell viability under different conditions, NP cells were cultured with 15 mg/mL glucose for three days to induce cell injury. In addition, NP cells were treated with Belnacasan (Beln, Selleck, China) to inhibit caspase-1 expression, Tunicamycin (TM, Sigma-Aldrich, St. Louis, MO, USA) to induce ERS, and 4-Phenylbutyric Acid (4-PBA, Sigma-Aldrich, St. Louis, MO, USA) to suppress ERS. Further details of the treatments are described in the Results section.

Immunofluorescence (IF)

We assessed the cellular expression of CHOP and caspase-1 using immunofluorescence (IF) staining. NP cells were seeded onto coverslips in 24-well plates at a density of 104 cells per well. After treatment, the coverslips were washed 3 times with PBS, fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton-X, and blocked with 5% bovine serum albumin (Beyotime, Shanghai, China) at room temperature. The coverslips were then incubated with primary antibodies, including Mouse monoclonal CHOP (ab11419, Abcam, Cambridge, MA, USA) and Rabbit monoclonal caspase-1 (Cell Signaling Technology, Danvers, MA, USA), overnight at 4°C. Afterwards, the cells were incubated with a rabbit monoclonal Alexa Fluor 647/mouse IgG and monoclonal Alexa Fluor 488-conjugated secondary antibody (Invitrogen, Carlsbad, CA, USA) in the dark at room temperature. The fluorescence intensity was measured using the Image-Pro Plus software.

Reverse transcription-polymerase chain reaction (RT-PCR)

We isolated total RNA from NP cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The RNA was then reverse-transcribed into cDNA and subjected to PCR using SYBR Green Master (TOYOBO, Osaka, Japan) following the manufacturer's instructions. The PCR primers used are listed in Table 1. The relative gene expression was normalized by the amount of GAPDH and calculated using the method of $2^{-\Delta\Delta Ct}$.

Enzyme-linked immunosorbent assay (ELISA)

The contents of IL-1β and IL-18 in the culture medium were determined by the ELISA kit (ab214025, ab215539, Abcam, Cambridge, MA, USA) according to the manufacturer's instructions. The final OD values were captured using a microplate reader.

Cell counting kit-8 (CCK-8) assay

We used the CCK8 test to determine cell viability. NP cells were seeded in 96-well plates at a density of 5000/well and treated as designed. After treatments, CHs were incubated with a CCK8 kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. The intensity of the CCK8 product was measured at OD of 450 nm using a microplate reader. We set the essential 1 mg/mL glucose treatment as a control group, and the other cell viability was shown relative to the control.

Glucose uptake assay

As the structure of 2-deoxyglucose (2-DG) is similar to glucose, glucose transporters take up 2-DG and

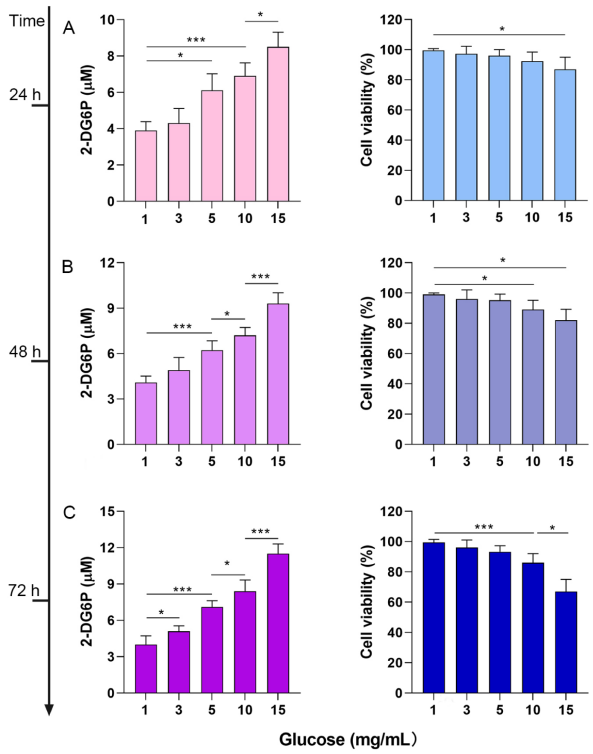

Figure 1. High-glucose injures NP cells' viability.
(**A**) The glucose uptake and cell viability of NP cells after 24-hour culture with ranged concentration of glucose. (**B**) The glucose uptake and cell viability of NP cells after 48hour culture with ranged concentration of glucose. (**C**) The glucose uptake and cell viability of NP cells after 72-hour culture with ranged concentration of glucose. Results are expressed as mean ± S.D. (**P*<0.05, ****P*<0.001)

metabolize it to 2-DG-6-phosphate (2-DG6P). The accumulation of 2-DG6P is therefore directly proportional to glucose uptake by cells. We treated NP cells with different concentrations of glucose and 1 mM 13 C-labeled 2-DG. After collecting the metabolites with 70% methanol extraction for LC-MS/MS, we measured glucose uptake using the Glucose Uptake Assay Kit (Colorimetric) (ab136955, Abcam, Cambridge, MA, USA) following the manufacturer's instructions.

