Journal of Orthopaedic Surgery and Research

RESEARCH

Open Access

Autophagy activation alleviates annulus fibrosus degeneration via the miR-2355-5p/mTOR pathway

Zilin Yu[1,2†], Chunyang Fan[1†], Yubo Mao[2*], Xiexing Wu[1*] and Haiqing Mao[1*]

Abstract

Background Intervertebral disc degeneration disease (IVDD) is a major cause of disability and reduced work productivity worldwide. Annulus fibrosus degeneration is a key contributor to IVDD, yet its mechanisms remain poorly understood. Autophagy, a vital process for cellular homeostasis, involves the lysosomal degradation of cytoplasmic proteins and organelles. This study aimed to investigate the role of autophagy in IVDD using a hydrogen peroxide (H_2O_2)-induced model of rat annulus fibrosus cells (AFCs).

Methods AFCs were exposed to H_2O_2 to model oxidative stress-induced degeneration. Protein expression levels of collagen I, collagen II, MMP3, and MMP13 were quantified. GEO database analysis identified alterations in miR-2355-5p expression, and its regulatory role on the mTOR pathway and autophagy was assessed. Statistical tests were used to evaluate changes in protein expression and pathway activation.

Results H_2O_2 exposure reduced collagen I and collagen II expression to approximately 50% of baseline levels, while MMP3 and MMP13 expression increased twofold. Activation of autophagy restored collagen I and II expression and decreased MMP3 and MMP13 levels. GEO analysis revealed significant alterations in miR-2355-5p expression, confirming its role in regulating the mTOR pathway and autophagy.

Conclusions Autophagy, mediated by the miR-2355-5p/mTOR pathway, plays a protective role in AFCs degeneration. These findings suggest a potential therapeutic target for mitigating IVDD progression.

Introduction

Intervertebral degenerative disc disease (IVDD) is a pathological process that destroys the structure of the intervertebral discs. The progression of this disease predisposes to the risk of disc herniation, spinal stenosis, spinal slippage and spinal instability, which uniformly presents clinically as low back pain and radiating pain in the lower limbs [1, 2]. Globally, about 700 million people are experiencing IVDD, with annual medical expenditures amounting to billions of RMB, putting enormous pressure on family finances and national finances [3–5]. Finding effective treatment is crucial for IVDD.

†Zilin Yu and Chunyang Fan contributed equally to this work.

*Correspondence:
Yubo Mao
543256031@qq.com
Xiexing Wu
wuxiexing@163.com
Haiqing Mao
maohq@suda.edu.cn
[1]Orthopedic Institute, Department of Orthopaedic Surgery, The First Affiliated Hospital, Suzhou Medical College, Soochow University, Suzhou 215006, Jiangsu, China
[2]Department of Orthopedics, Wuxi Ninth People's Hospital Affiliated to Soochow University, Wuxi 214026, Jiangsu, China

The pathogenesis of IVDD is primarily associated with the degeneration and structural destruction of the intervertebral disc tissue. The intervertebral disc is an invaluable tissue in the structure of the human spine and plays a key role in the normal physiological activity of the spine by increasing joint mobility, stress tolerance, shock absorption and protection of the brain and spinal column. Degenerative disc disease is the degenerative process of water loss and functional failure in the disc tissue [6, 7]. The function of the annulus fibrosus is to limit the position of the nucleus pulposus and provide axial tension [8]. As the intervertebral disc degenerates, the content of collagen type 1 and aggrecan, which mainly constitutes the annulus fibrosus tissue, decreases, causing changes in the mechanical properties of the tissue (e.g., properties such as modulus of elasticity and energy storage modulus), which ultimately leads to rupture of the annulus fibrosus and extrusion of the nucleus pulposus, resulting in clinical manifestations such as pain and nerve damage [9]. The mechanisms underlying annulus fibrosus degeneration remain poorly understood, and no effective treatment currently exists. Therefore, identifying pathways to mitigate or treat annulus fibrosus degeneration is of critical importance.

Autophagy is a self-degradation process that occurs within the cell and plays an important role in both healthy and pathological states by transporting cargoes such as misfolded or aggregated proteins, damaged organelles, and intracellular pathogens via autophagic vesicles (a type of double-membrane-bound vesicles) to the lysosome for degradation and by transporting the degraded molecular products (e.g., amino acids, ATP, etc.) back to the cytoplasm of the cell [10–13]. In normal cellular physiological activities, autophagy achieves intracellular homeostasis and intracellular protein homeostasis through the degradation and recycling of cellular products by lysosomes [10, 14]. Previous studies have found that the progression of IVDD is accompanied by an increase in this autophagy deficiency [15–17]. The deficiency of this autophagic system is in association with the degradation of the extracellular matrix (ECM) and the apoptosis of nucleus pulposus (NP) cells [18, 19]. In a previous study by Ye et al. [20] it was shown that autophagic activity in rat nucleus pulposus cells decreased with age and that there was a large intracellular hoarding of autophagic vesicles as well as an increase in autophagy-related proteins. Increasingly, studies have shown that proper autophagy activation can protect NP cells, such as Lin Xie et al. [21] who concluded that activation of mitochondrial autophagy and inhibition of apoptosis using cyclic RNA could improve IVDD. Whereas most studies have focused on NP cells, the intervertebral disc as a whole, its annulus fibrosus component and cellular autophagy have been poorly studied. Therefore, in our work, we investigate the degeneration and autophagy of the annulus fibrosus cells (AFCs).

MicroRNA (miRNA) is a crucial player in gene expression. It restrains translation by binding to mRNA molecules and destroys them [22, 23]. In past studies, miRNAs have been shown to contribute to disc degeneration by inhibiting structural protein expression and activating ECM degradation enzymes [24–27]. For instance, miR-132 and miR-7 were found to be highly expressed in degenerating discs, directly inhibiting GDF5, which promotes disc ECM synthesis. miR-27b was shown to be reduced in degenerating discs in a study by Ming Ji et al. and its target gene, matrix metalloproteinases 13 (MMP13), was synthesized in large quantities to degrade ECM. These evidences have suggested a possible role of miRNAs in the pathogenesis of IVDD.

This study focuses on the interaction between autophagy and IVDD induced by oxidative stress. To explore the protective role of autophagy in AFC degeneration, researchers conducted bioinformatic analyses to identify specific miRNA pathways associated with autophagy in AFCs. The ultimate goal is to identify potential therapeutic modalities for annulus fibrosus degeneration, which may offer new avenues for the treatment of IVDD.

Methods and materials

Isolation and culture of Rat AF cells

Five-week-old male Sprague-Dawley rats had the caudal annulus fibrosus tissue removed with euthanasia. It was minced and digested with collagenase for 6 h. The digest was carefully sieved through a 50 μm cell strainer. The collected AF cells were cultured in F12 medium. The culture conditions were maintained at 37 °C with a controlled atmosphere of 5% CO_2 and 5% O_2. Upon reaching a cellular confluence of 90%, the cells were subjected to dissociation via treatment with a 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA) solution, and subsequently seeded in culture plates appropriate for their growth conditions. The entirety of cells employed in this investigation were cultivated as monolayers during the initial third generation of AF cells, and all experimental procedures were meticulously conducted in triplicate, constituting a total of three replicates ($n = 3$).

The animal experiments required for this study were conducted under the regulations of the Animal Protection and Utilization Committee of Soochow University. This study was approved by the Ethics Committee of Soochow University (SUDA20230911A02).

AF cells treatments

AF cells were added to 96-well plates with a concentration of 700 cells per well. After cells reached 90% confluence, H_2O_2 (100 μM) was added to cultural medium and incubated for 24 h, respectively. In the group with

rapamycin (RAPA) treatment, the AF cells were first treated with a complete medium containing rapamycin for 3 h, then followed by the above steps.

CCK8

Cellular Activity Tests were performed by using a cytotoxicity assay kit according to instructions. AF cells were seeded in 96-well plates at 2×10^3 cells per well. After 1, 3, and 5 days of incubation respectively, they were incubated with 90 μL complete medium and 10 μl CCK-8 reagent solution for 2 h. The automated microplate reader was used to measure the optical densities (OD) at 450 nm for both the control and treatment groups.

