INTRODUCTION

Intervertebral disc degeneration is an ageing-related disease. Nowadays, it is characterized by the reduction of the extracellular matrix, inflammatory activation or cell senescence, thereby fail to support spine structure and function, leading to many spine-related disorders such as low back pain and disc herniation. The healthy intervertebral disc (IVD) is a tissue that is composed of inner soft nucleus pulposus (NP) cells and surrounding fibrocartilaginous ring—annulus fibrosus (AF), as well as cartilage endplates. The NP cells could promote the production of extracellular matrix to maintain the IVD hydrated. Researchers believe NP cell dehydration triggers the loss of disc height, disc deformation, segmental instability and even pain generation. Further study has confirmed that inhibit NP cell
apoptosis could slow the ECM degeneration to delay the progression of IVDD. Therefore, it is essential further to understand the cell death mechanisms of NP cells to investigate new therapeutic strategies for IVDD.

Pyroptosis is a form of programmed cell death, which is triggered by various inflammasomes such as NOD-, LRR- and pyrin domain-containing proteins (NLRP), AIM2-like receptor proteins and tripartite motif-containing proteins. Once these inflammasomes are stimulated, the downstream inflammatory caspase 1 or caspase 11 will be cleaved and further promoting cells to release pro-inflammatory cytokines IL-1β and IL-18. This process is involved in various diseases, such as alcoholic liver disease and cancer. Previous studies have reported that many pro-inflammatory molecules such as interleukin-1β (IL-1β) and interleukin-17 take part in the progress of primary catabolic enzymes expression, and subsequently influence many kinds of substrates such as proteoglycans, collagens and gelatins, which is highly related to IVDD formation. To date, Hu et al first presented that NLRP3-mediated NP cell pyroptosis take part in the IVDD process both in vivo and in vitro under the propionibacterium acnes stimulation. Moreover, Tang et al reported that NOD-, LRR- and pyrin domain-containing proteins (NLRP3) inflammasome could be activated in nucleus pulposus cells after H2O2 stimulation. Another group observed activation of NLRP3 in the patients, which indicated that the dysregulation of NLRP3 might play a vital role in the development of IVDD.

The exosome is one type of extracellular vesicles with a diameter of 50-100 nm, which is originated from multivesicular endosomes and released by almost all kinds of cells. It contains various molecules, including cytokines, proteins, lipids and non-coding RNAs. It is noteworthy that exosomes take part in cell-cell communication by transferring its contents among different cells. Therefore, exosomes have emerged as important mediators for multiple disease therapy. Some groups have reported that exosomes could be used to restrain pyroptosis. For example, Singla et al found that doxorubicin exposure significantly stimulated the expression of NLRP3 to induce pyroptosis in H9c2 cells. With the treatment of exosomes derived from embryonic stem cells, pyroptosis could be inhibited. They further demonstrated that exosome treatment showed a similar effect in vivo, which is beneficial for ameliorating doxorubicin-induced cardiomyopathy. Lu et al considered exosomes could be used as an alternative to stem cell therapy for IVDD. They found that stem cell-derived exosome treatment showed the same effect on NP cell proliferation with stem cell therapy. Further studies confirmed the MSCs-derived exosomes have antioxidant and anti-inflammatory effects, as well as alleviated NP cell apoptosis, which is a benefit for IVDD treatment. However, whether exosomes inhibit pyroptosis in IVDD remains unknown.

For this purpose, we developed an IVDD model using LPS-treated NP cells in this study. Then, we evaluated the therapeutic ability of MSCs and related exosomes on NP cell pyroptosis. Our data indicated that miR-410 was enriched in MSCs-derived exosomes and could target NLRP3 to suppress NP cell pyroptosis to prevent IVDD.

2 MATERIALS AND METHODS

2.1 Animals

Wild-type C57BL/6 mice were obtained from Charles River Laboratory Animal Technology Co. Ltd. The IVDD model was established by annulus fibrosus (AF) needle puncture, as described in the previous study. In brief, after general anaesthesia with ketamine (100 mg/kg), the coccygeal discs were punctured by a syringe needle for 10 seconds. The needle needs to pass through the AF into the NP tissue to depressurize the nucleus. Then, the discs were harvested after 6 weeks. The sham group was subjected to the same operation without puncture. All procedures were conformed to the Guide for the Care and Use of Laboratory Animal. There are two reasons why we choose this model. First, it has been proved that the structure of C57BL/6 mice’s tail discs is similar to that in human lumbar discs. Second, the morphologic characteristics of our IVDD model are similar to many human age-related IVDD features, especially the changes in NP cells.

2.2 H&E staining

IVD tissue was fixed in 4% paraformaldehyde for 48 hours, and then embedded by paraffin and cut into five-micrometre sections. Then, the sections were incubated in haematoxylin solution for 15 minutes, followed by stained using eosin solution for 15 seconds. Finally, the slices were dehydrated, transparentized and sealed. The images were captured under an optical microscope (Olympus).