Statistical analysis

Data were expressed as mean ± standard deviation (S.D.) of three independent experiments. All statistical analyses were performed by the Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) package and GraphPad Prism 8 (La Jolla, CA, USA). Unpaired one-way ANOVA was used to compare the differences among the groups. *P*-value <0.05 was statistically significant between groups.

RESULTS

High-glucose injures NP cells' viability

To confirm that glucose had an impact on the viability of NP cells, we cultured cells in a range of glucose concentrations and measured glucose uptake and cell viability for each condition. The culture medium was initially supplemented with 1 mg/mL glucose, and additional glucose was added to obtain final concentrations of 3/5/10/15 mg/mL. Compared to the control group, glucose uptake levels began to increase after 72 hours of culture when the glucose concentration was 3 mg/mL, and intracellular glucose uptake increased after 24 hours of culture when the glucose

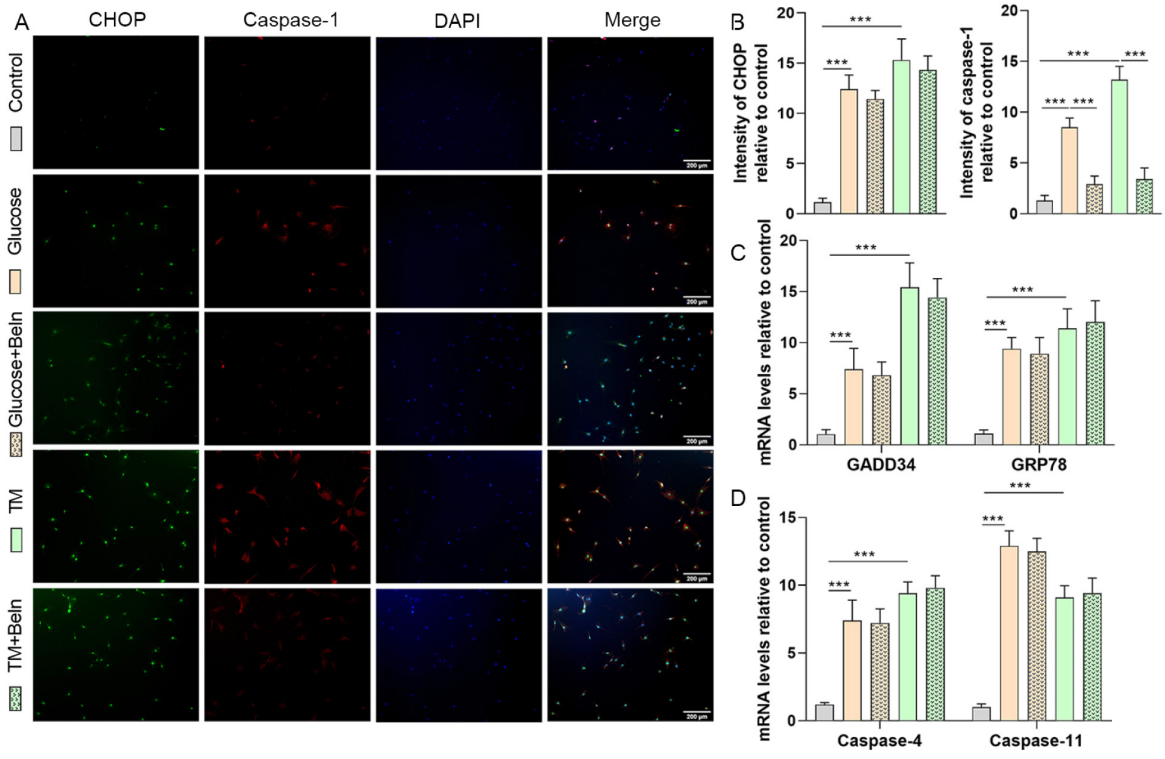

Figure 2. High-glucose induces the ERS and pyroptosis of NP cells.
(**A**) Immunofluorescence analysis of CHOP and caspase-1 expression, and (**B**) quantification by ImageJ software. (**C, D**) RT-PCR analysis for indicated RNA expression by normalization to GAPDH expression. Results are expressed as mean ± S.D. (****P*<0.001)