Western blot analysis

The quantification of protein extraction from rat spinal tissues for the assessment of aggrecan fragmentation was performed utilizing the Western blot technique, following a previously established protocol. Briefly, AF cells were seeded in 6-well plates at 5×10^5 cells per well. when cells reached 90% confluence, whole-cell extracts were separated by RIPA lysate (supplemented with phosphatase inhibitors and protease inhibitors). The entire protein extracts underwent separation through 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer onto a nitrocellulose (NC) membrane. Subsequently, the NC membrane was subjected to incubation in a solution containing 10 mM Tris-buffered saline with 1.0% Tween 20 and 5% dehydrated skim milk to effectively obstruct non-specific protein interactions. The membranes were subsequently incubated overnight with 1:1000 rabbit polyclonal anti-Aggrecan primary antibody (with rabbit polyclonal antibodies against Collagen I (1:1000 dilution, ab34710), Collagen II (ab188570), matrix metalloproteinases 3 (MMP 3) (ab53015), MMP 13 (ab39012), p62 (ab109012), Beclin 1 (ab217179), LC3B (ab192890), beta Actin (ab8227) mTOR (ab32028), (Abcam, UK) and phospho-mTOR (p-mTOR, 09-343, Sigma-Aldrich, Germany), followed by anti-rabbit goat secondary antibody with HRP, for 1 h. Membrane visualization was accomplished through the application of an enhanced chemiluminescence reagent. Protein expression levels, normalized to β-actin, were assessed through analysis conducted with ImageJ software, developed by the National Institutes of Health, Bethesda, MD, USA.

mCherry-GFP-LC3B adenovirus transfection

To ascertain autophagic flux, AFCs were transfected with an adenoviral vector carrying the mCherry-GFP-LC3 fusion protein. AFCs were seeded in 24-well plates, which included slides for cell adhesion, at a density of 1×10^4 cells per well. Upon achieving 50% confluence, the mCherry-GFP-LC3B adenovirus was diluted in a culture medium and subsequently administered to the cells at a multiplicity of infection of 20, in accordance with the manufacturer's instructions. Three replicates were set up for each group and incubated for 24 h. After decanting the supernatant, the cells were transferred into 1 ml of fresh medium and incubated for an additional 24 h. Subsequently, they were fixed with 4% paraformaldehyde at room temperature for a duration of 20 min. Subsequently, the cells on the slides were treated with drops of anti-fluorescence quenching mounting solution. Once mounted, the cells were shielded from light and subjected to imaging using a fluorescence microscope (Carl Zeiss, Germany) to observe LC3 puncta.

Reverse transcription-polymerase chain reaction (RT-PCR)

For the detection of messenger RNA, AF cells underwent lysis, and the extraction of total RNA was performed employing the Trizol reagent. Complementary DNA templates were synthesized utilizing the PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan). Utilizing the SYBR Premix Ex Taq™ II Kit (Takara Shiga, Japan), reverse transcription-polymerase chain reaction was carried out on the Mx3000P system (Stratagene, US), adhering to the manufacturer's instructions. RT-PCR was carried out utilizing the All-In-One™ miRNA First-Strand cDNA Synthesis Kit (GeneCopoeia, US) in combination with the All-In-One™ miRNA qPCR Kit (GeneCopoeia, US), using GADPH as the internal reference. The assessment of relative mRNA expression levels was performed using the 2-ΔΔCt method.

Immunofluorescence examination

Immunofluorescence staining was conducted following previously established protocols [28]. Following the removal of the culture medium, AF cells were fixed for 20 min using 4% paraformaldehyde. Subsequently, they were permeabilized for 60 min with 0.03% Triton X-100 and blocked for 60 min with 5% BSA. Subsequently, the cells were exposed to a primary antibody and incubated overnight at 4 °C in a controlled humidity chamber. Following this, the cells underwent three washes and were then exposed to fluorescein-conjugated secondary antibodies for a duration of 1 h, followed by labeling with DAPI for 5 min. The fluorescence intensity was analyzed by fluorescence microscopy (Carl Zeiss, Germany).

Histological staining

Rat intervertebral disc specimens were initially fixed in a 4% buffered paraformaldehyde solution for 48 h to facilitate preservation, followed by a one-month decalcification process using 10% EDTA. Subsequently, the tissues underwent dehydration through sequential graded ethanol solutions before being embedded in paraffin. Three serial sections (5-μm thick) were cut from each specimen

using a microtome. Hematoxylin and eosin (H&E) staining or Safranin-O&Fastgreen staining was performed to observe morphology and matrix degeneration, with specimens examined and photographed under high-quality microscopy.

The disc samples used in this study were subjected to immunohistochemical staining. To prepare the samples, they were first blocked by incubating them with 5% (w/v) horse serum. Then, primary antibodies specific to LC3B and mTOR were applied to the samples. Staining procedures were meticulously followed in accordance with the protocol provided in the user manual accompanying the DAB Horseradish Peroxidase Color Development Kit obtained. Once the staining was completed, the histological sections were examined under a microscope. The researchers counted the number of cells that showed positive staining in the AF tissues of each disc in the different experimental groups. This approach allowed the researchers to assess the expression levels of LC3B and mTOR in the AF tissues and compare them between the groups. By quantifying the positively stained cells, the researchers could gain insights into the distribution and abundance of these specific markers in the different experimental conditions.

miRNA prediction and expression analysis

miRNA prediction upstream of mTOR was performed using miRmap (mirmap.ezlab.org), miRWalk (mirwalk.umm.uni-heidelberg.de) and miRDB (mirdb.org) online databases. The prediction of gene binding sites was conducted using TargetScanHuman (www.Targetscan.org). The screening and comparison of a predicted miRNA-mRNA data pair were conducted. Subsequently, cells were treated with miRNA inhibitors to examine their effects on mTOR and autophagy levels after miRNA inhibition.

Statistical analysis

The experiments were performed in triplicate, and the results were reported as the mean ± standard deviation. Statistical analyses were conducted using SPSS statistical software version 26.0.

Moreover, significant differences between two independent groups were evaluated employing Student's t-test, while distinctions among multiple groups were assessed through one-way analysis of variance (ANOVA). The statistical graphs were generated using the software GraphPad Prism 9.0. The significance levels were indicated as follows: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$.

Result

Autophagy activation after annulus fibrosus degeneration

The previous studies conducted by our research group have demonstrated a reduction in autophagy levels during the degeneration of cartilage tissue. By reinstating the autophagic expression of the tissue, it is possible to ameliorate its degenerative process [29]. The expression of autophagy was thus investigated in a rat model of degeneration. HE staining of the rat disc degeneration model revealed a reduction in the nucleus pulposus and disorganization of the annulus fibrosus within the degenerated disc. The degenerated annulus fibrosus exhibited a decrease in collagen content as observed through Safranin-O&Fastgreen staining. The immunohistochemical staining results indicated a decrease in autophagy levels in the degenerated tissues, as evidenced by an increase in P62 expression and a decrease in LC3 expression (Fig. 1A). In the established model of AFC degeneration, a decrease in the synthesis of collagen type I was observed at both the protein and gene levels, with their expression being less than half that of the normal group ($P<0.05$, 95% confidence interval: -0.7771 to -0.1360). The expression of both MMP3 and MMP13 proteins was significantly upregulated by more than 80%, accompanied by a robust synthesis of their respective mRNAs, as well as an enhanced metabolic activity for degradation of the extracellular matrix (Fig. 1B-C).

Activation of autophagy decelerates H_2O_2-induced degeneration of AFCs

In order to investigate the role of autophagy in the degeneration of fibrous annulus, 50 nM was picked as the intervention concentration of RAPA and 4 h was suggested as the intervention time of RAPA in view of the relevant literature [30]. The Western Blot results revealed a significant reduction of approximately 50% in the levels of collagen I and collagen II in the H_2O_2 group compared to the Ctrl group ($P<0.05$, respectively, 95% confidence interval: -0.4773 to -0.3570, -0.5045 to -0.4276) (Fig. 2B-C). However, no statistically significant difference was observed between the RAPA group and the Ctrl group. Extracellular matrix levels in the H_2O_2 + RAPA group were significantly higher compared to the H_2O_2 group, but still lower than the Ctrl group. The results of RT-qPCR also corroborated the protein alterations (Fig. 2A). The expression of MMP13 in the H_2O_2 group was approximately four times higher compared to that in the Ctrl group, whereas its levels were reduced upon addition of RAPA, thereby leading to a significant amelioration in extracellular matrix degradation (Fig. 2D-E). This further demonstrated the effectiveness of RAPA in retarding the degeneration of AFCs.

