Original Article

Mechanism of protective role of miR-874-3p in intervertebral disc degeneration

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Background: The intervertebral disc can increase the amplitude of spinal motion, withstand pressure, buffer vibration, and protect the brain and spinal cord. It is also the main reason why height changes, but the regulatory mechanism is still unclear, and this study mainly explored the role of miR-874-3p in intervertebral disc degeneration (IDD).

Methods: The mechanism and perform correlation analysis of miR-874-3p and the pathological degree and prognosis of patients with IDD. miR-874-3p is involved in the progression of several diseases, such as cell differentiation, proliferation, apoptosis and extracellular matrix degradation, overexpressing cell line GV369-miR-874-3p-NP was obtained by infected nucleus pulposus (NP) cells, and the empty vector GV369-NP group was set with the blank group. The expression of the tag protein (green fluorescent protein, GFP) was visualized by fluorescence microscopy, followed by real-time polymerase chain reaction (PCR) method to detect miR-874-3p expression, apoptosis by flow cytometry, luciferase reporter analysis for verifying the targeting relationship between miR-874-3p, caspase-3, B-cell lymphoma-2 (Bcl-2) and Bax in cells, and examined the changes in cellular mitochondrial membrane potential using kits.

Results: The expression of miR-874-3p was significantly reduced in IDD patients, and was negatively correlated with matrix metallopeptidase 2 (MMP2) and downregulated matrix metallopeptidase 3 (MMP3) in the NP cells. In addition, western blot revealed that overexpression of miR-874-3p increased the aggregation protein level in the NP cells.

Conclusions: miR-874-3p can inhibit the cell death of IDD, and not only participate in caspase-3 and Fas-associating protein with a novel death domain (FADD)-mediated apoptosis through targeted regulation of exogenous MMP2/MMP3 pathway, but also play a role in cell apoptosis through mitochondrial pathway.

Keywords: miR-874-3p; intervertebral disc degeneration (IDD); nucleus pulposus cells; cell apoptosis

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Introduction

Intervertebral disc degeneration (IDD) affects the fibrocartilage disc (i.e., the intervertebral disc), causing instability (1). IDD is decreased musculoskeletal function, but its pathogenesis is not fully defined (2). Therefore, the intervertebral disc (IVD) is the fibrocartilage tissue connecting the two adjacent vertebrae. The stability provided by the IVD is important for the whole spine, and the central part of the IVD is the nucleus pulposus (NP), forming a hydrogel-like nucleus in the IVD (3). NP is mainly composed of proteoglycans and collagen fibers, and its elastic function can be distributed in all directions (4). Mymyeloid cells have two cell types, chordate and mature myeloid cells (5). The NP is surrounded by a fiber ring (A nnulus Fibrosus, AF). Excessive destruction of the outer disc extracellular matrix (ECM), plays a crucial role in the development of IDD and the major catabolic enzymes mediating this destruction are the matrix metalloproteinases (MMPs). In the process of disc degeneration, the disc

has complex biochemistry and molecular level changes, including the reduction of proteoglycan content, type collagen to collagen I transition and NP cell density (6). These changes directly lead to the reduction of IVD machinery and eventually lead to structural damage, such as fiber ring rupture, NP cell protrusion, etc. In sharp contrast to the factors causing the extracellular matrix breakdown are the synthetic factors that stimulate the extracellular matrix, and they play a role in regeneration (7-9). However, the regulators of matrix metallopeptidase 2 (MMP2) and matrix metallopeptidase 3 (MMP3) and their underlying mechanisms are still unknown (10). miRNA was formally recognized as a short-segment noncoding RNA in 2001 as one of the classical gene regulators in eukaryotic cells (11). miRNA is an endogenous small RNA that plays an important role in cell proliferation, development, and metabolism by acting on other genes (12). miRNA, as a single-stranded RNA in a noncoding region composed of 18 to 22 nucleotides, is transcribed by miRNA (13). It is generally believed that pri-miRNA has two sources: (I) genes encoded by special miRNA are transcribed by type RNA polymerase, and then these pri-miRNA pass more in the nucleus Proteins cleave together, which contain an anchor protein DGCR8 that contributes to pre-miRNA composed of about 70 nucleotides; (II) transcribed from intrinsic mRNA fragments, and their maturation process does not require Drosha/DGCR8 from lariat debranching enzyme (Ldbr) and co-transcribed with host protein-coding genes to form hairpin pre-miRNA, these intrinsic miRNA will often appear in the same biological pathway as the host protein-encoded genes (14-16), and a very recent study found that miR-874-3p targets and inhibits MMP2 in non-small cell lung and gastric cancer . The role of tumor suppression.