2.3 Immunohistochemistry staining

According to the manufacturer’s instructions, the slices were blocked using 1% horse serum solution for 30 minutes at room temperature. The slices were then incubated with the primary antibody against NLRP3 (1:500, Abcam, ab214185) overnight at 4°C. After incubation with high sensitivity streptavidin-HRP conjugate for 30 minutes, DAB/AEC chromogen solution was added. In the end, the slices were visualized with a microscope at the bright field. The IHC kit was purchased from CST.

2.4 Cell culture and treatment

NP cells were isolated and cultured according to the previous study. In brief, the NP tissues were isolated from lumbar IVD of the normal mice under a stereotaxic microscope. After washed with PBS, the NP tissues were cut into small pieces and digested with a 0.5% type II collagenase (Roche Diagnosis) for 2 hours. The cells were obtained and maintained in F12 basal medium containing 10% foetal bovine serum.
serum and 100 U/mL penicillin-streptomycin in a humidified incubator containing 5% CO₂.

Human MSCs (hMSCs), fibroblasts and 293 cells (American Type Culture Collection) were maintained in DMEM medium added by 10% foetal bovine serum and 100 U/mL penicillin-streptomycin in a humidified incubator containing 5% CO₂. Passages 3 and 5 of NP cells, MSCs, 293 cells and fibroblasts were used for further experiments.

2.5 | LPS treatment

Lipopolysaccharide was purchased from MedChem Express. The NP cells were treated with LPS (5 mmol/L) for 24 hours according to the manufacture’s protocol. Cells were then collected for further experiments.

2.6 | Co-culture of MSCs and NP cells

Before co-culture, the NP cells were treated with LPS. Then, the cells were seeded on the lower chambers of transwell plates. The MSCs were plated on the upper chambers of the transwell plates. After 24 hours, the NP cells were harvested for further study. In one group, the GW4869 (20 µmol/L) was added into the culture media of MSCs and incubated for 48 hours before co-culture. GW4869 was purchased from MedChem Express.

2.7 | Enzyme-linked immunosorbent assay (ELISA)

After different treatments, the NP cell culture supernatant was collected. The level of cytokines IL-18 and IL-1β was quantified using

![Image](image.png)

**FIGURE 1** Pyroptosis occurred during intervertebral disc degeneration (IVDD). A, Representative photomicrographs of H & E stained intervertebral disc tissue of sham and IVDD mice. B, Representative photomicrographs of IHC for NLRP3 expression in sham and IVDD groups. The brown points were performed as positive signals. C and D, Representative blots and densitometric analysis were shown for NLRP3, cleaved caspase-1, GAPDH, IL-18, GSDMD and cleaved GSDMD expression in NP tissues of three mice with sham or IVDD operation. Values are presented as mean ± SD, n = 3, *P < .05, **P < .01, ***P < .001 vs the sham group.
specific ELISA kits (Abcam) according to the manufacture’s protocol. Three independent repeats were performed.

2.8 | Western blot

Protein lysates of cell samples or NP tissues were prepared by RIPA lysis buffer plus 1% phenylmethanesulfonfyl fluoride. The total protein concentrations were measured with a BCA protein assay kit (Santa Cruz). Proteins were then separated by 10% SDS-PAGE and transferred onto polyvinylidene fluoride membranes (Millipore). The membranes were then blocked by 5% bovine serum albumin for 2 hours at room temperature, followed by incubation with primary antibodies at 4°C overnight. After that, the membranes were incubated with secondary antibodies (1:5000, Abcam, ab150077) at room temperature for 2 hours. Protein bands were then detected using an enhanced chemiluminescence kit (ThermoFisher) and quantified by Image J software. Antibodies against NLRP3 (1:2000, ab214185), IL-18 (1:1000, ab71495), IL-1β (1:2000, ab234437), Gassdermin D (GSDMD, 1:1000, ab219800), TSG101 (1:2000, ab125011), and GAPDH (1:5000, ab181602) were purchased from Abcam. Antibodies against CD9 (1:2000, #13403), CD81 (1:2000, #56039), CD63 (1:2000, #55051), GM130 (1:1000, #12480), Cleaved caspase-1 (1:1000, #89332), cleaved GSDMD (1:1000, #50928) and Calnexin (1:1000, #2679) were purchased from CST.

2.9 | Immunofluorescence

The cells were fixed using 4% paraformaldehyde for 20 minutes at room temperature, followed by permeation using the 0.1% Triton for 5 minutes. Cells were then blocked by 5% bovine serum albumin for 1 hour at room temperature, followed by incubation with the primary antibodies against NLRP3 (1:500, Proteintech, 19771-1-AP) or caspase-1 (1:500, Proteintech, 22915-1-AP) at 4°C overnight. Finally, cells were incubated with second antibodies (1:500, Abcam, ab150077) for 1 hour and stained with 4′,6-Diamidine-2′-phenylindole dihydrochloride (DAPI, Roche) for 5 minutes at room temperature. Images were captured by a fluorescence microscope.

