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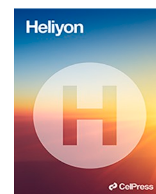

Research article

Overactivation of NF-kB pathway can induce apoptosis by down-regulating glycolysis in human degenerative nucleus pulposus cells

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A B S T R A C T

Intervertebral disc herniation, a prevalent condition in spinal surgery that frequently results in low back pain and lower limb dysfunction, significantly impacting patients' quality of life. Several factors, including spine biomechanics, biology, nutrition, injury, and abnormal inflammatory responses, have been associated with the development of intervertebral disc herniation. Among these factors, abnormal inflammatory responses have received considerable attention as a crucial mediator of both clinical symptoms and disease progression during the intervertebral disc herniation process. However, the underlying mechanisms of inflammation-induced intervertebral disc herniation remain inadequately explored. The NF-κB (Nuclear Factor-κB) pathway plays a central role in regulating the expression of proinflammatory cytokines. Research on intervertebral disc herniation has suggested that NF-κB can activate the NLRP3 inflammasome, thereby exacerbating intervertebral disc degeneration. Targeting the NF-κB pathway has shown promise in alleviating disc degeneration and associated pain. Previous research indicated that the upregulation of the NF-κB pathway, achieved through the inhibition of A20 (zinc finger protein A20), accelerated intervertebral disc herniation. In the present study, we observed that increased activation of NF-κB pathway activation suppressed the glycolysis process in nucleus pulposus cells (NPCs), leading to NPC apoptosis. Conversely, inhibition of the NF-κB pathway overactivated promoted the restoration of glycolysis and reversed NPC apoptosis, especially when treated with Lipopolysaccharide (LPS).

1. Introduce

Intervertebral disc herniation is a prevalent condition in spinal surgery, which often causes low back pain and lower limb dysfunction, significantly impacts the quality of life of patients. Most scholars believe that intervertebral disc degeneration is caused by various factors, such as aging, stress, infection, smoking, genetic factors, and disruptions in biological rhythm. Inflammatory signaling pathways, such as NF-κB and MAPK, are known to play a crucial role in this process, leading to abnormal activation. Additionally, there is an increase in signal proteins associated with the apoptosis pathway, as well as a notable rise in indicators of cellular senescence [1,2].

The nucleus pulposus tissue is located in the interior of the intervertebral disc and lacks a vascular supply, leading to a state of hypoxia. It relies on the infiltration of the endplate to provide nutrients for cell growth. Anaerobic glycolysis provides energy and produces lactic acid. Nucleus pulposus cells have a high tolerance for acidity, but excessively low pH levels can result in a decrease in extracellular matrix [3]. Previous studies have indicated that an excessive increase of intracellular or extracellular lactic acid can lead to cell degeneration [4–6]. In MCT4(monocarboxylate transporter 4) knockout rat intervertebral disc, lactate efflux is inhibited,

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leading to intervertebral disc degeneration and phenotypic changes of nucleus pulposus cells [7]. In the studies of osteoarthritis and rheumatoid arthritis, the inhibition of partial glycolysis or galactose replacement therapy has been shown to alleviate cartilage degeneration [8]. It has been reported that the level of lactic acid is significantly increased in mice with ARDS(Acute respiratory distress syndrome) induced by LPS stimulation, accompanied by cell apoptosis and necrosis [9]. Numerous studies have highlighted the important pathological and physiological role of inflammatory stimulation and the inflammatory pathway in glycolysis reprogramming [10–12].

At present, there is a belief that the degeneration of intervertebral disc is associated with the abnormal inflammation of nucleus pulposus [13–15], and an increase of lactic acid level has been observed in degenerative intervertebral disc [3,16]. The impact of excessive inflammatory stimulation on the energy metabolism and extracellular lactic acid of nucleus pulposus cells remains underexplored in existing literature. In our research, we focused on investigating the influence NF-κB inflammatory signal pathway and its natural endogenous inhibitor A20 on intervertebral disc degeneration [17–19]. Our findings have shown that A20 has the ability to mitigate excessive inflammation and reduce nucleus pulposus cell degeneration by inhibiting NF-κB pathway and promote autophagy in nucleus pulposus cells [20,21]. It is hypothesized that an overactivation of NF-κB inflammatory signals may suppress the glycolysis of nucleus pulposus cells and lead to apoptosis.

2. Materials and methods

2.1. Primary NPC isolation and culture

The collection and utilization of human intervertebral disc (IVD) specimens were approved by the Ethics Committee of Chongqing Medical University prior to surgery. All samples were obtained in accordance with the World Medical Association's Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. Written informed consent was obtained from all participants. The study included 20 patients with chronic hernia., comprising 9 males and 11 females, with an average age of 42.10 ± 12.07 years. All patients with intervertebral disc degeneration were classified as Pfirrmann grades III or IV based on preoperative MRI scans. Degenerative discs were harvested from these patients during discectomy and intervertebral fusion surgery. Nucleus pulposus (NP) tissues from DDD patients were isolated immediately after surgery. The tissues were sequentially digested at 37 °C with a 0.25 % trypsin solution for 30 min, followed by a 0.2 % type II collagenase solution for 4 h. A 200-μm filter was employed to remove tissue debris. The digested NP cells were cultured in DMEM/F12 medium (Gibco, USA) supplemented with 10 % fetal bovine serum (FBS) (Gibco, USA) and 1 % penicillin-streptomycin at 37 °C in a humidified atmosphere containing 5 % CO2. The second generation of nucleus pulposus cells (NPCs) was utilized for all experiments.

