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Melatonin protects human nucleus pulposus cells from pyroptosis by regulating Nrf2 via melatonin membrane receptors

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Aims

This study was performed to explore the effect of melatonin on pyroptosis in nucleus pulposus cells (NPCs) and the underlying mechanism of that effect.

Methods

This experiment included three patients diagnosed with lumbar disc herniation who failed conservative treatment. Nucleus pulposus tissue was isolated from these patients when they underwent surgical intervention, and primary NPCs were isolated and cultured. Western blotting, reverse transcription polymerase chain reaction, fluorescence staining, and other methods were used to detect changes in related signalling pathways and the ability of cells to resist pyroptosis.

Results

Western blot analysis confirmed the expression of cleaved CASP-1 and melatonin receptor (MT-1A-R) in NPCs. The cultured NPCs were identified by detecting the expression of CD24, collagen type II, and aggrecan. After treatment with hydrogen peroxide, the pyroptosis-related proteins NLR family pyrin domain containing 3 (NLRP3), cleaved CASP-1, N-terminal fragment of gasdermin D (GSDMD-N), interleukin (IL)-18, and IL-1β in NPCs were upregulated, and the number of propidium iodide (PI)-positive cells was also increased, which was able to be alleviated by pretreatment with melatonin. The protective effect of melatonin on pyroptosis was blunted by both the melatonin receptor antagonist luzindole and the nuclear factor erythroid 2–related factor 2 (Nrf2) inhibitor ML385. In addition, the expression of the transcription factor Nrf2 was up- or downregulated when the melatonin receptor was activated or blocked by melatonin or luzindole, respectively.

Conclusion

Melatonin protects NPCs against reactive oxygen species-induced pyroptosis by upregulating the transcription factor Nrf2 via melatonin receptors.

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Keywords: Melatonin, Intervertebral disc degeneration, Pyroptosis, Transcription factor, Nrf2

Article focus

- This study was performed to explore the effect of melatonin on the pyroptosis of nucleus pulposus cells (NPCs) and the underlying mechanism of that effect.

Key messages

- Pyroptosis, a newly discovered form of cell death, can be induced by reactive oxygen species. Melatonin protects NPCs from pyroptosis via the melatonin receptor and upregulates nuclear factor erythroid 2–related factor 2.

Strengths and limitations

- We extracted NP tissue from intervertebral disc degeneration patients treated with percutaneous transforaminal endoscopic discectomy (PTED), which more closely approximates the clinical

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situation. We have shown that hydrogen peroxide induces pyroptosis in NPCs. However, our work lacks in-depth research.

Introduction

Low back pain, usually caused by intervertebral disc degeneration (IDD), creates a large social and economic burden.[1,2] Histopathology and imaging studies of clinical samples have indicated that most IDD-related low back pain begins with a reduction in number and functional disorder of nucleus pulposus cells (NPCs).[3,4] Reduction of NPCs may induce the imbalance of synthesis and degradation of the extracellular matrix (ECM), accelerating the IDD process.[5] However, the underlying mechanisms of the reduction in NPCs are still largely unclear.

Pyroptosis, a newly discovered form of cell death,[6] can lead to cell number reduction. As generally accepted, apoptosis depends on the activation of CASP-3,8,9 but does not cause inflammation. In contrast to apoptosis, pyroptosis depends on the activation of CASP-1 and is accompanied by an increase in inflammatory cytokines such as cleaved interleukin (IL)-1β and IL-18.[7] It was previously revealed that IL-1β was highly expressed in degenerated discs and closely related to IDD progression.[8] However, pyroptosis of NPCs has rarely been reported. Exploring the pyroptosis of NPCs is helpful for further understanding NPC reduction and exploring new targets for the treatment of IDD.

In recent years, the protective effect of melatonin (N-acetyl-5-methoxytryptamine) on human degenerative diseases has been a research hotspot. Melatonin was first isolated from the bovine pineal gland and was reported to be distributed in various organs and organisms.[9,10] In addition to modulating circadian rhythms, melatonin has also shown beneficial effects in sleep disorders,[11,12] osteoporosis,[13] cancers,[14] diabetes,[15] brain injury,[16] neuroprotection,[17] and bone marrow mesenchymal stem cell proliferation[18] through activation of melatonin receptors. The underlying mechanisms involved in the protective effects of melatonin include but are not limited to inhibiting the mTOR signalling pathway,[19] reducing the inflammatory response,[20] activating the AMPK signalling pathway,[21] disturbing SUMOylation,[14] and enhancing antioxidant capacity.[22] However, the effect of melatonin on NPC pyroptosis has not been reported. Exploring the potential effect of melatonin on NPC pyroptosis would help to identify new strategies for the treatment of IDD.

An underlying association between IDD and melatonin was first implicated by the finding that melatonin reduces the cartilage endplate vascularity of degenerated intervertebral discs.[23] Surgical pinealectomy was reported to accelerate IDD in chickens.[24] In rat IDD models, melatonin was found to activate the recovery process in degenerated IVD tissue, possibly by stimulating transforming growth factor (TGF)-beta activity.[23] In addition, melatonin also protects NPCs[25,26] and endplate chondrocytes[27] from apoptosis, delaying IDD progression. However, the potential association between melatonin and pyroptosis of NPCs remains to be determined. This study was performed to explore the effects of melatonin on the pyroptosis of NPCs and the likely signal transduction pathways involved.