concentration was higher than 5 mg/mL (Fig. 1A–C, left). Thus, providing more soluble glucose in the medium significantly affected the glucose metabolism of NP cells. However, only the concentrations of 10 and 15 mg/mL had an impact on cell viability compared to the control, with a concentration of 15 mg/mL being more sensitive to NP cells in a short period of time (Fig. 1A–C, right).

High-glucose induces the ERS and pyroptosis of NP cells

To induce cell injury in subsequent experiments, we used a glucose concentration of 15 mg/mL, which quickly and effectively reduces the survival of NP cells. To investigate whether ERS aggravates pyroptosis, we used an ERS inducer in cell culture. NP cells were divided into five groups: 1) control: cells without any treatment, 2) glucose: cells were cultured with 15 mg/mL glucose, 3) glucose + Beln: cells were cultured with 15 mg/mL glucose and 1 nM Beln, 4) TM: cells were cultured with 5 μg/mL TM, 5) TM + Beln: cells were cultured with 5 μg/mL TM and 1 nM Beln. All cells were cultured in the indicated conditions for three days. We tested the ERS markers CHOP, GADD34, GRP78, pyroptosis markers caspase-1, caspase-4, and caspase-11 expression by IF or RT-PCR. Compared to the control, CHOP and caspase-1 were massively increased as a result of both high glucose and TM treatments (Fig. 2A, 2B). The supplement of Beln significantly reduced caspase-1 expression but did not affect the CHOP level. Additionally, the expression of GADD34, GRP78, caspase-4, and caspase-11 was also increased by the treatment of high glucose and TM, which was not affected by the supplement of Beln (Fig. 2C, 2D). Therefore, high glucose triggers the ERS and pyroptotic progress of NP cells, and ERS can trigger the pyroptosis of NP cells. The suppression of caspase-1 would not alleviate the ERS and other pyroptosis markers' expression.

Suppressing caspase-1 alleviates highglucose- induced NP cells degeneration

Continuously, we tested the cell function and pyroptosis-related inflammation under the high-glucose and ERS conditions. In addition to high glucose, TM-induced ERS also injured cell viability. However, the suppression of caspase-1 by Beln was effective in improving the cell viability in high-glucose, but not in terms of ERS (Fig. 3A). In the aspect of the secretory phenotype, high glucose influenced collagen II and aggrecan production, which can be rescued by Beln. Moreover, Beln also alleviated the ERS-caused collagen II and aggrecan downregulation (Fig. 3B). As one of the characteristics, IL-1β and IL-18 are largely synthesized and enter the intercellular substance during cell pyroptosis. Therefore, we tested the IL-1β and IL-18 content in the medium after three days' culture. The results indicated that high-glucose and ERS both raised the IL-1β and IL-18 levels, and the Beln was efficient to suppress the IL-1β and IL-18 production under both high-glucose and ERS conditions (Fig. 3C). Besides, we also analyzed the cellular MMP3 and MMP9 gene expression to value the degenerative degree of NP cells, which were all increased by glucose and TM stimulation, and the suppression of caspase-1 presented an inhibiting effect on MMP3 and MMP9 expression (Fig. 3D). Thus, Beln alleviated the pyroptosis and the resulting NP cell degeneration.

Suppressing ERS alleviates high-glucose-induced pyroptosis

We have ensured that high glucose trigged the ERS and pyroptosis; meanwhile, ERS could naturally result in pyroptosis. However, whether the high glucose aggravated the pyroptosis via the mediation of ERS remains unknown. We further applied the inhibitor of ERS in the high-glucose medium and tested the pyroptotic status. NP cells were divided into four groups, 1) control: cells without any treatments, 2) glucose: cells were cultured with 15 mg/mL glucose. 3) glucose + 4-PBA (+): cells were cultured with 15 mg/mL glucose and 1 mM 4-PBA 4) glucose + 4-PBA (++): cells were cultured with 15 mg/mL glucose and 2 mM 4-PBA. All the cells were cultured in the indicated condition for three days. The result of IF indicated that 4-PBA suppressed CHOP and caspase-1 with a dose-independence (Fig. 4A, 4B). Additionally, the expression of GADD34, GRP78, caspase-4, and caspase-11 was also decreased resulting from the presence of 4-PBA. Similarly, when the inhibitor dose increased, the efficiency became more obvious (Fig. 4C, 4D). Therefore, the 4-PBA successfully suppressed the high-glucose caused ERS and the resulting pyroptosis in NP cells.