2.2. Transfection

Transfection was performed according to the manufacturer's protocol (Tsingke Biotechnology, Beijing, China). The sequences of the specific small interfering RNAs (siRNAs) were as follows: *A20*, siRNA1 sense (5'-3') AACAAAUUCCUGAGAUUUGAG antisense (5'-3') CAAAUCUCAGGAAUUUGUUGA. siRNA2 sense (5'-3') UUCAAAGUAAUCAUCUACCAG antisense (5'-3') GGUAGAUGAUUACUUUGAACU. siRNA3 sense (5'-3') CUACUAAUGGGAUCAUUCATT; antisense (5'-3') UGAAUGAUCCCAUUAGUAGTT. Non-targeting siRNA was utilized as a control. The NPCs were transferred to a 6 cm dish containing 4 × 10ˆ5 cells one day prior to the experiment. Lipofectamine 6000 (Beyotime) was employed as the transfection reagent. A total of 0.2 nmol of siRNA and 10 μl of Lipofectamine were diluted in 250 μl of serum-free DMEM/F12 medium. The mixture was added to the cell culture medium, and after 6 h of transfection, the cells were maintained in complete medium for an additional 24 h before being treated with various test compounds. Subsequently, the NPCs were harvested for subsequent experiments.

2.3. Detection of apoptosis by flow cytometry

Cells were cultured in six-well plates at a density of 8 × 10ˆ4 cells per well. The incidence of apoptosis was assessed using the Annexin V/PI apoptosis detection kit (Keygen, China). Following various treatments for 24 h, the neural progenitor cells (NPCs) were harvested, washed, and suspended in binding buffer supplemented with 5 μL of Annexin V and propidium iodide (PI) in the dark for 15 min at room temperature. Fluorescence intensities were measured using flow cytometry immediately after staining. The apoptotic incidence, including the percentages of early apoptotic cells (Annexin V+/PI-) and late apoptotic cells (Annexin V+/PI+), was expressed as a percentage of the total apoptotic cell population.

2.4. Western blot

Tissues and whole cell proteins were extracted using RIPA lysis buffer, and the protein concentration was subsequently analyzed using the BCA Protein Assay Kit (Beyotime, P0010S). After the proteins were transferred to a PVDF membrane (0.22 mm), the membranes were blocked with 5 % nonfat dry milk. Sequentially, membranes were incubated with primary anti-A20, P-P65, LC3B, mTOR, HIF-1α, (Cell Signaling Technology, USA), Bcl-2, BID, GLUT1,KH-2 (ZEN-BIO, China), P65, β-actin (Boster, China), BAX (Abcam, USA) overnight at 4 °C. The membranes were incubated with the respective secondary antibodies for 2 h at room temperature. Finally, the membranes were measured by an ECL plus reagent (Millipore, USA) and the results were detected by the software. The protein expression of different groups were compared by the ratio of the target protein to the internal reference.

2.5. Measurement of lactate

Lactate concentration was quantified using a Lactate Assay Kit (Sigma-Aldrich, UK) according to the manufacturer's protocol. The primary reaction mixture consisted of 26 μL of lactate assay buffer, 2 μL of lactate probe, 2 μL of lactate mix, and 26 μL of the sample solution. Following a 30-min incubation at room temperature, the sample absorbance was measured using colorimetric detection at 570 nm and fluorometric detection with excitation at 535 nm and emission at 587 nm, employing a Varioskan Flash (Thermo Fisher Scientific, Waltham, MA).

2.6. ATP measurement

Intracellular ATP levels were quantified using an ATP detection kit, following the manufacturer's instructions. Briefly, 50 μl of lysis buffer from the ATP detection kit was added to each well of a 48-well plate. The lysates were then centrifuged at 12,000 g for 5 min at 4 °C. Subsequently, 20 μl of the supernatant was transferred to 100 μl of ATP reaction reagent in a 96-well plate. Absorbance was measured using a spectrophotometer plate reader, and all values were normalized to the control group.