Methods

Cell culture and treatment. Nucleus pulposus tissue was isolated from three patients suffering lumbar disc herniation who were being treated with surgical intervention.[28] All written informed consent was obtained, and the research was approved by the ethics committee of The First Affiliated Hospital of Wenzhou Medical University. According to symptoms, signs, and imaging data, three patients (one male, two female) who were diagnosed with lumbar disc herniation and had waist and leg symptoms after the failure of conservative therapy for three months were included. Patients with other diseases of the spine or systemic diseases, such as diabetes mellitus, hypertension, and heart disease, were excluded from this study. In this study, the three included patients had an IDD of grade IV based on Pfirrmann's classifications. The degenerative segments of two patients were L5/S1 and those of the other were L4/L5. The mean age of the patients was 47.6 years (standard deviation (SD) 2.5). NPCs were isolated and cultured as previously described.[4,8] Briefly, after the intervertebral disc tissue was washed with sterile saline three times, the annulus fibrosus tissue and the tough cartilage endplate were carefully removed. The remaining nucleus pulposus tissue was then digested with 0.25% trypsin for 30 minutes, followed by 0.2% type II collagenase (Sigma-Aldrich, USA) for four hours in a sterile 15 ml centrifuge tube. The cells were then filtered through 200 mesh sieves and cultured in conditioned medium made up of 84% DMEM/F12 medium and 16% foetal bovine serum (Gibco, USA, CAS No.: 10099141). The cells were cultured in a humidified incubator with 1% O_2, 5% CO_2, and 94% N_2 at 37°C. NPCs were identified by detecting the expression of CD24, collagen type II, and aggrecan.[29] The second-passage NPCs were used for further experiments.[30] To induce oxidative stress, NPCs were treated with 200 μM hydrogen peroxide for three hours.[31] Melatonin (50 nM, MCE, USA, CAS No.: 73-31-4) and luzindole (10 μM, Santacruz Biotechnology, USA, CAS No.: sc-202700) were used to activate and inhibit the melatonin receptor.[26] ML385 (20 μM MCE, USA, CAS No.: 846557-71-9) was used to inhibit the expression of Nrf2, according to the cell counting kit-8 (CCK) test (Beyotime, China, CAS No.: C0038).

Cell viability assay. The CCK-8 assay was performed according to the manufacturer's instructions. Briefly, 1×10^4 cells/well were seeded in 96-well plates and treated with different drugs. Then, the medium was replaced with 100 μl basic medium containing 10 μl CCK-8 solution for another two hours in the incubator at 37°C. Finally, the absorbance at 450 nm of each well was measured using an enzyme labelling measuring instrument (Infinite 200 Pro; Tecan, USA). There were three replicates for each group.

Table I. Primer sequences used for reverse-transcription polymerase chain reaction analyses, as also reported in Bai et al.[33]

Gene	Sequence
CD24	
Forward	5'-CCCACGCAGATTTATTCCAG-3'
Reverse	5'-GACTTCCAGACGCCATTTG-3'
β-actin	
Forward	5'-GGACTCGTCATACTCCTGCTTG-3'
Reverse	5'-GGAAATCGTGCGTGACATTAAG-3'

ROS detection. The ROS level of NPCs was evaluated by 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) (Beyotime, China, CAS No.: S0033) according to the instructions. After the DCFH-DA was diluted with serum-free medium to a final concentration of 10 μmol/l, the collected cells were suspended in diluted DCFH-DA and incubated in a cell incubator for 20 minutes to allow the probe to fully contact the cells. Before detection by flow cytometry (BD Biosciences, USA), the cells were washed with serum-free cell culture medium three times to remove the remaining DCFH-DA. Experiments were repeated three times independently.

RNA extraction, retrotranscription, and real-time PCR. Target gene primers were designed and synthesized by Shanghai Shenggong Biological Co (China), and β-actin was used as an internal reference control (Table I). The total RNA from the NPCs was extracted using kit reagents (RC112-01; Vazyme, China) according to the manufacturer's instructions. Reverse transcription of 1 μg of RNA enables the amplification of complementary products.[32] Quantitative polymerase chain reaction (PCR) was performed in a 20 μl reaction system containing specific primers and ChamQ SYBR qPCR Master Mix (Q321-02; Vazyme). Amplification was performed in the Roche LightCycler 480 System (Roche, Switzerland). The PCR extension conditions were 95°C for 30 seconds, 95°C for five seconds, 60°C for 34 seconds, and 40 cycles. There were three replicates for each group. The Ct value obtained for each group is represented by $2^{-\Delta\Delta Ct}$ or $2^{-\Delta Ct}$.