AFCs autophagy activation is activated by mTOR pathway

To further explore the relationship between autophagy and the regression of AFCs, the level of autophagy was examined. The findings indicated a marginal elevation in autophagy levels of AFCs following hydrogen peroxide

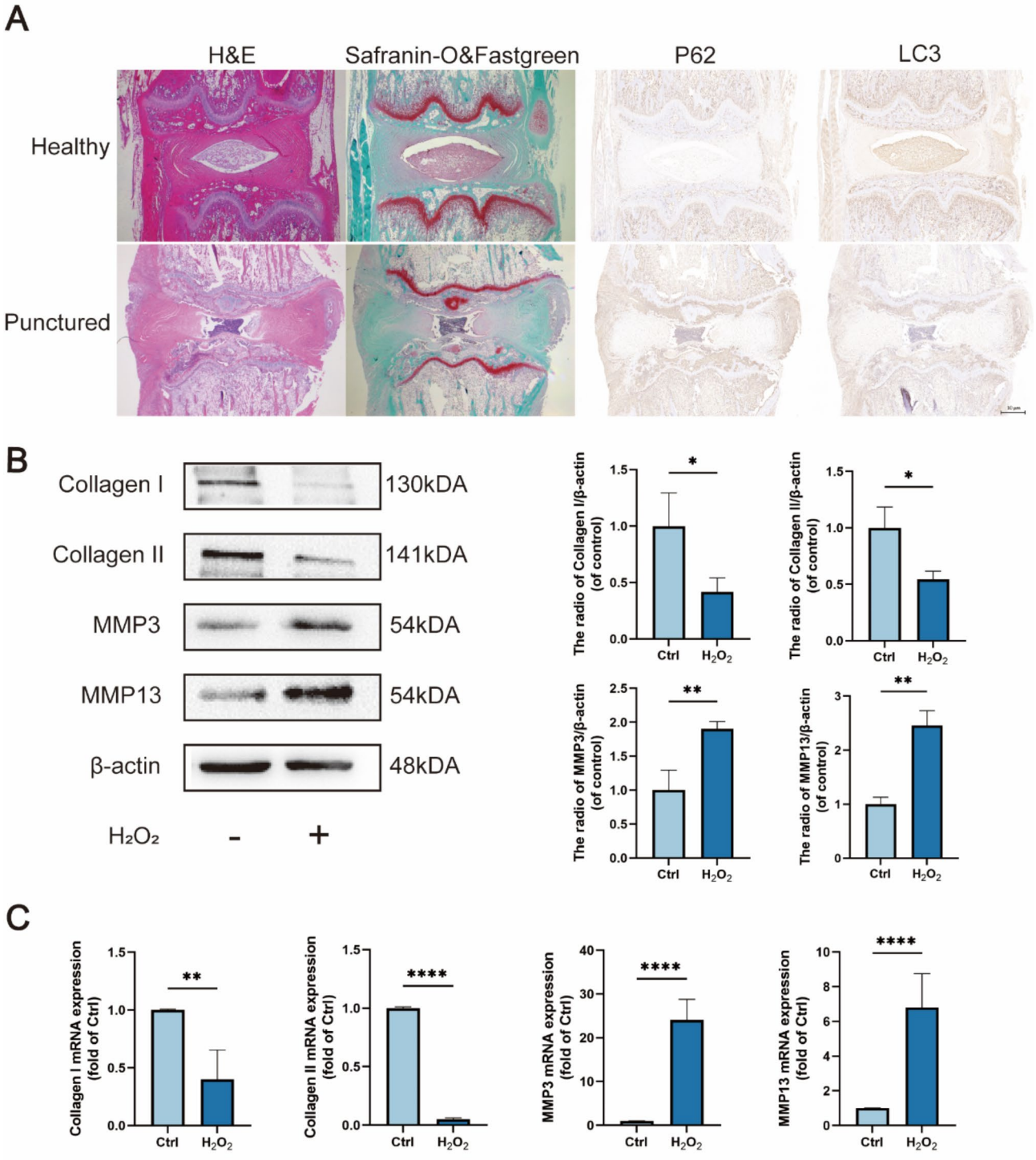

Fig. 1 H_2O_2 induced degeneration of annulus fibrosus. (**A**) Tissues' staining and immunohistochemical staining of different degenerated discs (Scale bar = 10 μm). (**B**) The effects of H_2O_2 on the protein levels of COL I, COL II, MMP3 and MMP13 in AFCs were determined using Western blot assays. (**C**) RT-PCR analysis for the expression of COL I, COL II, MMP3 and MMP13 mRNA levels in AFCs after H_2O_2 treatment. H2O2 hydrogen peroxide, COL I collagen I, COL II collagen II, MMP3 matrix metalloproteinases 3, MMP13 matrix metalloproteinases 13, RT-PCR reverse transcription-polymerase chain reaction. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$; between the indicated groups)

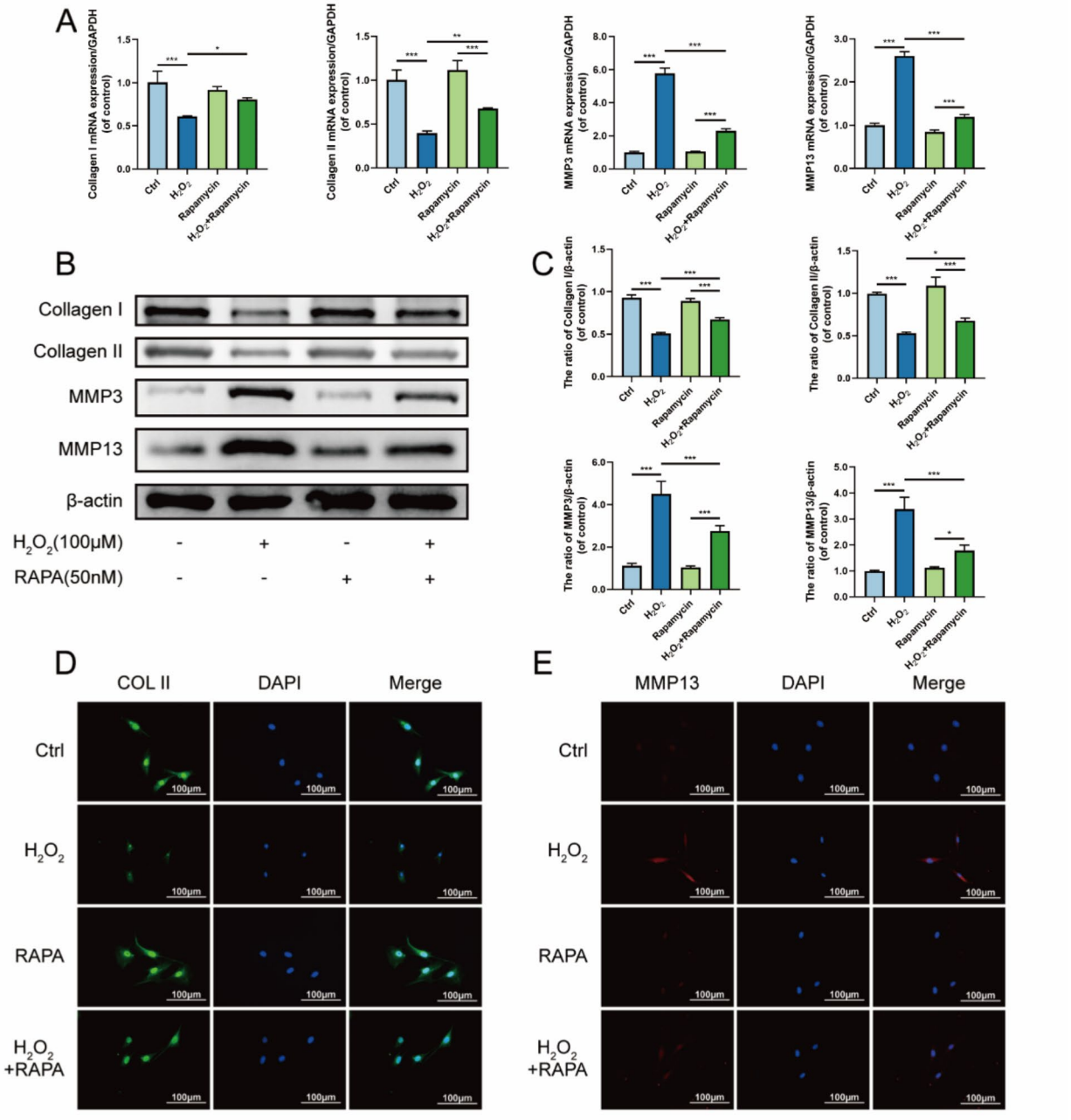

Fig. 2 Effect of RAPA on degeneration of AFCs after H_2O_2 treatment. **A**) RT-PCR analysis for the expression of COL I, COL II, MMP3 and MMP13 mRNA levels in AFCs after H_2O_2 and RAPA treatment. **B**-**C**) The effects of H_2O_2 and RAPA on the protein levels of COL I, COL II, MMP3 and MMP13 in AFCs were determined using Western blot assays. **D**-**E**) Immunofluorescence analysis for the expression of COL II and MMP13 proteins in AFCs after H_2O_2 and RAPA treatment. (Scale bar = 100 μm). RAPA rapamycin. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$; between the indicated groups)