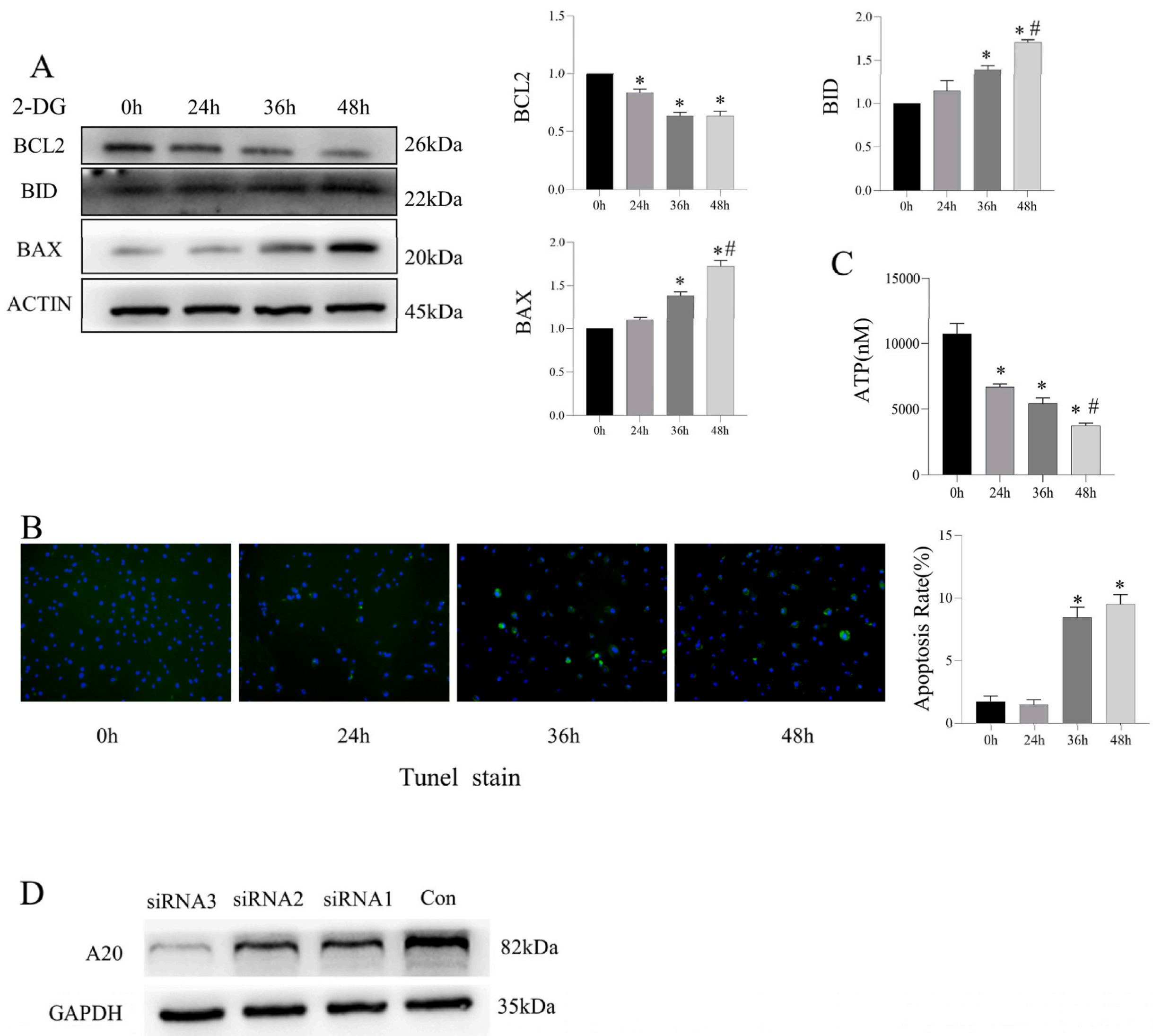

Fig. 1. Inhibition of glycolysis can induce apoptosis in human degenerative nucleus pulposus cells
A The nucleus pulposus cells were treated with 2-DG. At the time point of 24 h, 36 h and 48 h, the expression of apoptosis-related proteins BCL2, BID and BAX were detected by WB and semi-quantitative analysis. B Apoptotic cells were detected by TUNEL staining and quantitatively analyzed. C Total ATP content of cells was detected by kit and quantitatively analyzed. D Three different siRNA-A20 sequences were used to down-regulate the expression of A20 protein, and siRNA3 had the best interference effect. In this experiment, siRNA3 sequence was selected.*$P < 0.05$ v. s. control group, #$P < 0.05$ v. s. 36h group.

2.7. Tunel assay

The TdT-UTP nick end-labeling (TUNEL) method was employed to label the 3′-ends of fragmented DNA in apoptotic NPCs. Cells cultured in 6-well plates were treated as described in the mitochondrial depolarization assay and subsequently fixed with 4 % paraformaldehyde in phosphate-buffered saline (PBS). Following fixation, the cells were rinsed with PBS and permeabilized using 0.1 % Triton X-100 for 2 min on ice. TUNEL labeling was conducted for 1 h at 37 °C. The fluorescein isothiocyanate (FITC)-labeled TUNEL-positive cells were imaged using fluorescence microscopy with 488 nm excitation and 530 nm emission. Cells exhibiting green fluorescence were classified as apoptotic.

2.8. Statistical analysis

Statistical analysis was performed by one-way ANOVA or Student's t-test using software SPSS 19.0. Data points from three independent experiments are expressed as mean ± standard deviation (SD). *P* value less than 0.05 was considered significant.

3. Results

3.1. Inhibition of glycolysis induces apoptosis in human degenerative nucleus pulposus cells

Previous research has emphasized the critical role of glycolysis as the primary energy source for nucleus pulposus cells, which is essential for maintaining physiological homeostasis. In this study, we exposed nucleus pulposus cells to 2-Deoxy-D-glucose(2-DG), a glycolysis inhibitor, for 48 h. As expected, the total ATP levels in the 2-DG-treated cells notably decreased at 24, 36, and 48-h time points compared to the control group(Fig. 1C). Additionally, the expression of pro-apoptotic proteins BID and BAX proteins increased, while the anti-apoptotic protein BCL2 protein decreased in the 36 and 48-h groups, indicating enhanced apoptosis in nucleus pulposus cells following 2-DG treatment(Fig. 1A). TUNEL staining further confirmed a substantial increase in the percentage of apoptotic cells in the 36 and 48-h groups(Fig. 1B). Therefore, the result suggest that inhibiting glycolysis can trigger apoptosis in nucleus pulposus cells.