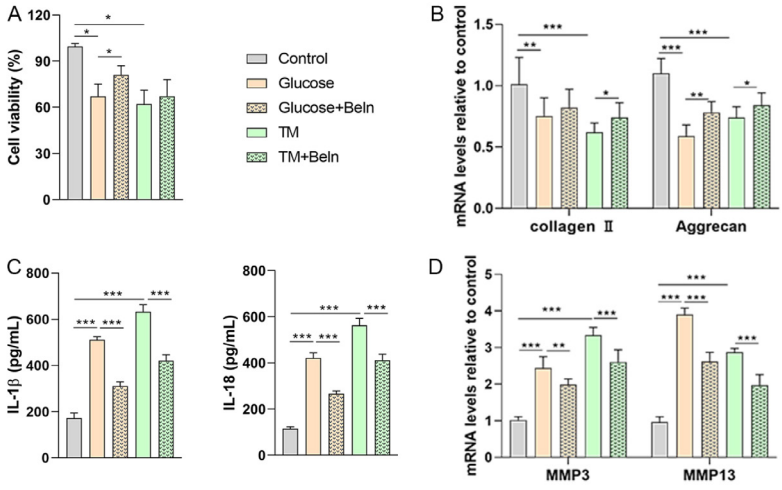

Figure 3. Suppressing caspase-1 alleviates high-glucose induced NP cell degeneration.
(**A**) Cell viability was determined by the CCK8 test. (**B, D**) RT-PCR analysis for indicated RNA expression by normalization to GAPDH expression. (**C**) The IL-1β and IL-18 content in the medium was tested by ELISA. Results are expressed as mean ± S.D. (**P*<0.05, ***P*<0.01, ****P*<0.001).

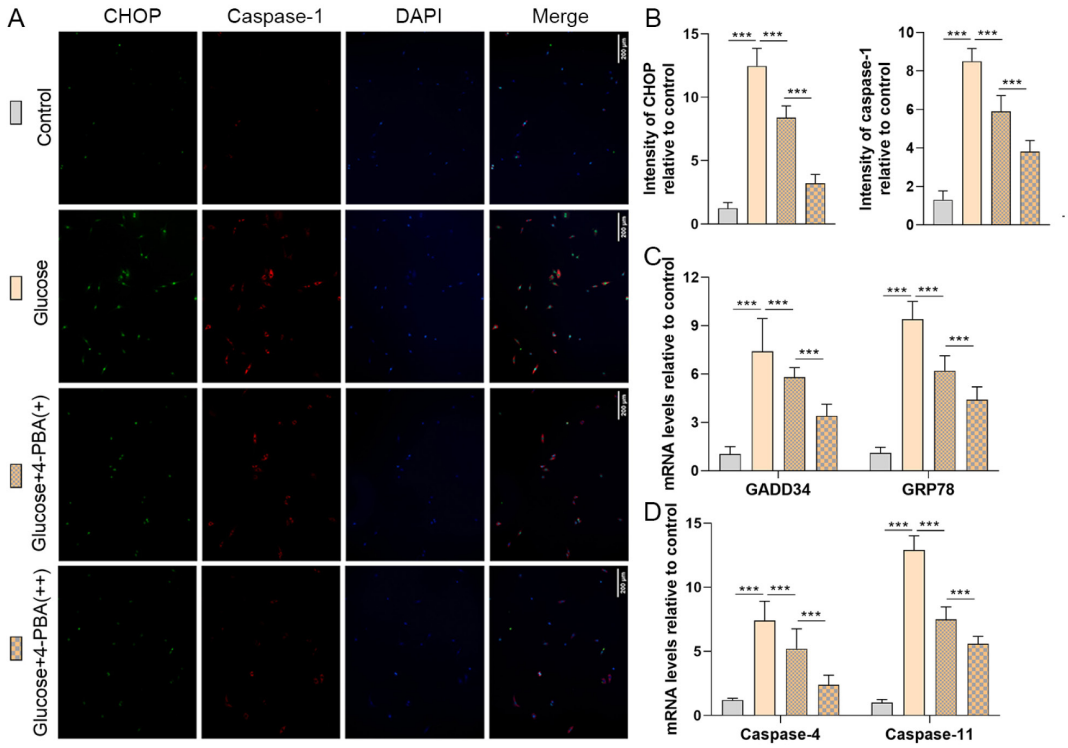

Figure 4. Suppressing ERS alleviates high-glucose induced pyroptosis.
(**A**) Immunofluorescence analysis of CHOP and caspase-1 expression, and (**B**) quantification by ImageJ software. (**C, D**) RT-PCR analysis for indicated RNA expression by normalization to GAPDH expression. Results are expressed as mean ± S.D. (****P*<0.001)