Protein isolation and Western blot. Total cellular protein was extracted from NPCs with modified radioimmunoprecipitation assay (RIPA) buffer (Beyotime, China, Cat. No.: P0013B) supplemented with 1 mmol/l phenylmethylsulfonyl fluoride (PMSF) following the manufacturer's protocol. Proteins (40 μg) of each group were resolved by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS–PAGE) (12%) and transferred to polyvinylidene fluoride (PVDF). After transfer, the membranes were blocked in Tris-buffered saline (TBST) containing 5% non-fat milk for two hours at 37°C and then incubated overnight with primary anti-NLRP3 (1:1,000; Abcam, USA, Cat. No.: ab210491), CASP-1 (1:1,000, Proteintech, USA, Cat. No.: 22915-1-AP), GSDMD (1:1,000, Affinity Biosciences, USA, Cat. #: AF4012), GSDMD-N (1:1,000, Affinity Biosciences, Cat. #: DF12275), IL-18 (1:1,000, Affinity Biosciences, Cat. #: DF6252), IL-1β (1:500, ABclonal, USA, Cat. No.: A1112), Nrf2 (1:1,000, Abcam, Cat. No.: ab62352), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:10,000, Proteintech, Cat. No.: 10494-1-AP) at 4°C. Before incubation with secondary antibodies for one to two hours at 37°C, the membranes were washed with TBST solution three times. The bands were visualized by an ECL-Plus detection kit (P10100; New Cell & Molecular Biotech Co). The membranes were analyzed by Quantity One software (Bio-Rad, USA). Experiments were repeated three times independently.

Immunohistochemical staining. The paraffin-embedded nucleus pulposus tissues were processed for immunohistochemical staining as previously described.[33] The primary antibody used was caspase-1 (1:150, Proteintech, Chicago, USA, Cat. No.: 22915-1-AP) and the secondary antibody was goat anti-rabbit IgG (H+L) HRP (1:200, Affinity Biosciences, USA, Cat. #: S0001). The sections were visualized using a microscope (CTR4000B, Leica). Experiments were repeated three times independently.

Immunofluorescence staining. NPCs were cultured on 14 mm-diameter round glass plates (801010; Nest, China) and treated as appropriate. After 24 hours, the cells were fixed with 4% paraformaldehyde.[34] Cells were permeabilized with 0.1% Triton in PBS, blocked with QuickBlock (P0260, Shanghai, China, Beyotime) blocking buffer, and incubated with primary antibodies against aggrecan (1:500, Abcam, USA, Cat. No.: ab3778)) and collagen II (1:500, Abcam, USA, Cat. No.: ab34712) overnight at 4°C. Collagen II primary antibodies were detected using goat anti-rabbit IgG labelled by fluorescein isothiocyanate isomer (FITC) (1:500, Affinity Biosciences, USA, Cat. #: S0008), and aggrecan primary antibodies were detected using goat anti-mouse IgG labelled by Cyanine 3 (CY3) (1:500, Affinity Biosciences, USA, Cat. #: S0012). Cells were stained with 4′,6-diamidino-2-phenylindole (DAPI), and the glass slides were sealed after washing three times. Sections were acquired and imaged using laser scanning confocal microscopy (LSMC).

Fluorescence staining. Hoechst 33342/propidium iodinate (PI) (CA1120; Solarbio, China) double staining observed by fluorescence microscopy (CTR4000B; Leica, Germany) was used to detect the membrane destruction of the cells according to the manufacturer's instructions. Briefly, treated cells in each well of the six-well plates were incubated with 1 ml of staining buffer containing 5 μl of Hoechst 33,342 and PI dye for 30 minutes at 4°C before observation. The stained cells were visualized using LSMC (A1; Nikon, Japan). Experiments were repeated three times independently.

Statistical analysis. Statistical analyses were performed with GraphPad Prism 6.0 (GraphPad, USA). The results are expressed as the mean and standard deviation (SD). In addition, the measurement data were compared with independent-samples *t*-test. The graphs were produced by GraphPad 6.0 software. A p-value < 0.05 was considered significant. Unless otherwise stated, all in vitro experiments were performed at least three times, and three independent experiments used cells from three different donors.