With the deepening of IDD development mechanism research in recent years, it is found that degeneration-related genes could activated the development of IDD, but their interaction and their abnormal expression mechanism remain unclear. Small RNA (microRNA), as one of the important regulatory molecules of gene expression, has been shown to play a key role in multiple disease initiation and progression stages, so it is believed that it may also play an important role in disc degeneration. Current studies of miRNA affecting disc degeneration have found that many kinds of miRNA are mainly available Participate in influencing the development of IDD through its action on apoptosis, inflammatory signaling response, and ECM components.

Significant abnormalities in the expression of many miRNA in the nuclear medullary tissue of the degenerative disc, suggesting that miRNA may be involved in the pathophysiology of IDD (17). Although increasingly it is being reported that abnormal miRNA leads to nuclear myeloid cell decline in IDD patients, and if miR-874-3p expression levels change in IDD (18). Whether there is a MMP2 alveolar pathway that also functions in the nuclear myeloid cells of IDD, and whether there are other relevant target genes are unknown (19,20). In this study, the correlation of disease severity in patients with IDD expressing miR-874-3p, and a possible protective role of miR-874-3p may play through inhibition of MMP2 and MMP3 expression were investigated.

I present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-91/rc).

Methods

Sample collection

Fifty-four clinical NP tissue samples were collected from either the Department of Pathology or the operating room with the informed consent of all subjects. Of the 54 patients, 28 had significant disc degeneration and underwent laminectomy fusion. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Orthopaedic Clinical Research Ethics Committee of the Yingkou Central Hospital (2021-146) and informed consent was taken from all the patients.

Real-time quantitative and qualitative polymerase chain reaction (qRT-PCR) analysis

For mRNA detection, cDNA was obtained using a RT kit (Promega) with oligomeric-dt primers (21). The qRT-PCR analysis was performed using SYBRGreen PCRMas systems (Applied Biosystems) using standard protocols and GAPDH served as control group. miR-874-3p is significantly upregulated in cervical disc degeneration, and miR-874-3p may serve as a new therapeutic target in cervical disc degeneration (22).

Isolation and culture of NP cells

Cells were cultured in medium containing fetal bovine serum (23). NP cells from a patient with

immunofluorescence assay characteristics for subsequent experiments. Human IDD NP cells from log growth period were spread on 48-well cell culture plates. After overnight culture, appropriate Dulbecco's modification of Eagle's medium containing 10% fatal bovine serum containing different gradient concentrations of puromycin was added to each well, and fluid was changed every 48 h for 1 week to observe cell growth, and the concentration of all death was the minimum screening concentration.

Lentiviral infection

miR-874-3p inhibitory lentiviral vector (LV) contained the anti-miR-874-3p target sequences (TCGGTCCGGGGCAG) (24). Human IDD NP cells prepared and stored in this laboratory were stored in 6-well plates for cell growth at 37 ℃ and 5% CO_2 overnight. When cells grew to about 75% the next day, GV369-miRNA-326 lentiviral medium and human IDD NP cells for 8 h, changed to culture for 2 d and observed green fluorescent label expression under a fluorescence microscope.

Carrier construct and double-luciferase assay

The pmiR-report vector construct that amplified the MMP3 gene and cloned the downstream luciferase gene. We again performed the reporter gene activity measurements using the dual-luciferase assay system (Promega). The target fragments were amplified by polymerase chain reaction (PCR), digested and purified using a gel recycling kit. Connecting the linearized lentiviral expression vector GV369, transformed into E. coli competent cell DH5, coated with LB plates with puromycin resistance, colonies were picked for identification, then positive clones were expanded in culture after plasmid extraction.

Apoptosis detection and cell counting kit-8 (CCK-8) detection

CCK-8 is a rapid and highly sensitive Kit based on WST-8 that is widely used in Cell activity and cytotoxicity detection. We also examined apoptosis using flow cytometry. Cell proliferation or cell viability (CCK-8 assay) (Beyotime, China) was analyzed.