Suppressing ERS alleviates high-glucose induced NP cells degeneration

In addition, the cell viability in the high-glucose environment was rescued by suppressing ERS with 4-PBA (2 mM) (Fig. 5A). Compared to the high-glucose group, collagen II and aggrecan synthesis were protected by adding 4-PBA (2 mM) (Fig. 5B). Furthermore, 4-PBA exhibited excellent anti-inflammatory properties by dose-dependently suppressing IL-1β and IL-18, providing further evidence that suppressing ERS alleviated pyroptosis (Fig. 5C). Finally, we tested the RNA expression of MMP3 and MMP13, which were also inhibited

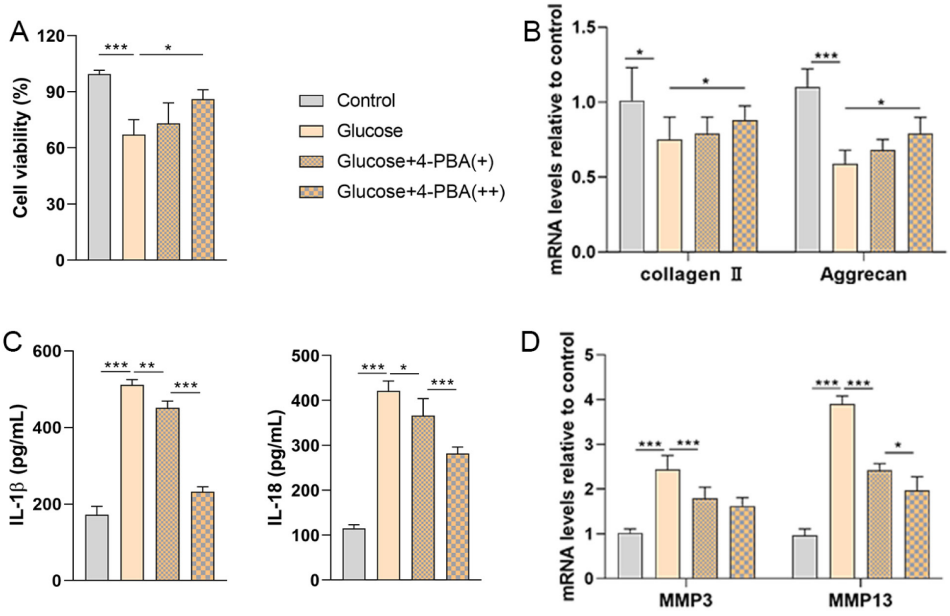

Figure 5. Suppressing ERS alleviates high-glucose induced NP cells degeneration.
(**A**) Cell viability was determined by the CCK8 test. (**B, D**) RT-PCR analysis for indicated RNA expression by normalization to GAPDH expression. (**C**) The IL-1β and IL-18 content in the medium was tested by ELISA. Results are expressed as mean ± S.D. (**P*<0.05, ***P*<0.01, ****P*<0.001).

by 4-PBA treatment (Fig. 5D). Therefore, suppressing ERS could prevent cell degeneration.

DISCUSSION

This study investigated the current evidence that high glucose is a risk factor for pyroptosis of NP cells and explored the role of ERS in this process. Previous studies have identified various factors contributing to intervertebral disc degeneration (IDD), such as inflammation, oxidative stress, and an imbalance in extracellular matrix catabolism. The dysfunction and decreased number of NP cells are considered the main and most direct causes of IDD (Ding *et al*., 2013). While the specific mechanism is not entirely clear, apoptosis, autophagy, and decreased cell proliferation have been implicated (Feng *et al*., 2016). However, the role of pyroptosis in IDD remains poorly understood. Pyroptosis is a highly inflammatory programmed cell death process that is triggered by inflammasome-mediated activation of proinflammatory cysteine proteases. In contrast to apoptosis, pyroptosis is typically accompanied by the release of large amounts of IL-1β and IL-18. Currently, caspase-1 mediates pyroptosis in the classical pathway, while the pyroptosis caused by caspase-4, 5, and 11 is defined as a non-classical pathway (Vande & Lamkanfi, 2016; Man *et al*., 2017).