2.10 | Isolation of exosomes

The MSCs and fibroblasts were washed by PBS for several times and cultured in DMEM medium supplemented with 10% exosome-free foetal bovine serum for 48 hours. The culture medium was collected and centrifuged at 300 × g for 15 minutes at 4°C, followed by centrifugation at 2500 × g for 30 minutes. The supernatant was then filtered and ultra-centrifuged at 100 000 × g for 4 hours at 4°C. Then, the pellets were placed on top of a 30% sucrose/D₂O cushion and ultra-centrifuged at 100 000 × g for 1 hour at 4°C. The pellets were then resuspended in 15 mL PBS and centrifuged at 100 000 × g for 1 hour at 4°C. Finally, the pellets were resuspended in 200 µL PBS. For the exosome treatment, 20 µg/mL exosomes in 100 µL PBS were added to the NP cell culture system. After 24 hours, the NP cells were collected for further study. For the in vivo treatment, 20 µg/mL exosomes in 500 µL PBS were injected into the mice through tail vein. After 48 hours, the mice were used for further study.

2.11 | Identification and labelling of MSCs-derived exosomes

According to previously reported, transmission electron microscopy (TEM) was used to observe purified MSCs exosomes double-layer capsule ultrastructure. The average diameter and concentration of exosome were measured by dynamic light scattering (DLS). MSCs-derived exosomes were labelled using a PKH26 red fluorescent labeling kit (Sigma-Aldrich) according to the manufacturer’s protocol. The protein markers were detected by Western blotting, as described previously herein.

2.12 | Cell transfection

The mimic control (mimic-NC), miR-410 mimic, inhibitor control (inhibitor-NC) and miR-410 inhibitor were purchased from Synthegene. 100 nmol/L of each item was transfected into NP cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. The NP cells were harvested for further study after 48 hours. To obtain the miR-410 OE or miR-410 KD exosomes, MSCs were incubated with 100 nmol/L of miR-410 mimic and miR-410 inhibitor for 48 hours using the same reagent. The exosomes were isolated by series centrifugation, as described previously herein.

2.13 | qPCR

Total RNA was extracted and purified using mirVana™ PARIS™ RNA and Native Protein Purification Kit (ThermoFisher) following the manufacturer’s instructions. cDNA was synthesized using a TaqMan™ Advanced miRNA cDNA Synthesis Kit following the manufacturer’s protocol. qRT-PCR was then performed using TaqMan™ Fast Advanced Master Mix (Thermo Fisher). The relative expression level of the individual genes was

**FIGURE 2** MSCs treatment inhibits NLRP3-mediated pyroptosis in the LPS-treated NP cells. A, Representative blots and densitometric analysis are shown for NLRP3, Cleaved caspase-1, GSDMD and cleaved GSDMD in control, LPS, LPS + MSCs and LPS + MSCs + GW4869 groups. B, Representative photomicrographs of immunofluorescence assay for NLRP3 and Cleaved caspase 1 expression in the NP cells after different treatments. C, Quantitative analysis for IL-1β and IL-18 in NP cell culture supernatants under different conditions. Values are presented as mean ± SD, n = 3, *P < .05, **P < .01, ***P < .001 vs the sham group. #P < .05, ##P < .01, ###P < .001 vs the LPS group.
normalized by U6 via using 2\(^{-\Delta\DeltaCT}\) cycle threshold method. The primer of miR-410: forward: 5’-CGCGCCGGAATACAGCAT-3’; and reverse: 5’-AGTGCAGGCTTCCGATT-3’; U6: forward 5’-GCTCGCGTCGGCAGCAT-3’, reverse 5’-ATGGAACGCTTCA CGAAT-3’.

2.14 | Luciferase assay

A fragment of NLRP3 3’UTR, including the miR-410 binding site, was predicted via TargetScan7.2 (http://www.targetscan.org/). pMIR-WT/Mut NLRP3 3’-UTR-Luc reporter plasmids were purchased from Synthgene. The 293 cells were transfected with plasmids, and miR-410 mimic as well as mimic-NC. After 48 hours, cells were lysed, and the luciferase activities were detected by the Dual-Glo Luciferase Assay System (Promega) following the manufacturer’s protocol. The luciferase activity was calculated by renilla. The experiments were repeated at least three times independently.

2.15 | Statistical analysis

For statistical analysis, SPSS software was used. A Student t test was performed to determine the significance between two groups, one-way or two-way ANOVA with Bonferroni post hoc tests for multiple groups. Quantitative results are represented as the means ± standard deviation (SD). P < .05 was considered significant.

3 | RESULTS

3.1 | NP cell pyroptosis was induced by IVDD

We first determined the severity of intervertebral disc degeneration by histology assay. As shown in Figure 1A, compared with the sham group, the IVDD group showed lesser chondrocyte-like cells and disorganized hypocellular fibrocartilaginous tissue. The expression of NLRP3 was further detected by IHC assay. As shown in Figure 1B, the NLRP3 signal in the IVDD group was significantly higher than those in the sham group. Then, we determined the protein level of NLRP3 in the NP tissues. As expected, the Western blot results showed that the level of NLRP3 was significantly up-regulated in the IVDD group than that in the sham group. Consistently, the expression level of downstream proteins, including cleaved caspase-1, IL-18 and IL-1β, was all markedly increased when the mice were subjected to IVDD (Figure 1C). Consistently, the protein level of GSDMD cleavage was remarkably increased by IVDD (Figure 1D). These data indicated that NLRP3-mediated pyroptosis was activated during IVDD formation.