Cellular immunoassay analysis

Human IDD NP cells infected with lentivirus were collected, and total protein was extracted from the cells by using the SD-001/SD-002 Animal Cell/Tissue Total Protein Extraction Kit, and were subjected to SDS-PAGE gel electrophoresis, transferred to a polyvinylidene difluoride membrane and blocked with a blocking solution containing 5% bovine serum albumin for 2 h at room temperature. Membranes were washed three times in Phosphate Buffer Saline Tween (PBST) for 10 min each, supplemented with HRP-labeled sheep anti-rabbit IgG secondary antibody, incubated at room temperature for 2 h, counterstained with PBST, washed with ECL, and photographed with dark chamber exposure.

Statistical analysis

Our study analyzed relevant experimental data by SPSS software (23.0), measurement data were expressed as mean ± standard deviation ($\bar{x}$±s), one-way ANOVA, LSD-*t* test for pairwise comparisons, $P<0.05$ was statistically significant.

Results

Expression of miR-874-3p and MMP2 in IDD

The level of miR-874-3p differed from those in normal subjects, the qRT-PCR results suggested that in IDD patients compared with the controls, the expression level of miR-874-3p were obviously decreased. The MMP2 expression levels in the IDD and control patients were also evaluated and the results showed they were elevated in the IDD patients as compared with the control group. There was also a significant increase in MMP3 as another important enzyme for ECM degradation in the IVD, lower expression levels of MMP2 and MMP3 (*Figure 1*).

Downregulation of MMP2 in IDD

The experimental results showed that the cells had the markers. Lentivirus expressing miR-874-3p, lentivirus with miR-874-3p inhibition and negative control (NC) lentivirus were generated and activated by the infected NP cells' response, ensuring that infection efficiency was sufficiently high. By qRT-PCR analysis, lentiviral infection with miR-874-3p led to significantly downregulated level of expression of MMP2 (*Figure 2*).

Downregulation of MMP3 in IDD

miR-874-3p is an extremely important regulator of gene

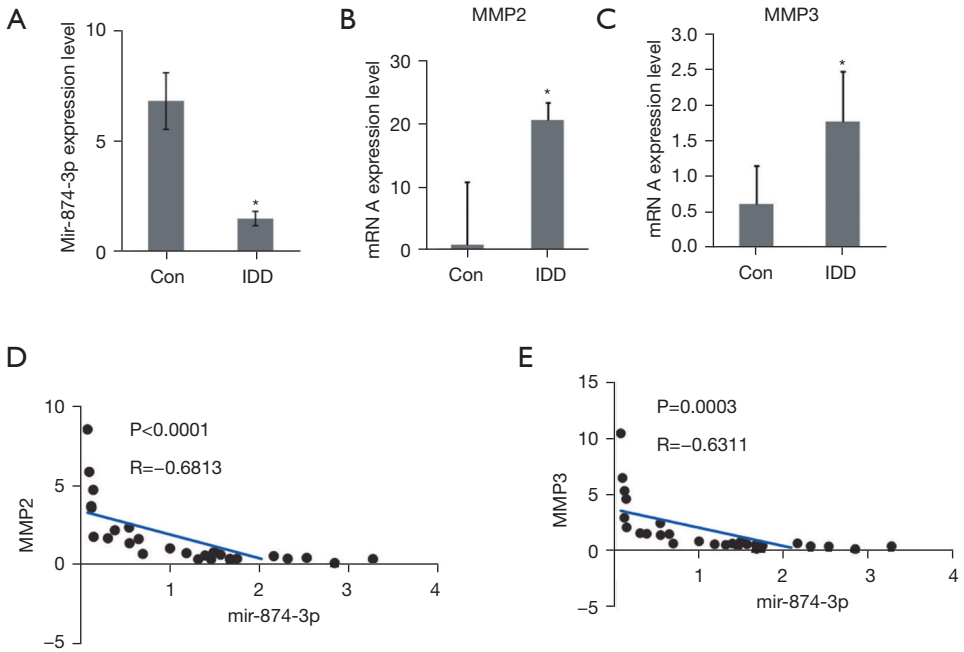

Figure 1 The expression of miR-874-3p is significantly reduced in tissue specimens from IDD patients, and inversely correlated with MMP2. (A) Relative expression levels of miR-874-3p in the IDD group (n=28) and controls (n=26); (B,C) relative expression levels of MMP2 and MMP3 in MMP3 [IDD patients (n=28) and controls (n=26)]; (D,E) miR-874-3p expression levels between MMP2/3 and IDD by real-time PCR in IDD patients (n=28). *P<0.05. IDD, intervertebral disc degeneration; PCR, polymerase chain reaction; MMP2, matrix metallopeptidase 2; MMP3, matrix metallopeptidase 3.