3.2. Induction of apoptosis and suppression of glycolysis in nucleus pulposus cells following treatment with siRNA-A20+LPS

Persistent and dysregulated inflammatory signals are widely acknowledged as contributors to intervertebral disc degeneration by promoting apoptosis. Previous research as well as our own studies have highlighted the abnormal activation of the NF-κB inflammatory signaling pathway in intervertebral disc degeneration. The negative feedback inhibitor protein A20 play a crucial role in delaying this degenerative process. Therefore, we down-regulated A20 expression using siRNA-A20 and exposed the cells to 200 μg/mL LPS stimulation. The apoptosis-related proteins BAX and BID significantly increased, while BCL2 substantially decreased in the siRNA-A20+LPS group, with statistically significant differences observed. Compared to the control group, only the BID protein expression increased in both the LPS and siRNA-A20 groups (Fig. 2A). Results from AnnexinV-PI double staining revealed significantly higher levels of apoptosis in the LPS stimulation group and the siRNA-A20 group compared to the control group, with no significant difference between the two. Importantly, treatment with siRNA-A20+LPS resulted in a significant increase in apoptosis of nucleus pulposus cells, surpassing the apoptosis levels induced by either treatment alone (Fig. 2B).

It is well-documented that the NF-κB pathway plays a critical role in regulating cellular energy and metabolic processes, which may impact NPCs apoptosis through its effects on glycolysis. To test this hypothesis, we measured ATP levels in control group and those treated with LPS siRNA-A20 or siRNA-A20+LPS. The results revealed a slight decrease in total cell ATP in the LPS group, a significant decrease in the siRNA-A20 group, and a further notable decrease in the siRNA-A20+LPS group (Fig. 2C). Furthermore, while lactic acid concentrations slightly increased in the LPS group, decreased in the siRNA-A20 group and were even more pronounced in the siRNA-A20+LPS group (Fig. 2D). The level of the glycolysis-related protein HIF-1α were significantly elevated in the LPS group, showing no significant difference in siRNA-A20 compared to control group. In contrast, its levels slightly increased in the siRNA-A20+LPS group but were significantly lower than those in the LPS stimulation group. No significant differences were observed in the levels of Hexokinase II(HK-2) and Glucose Transporter 1(GLUT-1) levels among the three groups (Fig. 2E). Therefore, our results suggest that apoptosis and the inhibition of glycolysis are modulated by siRNA-A20+LPS treatment in nucleus pulposus cells, potentially involving HIF-1α.

3.3. Inhibition of NF-κB or mTORC1 reduces apoptosis and modulates glycolysis in NPCs following treatment with siRNA-A20+LPS

Previous studies have suggested that inhibition the hyperactivated NF-κB pathway may enhance glycolysis, with mTOR also playing a significant role in this process. In our prior investigations, we observed that downregulation of A20 could activate mTOR activity, particularly under LPS stimulation, which could further exacerbate NF-κB hyperactivation. Subsequently, NPCs were pretreated with inhibitor of NF-κB or mTOR before being exposed to 200 μg/mL LPS for 24 h. Compared to the siRNA-A20+LPS group, the drug pretreatment group showed BID and BAX significantly decreased, while BCL2 increased (Fig. 3A). Apoptosis was evaluated using the AnnexinV-PI double labeling method, revealing a notably lower apoptosis rate in the drug pretreatment group compared to the non-pretreatment group (siRNA-A20+LPS). Although the apoptosis rates in both groups were higher than those in the control group, there was no significant difference between the two treatment groups (Fig. 3B). These findings suggest that inhibiting NF-κB may reduce apoptosis under siRNA-A20+LPS treatment. Furthermore, after pretreatment with Caffeic acid phenethyl ester