induction, which was consistent with the observations made in tissues. In the H_2O_2 + RAPA group, the P62 protein was significantly depleted, while the production of Beclin-1, LC3B, ATG5, and ATG7 was markedly increased in the AFCs. These findings indicate a substantial enhancement of autophagy in the cells, which corresponds to the alleviation of AFC degeneration observed in the previous analysis (Fig. 3A–C). These were shown that RAPA activates AFC autophagy to improve the H_2O_2-induced degeneration (Fig. 3D). Researchers also further analyzed the expression of the mTOR pathway and found that there was a reduction in the amount

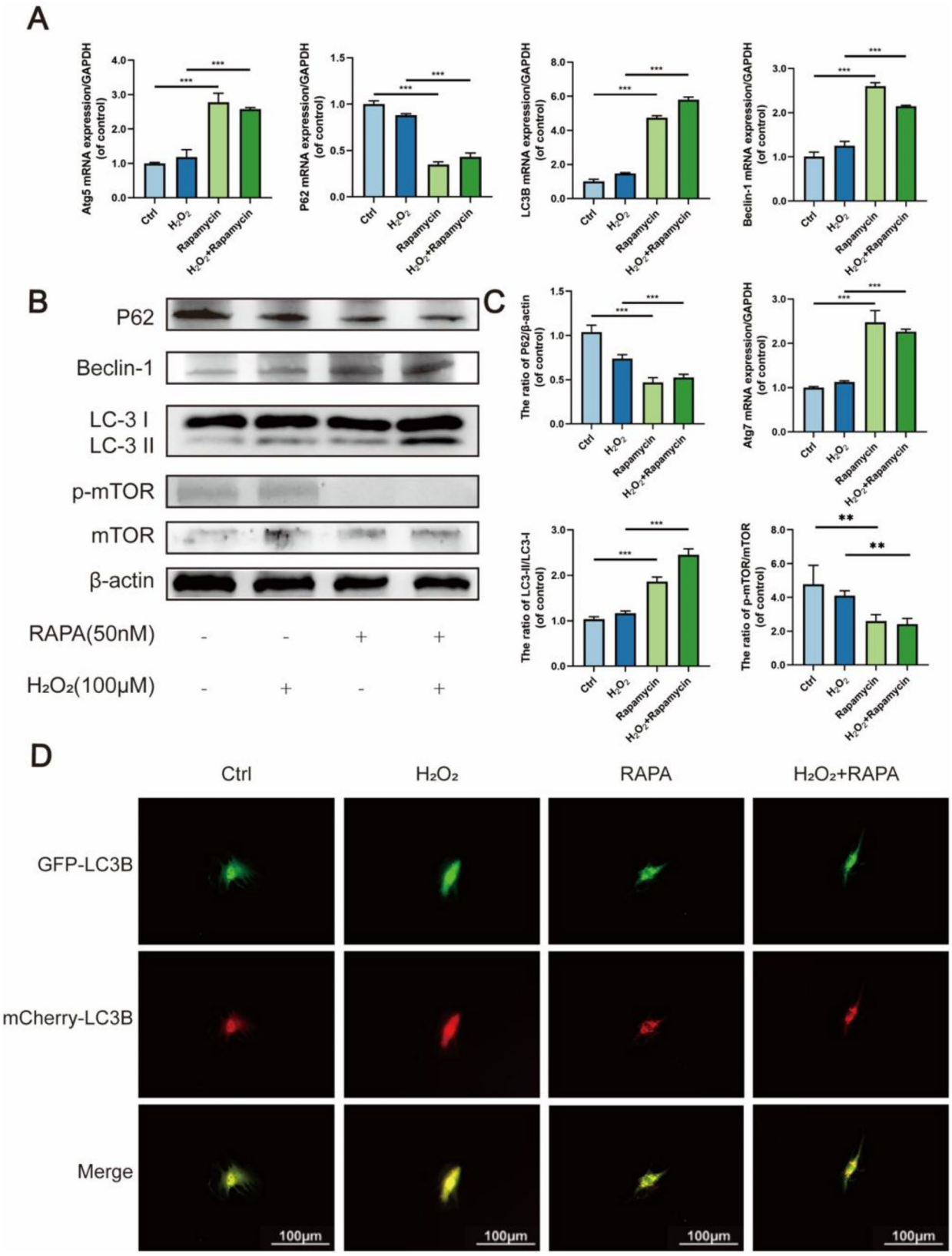

Fig. 3 Effect of RAPA on autophagy in AFCs. **A**) RT-PCR analysis for the expression of P62, Beclin-1, LC3B, Atg5 and Atg7 mRNA levels in AFCs after H_2O_2 and RAPA treatment. **B**-**C**) The effects of H_2O_2 and RAPA on the protein levels of P62, Beclin-1, LC3, p-mTOR and mTOR in AFCs were determined using Western blot assays. **D**) Fluorescence analysis for the expression of LC3B protein in AFCs after adenovirus expressing mCherry-GFP-LC3B fusion protein treatment. (Scale bar = 100 μm). (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$; between the indicated groups)

of phosphorylated mTOR in the AFCs after the addition of RAPA treatment (Fig. 3B). The ratio of p-mTOR to mTOR was reduced compared to the CTRL group. This suggests that activation of AFC autophagy by the mTOR pathway ameliorates H_2O_2-induced degeneration.

microRNAs may play an important role in the activation of AFC autophagy

The mechanism underlying the involvement of autophagy in intervertebral disc degeneration was further investigated. The prediction of miRNAs upstream of mTOR was performed using online databases including miRDB (91), miRmap (479) and miRWalk (32) respectively, as shown in Fig. 4A. By conducting a comprehensive analysis and comparison of prediction results obtained from various databases, researchers successfully identified a specific miRNA-RNA pair. Subsequently, an in-depth binding site prediction was performed for the interaction between miR-2355-5p and mTOR genes (Fig. 4B). For this purpose, we retrieved the sequencing results pertaining to AFC from the GEO database. Upon reanalysis of the transcriptome sequencing data of GSE45856, miR-2355-5p exhibited differential expression in AFC with varying degeneration grades. The expression of miR-2355-5p was observed to be downregulated in AFC samples with degeneration grade II. In contrast, it showed a significant upregulation, exceeding 50%, in AFC samples with degeneration grades IV and V (Fig. 4C–E). The cell degeneration model also demonstrated similar findings of elevated miR-2355-5p expression. Furthermore, the administration of RAPA resulted in a further increase in miR-2355-5p levels, while its inhibition by the miR-2355-5p inhibitor (YI) showed some degree of suppression (Fig. 4F).