expression regulation, which is involved in various aspects of cell function such as development, aging and disease. miRNAs work by regulating mRNA expression levels. Abnormalities of immune system are closely related to miRNA, which can lead to the occurrence and development of a variety of diseases. Two homologous miRNAs, MMP3 and miR-874-3p, were found to play an important role in maintaining joint homeostasis and inhibiting the pathogenesis of osteoarthritis. At present, it is quite clear that miRNA will intervene in the occurrence, development and maintenance of diseases in a variety of ways, which is of great help for us to master and treat diseases. It is known from the latest research progress that the regulatory network of MMP3 is extremely complex and penetrates into all aspects of the body response. It is extremely difficult to fully identify target-related regulatory genes in IDD (*Figure 3*).

Role of abnormal miR-874-3p expression in IDD pathology

Decomposition of the polymerization glue and collagen, which mainly correlates. After finding that miR-874-3p can regulate cell death and that there is decreased miR-874-3p in IDD patients, whether miR-874-3p affects the development of IDD was further explored using western blot, which found that overexpression of miR-874-3p increases aggregated protein levels in NP cells. On the other hand, the inhibition of miR-874-3p reduced the expression of Aggr in NP tissue. More importantly, no significant degradation of collagen was found. It is precisely because the reporter gene test results are affected by a variety of factors, such as carrier state, cell state, transfection quantity, transfection efficiency, lysis efficiency, sample addition accuracy, and detection process, that improper processing of a certain detail may lead to experimental failure or inaccurate results. In this study, the results of the dual-luciferase reporter gene test to detect the effect of miR-874-3p on MMP2 were not ideal, so the results were not further analyzed in the results section. The changes were consistent and suggested that regulation of miR-874-3p expression may play a role in developmental disorders such as IDD. Therefore, apoptosis of NP cells expressing miR-874-3p or with miR-874-3p infection was analyzed by flow cytometry. These experimental data suggested that