Although people are typically more familiar with the complications of diabetes affecting the heart, brain, kidneys, peripheral nerves, eyes, and feet, diabetes is also a significant risk factor for intervertebral disc disease (Fletcher *et al*., 2002; Vijan, 2015). The high-glucose microenvironment can cause IDD due to nutritional metabolism disorder. Long-term hyperglycemia significantly alters the capillaries of the vertebral body's cartilage endplates, leading to the reduced blood supply and nutrient metabolism in the intervertebral discs (van Sloten & Schram, 2018). Additionally, the high-glucose microenvironment increases the production of advanced glycation end products (AGEs), which contribute to NP cell apoptosis (Yamamoto & Sugimoto, 2016). The combination of AGEs and their receptors results in local chronic oxidative stress and induces NP cell death. However, the role of the high-glucose microenvironment in NP cell pyroptosis remains unclear. In our study, high glucose impaired cell viability and upregulated the expression of caspase-1/4/11 and IL-1β/18, suggesting a correlation between them. To further elucidate the potential mechanism linking them, our attention is focused on ERS.

The endoplasmic reticulum (ER) is a crucial cellular organelle responsible for synthesizing, folding, and modifying secreted proteins, regulating lipid synthesis, and maintaining intracellular calcium ion homeostasis (Wang & Kaufman, 2016). However, when the ER's normal function is impaired, ER stress (ERS) occurs. High glucose, which mimics maternal diabetes, is widely reported to be a risk factor that induces ERS in many cell types (Chen *et al*., 2018; Hadley *et al*., 2018; Zhang *et al*., 2014). Consistent with previous studies, we also found that high glucose increased ERS markers in NP cells, including CHOP, GADD34, and GRP78 (Huang *et al*., 2019). The secreted protein CHOP is a transcription factor specific to the ER, which participates in various cell activities, especially the regulation of energy metabolism, proliferation, differentiation, and apoptosis (Li *et al*., 2014). Under normal circumstances, CHOP expression is low, but it is significantly increased when ERS occurs, which further activates downstream GADD34 and GRP78. In addition to mediating apoptosis (Oakes & Papa, 2015; Almanza *et al*., 2019), ERS can also lead to pyroptosis (Chou *et al*., 2019; Cheng *et al*., 2019). By confirming that ERS can induce pyroptosis in NP cells, we found that the pyroptotic response was activated by TM treatments (Yakin *et al*., 2019). Furthermore, the inhibitor of ERS also played a role in suppressing high-glucose-induced pyroptosis, indicating that high glucose caused ERS in NP cells, which led to subsequent pyroptosis. Suppressing ERS is an effective strategy to alleviate high-glucose-induced pyroptosis. To determine whether suppressing pyroptosis would backwards restrain ERS, we used the caspase-1 inhibitor, which rescued cell viability, protected collagen II and aggrecan expression, and reduced IL-1β/18 but did not affect the ERS response. Therefore, pyroptosis is a consequence of ERS, rather than the cause.

However, there are some limitations to this study. We collected only 9 intervertebral discs from patients, and this sample size may be insufficient. For the concentration and duration of glucose treatment, our in vitro model is a little different, with lower but longer periods of hyperglycemia. Therefore, further studies should be performed on diabetic mice in vivo.

In conclusion, our data indicate that high-glucose induces pyroptosis through ERS in NP cells. Suppressing ERS alleviates the pyroptotic response and reverses the degenerated NP cells under high-glucose conditions. Diabetes is involved in a variety of disease processes, and an in-depth study of the molecular mechanism of a high-glucose environment on NP cell metabolism may help us further understand diabetic IDD.

Declarations

Conflict of Interests. The authors declared no conflict of interest.

Authors' contributions. XX, YW and GL designed the study and performed the experiments, HL and TL collected the data, WL and XH analyzed the data, XX, YW and GL prepared the manuscript. All authors read and approved the final manuscript.