Autophagy activation via the hsa-miR-2355-5p/mTOR pathway to exert a protective effect on the fibrous annulus

The inclusion of YI in the protein assay related to extracellular matrix metabolism attenuated the extracellular matrix salvage effect of RAPA on AFC. The expression of extracellular matrix proteins (Collagen I and Collagen II) at the protein and gene levels was found to be reduced in the H_2O_2 + Rapamycin + YI (HRYI) group compared to the HR group (Fig. 5A). Moreover, a reduction in the autophagic flux (Beclin-1 and LC-3) was observed in the HRYI cohort relative to the HR group. Meanwhile, Western Blot showed an increase in mTOR protein in the HRYI group compared to the HR group, suggesting that their mRNA may be inhibited by miR-2355-5p, which in turn inhibits the entire mTOR autophagic pathway (Fig. 5B-C). Consistently, RT-PCR also showed similar results, with downstream genes of the mTOR pathway being variously lowered in the HRYI group. Expression of the extracellular matrix decreased with lower levels of autophagy as well. This suggests the possibility

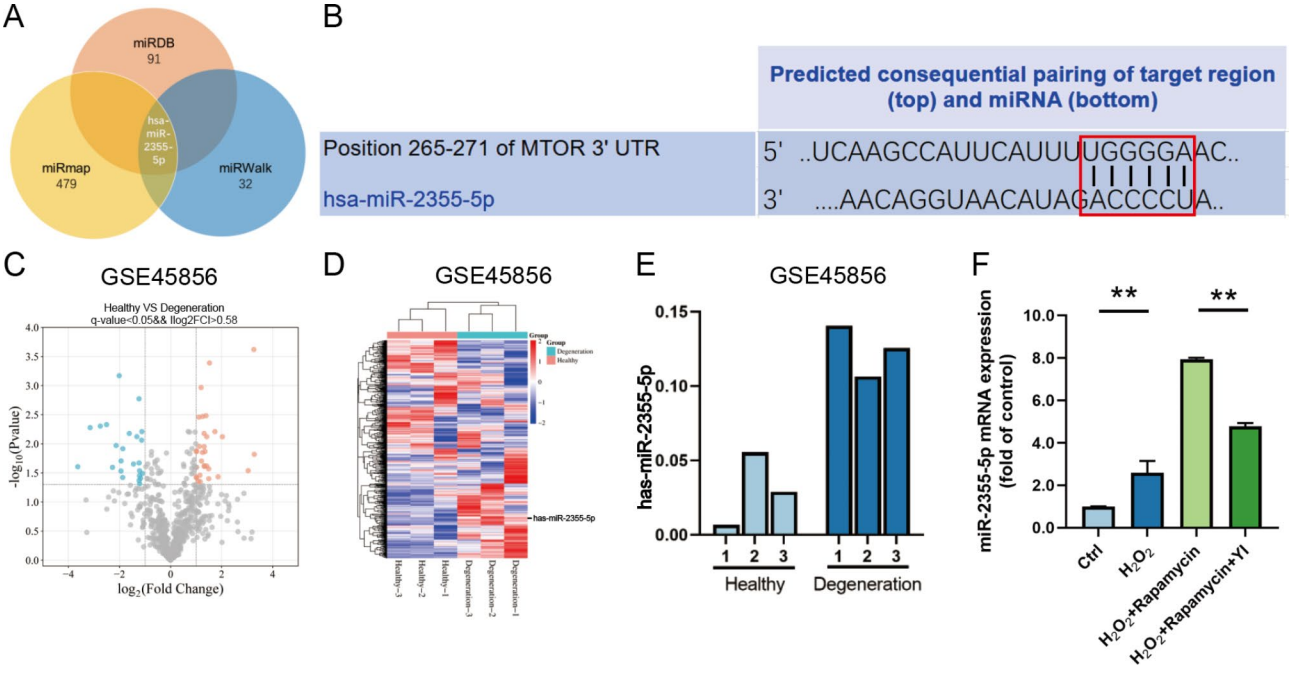

Fig. 4 Expression of miR-2355-5p in human tissues and animal models. (**A**) Prediction, screening and analysis of miRNA-mRNA pairs were found the best pair of upstream of mTOR. (**B**) MTOR and miR-2355-5p predicted binding sites. **C**-**D**) Volcano and heat maps of gene expression in GSE45856. **E**) Expression of miR-2355-5p in GSE45856 in different degenerating tissues of the human body. **F**) RT-PCR analysis of miR-2355-5p expression in AFCs. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$; between the indicated groups)

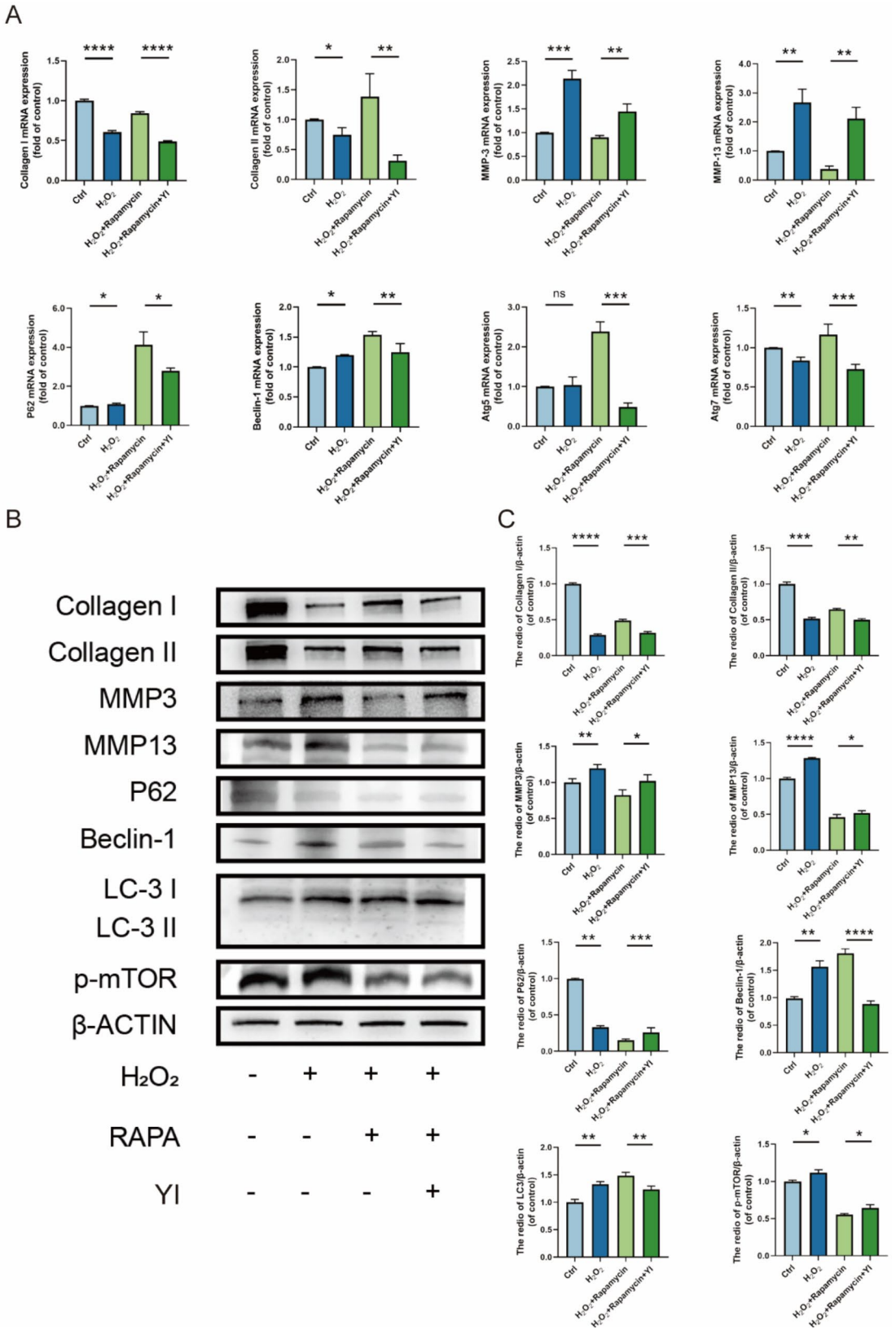

Fig. 5 Expression of autophagy-related and degeneration-related proteins after inhibition of miR-2355-5p. **A**) RT-PCR analysis for the expression of COL1, COL2, Beclin-1, LC3B, Atg5 and Atg7 mRNA levels in AFCs after H_2O_2 and RAPA treatment. **B**-**C**) The effects of RAPA and miR-2355-5P inhibitor on the protein levels of COL1, COL2, MMP3, MMP13, P62, Beclin-1, LC3 and p-mTOR in AFCs were determined using Western blot assays. YI miR-2355-5p inhibitor. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$; between the indicated groups)

that miR-2355-5p may be a way for RAPA to inhibit the mTOR pathway and, in consequence, the autophagy of AFC.