3.2 | Effect of MSCs on pyroptosis in IVDD model

To investigate the effect of MSCs on NLRP3 inflammasome activity, LPS-treated NP cells were co-cultured with MSCs. The result showed that LPS treatment significantly increased the expression level of NLRP3, caspase-1 and cleaved GSDMD in NP cells. However, this phenomenon was dramatically reversed after co-culturing with MSCs (Figure 2A). Consistently, similar results had been detected by immunofluorescence (Figure 2B). Subsequently, we evaluated the level of IL-18 and IL-1β expression in NP cell culture supernatants. They were increased after LPS stimulation, but both were declined when co-cultured with MSCs (Figure 2C). It suggested that MSCs treatment inhibited LPS-induced pyroptosis in NP cells. Interestingly, when we further treated the MSCs with GW4869 to inhibit exosomes secretion, the effect of MSCs was abolished. Moreover, this operation not only inclined the expression level of NLRP3, cleaved GSDMD and caspase-1 but also reversed the level of IL-18 and IL-1β expression in LPS-treated NP cells (Figure 2). Hence, we have been suggested that the effect of MSCs on pyroptosis might mainly cause by its derived exosomes.

3.3 | The function of MSCs-derived exosomes on LPS-induced NP cell pyroptosis

Exosomes derived from MSCs were isolated by centrifugation, and the characteristics were identified. Compared with the cell lysates, the protein levels of the positive markers such as CD63, CD9, CD81 and TSG101 were enriched in MSCs-derived exosomes. Adversely, neither GM130 nor the Calnexin expressed in the exosomes (Figure 3A). Moreover, as shown in Figure 3B, C, the shape and size were as same as the description of previous data, with a typical size around 100 nm in diameter. Furthermore, we found the PKH26-labelled exosomes could be uptake by NP cells after 24 hours, with a high level of red fluorescence in the cells (Figure 3D). Subsequently, we further checked the effect of exosomes on LPS-induced pyroptosis. Compared to that in the LPS-treated group, the LPS + MSCs-exosome group showed a decrease in the IL-18 and IL-1β level of IL-18 and IL-1β, was all markedly increased when the mice were subjected to IVDD (Figure 1C). Consistently, the protein level of GSDMD cleavage was remarkably increased by IVDD (Figure 1D). These data indicated that NLRP3-mediated pyroptosis was activated during IVDD formation.

**FIGURE 3** MSCs-derived exosome treatment inhibits LPS-induced pyroptosis in NP cells. A, Representative blot for CD63, CD9, CD81, and TSG101, GM130 and Calnexin expression in the MSCs cells and MSCs-derived exosomes were performed by Western blot. B, The representative TEM image of exosomes. Scale bar = 200 µm. C, The size of MSCs-derived exosomes was measured by DLS. D, Immunofluorescence indicated MSCs-derived exosomes were taken up by NP cells. Scale bar = 20 µm. E, Quantitative analysis of IL-1α and IL-18 in the NP cell supernatants after different treatments were determined by ELISA. F, Western blot assay showed the expression level of NLRP3, cleaved caspase-1, GSDMD and cleaved GSDMD in NP cells after treatment with LPS, LPS + MSCs-exosomes and LPS + fibroblast-exosomas. Quantification of different signal intensities was performed. Values are presented as mean ± SD, n = 3, *P < .05, **P < .01, ***P < .001 vs the control group. #P < .05, ##P < .01, ###P < .001 vs the LPS group.
IL-1β levels. However, the levels of these inflammatory cytokines were not changed after fibroblast-exosome treatment (Figure 3E). Consistently, the Western blot analysis revealed that the level of NLRP3, caspase-1 and cleaved GSDMD increased notably in NP cells after LPS treatment. However, the expression levels of these
markers were significantly reduced by MSCs-derived exosome treatment rather than fibroblast-derived exosome treatment (Figure 3F).

3.4 | MSCs-derived exosomal miR-410 inhibits pyroptosis response in NP cells

The expression level of miR-410 was subsequently examined in the sham and IVDD groups. As shown in Figure 4A, significantly decreased miR-410 level was detected in the IVDD samples as compared with the sham group. In contrast, we found that the expression of miR-410 was dramatically higher in exosomes derived from MSCs than that in the fibroblast-derived exosomes. Subsequently, we checked the miR-410 level in NP cells with different treatments. As shown in Figure 4B, LPS treatment decreased the expression of miR-410 in NP cells as compared with the control group. Moreover, comparing with fibroblast exosomes, MSCs-derived exosome treatment re-up-regulated the expression of miR-410 in LPS-treated NP cells. Thus, our data indicated that up-regulated miR-410 might play a positive effect on IVDD.