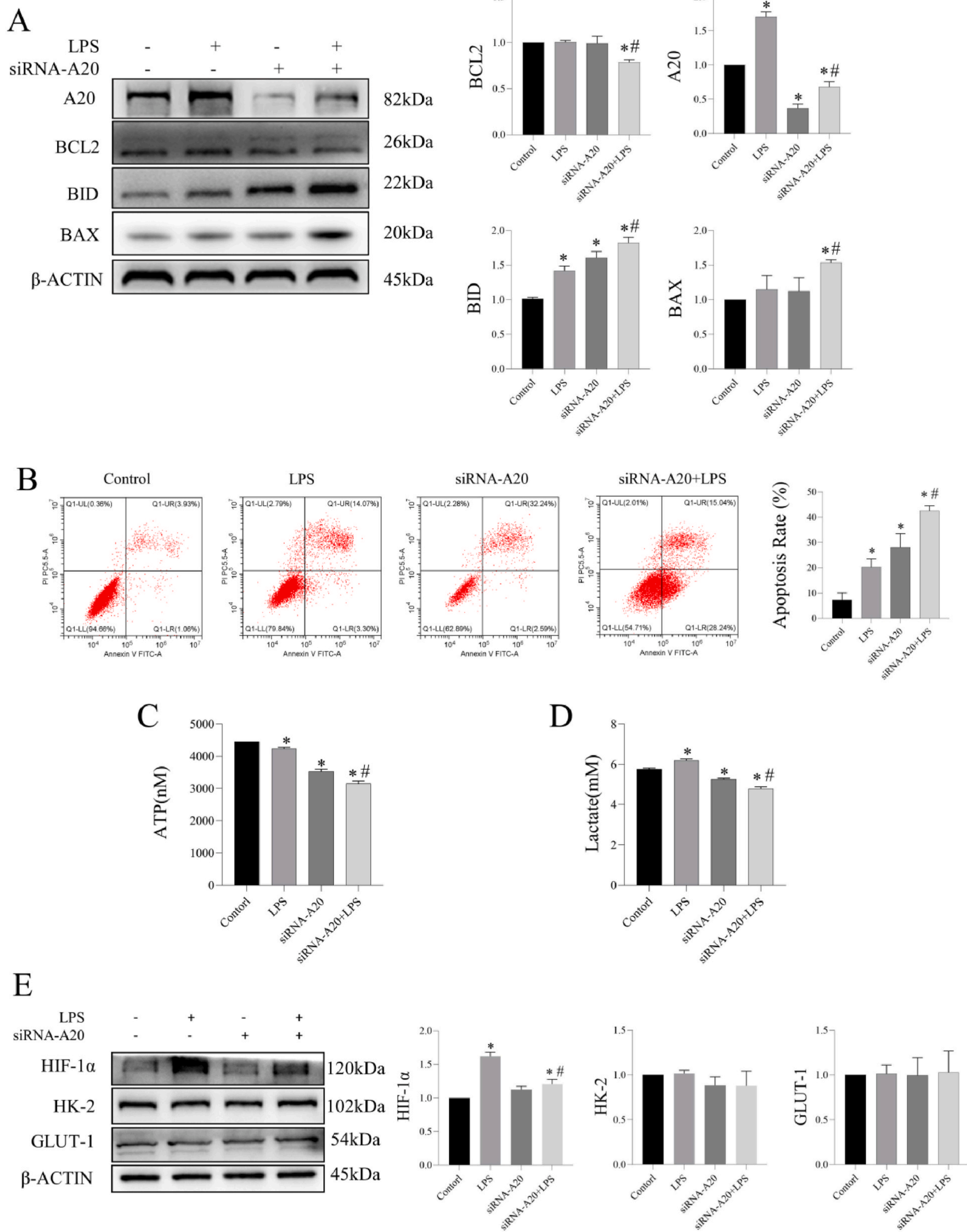

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Fig. 2. LPS stimulation can induce apoptosis and inhibit glycolysis in nucleus pulposus cells treated by siRNA-A20.
A The expression of A20 BCL2 BID and BAX was detected by WB and semi-quantitative analyzed. B Apoptotic cells were detected by PI-AnnexinV double staining and quantitatively analyzed. C Total ATP content of cells was detected by kit and quantitatively analyzed. D The concentration of lactic acid was detected by kit in cell culture medium and quantitatively analyzed. E The expression of HIF-1αHK-2 and GLUT-1 was detected and semi-quantitative analyzed. *$P < 0.05$ v. s. control group, #$P < 0.05$ v. s. LPS group.

(CAPE), there was a significant increase in both total ATP and lactic acid concentrations, indicating increased glycolysis and a notable increase in the expression of the glycolysis-related protein HIF-1α. Conversely, treatment with Rapamycin (RAPA) resulted in decreased total ATP and lactic acid concentrations, a significant decrease in HIF-1 α expression, a substantial increase in autophagy levels, and a marked reduction in apoptosis. This suggests that, in addition to glycolysis, autophagy also plays a role in anti-apoptosis by providing cellular energy in a discrete form. These differences were statistically significant (Fig. 3C–E). Importantly, no significant variations were observed in the levels of glycolysis-related proteins HK-2 and GLUT-1 between the two treatment groups.

3.4. Inhibition of glycolysis in nucleus pulposus cells can counteract the anti-apoptotic effects of CAPE

To further investigate the impact of glycolysis on apoptosis after inhibiting the NF-κB pathway, cells were pre-treated with the glycolysis inhibitor 2-DG. Compared to the siRNA-A20+LPS + CAPE group, the levels of apoptosis-related proteins BID and BAX significantly increased, while BLC2 decreased following 2-DG treatment. As a result, the apoptosis rate markedly increased, accompanied by a substantial decrease in both total ATP and lactic acid concentrations (Fig. 4A–D). These findings suggest that inhibiting glycolysis can counteract the anti-apoptotic effects of CAPE in nucleus pulposus cells, highlighting the crucial role of glycolysis in the anti-apoptosis mechanisms of nucleus pulposus cells under siRNA-A20+LPS treatment.