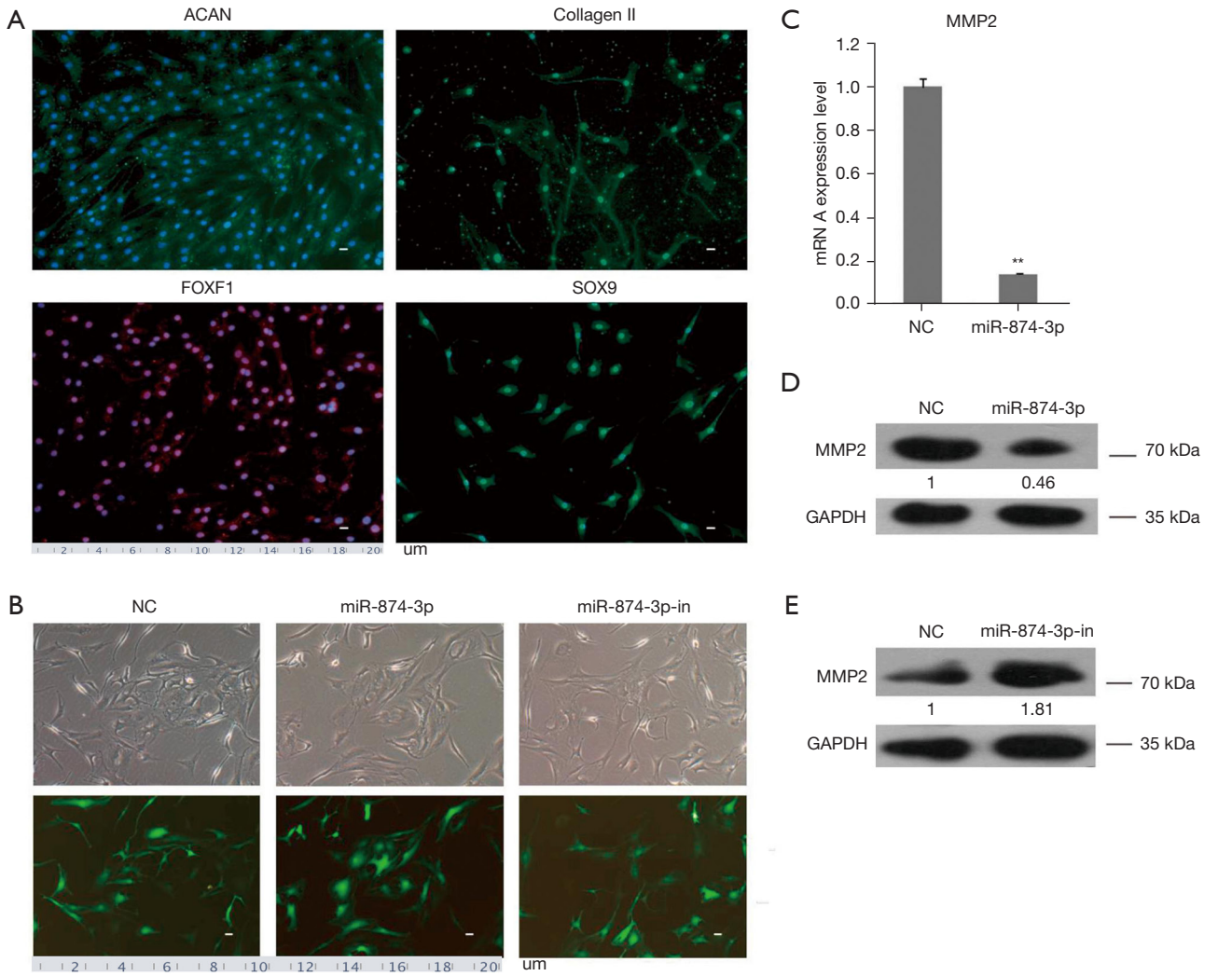

Figure 2 Downregulation of MMP2 in NP cells by miR-874-3p. (A) Immunofluorescence assays show the cultured cells expressing NP cell markers (HE staining method). (B) Shape and infection efficiency of the cultured NP cells with a NC, miR-874-3 expression, and lentivirus expression with miR-874-3p inhibition (HE staining method). (C) qRT-PCR analysis shows the downregulated expression levels of MMP2 after lentivirus expressing miR-874-3p and (D,E) western blot results of MMP2 expression levels after miR-874-3p overexpression or inhibition. **P<0.01. MMP2, matrix metallopeptidase 2; NP, nucleus pulposus; NC, negative control; qRT-PCR, real-time quantitative and qualitative polymerase chain reaction.

dysregulation of miR-874-3p expression plays an important role in IDD pathology (*Figure 4*).

Discussion

IDD disease has become a common and high-incidence of chronic disease, suffering from both middle-aged and elderly people and young people (25-27). IDD can cause a variety of symptoms, such as waist and leg pain, spinal stenosis, protruding disc and compression of nerve roots leading to pain in other parts. Currently, age, drinking, overwork and so on are considered to be extrinsic factors contributing to disc degenerative diseases (28). There are also numerous views on the molecular mechanisms and transduction pathways of its pathogenesis, and inflammatory cytokines, genetic genes, oxidative stress, Nitric oxide induction, and miRNA play important roles in IDD (29). miR-874-3p plays an important role in cell and mitochondrial energy metabolism. With the deepening of research, more and more studies have found that miR-