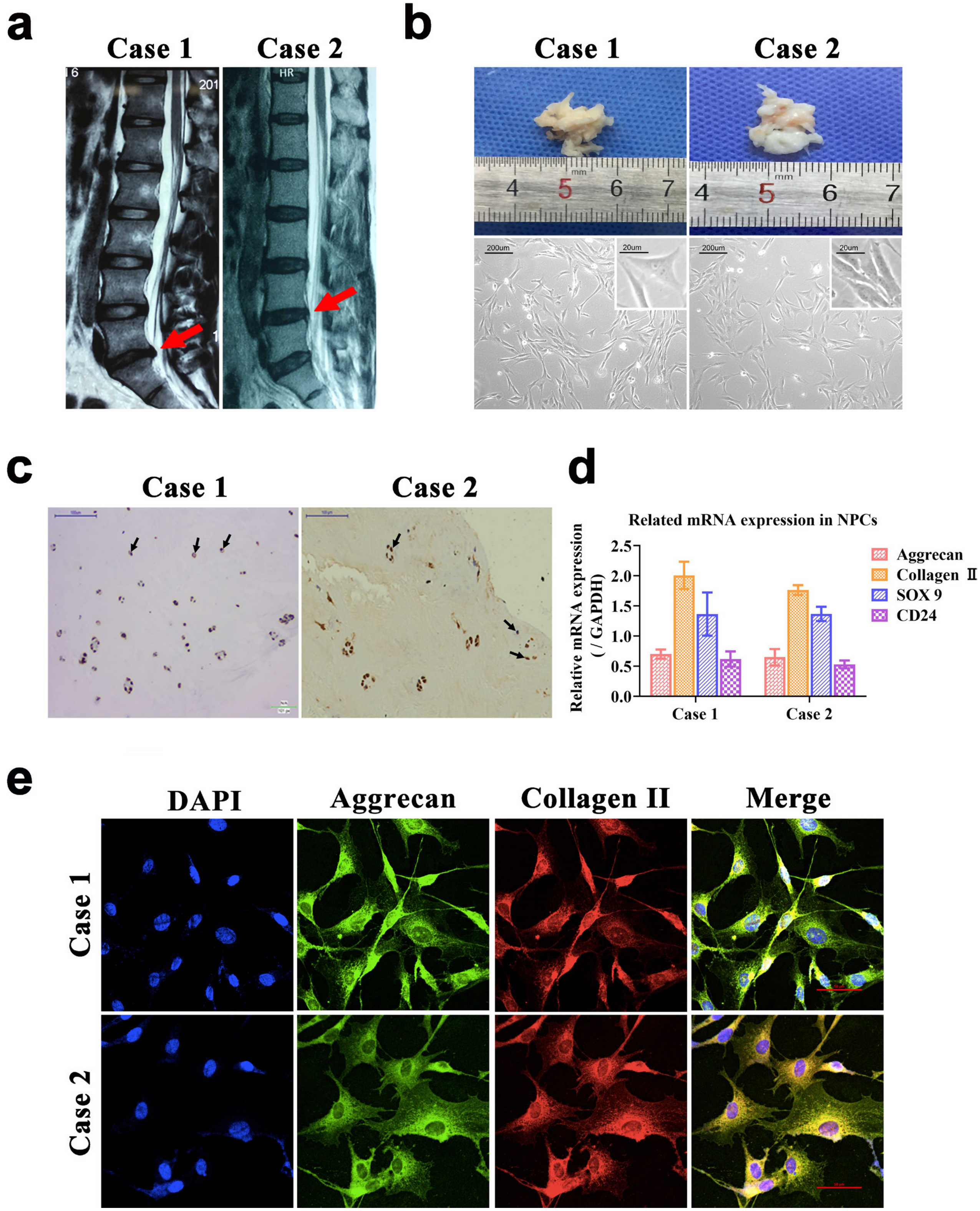

Fig. 1

Identification of nucleus pulposus cells (NPCs) isolated from patients with lumbar disc herniation (LDH). a) The MRI T2 weighted images show the intervertebral disc of the patients with LDH. Red arrows indicate the herniated disc. b) Macroscopic observation of nucleus pulposus tissues and microscopic observation of the cultured NPCs (magnification: ×100, scale bars = 200 μm). c) Caspase-1 in the nucleus pulposus tissue was detected by immunohistochemistry (magnification: ×200, scale bars = 100 μm). d) The mRNA expression of aggrecan, Collagen II, SOX9, and CD24 was detected by reverse-transcription polymerase chain reaction. e) The immunofluorescence assay of collagen II and aggrecan of NPCs (magnification: ×600, scale bar = 50 μm). GAPDH, glyceraldehyde 3-phosphate dehydrogenase; mRNA, messenger RNA.