4. Discussion

Cell energy acquisition involves the breakage of high-energy phosphate bonds in ATP, with storage primarily in the form of ADP, which obtains high-energy phosphate bonds. This process provides the necessary energy and rich energy storage for various physiological and pathological activities of cells [22]. Intracellular ATP serves not only as a crucial source of energy co-supply but is also essential for the synthesis of biological macromolecules and is consumed in the initial step of protein synthesis, specifically the activation of amino acids. Additionally, ATP also participates in intracellular and intercellular signal transduction. Acting as a reactant in protein phosphorylation reactant, adenosine triphosphate is involved in intracellular protein kinase signal transduction pathways, such as mitogen activated protein kinase (MAPK) cascades that control cell proliferation, differentiation and death [22,23]. Recently, another function of ATP has been discovered as a hydrophilic substance that prevents harmful aggregation of macromolecules and regulates the phase separation of cells in the cytoplasm and nucleus [24]. ATP plays a crucial role in the survival and maintenance of cell function. Most of the intracellular ATP mainly comes from aerobic respiration. The majority of intracellular ATP is derived from aerobic respiration, with a significant amount being produced through the tricarboxylic acid cycle and oxidative phosphorylation to supply cell energy. However, some cells primarily rely on anaerobic respiration, producing a smaller amount of ATP and lactic acid, such as nucleus pulposus cells. Inhibition of glycolysis in cells that primarily rely on glycolysis or under hypoxia conditions can induce cell apoptosis [25,26]. Nucleus pulposus cells are predominantly supplied by anaerobic respiration even under aerobic conditions, therefore we utilized 2-DG (glucose analogue) to block the anaerobic respiratory energy supply of cells and observe the changes of apoptosis. The percentage of positive cells significantly increased in 36 and 48 h after inhibition of anaerobic respiratory energy supply of nucleus pulposus cells, with apoptosis-related proteins BID and BAX increased significantly, while BCL2 decreased significantly. These findings suggest that inhibition of glycolysis can induce apoptosis, particularly after 48h treatment.

Zinc finger protein A20 is an endogenous inhibitor of NF-κB pathway, capable of providing negatively feedback on the activity of this pathway and inhibiting inflammatory signal transduction. Previous research conducted by our team has demonstrated that A20 effectively suppress inflammation and degeneration of nucleus pulposus cells induced by LPS [18–21]. However, there is a dearth of reports on its role in cell energy metabolism and apoptosis. In this study, we utilized siRNA-A20 to downregulate the expression of A20 protein in nucleus pulposus cells, along with LPS as an inflammatory stimulator. Our findings demonstrated that treatment with siRNA-A20+LPS significantly induced cell apoptosis and inhibited glycolysis. It was observed that LPS treatment led to an increase in lactic acid concentration, consistent with previous research [27], but the overall ATP level in cells show a slight decrease. Our previous investigations revealed that LPS stimulation could result in mitochondrial damage. Although nucleus pulposus cells primarily rely on anaerobic respiration, their aerobic respiration also contributes significantly to energy production. Therefore, we hypothesized that 200ug/mL concentration of LPS induced mitochondrial damage, consequently impairing aerobic respiratory energy supply. This disruption in energy production ultimately led to a reduction in total ATP levels, despite an increase in anaerobic respiration. Notably, the level of glycolysis decreased significantly in the siRAN-A20 group, necessitating further exploration of the underlying mechanisms. Previous studies have reported that activating NF-κB can increase the level of HIF-1 α, promoting glycolysis [10]. Furthermore, recent studies suggest that LPS induces HIF-1 α expression in human ovarian epithelial cells through Toll-like receptor 4(TLR4)/NF-κB [28]. HIF-1 α remains expressed in nucleus pulposus cells at 21 % oxygen concentration, unlike in other mammalian cells, and is not substantially induced under hypoxia, although transcriptional activity increases slightly. This unique feature may be attributed to the distinct energy metabolism of NPC [29,30], where glycolysis is the predominant pathway even in the presence of oxygen [29]. The expression of HIF-1α increased in nucleus pulposus cells stimulated by LPS. However, the expression of HIF-1α decreased significantly