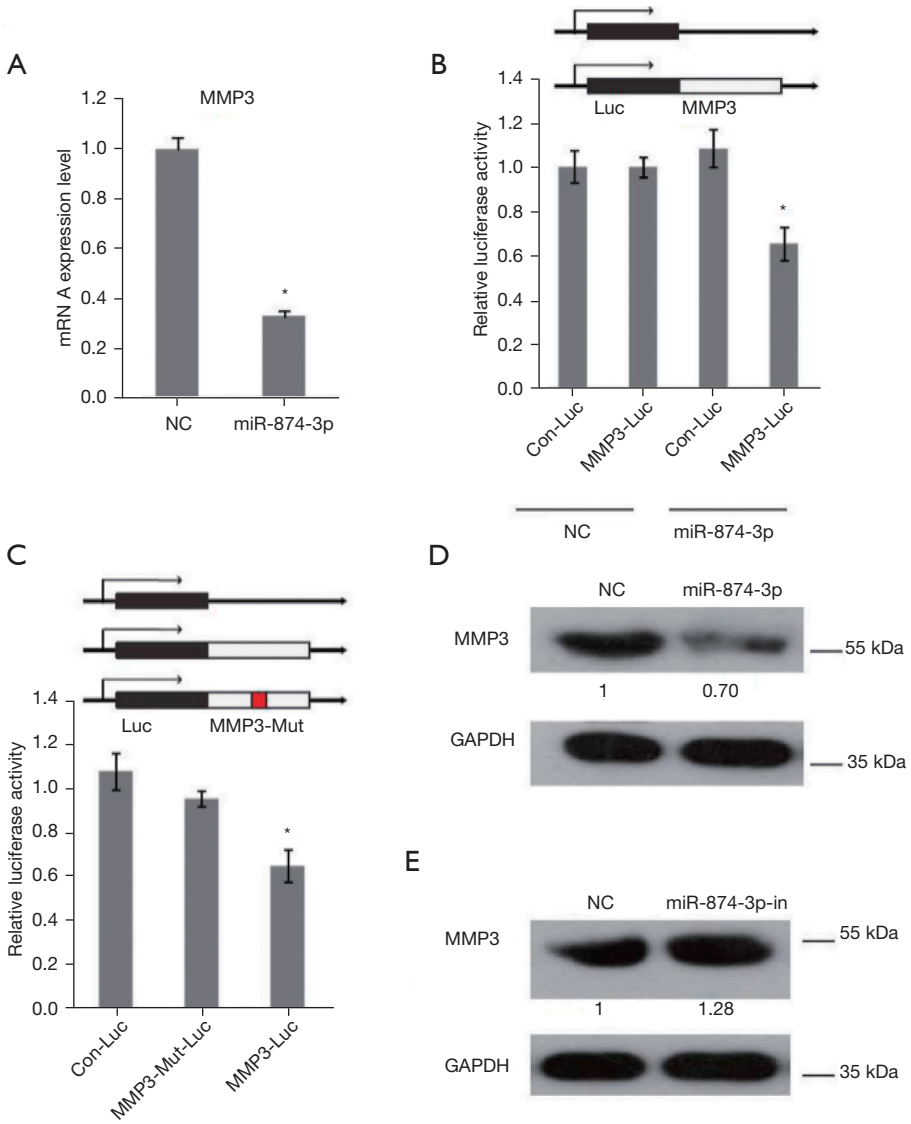

Figure 3 miR-874-3p downregulates MMP3 in NP cells. (A) qRT-PCR results for MMP3 in NP cells following lentiviral infection expressing miR-874-3p. (B) Double-luciferase reporter analysis shows that MMP3 can be inhibited by miR-874-3p. (C) Double-luciferase reporter experiments show that miR-874-3p inhibits MMP3 through predicted binding sites. (D,E) Western blot results for MMP3 protein levels in NP cells after overexpression or inhibition of miR-874-3p. *P<0.05. MMP3, matrix metallopeptidase 3; NC, negative control.

874-3p not only participates in the control of energy metabolism, but also is closely related to the abnormal electronic respiratory chain in gene transcription, protein synthesis, mitochondrial oxidative stress, and the activity of intracellular phosphorylation of miR-874-3p is strongly correlated with the aging state of chondrocytes. Although miR-874-3p has been previously stated to be involved in cell energy generation, some studies have shown that miR-874-3p is involved in inflammatory response mediated by NF-kB signaling pathway. There was no significant difference between miR-874-3p gene level and miR-874-3p regulatory protein level in early cartilage stage, but the phosphorylation level of miR-874-3p gradually decreased with aging. Gene energy metabolism is related to oxidative stress in mitochondria (30). We speculated that miR-874-3p signaling pathway not only interferes with energy metabolism, but also mediates the corresponding oxidative system and antioxidant system in mitochondria. Abnormal