Discussion

Annulus fibrosus is a key part of the histological structure and mechanical composition of the intervertebral disc [31]. A healthy annulus fibrosus maintains the normal structure of the intervertebral disc by wrapping around the nucleus pulposus [32, 33]. The annulus fibrosus supports the nucleus pulposus in all directions to accommodate different postures of the spine [34, 35]. In the process of IVDD, the annulus fibrosus breaks down and the nucleus pulposus herniates, causing damage to the nerves and leading to a range of clinical symptoms [36, 37]. At the same time, the disruption of the mechanical structure causes it to lose its function of cushioning and regulating the spinal lines of force, leading to instability of the corresponding vertebral structures and accelerating the degeneration of other healthy discs [38–40]. However, the focus of disc research to date has been on the nucleus pulposus and cartilage endplates, with limited research on the annulus fibrosus, which has restricted therapy for IVDD. Therefore, this paper focuses on the changes in the fibrous annulus during disc degeneration. Building on a previously established model of nucleus pulposus cell degeneration [41], this study designed and developed a model of H_2O_2-induced degeneration in AFCs. This model successfully mimics the extracellular matrix degradation characteristic of the annulus fibrosus during disc degeneration. Notably, significant changes in autophagy were observed within the AFCs during this process, underscoring the potential of autophagy as a pivotal area for further investigation.

In the development of IVDD, most previous studies have found a sustained increase in autophagy in disc tissue [20, 42], whereas in more recent studies it has been found that autophagy in disc tissue increases to a certain level and persists for a period of time, and then returns to basal levels [17, 43, 44]. This is in contrast to the previous belief that autophagy is a line of defense for the body [45] and is maintained at a high level during the ongoing inflammation of IVDD [46]. Apparently, these are more in line with the dynamic regulation of the intervertebral disc microenvironment, where autophagy is stimulated to slow down oxidative stress-induced apoptosis in the early stages of IVDD, functioning as a stress regulator. Autophagy, the body's emergency response, does not remain at high levels for long and sustained high levels of autophagy lead to the impairment of individual cell functions and ultimately to apoptosis [47, 48]. Intervertebral discs are nutrient-deprived tissues with relatively inactive cells proliferating and differentiating in their various parts [49]. Maintaining high levels of autophagy for a long time, resulting in massive apoptosis and poor cell replacement, can lead to disc tissue destruction [50]. In the present study, we observed a decrease in autophagy levels within animal tissues, whereas in the cellular model, autophagy levels were slightly elevated in the H_2O_2 group compared to the healthy group. This suggests that there are dynamic fluctuations in autophagy levels within degenerating discs, which aligns with current research on autophagy in the annulus fibrosus [51, 52]. The upregulation of miRNA expression observed in the sequencing results from human tissue databases suggests that AFCs maintain an elevated level of autophagy to counteract the oxidative stress microenvironment during the degeneration process. The in vitro cellular model utilized in this study aligns more closely with the typical stages of IVDD that can induce clinical symptoms in medical practice. It offers valuable insights for the current investigation into the role of autophagy in AFC degeneration.

In our study, autophagy of AFCs stimulated via the mTOR pathway showed significant alleviation of oxidative stress-induced degeneration. It suggests that during tissue degeneration, the self-regulatory capacity of the tissue limits the alteration of its autophagy level. Topical drugs, which enable them to increase autophagy levels more effectively, can better exert their anti-oxidative stress capacity. In studies related to cartilage endplates, type II collagen expressed by chondrocytes induced to degenerate was found to rebound effectively upon increasing their autophagic levels [16, 53, 54]. Reduced expression of MMP3, which destroys the extracellular matrix, has also been shown in studies of NP cells following increased autophagy [18, 55–57]. These studies certainly remind us of the relationship between fibroblasts and autophagy. In early related explorations, only autophagy was found to potentially alleviate IVDD-induced senescence and apoptosis of AFC [58]. In recent work, its associated possible mechanisms have only gradually emerged. In Gao et al. [30] the mTOR pathway became a possible pathway to induce autophagy in AFCs, and in Ni et al. the ERK pathway was indicated to be able to be a pathway to induce autophagy in AFCs [43]. This well illustrates the reticulation of signaling pathways and suggests that autophagy induced by different pathways can likewise mitigate oxidative stress-induced degradation of annulus fibrosus. When certain key molecules of intervertebral disc autophagy are inhibited, the disc tissue alternatively maintains its autophagy levels through other pathways [59, 60]. This well illustrates the important role of autophagy in the physiopathological activity of the intervertebral disc and the potential of modulating autophagy in the therapeutic field of IVDD.

MiRNAs represent a category of non-coding, single-stranded RNA molecules with an approximate length

of 22 nucleotides [61]. Originating from endogenous genes, these molecules play a central role in governing gene expression [62, 63]. In the context of investigating miRNA-mediated gene regulation upstream of the mTOR pathway, the researchers employed online bioinformatic databases to predict miRNAs and identified miR-2355-5p as a potential key gene upstream of mTOR, based on their respective weights. Previous studies have demonstrated that miR-2355-5p can inhibit cancer cell proliferation in various cancer cells [64, 65] and promote angiogenesis [66, 67]. However, its association with cellular autophagy in NP cells has only been confirmed in the study conducted by Yu Guo et al. [68], and limited research has been conducted on its involvement in mTOR pathway-mediated autophagy. The researchers of the current study reveal that miR-2355-5p may function as a potential gene upstream of mTOR by suppressing its expression. This finding suggests that cellular autophagy can be regulated by modulating the miR-2355-5p/mTOR pathway in AFC, potentially providing relief from the cellular damage caused by IVDD.

This study has several limitations. In the experimental design, male rats were chosen for the experiments to control for a single variable and ensure consistency and reproducibility of the results. Using male rats also minimizes the potential impact of sex hormones on degeneration and autophagy. However, the lack of investigation into the effects of gender limits the generalizability of this study. The rat model used in this study has several advantages, including its moderate size, docile temperament, large blood volume, and high reproductive capacity. Additionally, the genetic similarity between rats and humans is relatively high. However, since rats are quadrupeds, the mechanical stress on their intervertebral discs differs significantly from that in humans, limiting the applicability of this model to disc-related research. Furthermore, the study's analysis of human miRNAs from the GEO database was limited, focusing only on mTOR-related molecular pathways. A broader exploration of other potential signaling pathways would provide a more comprehensive and accurate understanding. Additionally, this research is confined to cell and animal experiments, and does not extend to studies of human annulus fibrosus tissue or cells, which remains a limitation of this study. Lastly, the local application of autophagy modulation to intervertebral discs and ensuring that the autophagy levels remain within a safe range will be the focus of further research by our team.

In conclusion, the researchers propose that targeting the miR-2355-5p/mTOR pathway could be a promising strategy for regulating cellular autophagy and mitigating the cellular damage associated with IVDD.

Conclusion

In this experiment, we demonstrated that autophagy of fibroblasts mediated by increasing miR-2355-5p/mTOR pathway can alleviate degeneration caused by hydrogen peroxide-induced oxidative stress. This provides a reference and idea for the next basic and clinical studies.

Abbreviations

AFCs	Annulus fibrosus cells
ECM	Extracellular matrix
EDTA	Trypsin-ethylenediaminetetraacetic acid
H&E staining	Hematoxylin and Eosin staining
H2O2	Hydrogen peroxide
HRYI group	H2O2 + Rapamycin + YI group
IVDD	Intervertebral disc degeneration disease
miRNA	MicroRNA
MMP13	Matrix metalloproteinases 13
MMP3	Matrix metalloproteinases 3
NP	Nucleus pulposus
OD	Optical densities
RAPA	Rapamycin
YI	MiR-2355-5p inhibitor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13018-025-05492-x.

Supplementary Material 1

Acknowledgements

Thank to Prof. Jiale Wang, Prof. Di Hua, Prof. Wei He, and Prof. Dechun Geng for their assistance in methodology, data statistics, and article writing of this study.