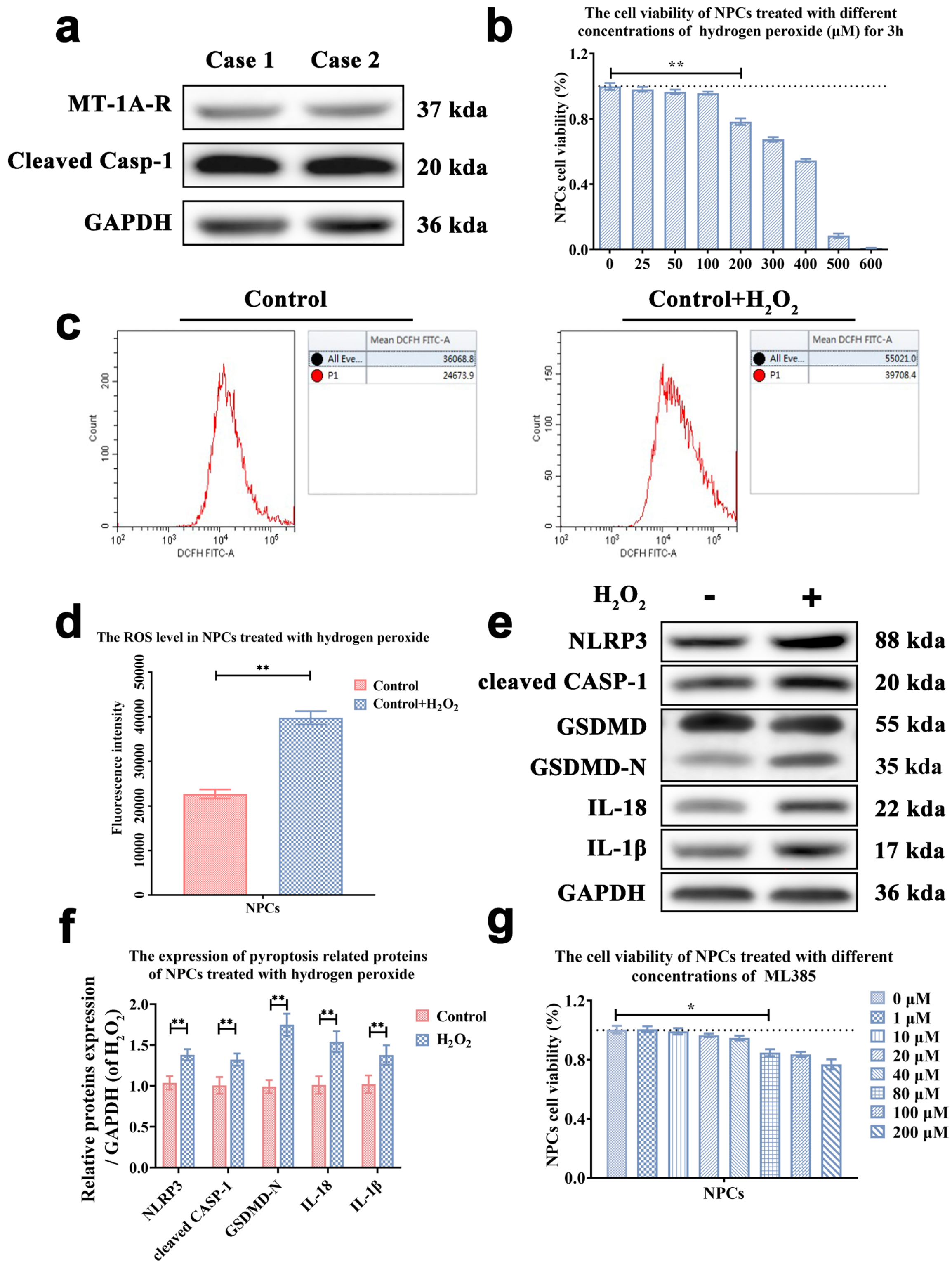

Fig. 2

Detection of pyroptosis-related proteins in human nucleus pulposus cells (NPCs). a) The expression of melatonin receptor type 1A (MT-1A-R) and cleaved caspase-1 (CASP-1) of NPCs was detected by Western blot. b) The influence of hydrogen peroxide with different concentrations on NPC viability was detected by the cell counting kit-8 test. c) The intracellular reactive oxygen species (ROS) levels of NPCs were detected by flow cytometry through DCFH-DA staining. The P1 value represents the fluorescence intensity of each 1×10⁴ NPCs. d) The comparison of the fluorescence intensity of NPCs treated with and without hydrogen peroxide. e) The expression of pyroptosis-related protein NLRP3, cleaved CASP-1, N-terminal fragment of gasdermin D (GSDMD-N), interleukin (IL)-18, and IL-1β expressed in NPCs with and without treatment of hydrogen peroxide (200 μM for three hours) was detected by Western blot. f) The panel showed the gray histogram of the Western blot band in Figure 2e. g) The influence of ML385 with different concentrations on cell viability of NPCs. *$p < 0.05$, **$p < 0.01$. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Results

Pyroptosis-related proteins were upregulated in human NPCs treated with hydrogen peroxide. The MRI of two patients with IDD is shown in Figure 1a. The nucleus pulposus tissue extracted from the patients with IDD was ivory white, and the NPCs displayed a long fusiform shape under the light microscope (Figure 1b). Immunohistochemical staining showed that CASP-1 was present in the cellular portion of intervertebral disc tissues (Figure 1c). The messenger RNA (mRNA) expression of aggrecan, collagen II, SOX9, and CD24 in NPCs was confirmed by real-time PCR (Figure 1d), and the expression of collagen type II and aggrecan was detected by immunofluorescence (Figure 1e). In parallel, identification of NPCs by the fluorescently labelled monoclonal antibody CD24 is shown in Supplementary Figure b. The presence of cleaved CASP-1 in NPCs was confirmed by Western blot (Figure 2a). The influence of different concentrations of hydrogen peroxide on the viability of nucleus pulposus cells was tested by the CCK-8 test. Hydrogen peroxide significantly reduced the viability of NPCs at concentrations greater than 200 μM (Figure 2b). After treatment with 200 μM hydrogen peroxide for three hours, the ROS level in NPCs was increased significantly (Figures 2c and 2d). Through Western blot analyses, the pyroptosis-related proteins NLRP3, cleaved CASP-1, GSDMD-N, IL-18, and cleaved IL-1β were found to be upregulated after treatment with hydrogen peroxide (Figures 2e and 2f). Under LSMC, more PI-positive NPCs were found in NPCs treated with hydrogen peroxide via Hoechst 33342/PI double staining (Figure 3a). Additional evidence of pyroptosis in NPCs is presented in Supplementary Figures c and d.