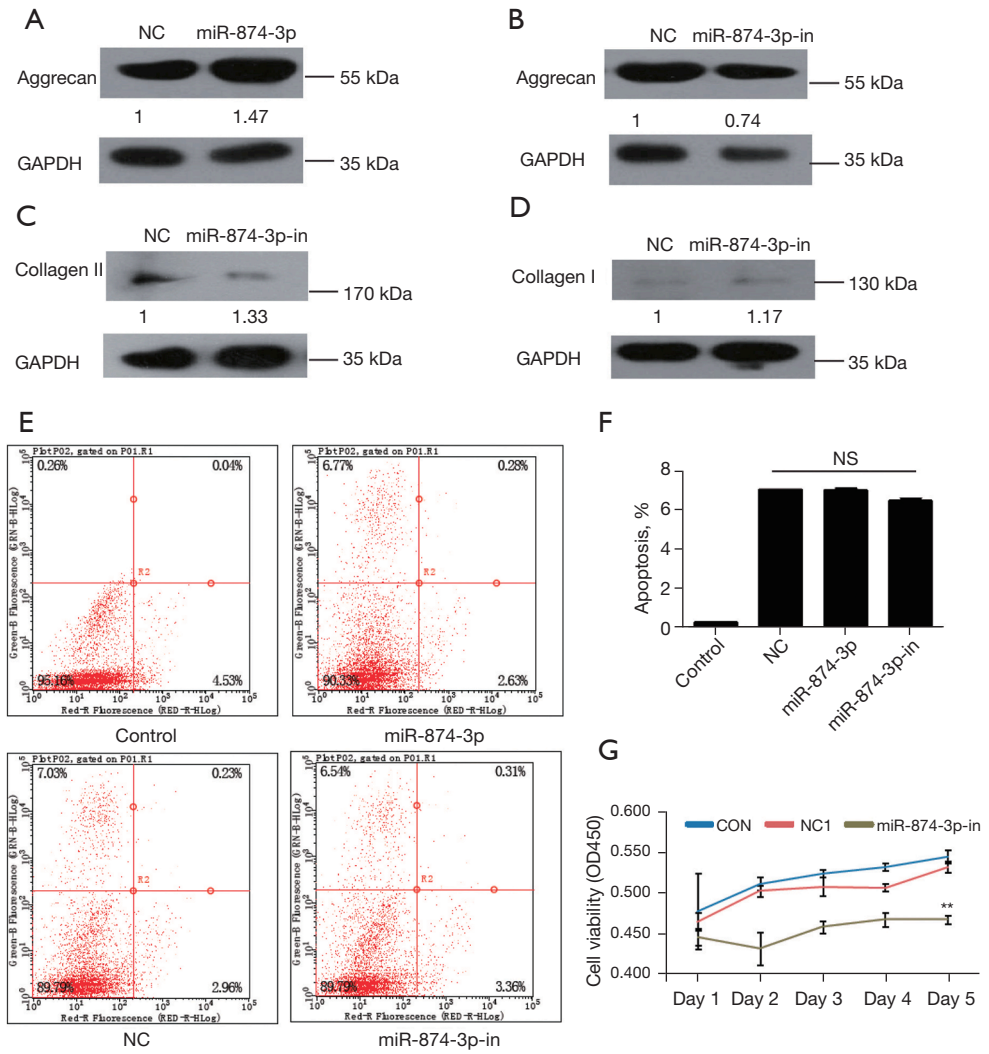

Figure 4 Important diagnostic role of miR-874-3p in IDD pathology. (A) Immunoblotting results of glycan protein levels in NP tissue after miR-874-3p overexpression. (B-D) Western blot results of polymeric gel (B), collagen II and collagen I (D) protein expression in NP cells after inhibition of miR-874-3p, and (E) representative scatter plot of apoptosis after miR-874-3p overexpression by flow cytometry. (F) Statistical results of the apoptosis assay by flow cytometry and (G) viability status of NP cells by CCK-8 assay. **P<0.01. IDD, Intervertebral disc degeneration; CCK-8, cell counting kit-8; NP, nucleus pulposus; NC, negative control; NS, not statistically significant.

miR-874-3p is closely related to cellular stress. Moreover, studies have shown that there is a strong correlation between the phosphorylation activation of miR-874-3p and the formation or degradation of cartilage. Studies on osteoarthritis have found that the activation of miR-874-3p is significantly reduced, and corresponding inhibitors or gene knockout can effectively alleviate the progression and inflammatory expression of osteoarthritis. miR-874-3p regulates the interaction of not only mTOR pathway but also HIF and other signaling pathways, which is closely related to the differentiation and maturation of cartilage endplate. Therefore, miR-874-3p is an important regulatory pathway in the formation, maintenance and aging of joint and cartilage endplates (31). The results also suggest biomarkers for the diagnosis of IDD, because miR-874-3p is significantly reduced in IDD, and also point to future exploration of restoring miR-874-3p expression in the NP cells of patients with IDD.