Author contributions

ZY, CF and HM designed the research strategy. ZY and CF worked with the animal models and performed immunohistochemical staining. ZY, CF and YM performed the in vitro assays. ZY, CF WH and YM performed the statistical analysis and wrote the manuscript. ZY, CF and XW revised the paper. XW, YM, and HM conceived the research and revised the paper. All the authors have read and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (82272547), the Postdoctoral Science Foundation project of China (2024M752336), the Natural Science Foundation of Jiangsu Province (BK20220252, BK20241795), Suzhou Science and Technology Fund Project (KJXW2021006, LCZX202203, SSD2024086), Clinical research project of the First Affiliated Hospital of Soochow University (BXLC017).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 2 November 2024 / Accepted: 12 January 2025
Published online: 23 January 2025

References

1. Cheung KM. The relationship between disc degeneration, low back pain, and human pain genetics. Spine J. 2010. https://doi.org/10.1016/j.spinee.2010.09.011
2. Livshits G, Popham M, Malkin I, Sambrook PN, Macgregor AJ, Spector T, Williams FM. Lumbar disc degeneration and genetic factors are the main risk factors for low back pain in women: the UK Twin Spine Study. Ann Rheum Dis. 2011. https://doi.org/10.1136/ard.2010.137836
3. Collaborators GDallaP. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the global burden of disease study 2017. Lancet. 2018. https://doi.org/10.1016/s0140-6736(18)32279-7
4. Jin Z, Wang D, Zhang H, Liang J, Feng X, Zhao J, Sun L. Incidence trend of five common musculoskeletal disorders from 1990 to 2017 at the global, regional and national level: results from the global burden of disease study 2017. Ann Rheum Dis. 2020. https://doi.org/10.1136/annrheumdis-2020-217050
5. Knezevic NN, Candido KD, Vlaeyen JWS, Van Zundert J, Cohen SP. Low back pain. Lancet. 2021. https://doi.org/10.1016/s0140-6736(21)00733-9
6. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. Nat Rev Rheumatol. 2014. https://doi.org/10.1038/nrrheum.2013.160
7. Huang YC, Urban JP, Luk KD. Intervertebral disc regeneration: do nutrients lead the way? Nat Rev Rheumatol. 2014. https://doi.org/10.1038/nrrheum.2014.91
8. Nerurkar NL, Mauck RL, Elliott DM. ISSLS prize winner: integrating theoretical and experimental methods for functional tissue engineering of the annulus fibrosus. Spine (Phila Pa 1976). 2008. https://doi.org/10.1097/BRS.0b013e31818e61f7
9. Taylor VM, Deyo RA, Cherkin DC, Kreuter W. Low back pain hospitalization. Recent United States trends and regional variations. Spine (Phila Pa 1976). 1994. https://doi.org/10.1097/00007632-199405310-00002
10. Kim KH, Lee MS. Autophagy–a key player in cellular and body metabolism. Nat Rev Endocrinol. 2014. https://doi.org/10.1038/nrendo.2014.35
11. Chung C, Seo W, Silwal P, Jo EK. Crosstalks between inflammasome and autophagy in cancer. J Hematol Oncol. 2020. https://doi.org/10.1186/s13045-020-00936-9
12. Mizushima N. Autophagy: process and function. Genes Dev. 2007. https://doi.org/10.1101/gad.1599207
13. Simonsen A, Tooze SA. Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes. J Cell Biol. 2009. https://doi.org/10.1083/jcb.200907014
14. Levine B, Kroemer G. Biological functions of autophagy genes: a disease perspective. Cell. 2019. https://doi.org/10.1016/j.cell.2018.09.048
15. Yang S, Zhang F, Ma J, Ding W. Intervertebral disc ageing and degeneration: the antiapoptotic effect of oestrogen. Ageing Res Rev. 2020. https://doi.org/10.1016/j.arr.2019.100978
16. Luo L, Jian X, Sun H, Qin J, Wang Y, Zhang J, et al. Cartilage endplate stem cells inhibit intervertebral disc degeneration by releasing exosomes to nucleus pulposus cells to activate Akt/autophagy. Stem Cells. 2021. https://doi.org/10.1002/stem.3322
17. Zhang TW, Li ZF, Dong J, Jiang LB. The circadian rhythm in intervertebral disc degeneration: an autophagy connection. Exp Mol Med. 2020. https://doi.org/10.1038/s12276-019-0372-6
18. He R, Wang Z, Cui M, Liu S, Wu W, Chen M, et al. HIF1A alleviates compression-induced apoptosis of nucleus pulposus derived stem cells via upregulating autophagy. Autophagy. 2021. https://doi.org/10.1080/15548627.2021.1872227
19. Tang Z, Hu B, Zang F, Wang J, Zhang X, Chen H. Nrf2 drives oxidative stress-induced autophagy in nucleus pulposus cells via a Keap1/Nrf2/p62 feedback loop to protect intervertebral disc from degeneration. Cell Death Dis. 2019. https://doi.org/10.1038/s41419-019-1701-3
20. Ye W, Zhu W, Xu K, Liang A, Peng Y, Huang D, Li C. Increased macroautophagy in the pathological process of intervertebral disc degeneration in rats. Connect Tissue Res. 2013. https://doi.org/10.3109/03008207.2012.715702
21. Xie L, Huang W, Fang Z, Ding F, Zou F, Ma X, et al. CircERCC2 ameliorated intervertebral disc degeneration by regulating mitophagy and apoptosis through miR-182-5p/SIRT1 axis. Cell Death Dis. 2019. https://doi.org/10.1038/s41419-019-1978-2
22. Giordano L, Porta GD, Peretti GM, Maffulli N. Therapeutic potential of microRNA in tendon injuries. Br Med Bull. 2020. https://doi.org/10.1093/bmb/ldaa002
23. Oliviero A, Della Porta G, Peretti GM, Maffulli N. MicroRNA in osteoarthritis: physiopathology, diagnosis and therapeutic challenge. Br Med Bull. 2019. https://doi.org/10.1093/bmb/ldz015
24. Ji ML, Zhang XJ, Shi PL, Lu J, Wang SZ, Chang Q, et al. Downregulation of microRNA-193a-3p is involved in invertebral disc degeneration by targeting MMP14. J Mol Med (Berl). 2016. https://doi.org/10.1007/s00109-015-1371-2
25. Jing W, Jiang W. MicroRNA-93 regulates collagen loss by targeting MMP3 in human nucleus pulposus cells. Cell Prolif. 2015. https://doi.org/10.1111/cpr.12176
26. Li HR, Cui Q, Dong ZY, Zhang JH, Li HQ, Zhao L. Downregulation of miR-27b is involved in loss of type II collagen by directly targeting matrix metalloproteinase 13 (MMP13) in human intervertebral disc degeneration. Spine (Phila Pa 1976). 2016. https://doi.org/10.1097/brs.0000000000001139
27. Xu YQ, Zhang ZH, Zheng YF, Feng SQ. Dysregulated miR-133a mediates loss of type II collagen by directly targeting matrix metalloproteinase 9 (MMP9) in human intervertebral disc degeneration. Spine (Phila Pa 1976). 2016. https://doi.org/10.1097/brs.0000000000001375
28. Ito S, Araya J, Kurita Y, Kobayashi K, Takasaka N, Yoshida M, et al. PARK2-mediated mitophagy is involved in regulation of HBEC senescence in COPD pathogenesis. Autophagy. 2015. https://doi.org/10.1080/15548627.2015.1017190
29. Yang C, Tao H, Zhang H, Xia Y, Bai J, Ge G, et al. TET2 regulates osteoclastogenesis by modulating autophagy in OVX-induced bone loss. Autophagy. 2022. https://doi.org/10.1080/15548627.2022.2048432
30. Gao C, Ning B, Sang C, Zhang Y. Rapamycin prevents the intervertebral disc degeneration via inhibiting differentiation and senescence of annulus fibrosus cells. Aging. 2018. https://doi.org/10.18632/aging.101364
31. Pattappa G, Li Z, Peroglio M, Wismer N, Alini M, Grad S. Diversity of intervertebral disc cells: phenotype and function. J Anat. 2012. https://doi.org/10.1111/j.1469-7580.2012.01521.x
32. Walmsley R. The development and growth of the intervertebral disc. Edinb Med J. 1953. 60(8):341–364.
33. Yu J, Tirlapur U, Fairbank J, Handford P, Roberts S, Winlove CP, et al. Microfibrils, elastin fibres and collagen fibres in the human intervertebral disc and bovine tail disc. J Anat. 2007. https://doi.org/10.1111/j.1469-7580.2007.00707.x
34. Best BA, Guilak F, Setton LA, Zhu W, Saed-Nejad F, Ratcliffe A, et al. Compressive mechanical properties of the human anulus fibrosus and their relationship to biochemical composition. Spine (Phila Pa 1976). 1994. https://doi.org/10.1097/00007632-199401001-00017
35. Setton LA, Chen J. Mechanobiology of the intervertebral disc and relevance to disc degeneration. J Bone Joint Surg Am. 2006. https://doi.org/10.2106/JBJS.F.00001
36. Zhou T, Yang X, Chen Z, Yang Y, Wang X, Cao X, et al. Prussian blue nanoparticles stabilize SOD1 from ubiquitination-proteasome degradation to rescue intervertebral disc degeneration. Adv Sci (Weinh). 2022. https://doi.org/10.1002/advs.202105466
37. Kamali A, Ziadlou R, Lang G, Pfannkuche J, Cui S, Li Z, et al. Small molecule-based treatment approaches for intervertebral disc degeneration: current options and future directions. Theranostics. 2021. https://doi.org/10.7150/thno.48987
38. Adams MA, Hutton WC. Gradual disc prolapse. Spine (Phila Pa 1976). 1985; https://doi.org/10.1097/00007632-198507000-00006
39. Heuer F, Schmidt H, Wilke HJ. The relation between intervertebral disc bulging and annular fiber associated strains for simple and complex loading. J Biomech. 2008. https://doi.org/10.1016/j.jbiomech.2007.11.019
40. Videman T, Nurminen M. The occurrence of anular tears and their relation to lifetime back pain history: a cadaveric study using barium sulfate discography. Spine (Phila Pa 1976). 2004. https://doi.org/10.1097/01.brs.0000146461.27105.2b
41. Cho H, Park SH, Lee S, Kang M, Hasty KA, Kim SJ. Snapshot of degenerative aging of porcine intervertebral disc: a model to unravel the molecular mechanisms. Exp Mol Med. 2011. https://doi.org/10.3858/emm.2011.43.6.036
42. Jiang L, Zhang X, Zheng X, Ru A, Ni X, Wu Y, et al. Apoptosis, senescence, and autophagy in rat nucleus pulposus cells: implications for diabetic intervertebral disc degeneration. J Orthop Res. 2013. https://doi.org/10.1002/jor.22289
43. Ni B, Shen H, Wang W, Lu H, Jiang L. TGF-β1 reduces the oxidative stress-induced autophagy and apoptosis in rat annulus fibrosus cells through the ERK signaling pathway. J Orthop Surg Res. 2019. https://doi.org/10.1186/s13018-019-1260-4
44. Zhang S, Liang W, Abulizi Y, Xu T, Cao R, Xun C, et al. Quercetin alleviates intervertebral disc degeneration by modulating p38 MAPK-mediated autophagy. Biomed Res Int. 2021. https://doi.org/10.1155/2021/6631562

45. Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, et al. Autophagy suppresses tumorigenesis through elimination of p62. Cell. 2009. https://doi.org/10.1016/j.cell.2009.03.048
46. Ye W, Xu K, Huang D, Liang A, Peng Y, Zhu W, Li C. Age-related increases of macroautophagy and chaperone-mediated autophagy in rat nucleus pulposus. Connect Tissue Res. 2011. https://doi.org/10.3109/03008207.2011.564336
47. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit beclin 1-dependent autophagy. Cell. 2005. https://doi.org/10.1016/j.cell.2005.07.002
48. Yousefi S, Perozzo R, Schmid I, Ziemiecki A, Schaffner T, Scapozza L, et al. Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. Nat Cell Biol. 2006. https://doi.org/10.1038/ncb1482
49. Roberts S, Evans H, Trivedi J, Menage J. Histology and pathology of the human intervertebral disc. J Bone Joint Surg Am. 2006. https://doi.org/10.2106/jbjs.F.00019
50. Liu L, Cash TP, Jones RG, Keith B, Thompson CB, Simon MC. Hypoxia-induced energy stress regulates mRNA translation and cell growth. Mol Cell. 2006. https://doi.org/10.1016/j.molcel.2006.01.010
51. Zou L, Lei H, Shen J, Liu X, Zhang X, Wu L, et al. HO-1 induced autophagy protects against IL-1 β-mediated apoptosis in human nucleus pulposus cells by inhibiting NF-κB. Aging. 2020. https://doi.org/10.18632/aging.102753
52. Li S, Hua W, Wang K, Gao Y, Chen S, Liu W, et al. Autophagy attenuates compression-induced apoptosis of human nucleus pulposus cells via MEK/ERK/NRF1/Atg7 signaling pathways during intervertebral disc degeneration. Exp Cell Res. 2018. https://doi.org/10.1016/j.yexcr.2018.06.012
53. Xiao L, Ding B, Gao J, Yang B, Wang J, Xu H. Curcumin prevents tension-induced endplate cartilage degeneration by enhancing autophagy. Life Sci. 2020. https://doi.org/10.1016/j.lfs.2020.118213
54. Luo L, Gong J, Wang Z, Liu Y, Cao J, Qin J, et al. Injectable cartilage matrix hydrogel loaded with cartilage endplate stem cells engineered to release exosomes for non-invasive treatment of intervertebral disc degeneration. Bioact Mater. 2022. https://doi.org/10.1016/j.bioactmat.2021.12.007
55. Zhou W, Shi Y, Wang H, Chen L, Yu C, Zhang X, et al. Exercise-induced FNDC5/irisin protects nucleus pulposus cells against senescence and apoptosis by activating autophagy. Exp Mol Med. 2022. https://doi.org/10.1038/s12276-022-00811-2
56. Chen D, Xia D, Pan Z, Xu D, Zhou Y, Wu Y, et al. Metformin protects against apoptosis and senescence in nucleus pulposus cells and ameliorates disc degeneration in vivo. Cell Death Dis. 2016. https://doi.org/10.1038/cddis.2016.334
57. Hu S, Zhang C, Qian T, Bai Y, Chen L, Chen J, et al. Promoting Nrf2/Sirt3-dependent mitophagy suppresses apoptosis in nucleus pulposus cells and protects against intervertebral disc degeneration. Oxid Med Cell Longev. 2021. https://doi.org/10.1155/2021/6694964
58. Shen C, Yan J, Jiang LS, Dai LY. Autophagy in rat annulus fibrosus cells: evidence and possible implications. Arthritis Res Ther. 2011. https://doi.org/10.1186/ar3443
59. Madhu V, Boneski PK, Silagi E, Qiu Y, Kurland I, Guntur AR, et al. Hypoxic regulation of mitochondrial metabolism and mitophagy in nucleus pulposus cells is dependent on HIF-1α-BNIP3 axis. J Bone Min Res. 2020. https://doi.org/10.1002/jbmr.4019
60. Madhu V, Guntur AR, Risbud MV. Role of autophagy in intervertebral disc and cartilage function: implications in health and disease. Matrix Biol. 2021. https://doi.org/10.1016/j.matbio.2020.12.002
61. Gargano G, Asparago G, Spiezia F, Oliva F, Maffulli N. Small interfering RNAs in the management of human osteoporosis. Br Med Bull. 2023. https://doi.org/10.1093/bmb/ldad023
62. Gargano G, Oliviero A, Oliva F, Maffulli N. Small interfering RNAs in tendon homeostasis. Br Med Bull. 2021. https://doi.org/10.1093/bmb/ldaa040
63. Gargano G, Oliva F, Oliviero A, Maffulli N. Small interfering RNAs in the management of human rheumatoid arthritis. Br Med Bull. 2022. https://doi.org/10.1093/bmb/ldac012
64. Xu H, Yu X, Yang Z, Song Q, Cheng S, He Z, Dai L. PAX5-activated lncRNA ARRDC1-AS1 accelerates the autophagy and progression of DLBCL through sponging mir-2355-5p to regulate ATG5. Life Sci. 2021. https://doi.org/10.1016/j.lfs.2021.119932
65. Zhou M, Dong J, Huang J, Ye W, Zheng Z, Huang K, et al. Chitosan-Gelatin-EGCG nanoparticle-meditated LncRNA TMEM44-AS1 silencing to activate the P53 signaling pathway for the synergistic reversal of 5-FU resistance in gastric cancer. Adv Sci (Weinh). 2022. https://doi.org/10.1002/advs.202105077
66. Cheng Y, Shang X, Chen D, Pang D, Zhao C, Xu X. MicroRNA-2355-5p regulates γ-globin expression in human erythroid cells by inhibiting KLF6. Br J Haematol. 2021. https://doi.org/10.1111/bjh.17134
67. Su SH, Wu CH, Chiu YL, Chang SJ, Lo HH, Liao KH, et al. Dysregulation of vascular endothelial growth factor receptor-2 by multiple miRNAs in endothelial colony-forming cells of coronary artery disease. J Vasc Res. 2017. https://doi.org/10.1159/000449202
68. 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. https://doi.org/10.1097/brs.0000000000003011

Publisher's note