To investigate the function of miR-410, we transfected LPS-induced NP cells with miR-410 mimic or miR-410 inhibitor and related controls. As expected, overexpression miR-410 in LPS-treated NP cells significantly inhibited pyroptosis via suppressing the NLRP3/caspase-1 pathway. Besides, the expression level of cleaved GSDMD was dramatically reduced by miR-410. Oppositely, transfection with miR-410 inhibitor shown no effect on the expression level of NLRP3, caspase 1 and cleaved GSDMD (Figure 4C). Accordingly, a similar trend was exhibited in the content of IL-1β and IL-18 (Figure 4D). To confirm whether the exogenous miR-410 derived from exosomes, loss or gain function experiment was performed. As shown in Figure 4E, overexpression (OE) miR-410 in MSCs-derived exosomes dramatically decreased NLRP3, caspase-1 and cleaved GSDMD protein expression in NP cells with LPS stimulation, which showed similar results with MSCs-exosome treatment. Nevertheless, the protein levels of these markers still maintained at a high level, if the cells were treated by miR-410 knockdown (KD) MSCs-exosomes. The RT-PCR analysis demonstrated that the level of IL-18 and IL-1β was elevated about two folds after LPS stimulation compared with the non-treated group, while it significantly dropped in the cells with miR-410-enriched exosome treatment. The silence of miR-410 in MSCs re-up-regulated the expression of such cytokines in the NP cells (Figure 4F). Together, these data clearly suggested that MSCs-derived exosomal miR-410 might inhibit pyroptosis in NP cells.

3.5 | Exosomal miR-410 is able to bind to NLRP3 directly

It is reported that multiple micro-RNAs, including miR-223, miR-133 and miR-22, could regulate NLRP3 inflammasome activation through binding to its 3’ untranslated regions or post-transcriptional regulation. To confirm the relationship between miR-410 and NLRP3, the potential binding site of miR-410 on the 3’UTR of NLRP3 was identified by bioinformatics analysis. The sequence of the wild-type or mutant NLRP3 3’UTR was designed and shown in Figure 5A. miR-410 was predicted to bind to NLRP3 3’UTR. To validate this result, a luciferase assay was performed. As shown in Figure 5B, the luciferase intensity was reduced obviously by miR-410 mimic in the wild-type group rather than the mutant one, which means miR-410 is able to bind to NLRP3 directly. Next, we further discussed the role of miR-410 in vivo. As shown in Figure 5C, MSCs-exosome treatment significantly reversed the increased protein levels of NLRP3, cleaved GSDMD and caspase-1 induced by IVDD. With the miR-410 inhibitor transfection, a higher level of such markers was observed in the IVDD mice. Consistently, a similar change of IL-1β and IL-18 was displayed in our model (Figure 5D); in addition, MSCs-exosomes and miR-410 treatment alleviated the severity degree of IVDD rather than the miR-410 inhibitor transfection (Figure 5E).

4 | DISCUSSION

Currently, many studies have reported that NP cell death is a crucial event during IVDD formation. Two major types of cell death have been demonstrated are apoptosis and necroptosis. They were caused by the increased levels of oxidative stress or inflammatory cytokines in the degenerated IVD tissues. The current study demonstrated whether the pyroptosis involved in the IVDD formation. We first established an IVDD model by IVD puncture and found that NLRP3-mediated NP cell pyroptosis was activated in the progression of IVDD, with the initiation of the NLRP3 inflammasome, up-regulation of caspase-1 and cleaved GSDMD, as well as increased secretion of downstream cytokines IL-1β and IL-18. Moreover, this study also investigated the initiation of pyroptosis as...
NLRP3 is a target of miR-410. A, Sequence alignment of the NLRP3 3′-UTR (WT and MUT) and miR-410-3p. B, Luciferase reporter assay was performed in 293 cells cotransfected with the plasmid containing WT or MUT NLRP3 3′-UTRs and miR-410 mimics or mimic-NC. *P < .05, **P < .01, ***P < .001. C, Representative blots and densitometric analysis are shown for NLRP3, cleaved caspase-1, GSDMD and cleaved GSDMD in mimic with different treatments. D, Quantitative analysis of serum IL-1β and IL-18 in the mice after different treatments was determined by ELISA. E, Representative photomicrographs of H&E stained intervertebral disc tissue of mice after different treatment. Bar = 200 μm. Values are presented as mean ± SD, n = 3. *P < .05, **P < .01, ***P < .001 vs the sham group. ##P < .01, ###P < .001 vs the IVDD group. WT, wild-type; MUT, mutant; NC, miRNA control.

FIGURE 5

NLRP3 is a novel participant in the LPS-treated NP cells. With the LPS treatment, the NLRP3 was also initiated, which caused activation of caspase-1 and GSDMD. In fact, previous studies have proved that LPS treatment could activate the inflammatory response and promote pro-inflammatory cytokines accumulation in NP cells.32,33 Moreover, the NLRP3-induced pyroptosis could be activated by LPS treatment in vivo.34 Therefore, our data showed that NP cell pyroptosis play a vital role in the IVDD formation.