Melatonin application inhibited the upregulation of pyroptosis-related proteins in NPCs via melatonin receptors. As confirmed by Western blotting, melatonin membrane receptors (MT-1A-R) were present in human NPCs (Figure 2a). Compared with NPCs treated with hydrogen peroxide, pretreatment with melatonin significantly upregulated the expression of Nrf2 and downregulated the pyroptosis-related proteins NLRP3, cleaved CASP-1, GSDMD-N, IL-18, and IL-1β (Figures 3b and 3c). In addition, the fluorescence staining test also showed that cells with cellular membrane rupture and PI-positive staining were also decreased by pretreatment with melatonin (Figure 3a).

Luzindole, a melatonin receptor antagonist, blunted the protective effects of melatonin in NPCs. When NPCs were pretreated with melatonin receptor antagonist luzindole at a concentration of 10 μM, the effect of melatonin on NPCs was blunted. The upregulation of Nrf2 and the inhibitory effect on ROS-induced upregulation of NLRP3, cleaved CASP-1, GSDMD-N, IL-18, and IL-1β were all blunted, as shown by Western blot analysis (Figure 3b). Under LSMC, more PI-positive NPCs were observed in the group treated with luzindole, melatonin, and hydrogen peroxide compared with the group treated with hydrogen peroxide and melatonin (Figure 3a).

ML385, an Nrf2 inhibitor, blunted the effects of melatonin on NPCs. Treatment with ML385 at a concentration of 40 μM did not significantly affect the viability of NPCs (Figure 2g). When NPCs were pretreated with ML385, an Nrf2 inhibitor, the effect of melatonin in NPCs was also blunted. The inhibitory effect of melatonin on the ROS-induced upregulation of NLRP3, cleaved casp-1, GSDMD-N, IL-18, and IL-1β and the increase in PI-positive cells was blunted (Figure 3). It was inferred that Nrf2 was involed in the protective effect of melatonin on the pyroptosis of NPCs.(Figure 4)

Discussion

In this study, we confirmed that hydrogen peroxide induces pyroptosis in NPCs.[33] Nucleus pulposus tissue was extracted from patients with IDD treated with PTED. Endoscopic observation and isolation make it possible to obtain nucleus pulposus tissue without other cell contamination. We identified nucleus pulposus cells by observing the cell morphology through a microscope and detecting the expression of the cell-specific proteins CD24, collagen type II, and aggrecan.[29] Pyroptosis, a newly discovered form of cell death, can be induced by lipopolysaccharide (LPS) or ROS. By activating the NLRP3 inflammasome, ROS increases the cleavage of CASP-1, leading to pore formation and rupture of the cellular membrane and the cleavage of IL-1β.[35] To explore the potential function of melatonin in pyroptosis, we identified the expression of CASP-1 in nucleus pulposus tissues and built a pyroptosis model of NPCs by using hydrogen peroxide.[33] The upregulated expression of the pyroptosis-related proteins NLRP3, cleaved CASP-1, GSDMD-N, IL-18, and IL-1β detected by Western blot proved that the pyroptosis signalling pathway was activated by hydrogen peroxide. Since PI is unable to penetrate the cells with a complete cellular membrane, the nuclei of cells in the late apoptotic stage show highlighted blue fluorescence with Hoechst 33342 staining. The dark blue- and red-positive cells during Hoechst 33342/PI double staining indicate that the cells are undergoing pyroptosis. Detection of the expression of cleaved CASP-1 and Hoechst 33342/PI via double staining has been reported as a convincing method to detect pyroptosis.[35]

Melatonin protects NPCs from pyroptosis via the melatonin receptor. Melatonin is an amine hormone produced by the pineal gland of mammals and reported to be effective in retarding degenerative and inflammatory diseases, but the potential mechanism remains to be elucidated.[36–38] In this study, when NPCs were pretreated with 50 nM melatonin for 24 hours before treatment with hydrogen peroxide, the upregulation of pyroptosis-related proteins and the increase in PI-positive cells were all alleviated, revealing the protective effect of melatonin on ROS-induced pyroptosis in NPCs. The biological effects of melatonin are mainly mediated by the specific binding of melatonin receptors and the downstream signalling pathways.[39] In addition, studies have found that melatonin can also bind to calmodulin