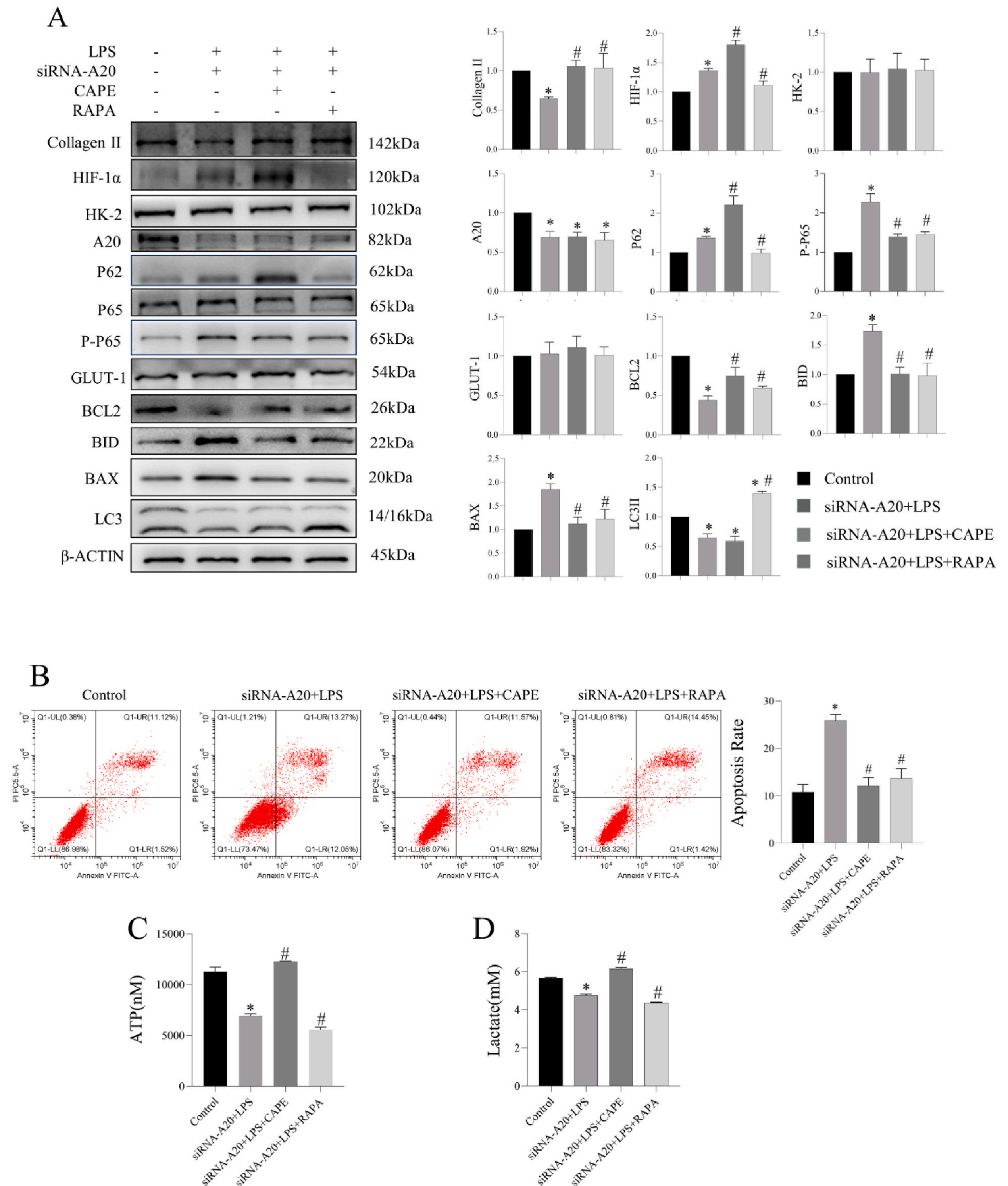

Fig. 3. Inhibition of NF-κB can enhance glycolysis and inhibit apoptosis of nucleus pulposus cells, inhibition of mTORC1 activity only attenuates apoptosis.

A The expression of protein was detected by WB and semi-quantitative analyzed. B Apoptotic cells were detected by PI-AnnexinV double staining and quantitatively analyzed. C Total ATP content of cells was detected by kit and quantitatively analyzed. D The concentration of lactic acid was detected by kit in cell culture medium and quantitatively analyzed. *$P < 0.05$ v. s. control group, #$P < 0.05$ v. s. siRNA-A20+LPS group.