Next, the question arose on how to inhibit pyroptosis from ameliorate IVDD. Nowadays, attention has been increasingly placed on the effect of stem cell therapy. Many pieces of evidence have successfully demonstrated that MSCs have the ability of self-renewal and differentiation into chondrocytes, osteoblasts and adipogenic lineages.35 MSCs based cell therapeutics showed a promised result for disc structure support, matrix synthesis in multiple animal models.36-39 Our study confirmed that MSCs could inhibit NP cell pyroptosis by suppressing the expression of NLRP3 in the LPS-induced model. However, when we further treated the MSCs with GW4869 to inhibit the secretion of exosomes, the anti-pyroptosis effect of MSCs had been abolished. It indicated that the effect of MSCs on pyroptosis might mainly cause by its derived exosomes. Recent discovery had proved that MSCs-derived exosomes prevented the progression of degenerative changes, although enhancing the antioxidant and anti-inflammatory effect in NP cells or against apoptosis.40 However, whether they could regulate NP cell pyroptosis is unknown. Therefore, we isolated the MSCs-derived exosomes to treat LPS-treated NP cells. Our data showed that MSCs-derived exosome treatment significantly decreased NLRP3 expression and reduced caspase activation, thereby suppressing the secretion of IL-1β and IL-18 in the NP cells under LPS stimulation. To date, accumulating evidence has explored that exosomal proteins or RNAs play a crucial role in changing the recipient’s activities or functions. An essential component of exosomes, miRNAs, has been concerned increasingly in many diseases. MiR-410 has been proven to regulate cell proliferation and apoptosis, and act as a prognostic biomarker in multiple inflammatory diseases.41,42 Significantly increased miR-410 expression levels have been reported to reduce the generation of cytokines, such as IL-10, TNF-α, IL-1β and IL-6.43,44 Accumulation of oxidative stress or inflammatory cytokines in the degenerated IVD tissues was the leading cause of IVDD. It provided insight into the potential of miR-410 in controlling IVDD formation. Moreover, compared with the healthy volunteer, miR-410 was decreased in patients with wet age-related macular degeneration.45 As another age-related degeneration disease, IVDD represents a major clinical problem. However, the relationship between miR-410 and IVDD remains unclear. Therefore, we focused on the role of miR-410 in this study. In our study, a decrease in the miR-410 level was found in our IVDD model compared with sham samples. Moreover, miR-410 levels in MSCs-derived exosomes were significantly higher than those in fibroblasts derived exosomes. These results provided us miR-410 might be a potential mediator of NP cell pyroptosis. Next, we confirmed that MSCs-derived exosomal miR-410 significantly inhibited the pyroptosis response via suppressing the NLRP3/caspase-1 pathway in LPS-treated NP cells. However, this specific role of miR-410 in pyroptosis progression we known is still limited.

NLRP3 inflammasome is a marker of pyroptosis and associated with several human chronic inflammatory diseases. NLRP3 silencing reduces a series of pyroptosis-related markers activation.46 For this reason, bioinformatic analysis (www.targetscan.org) was performed to investigate the potential relationship between miR-410 and NLRP3. It predicted that NLRP3 might be a target of miR-410. Besides, the result of the luciferase assay confirmed this hypothesis. Thus, our results demonstrated a novel mechanism by which the down-regulation of NLRP3 by exosomal miR-410 administration resulted in a declined caspase-1 and GSDMD, thereby weakening NP cell pyroptosis to alleviate IVDD. However, more evidence for the relationship between miR-410 and NLRP3 still required. In addition, establishment of a miRNA expression profile was still essential for reveal the mechanisms of IVDD-related NP cell pyroptosis in our future study.

In summary, we demonstrated that NP cell pyroptosis was initiated during IVDD formation. MSCs-derived exosomes could inhibit LPS-induced NP cell pyroptosis in vitro. In addition, MSCs-derived exosomal miR-410 directly binded to NLRP3 3′UTR to achieve this effect. All of our findings offer a new opportunity to prevent IVDD.

ACKNOWLEDGEMENTS
We would like to thank all the researchers and study participants for their contributions.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS
Jingwei Zhang: Writing-original draft (lead). Jieyuan Zhang: Visualization (equal). Yunlong Zhang: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal). Wenjun Liu: Visualization (equal). Weifeng Ni:
Writing-review & editing (equal). Xiaoyan Huang: Writing-review & editing (equal). Junjie Yuan: Writing-review & editing (equal). Bizeng Zhao: Investigation (equal). Haijun Xiao: Conceptualization (equal); Funding acquisition (equal). Feng Xue: Conceptualization (equal); Funding acquisition (equal).

ETHICAL APPROVAL
The study had approval from the Ethics Committee of Southern Medical University Affiliated Fengxian Hospital.

CONSENT FOR PUBLICATION
Not applicable.

DATA AVAILABILITY STATEMENT
Not applicable.