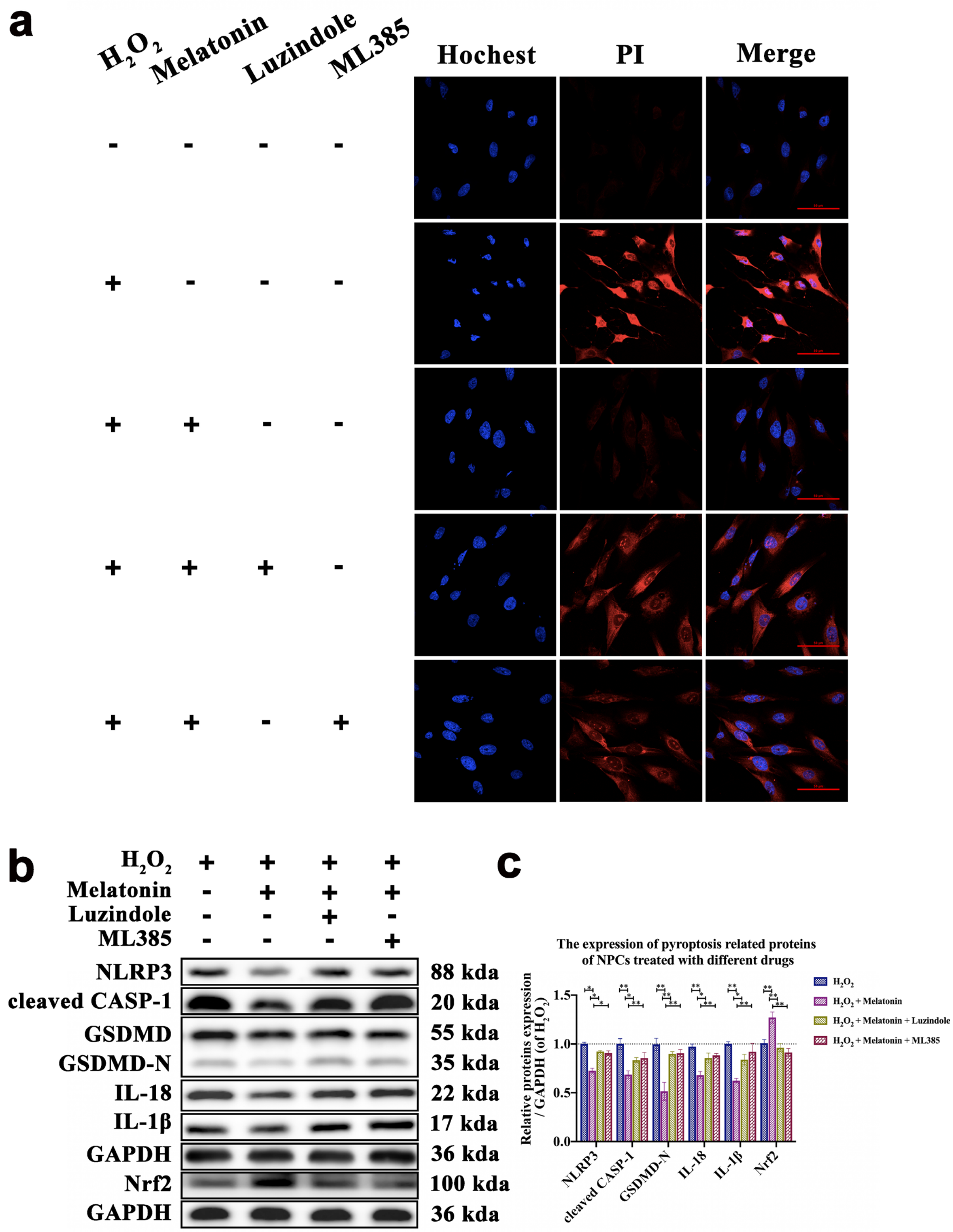

Fig. 3

Pyroptosis-related indexes of nucleus pulposus cells (NPCs) treated with different drugs. a) The Hochest33342/propidium iodide (PI) double staining of NPCs treated with different drugs (magnification: ×600, scale bar = 50 μm). b) Expression of pyroptosis-related proteins of NPCs treated with different drugs was detected by Western blot analysis. c) The panel showed the gray histogram of the Western blot analysis. *$p < 0.05$, **$p < 0.01$, . CASP-1, caspase-1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GSDMD-N, N-terminal fragment of gasdermin D; IL, interleukin; NLRP3, pyroptosis-related proteins NLR family pyrin domain containing 3; Nrf2, nuclear factor erythroid 2-related factor 2.