Availability of data and materials. The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

REFERENCES

Agius R, Galea R, Fava S (2016) Bone mineral density and intervertebral disc height in type 2 diabetes. *J Diabetes Complicat* **30**: 644–650. https://doi.org/10.1016/j.jdiacomp.2016.01.021

Almanza A, Carlesso A, Chintha C, Creedican S, Doultsinos D, Leuzzi B, Luis A, McCarthy N, Montibeller L, More S, Papaioannou A, Puschel F, Sassano ML, Skoko J, Agostinis P, de Belleroche J, Eriksson LA, Fulda S, Gorman AM, Healy S, Kozlov A, Munoz-Pinedo C, Rehm M, Chevet E, Samali A (2019) Endoplasmic reticulum stress signalling – from basic mechanisms to clinical applications. *Febs J* **286**: 241–278. https://doi.org/10.1111/febs.14608

Cannata F, Vadala G, Ambrosio L, Fallucca S, Napoli N, Papalia R, Pozzilli P, Denaro V (2020) Intervertebral disc degeneration: A focus on obesity and type 2 diabetes. *Diabetes-Metab Res* **36**: e3224. https://doi.org/10.1002/dmrr.3224

Che H, Li J, Li Y, Ma C, Liu H, Qin J, Dong J, Zhang Z, Xian CJ, Miao D, Wang L, Ren Y (2020) p16 deficiency attenuates intervertebral disc degeneration by adjusting oxidative stress and nucleus pulposus cell cycle. *Elife* **9**: e52570. https://doi.org/10.7554/eLife.52570

Chen X, Shen WB, Yang P, Dong D, Sun W, Yang P (2018) High glucose inhibits neural stem cell differentiation through oxidative stress and endoplasmic reticulum stress. *Stem Cells Dev* **27**: 745–755. https://doi.org/10.1089/scd.2017.0203

Cheng SB, Nakashima A, Huber WJ, Davis S, Banerjee S, Huang Z, Saito S, Sadovsky Y, Sharma S (2019) Pyroptosis is a critical inflammatory pathway in the placenta from early onset preeclampsia and in human trophoblasts exposed to hypoxia and endoplasmic re-

ticulum stressors. *Cell Death Dis* **10**: 927. https://doi.org/10.1038/s41419-019-2162-4

Chou R (2014) In the clinic. Low back pain. *Ann Intern Med* **160**: C1–C6. https://doi.org/10.7326/0003-4819-160-11-201406030-01006

Chou X, Ding F, Zhang X, Ding X, Gao H, Wu Q (2019) Sirtuin-1 ameliorates cadmium-induced endoplasmic reticulum stress and pyroptosis through XBP-1s deacetylation in human renal tubular epithelial cells. *Arch Toxicol* **93**: 965–986. https://doi.org/10.1007/s00204-019-02415-8

Ding F, Shao ZW, Xiong LM (2013) Cell death in intervertebral disc degeneration. *Apoptosis* **18**: 777–785. https://doi.org/10.1007/s10495-013-0839-1

Fang Y, Tian S, Pan Y, Li W, Wang Q, Tang Y, Yu T, Wu X, Shi Y, Ma P, Shu Y (2020) Pyroptosis: A new frontier in cancer. *Biomed Pharmacother* **121**: 109595. https://doi.org/10.1016/j.biopha.2019.109595

Feng C, Liu H, Yang M, Zhang Y, Huang B, Zhou Y (2016) Disc cell senescence in intervertebral disc degeneration: Causes and molecular pathways. *Cell Cycle* **15**: 1674–1684. https://doi.org/10.1080/15384101.2016.1152433

Fletcher B, Gulanick M, Lamendola C (2002) Risk factors for type 2 diabetes mellitus. *J Cardiovasc Nurs* **16**: 17–23. https://doi.org/10.1097/00005082-200201000-00003

Hadley G, Neuhaus AA, Couch Y, Beard DJ, Adriaanse BA, Vekrellis K, DeLuca GC, Papadakis M, Sutherland BA, Buchan AM (2018) The role of the endoplasmic reticulum stress response following cerebral ischemia. *Int J Stroke* **13**: 379–390. https://doi.org/10.1177/1747493017724584

Han CY, Rho HS, Kim A, Kim TH, Jang K, Jun DW, Kim JW, Kim B, Kim SG (2018) FXR inhibits endoplasmic reticulum stress-induced NLRP3 inflammasome in hepatocytes and ameliorates liver injury. *Cell Rep* **24**: 2985–2999. https://doi.org/10.1016/j.celrep.2018.07.068

Huang W, Han Z, Liu J, Yu L, Yu X (2016) Risk factors for recurrent lumbar disc herniation: a systematic review and meta-analysis. *Medicine* **95**: e2378. https://doi.org/10.1097/MD.0000000000002378