ORCID
Feng Xue https://orcid.org/0000-0002-1083-5023

REFERENCES
1. Zhao CQ, Wang LM, Jiang LS, Dai LY. The cell biology of intervertebral disc aging and degeneration. Ageing Res Rev. 2007;6(3):247-261.
2. Basso M, Cavagnero L, Zanirato A, et al. What is the clinical evidence on regenerative medicine in intervertebral disc degeneration? Musculoskelet Surg. 2017;101(2):93-104.
3. Sudo H, Minami A. Regulation of apoptosis in nucleus pulposus cells by optimized exogenous Bcl-2 overexpression. J Orthop Res. 2010;28(12):1608-1613.
4. Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. Nature. 2012;481(7381):278-286.
5. Jorgensen I, Rayamajhi M, Miao EA. Programmed cell death as a defence against infection. Nat Rev Immunol. 2017;17(3):151-164.
6. Heo MJ, Kim TH, You JS, Blaya D, Sancho-Bru P, Kim SG. Alcohol dysregulates miR-148a in hepatocytes through FoxO1, facilitating pyroptosis via TXNIP overexpression. Gut. 2019;68(4):708-720.
7. Zaki MH, Vogel P, Body-Malapel M, Lamkanfi M, Kanneganti TD. IL-18 production downstream of the Nlrp3 inflammasome confers protection against colorectal tumor formation. J Immunol. 2010;185(8):4912-4920.
8. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. Nat Rev Rheumatol. 2014;10(1):44-56.
9. Freemont AJ. The cellular pathobiology of the degenerate intervertebral disc and discogenic back pain. Rheumatology. 2009;48(5):1-10.
10. Tian Y, Yuan W, Fujita N, et al. Inflammatory cytokines associated with degenerative disc disease control aggrecanase-1 (ADAMTS-4) expression in nucleus pulposus cells through MAPK and NF-kappaB. Am J Pathol. 2013;182(6):2310-2321.
11. He D, Zhou M, Bai Z, Wen Y, Shen J, Hu Z. Propionibacterium acnes induces intervertebral disc degeneration by promoting nucleus pulposus cell pyroptosis via NLRP3-dependent pathway. Biochem Biophys Res Commun. 2020;526(3):772-779.
12. Tang P, Gu JM, Xie ZA, et al. Honokiol alleviates the degeneration of intervertebral disc via suppressing the activation of TXNIP-NLRP3 inflammasome signal pathway. Free Radic Biol Med. 2018;120:368-379.
13. Song Y, Wang Y, Zhang Y, et al. Advanced glycation end products regulate anabolic and catabolic activities via NLRP3-inflammasome activation in human nucleus pulposus cells. J Cell Mol Med. 2017;21(7):1373-1387.
14. Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol. 2002;2(8):569-579.
15. Andaloussi SEI, Mäger I, Breakfield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov. 2013;12(5):347-357.
16. Tavakoli Dargani Z, Singla DK. Embryonic stem cell-derived exosomes inhibit doxorubicin-induced TLR4-NLRP3-mediated cell death-pyroptosis. Am J Physiol Heart Circ Physiol. 2019;317(2):H460-H471.
17. Single DK, Johnson TA, Tavakoli Dargani Z. Exosome treatment enhances anti-inflammatory M2 macrophages and reduces inflammation-induced pyroptosis in doxorubicin-induced cardiomyopathy. Cells. 2019;8(10):1224.
18. Lu K, Li HY, Yang K, et al. Exosomes as potential alternatives to stem cell therapy for intervertebral disc degeneration: in-vitro study on exosomes in interaction of nucleus pulposus cells and bone marrow mesenchymal stem cells. Stem Cell Res Ther. 2017;8(1):108.
19. Xia C, Zeng Z, Fang B, et al. Mesenchymal stem-cell derived exosomes ameliorate intervertebral disc degeneration via anti-oxidant and anti-inflammatory effects. Free Radic Biol Med. 2019;143:1-15.
20. Ji ML, Jiang H, Zhang XJ, et al. Preclinical development of a microRNA-based therapy for intervertebral disc degeneration. Nat Commun. 2018;9(1):5051.
21. O’Connell GD, Vresilovic EJ, Elliott DM. Comparison of animals used in disc research to human lumbar disc geometry. Spine (Phila Pa 1976). 2007;32(3):328-333.
22. Showalter BL, Beckstein JC, Martin JT, et al. Comparison of animal discs used in disc research to human lumbar disc: torsion mechanics and collagen content. Spine (Phila Pa 1976). 2012;37(15):E900-E907.
23. Beckstein JC, Sen S, Schaer TP, Vresilovic EJ, Elliott DM. Comparison of animal discs used in disc research to human lumbar disc: axial compression mechanics and glycosaminoglycan content. Spine (Phila Pa 1976). 2008;33(6):E164-E173.
24. Yang F, Leung VY, Luk KD, Chan D, Cheung KM. Injury-induced sequential transformation of notochordal nucleus pulposus to chondrogenic and fibrocartilaginous phenotype in the mouse. J Pathol. 2009;218(1):113-121.
25. Han B, Zhu K, Li FC, et al. A simple disc degeneration model induced by percutaneous needle puncture in the rat tail. Spine (Phila Pa 1976). 2008;33(18):1925-1934.
26. Thery C, Amigorena S, Rapsogo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell Biol Chapter 3. 2006. Unit 3 22.
27. Pan Z, Sun H, Xie B, et al. Therapeutic effects of gefitinib-encapsulated thermosensitive injectable hydrogel in intervertebral disc degeneration. Biomaterials. 2018;160:56-68.
28. Zamani P, Oskuee RK, Atkin SL, Navashenaq JG, Sahebkar A. MicroRNAs as important regulators of the NLRP3 inflammasome. Prog Biophys Mol Biol. 2020;150:50-61.
29. Chen S, Lv X, Hu B, et al. Critical contribution of RIPK1 mediated mitochondrial dysfunction and oxidative stress to compression-induced rat nucleus pulposus cells necroptosis and apoptosis. Apoptosis. 2018;23(5-6):299-313.
30. Xu Y, Yao H, Wang Q, et al. Aquaporin-3 attenuates oxidative stress-induced nucleus pulposus cell apoptosis through regulating the P38 MAPK pathway. Cell Physiol Biochem. 2018;50(5):1687-1697.
31. Yu W, Fu J, Liu Y, Wu Y, Jiang D. Osteogenic protein-1 inhibits stress-induced nucleus pulposus cell apoptosis through regulating the NF-kB/ROS pathway in an inflammation environment. Biosci Rep. 2018;38(6):BSR20181530. https://doi.org/10.1042/BSR20181530
32. Ellman MB, Kim JS, An HS, et al. Toll-like receptor adaptor signaling molecule MyD88 on intervertebral disk homeostasis: in vitro, ex vivo studies. Gene. 2012;505(2):283-290.
33. Guo Y, Tian L, Liu X, He Y, Chang S, Shen Y. ERRFI1 inhibits proliferation and inflammation of nucleus pulposus and is negatively
regulated by miR-2355-5p in intervertebral disc degeneration. Spine (Phila Pa 1976). 2019;44(15):E873-E881.