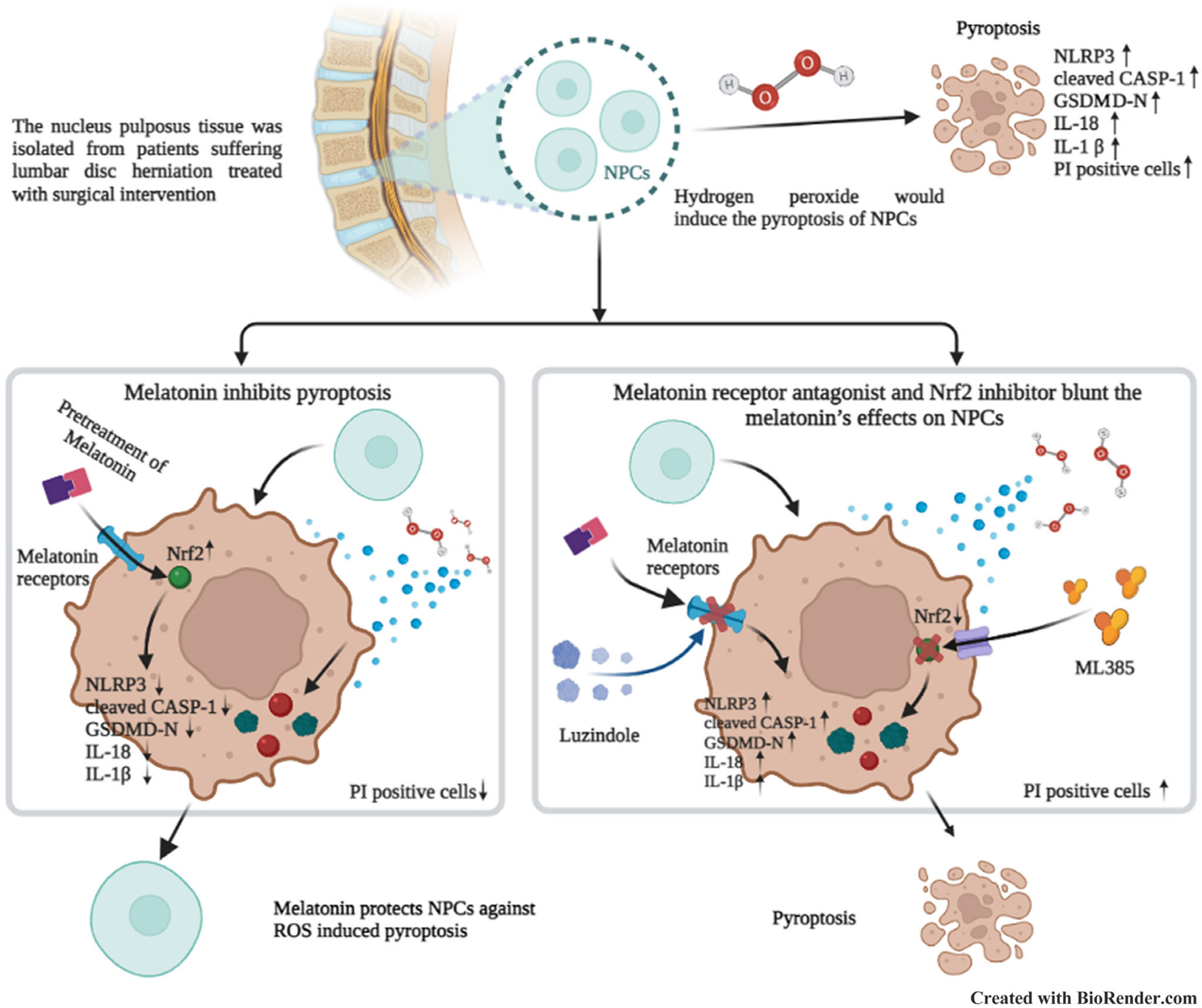

Fig. 4

Hydrogen peroxide induces the pyroptosis of nucleus pulposus cells (NPCs), and melatonin protects NPCs against reactive oxygen species (ROS)-induced pyroptosis by upregulating the transcription factor Nrf2 via melatonin receptors. CASP-1, caspase-1; GSDMD-N, N-terminal fragment of gasdermin D; IL, interleukin; NLRP3, NLR family pyrin domain containing 3; Nrf2, nuclear factor erythroid 2–related factor 2; PI, propidium iodide.

in cells and regulate the calmodulin signalling pathway through adenylate cyclase or other structural proteins, thereby participating in a variety of functions, including calcium ion transport.[40] In humans, melatonin receptors are mainly distributed in the central nervous system, intestine, ovary, blood, and skeletal system.[41] The melatonin receptor MT-1A-R was first proven to be present in human NPCs.[39] To further explore whether the melatonin receptor was involved in the protective effect of melatonin against pyroptosis of NPCs, luzindole – a melatonin receptor antagonist – was applied. Pretreatment with luzindole significantly blunted the protective effect of melatonin on NPC pyroptosis, indicating that melatonin functions via the melatonin receptor.

Melatonin protects NPCs from pyroptosis by upregulating Nrf2. Nrf2 is a transcription factor and regulates the expression of antioxidant proteins that protect against oxidative damage triggered by injury and inflammation.[42] Activation of Nrf2 results in the induction of many cytoprotective proteins, which include but are not limited to NAD(P)H quinone oxidoreductase 1 (Nqo1) and haem oxygenase-1 (HMOX1, HO-1).[43,44] To explore the potential mechanism of the protective effect of melatonin on ROS-induced pyroptosis, the expression of Nrf2 was detected when the melatonin receptor was activated and inhibited. From the Western blot analysis, the expression of Nrf2 was upregulated when the melatonin receptor was activated and downregulated when the melatonin receptor was inhibited, revealing that Nrf2 was downstream of the melatonin receptor. When cells were pretreated with an Nrf2 inhibitor, ML385, the protective effect of melatonin on pyroptosis was also inhibited, indicating that the protective effect of melatonin on pyroptosis was dependent on the upregulation of Nrf2. In this study, we have revealed that melatonin exhibited protective effects on ROS-induced pyroptosis of human NPCs at least partially through upregulating Nrf2. However, the further potential mechanism still needs to be explored by additional experiments in vitro and in vivo.

In summary, we have revealed for the first time that hydrogen peroxide induces pyroptosis in NPCs and that melatonin protects against ROS-induced pyroptosis, likely by upregulating the Nrf2 transcription factor via melatonin receptors .

Supplementary material

Figures displaying the pyroptosis process of nucleus pulposus cells (NPCs) and the expression of gasdermin D and N-terminal fragment of gasdermin D; NPC identified by fluorescently labelled monoclonal antibody CD24; dot blot results of pyroptosis-related proteins in NPCs treated with different drugs; pyroptosis-related indexes of NPCs treated with different drugs; NPCs isolated from patients with lumbar disc herniation; and a video demonstrating optical microscopy of the pyroptosis process of NPCs (magnification: ×600).

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