Huang XT, Liu W, Zhou Y, Sun M, Sun CC, Zhang CY, Tang SY (2019) Endoplasmic reticulum stress contributes to NMDA-induced pancreatic beta-cell dysfunction in a CHOP-dependent manner. *Life Sci* **232**: 116612. https://doi.org/10.1016/j.lfs.2019.116612

Jhawar BS, Fuchs CS, Colditz GA, Stampfer MJ (2006) Cardiovascular risk factors for physician-diagnosed lumbar disc herniation. *Spine J* **6**: 684–691. https://doi.org/10.1016/j.spinee.2006.04.016

Li Y, Guo Y, Tang J, Jiang J, Chen Z (2014) New insights into the roles of CHOP-induced apoptosis in ER stress. *Acta Bioch Bioph Sin* **46**: 629–640. https://doi.org/10.1093/abbs/gmu048

Man SM, Karki R, Kanneganti TD (2017) Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. *Immunol Rev* **277**: 61–75. https://doi.org/10.1111/imr.12534

Oakes SA, Papa FR (2015) The role of endoplasmic reticulum stress in human pathology. *Annu Rev Pathol-Mech* **10**: 173–194. https://doi.org/10.1146/annurev-pathol-012513-104649

Robinson D, Mirovsky Y, Halperin N, Evron Z, Nevo Z (1998) Changes in proteoglycans of intervertebral disc in diabetic patients. A possible cause of increased back pain. *Spine* **23**: 849–855, 856. https://doi.org/10.1097/00007632-199804150-00001

Shi J, Gao W, Shao F (2017) Pyroptosis: gasdermin-mediated programmed necrotic cell death. *Trends Biochem Sci* **42**: 245–254. https://doi.org/10.1016/j.tibs.2016.10.004

Toldo S, Mauro AG, Cutter Z, Abbate A (2018) Inflammasome, pyroptosis, and cytokines in myocardial ischemia-reperfusion injury. *Am J Physiol-Heart C* **315**: H1553–H1568. https://doi.org/10.1152/ajpheart.00158.2018

Vadala G, Russo F, Di Martino A, Denaro V (2015) Intervertebral disc regeneration: from the degenerative cascade to molecular therapy and tissue engineering. *J Tissue Eng Regen M* **9**: 679–690. https://doi.org/10.1002/term.1719

van Sloten T, Schram M (2018) Understanding depression in type 2 diabetes: a biological approach in observational studies. *F1000Res* **7**: F1000. https://doi.org/10.12688/f1000research.13898.1

Vande WL, Lamkanfi M (2016) Pyroptosis. *Curr Biol* **26**: R568–R572. https://doi.org/10.1016/j.cub.2016.02.019

Vijan S (2015) In the clinic. Type 2 diabetes. *Ann Intern Med* **162**: C1–C16. https://doi.org/10.7326/AITC201503030

Wagner M, Moore DD (2011) Endoplasmic reticulum stress and glucose homeostasis. *Curr Opin Clin Nutr* **14**: 367–373. https://doi.org/10.1097/MCO.0b013e32834778d4

Wang M, Kaufman RJ (2016) Protein misfolding in the endoplasmic reticulum as a conduit to human disease. *Nature* **529**: 326–335. https://doi.org/10.1038/nature17041

Yakin M, Seo B, Rich A (2019) Tunicamycin-induced endoplasmic reticulum stress up-regulates tumour-promoting cytokines in oral squamous cell carcinoma. *Cytokine* **120**: 130–143. https://doi.org/10.1016/j.cyto.2019.04.013

Yamamoto M, Sugimoto T (2016) Advanced glycation end products, diabetes, and bone strength. *Curr Osteoporos Rep* **14**: 320–326. https://doi.org/10.1007/s11914-016-0332-1

Zhang X, Yuan Y, Jiang L, Zhang J, Gao J, Shen Z, Zheng Y, Deng T, Yan H, Li W, Hou WW, Lu J, Shen Y, Dai H, Hu WW, Zhang Z, Chen Z (2014) Endoplasmic reticulum stress induced by tunicamycin and thapsigargin protects against transient ischemic brain injury: Involvement of PARK2-dependent mitophagy. *Autophagy* **10**: 1801–1813. https://doi.org/10.4161/auto.32136

Zhao CQ, Wang LM, Jiang LS, Dai LY (2007) The cell biology of intervertebral disc aging and degeneration. *Ageing Res Rev* **6**: 247–261. https://doi.org/10.1016/j.arr.2007.08.001