34. Lebeaupin C, Proics E, de Bieville CH, et al. ER stress induces NLRP3 inflammasome activation and hepatocyte death. Cell Death Dis. 2015;6:e1879.

35. Yim RL, Lee JT, Bow CH, et al. A systematic review of the safety and efficacy of mesenchymal stem cells for disc degeneration: insights and future directions for regenerative therapeutics. Stem Cells Dev. 2014;23(21):2553-2567.

36. Yang F, Leung YY, Luk KD, Chan D, Cheung KM. Mesenchymal stem cells arrest intervertebral disc degeneration through chondrocytic differentiation and stimulation of endogenous cells. Mol Ther. 2009;17(11):1959-1966.

37. Sakai D, Mochida J, Yamamoto Y, et al. Transplantation of mesenchymal stem cells embedded in Atelocollagen gel to the intervertebral disc: a potential therapeutic model for disc degeneration. Biomaterials. 2003;24(20):3531-3541.

38. Crevensten G, Walsh AJ, Ananthakrishnan D, et al. Intervertebral disc cell therapy for regeneration: mesenchymal stem cell implantation in rat intervertebral discs. Ann Biomed Eng. 2004;32(3):430-434.

39. Chik TK, Ma XY, Choy TH, et al. Photochemically crosslinked collagen annulus plug: a potential solution solving the leakage problem of cell-based therapies for disc degeneration. Acta Biomater. 2013;9(9):8128-8139.

40. Liao Z, Luo R, Li G, et al. Exosomes from mesenchymal stem cells modulate endoplasmic reticulum stress to protect against nucleus pulposus cell death and ameliorate intervertebral disc degeneration in vivo. Theranostics. 2019;9(14):4084-4100.

41. Wang Y, Jiao T, Fu W, et al. miR-410-3p regulates proliferation and apoptosis of fibroblast-like synoviocytes by targeting YY1 in rheumatoid arthritis. Biomed Pharmacother. 2019;119:109426.

42. Yeruva L, Myers GS, Spencer N, et al. Early microRNA expression profile as a prognostic biomarker for the development of pelvic inflammatory disease in a mouse model of chlamydial genital infection. MBio. 2014;5(3):e01241-e1314.

43. Liu D, Zhang N, Zhang X, Qin M, Dong Y, Jin L. MiR-410 down-regulates the expression of interleukin-10 by targeting STAT3 in the pathogenesis of systemic lupus erythematosus. Cell Physiol Biochem. 2016;39(1):303-315.

44. Wang Y, Xu N, Zhao S, et al. miR-410-3p suppresses cytokine release from fibroblast-like synoviocytes by regulating NF-kappaB signaling in rheumatoid arthritis. Inflammation. 2019;42(1):331-341.

45. Ertekin S, Yildirim O, Dinc E, Ayaz L, Fidanci SB, Tamer L. Evaluation of circulating miRNAs in wet age-related macular degeneration. Mol Vis. 2014;20:1057-1066.

46. Bauernfeind F, Rieger A, Schildberg FA, Knolle PA, Schmid-Burgk JL, Hornung V. NLRP3 inflammasome activity is negatively controlled by miR-223. J Immunol. 2012;189(8):4175-4181.

---

How to cite this article: Zhang J, Zhang J, Zhang Y, et al. Mesenchymal stem cells-derived exosomes ameliorate intervertebral disc degeneration through inhibiting pyroptosis. J Cell Mol Med. 2020;24:11742-11754. https://doi.org/10.1111/jcmm.15784