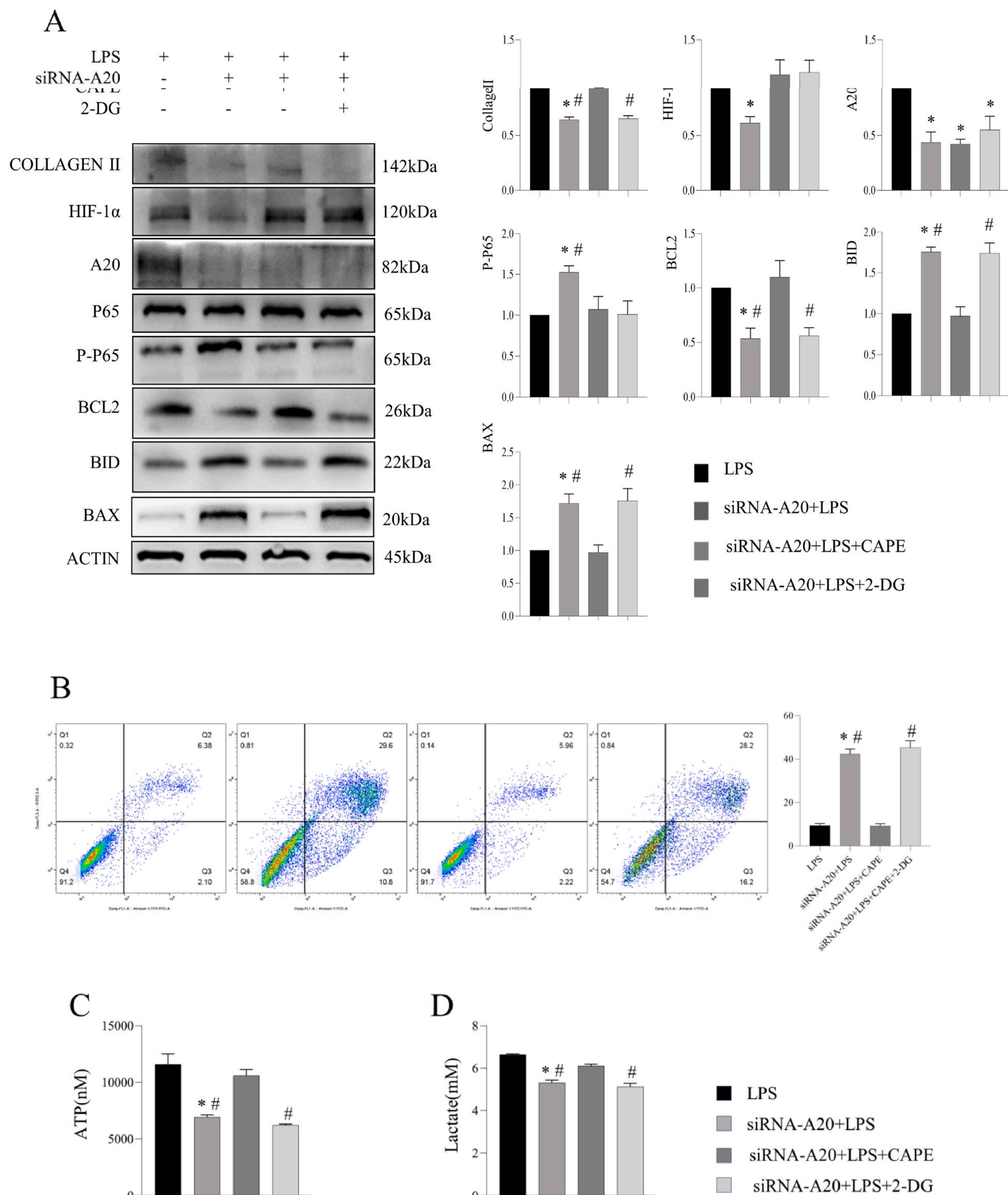

Fig. 4. Inhibition of glycolysis can reverse the anti-apoptosis effect of CAPE in nucleus pulposus cells.
A The expression of Collagen II HIF-1α A20 P-P65 BCL2 BID and BAX was detected by WB and semi-quantitative analyzed. B Apoptotic cells were detected by PI-AnnexinV double staining and quantitatively analyzed. C Total ATP content of cells was detected by kit and quantitatively analyzed. D The concentration of lactic acid was detected by kit in cell culture medium and quantitatively analyzed. *$P < 0.05$ v. s. LPS group, #$P < 0.05$ v. s. siRNA-A20+LPS + CAPE group.

after siRNA-A20+LPS treatment, leading to a reduction in anaerobic respiration and suggesting that overactivation of NF-κB may hinder glycolysis associated with HIF-1α.

A20 is known to positively regulate autophagy by directly acting on mTOR or indirectly acting on mTOR through DEPTOR [31,32]. Our previous studies have demonstrated that A20 inhibits NF-κB pathway or mTOR activity in nucleus pulposus cells, thereby delaying nucleus pulposus cell degeneration induced by LPS stimulation [20,21]. It has been reported that knockout RelA inhibits NF-κB activity can enhance aerobic glycolysis and induce cell necrosis during glucose deprivation conditions [33]. The results suggest that inhibition of basic NF-κB activity in mouse embryonic fibroblasts has been associated with increased anaerobic respiration levels. Additionally, increased mTORC1 activity can induce glycolysis by activating HIF-1 α and promoting glycolytic enzymes, thereby increasing anaerobic respiration [34]. Our findings suggest that overactivation of NF-κB may inhibit HIF-1-related glycolysis levels. Treatment with CAPE or RAPA to inhibit the activity of related pathways significantly reduced apoptosis. Specifically, inhibition of the NF-κB pathway resulted in decreased apoptotic cell numbers and increased anaerobic respiration, while inhibition of mTORC1 activity led to reduced apoptosis and anaerobic respiration levels. Notably, a decrease in NF-κB activity at low RelA levels was associated with alterations in energy metabolism [33]. In nucleus pulposus cells, LPS stimulation increased anaerobic respiration level, whereas siRNA-A20+LPS treatment significantly decreased anaerobic respiration level. Despite both conditions leading to increased NF-κB activity, the latter exhibited more pronounced activation. The expression of HIF-1 α increased significantly after CAPE treatment. Therefore, we propose that CAPE-mediated regulation of anaerobic respiration may counteract the reduction in HIF-1α-related glycolysis resulting from NF-κB hyperactivation. Conversely, the decrease of glycolysis induced by RAPA is consistent with that reported above [35,36], and autophagy can increase the level of autophagy. Furthermore, autophagy in macrophages serves as an energy-producing self-digestion process that inhibits apoptosis [37].

In order to elucidate the mechanism of CAPE on apoptosis. We utilized 2-DG to inhibit glycolysis in NPCs treated by siRNA + LPS + CAPE and observed that 2-DG treatment could promote cell apoptosis. Our findings indicate that inhibiting NF-κB pathway, when overactivated, can increase glycolysis that plays an crucial role in reducing NPCs apoptosis. In our study, we found that excessive NF-κB inflammatory signals can lead to the decrease of glucose metabolism, thereby accelerating the apoptosis of NPCs. Furthermore, our research highlights that inhibition of glycolysis of nucleus pulposus cells can promote apoptosis. In the context of inflammation promoting intervertebral disc degeneration. We propose that mitigating this degeneration in an inflammatory environment can be achieved by introducing drugs or genes that enhance glycolysis in nucleus pulposus cells and inhibit degeneration through various mechanisms. This conclusion also suggests a disruption in energy metabolism during the degeneration of nucleus pulposus cells, promoting us explore precise regulation points of glucose metabolism and identify areas of physiological metabolic lacking energy during degeneration process. This approach aims to provide targeted energy for cell repair and anti-degeneration.

We have previously determined that excessive activation of NF-κB can trigger apoptosis by suppressing glycolysis; however, our investigation was limited to degenerative nucleus pulposus cells from grade 3–4 intervertebral discs. It remains uncertain whether this finding is relevant to normal nucleus pulposus cells and mild degeneration. Therefore, further experiments involving normal nucleus pulposus cells and animal intervertebral discs are necessary to confirm the generalizability of this conclusion.

CRediT authorship contribution statement

Pan Tang: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bo Liu:** Writing – review & editing, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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