The function of miR-874-3p is important for IDD-related phenotypes, and warrants further exploration. A

limiting factor in this study was the relatively small number of samples from patients (32). miR-874-3p mediates cervical disc degeneration via MMP2/MMP3, thereby affecting protein synthesis and function. Studies have shown that the MMP2/MMP3, mammalian target of rapamycin and protein kinase B (Akt) activators proteins in hypothalamus of rainbow trout were found to have corresponding changes in rainbow trout fed with different nutrient levels of diets. It was found that the p-MMP2/MMP3 protein level of rainbow fish fed with high fat diet for 3 hours was significantly reduced. However, p-MTOR protein content increased significantly, reflecting the negative regulation of MMP2/MMP3 and mTOR. Relevant studies have shown that miR-217 regulates the proliferation and differentiation of A549 and H1299 cells through the MMP2/MMP3/mTOR pathway (33). Cervical and lumbar intervertebral disc degeneration is the most common degenerative degeneration of spine, and is also one of the important factors causing neck, low back pain. The endplate cartilage is a thin layer of hyaline cartilage located at both ends of the vertebral body anatomically. We have already explained that the endplate cartilage not only plays an important role in the nutritional supply of the intervertebral disc, but also plays an important role in the mechanical transmission between the vertebral body and the intervertebral disc. The intervertebral disc has been identified as the largest unvascular occluded structure in the body. As the embryo develops mature, the blood vessels in the intervertebral disc degenerate rapidly, and the circulation of the nucleus pulposus in the intervertebral disc is isolated from the attachment and becomes covert antigen. Endplate cartilage degeneration is the early morphological change of intervertebral disc degeneration (34). MMP2/MMP3 has been identified as an extremely important protein kinase in eukaryotes. It is expressed in multiple tissues closely related to metabolism and has tissue specificity. Different tissues have different subtypes. Studies have shown that MMP2/MMP3 is not only involved in the occurrence and development of tumors, but also closely related to the occurrence and development of diseases, such as the related pathways of protein synthesis and mitochondrial energy metabolism mentioned above (35,36).

All of the above miRNA molecules are involved in the regulation of the apoptotic pathway in myeloid cells, but none of the apoptosis-related pathways studied above were significantly disease-specific. Whether the above miRNA molecules are representative of IDD and whether they can respond to specific changes of IDD disease is still worth further research and analysis, and strive to find IDD specific changes of molecules and clear mechanism to provide important theoretical basis for future application research (37). MMP-mediated degradation of ECM promotes the metastasis of tumor cells from their primary site (38). Cazzanelli *et al.* (39) found that the content of chondroitin sulfate (CS) decreased significantly, while the five chondrothiol sulfate glycosyltransferases in the degenerative nuclear myeloid tissue was somewhat different (40). After comprehensive database analysis, it was believed that miR-29b, miR-194-515, miR-2355 may be able to act on chondroitinase sulfate and only miR-194 and miR-515 Significant increase, significant decrease in chondroitinase sulfate protein levels after miR-194 and miR-515 overexpression, no mRNA levels, miR-194 and miR-515 inhibitors without mRNA levels.

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Footnote

Reporting Checklist: The author has completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-91/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-91/dss

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-91/coif). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Orthopaedic Clinical Research Ethics Committee of the Yingkou Central Hospital (2021-146) and informed consent was taken from all the patients.

References

1. Liu WG, Zhuo L, Lu Y, et al. miR-874-3p inhibits cell migration through targeting RGS4 in osteosarcoma. J Gene Med 2020;22:e3213.
2. Xia B, Lin M, Dong W, et al. Upregulation of miR-874-3p and miR-874-5p inhibits epithelial ovarian cancer malignancy via SIK2. J Biochem Mol Toxicol 2018;32:e22168.
3. Yuan RB, Zhang SH, He Y, et al. miR-874-3p is an independent prognostic factor and functions as an anti-oncomiR in esophageal squamous cell carcinoma via targeting STAT3. Eur Rev Med Pharmacol Sci 2018;22:7265-73.
4. Jiang D, Sun X, Wang S, et al. Upregulation of miR-874-3p decreases cerebral ischemia/reperfusion injury by directly targeting BMF and BCL2L13. Biomed Pharmacother 2019;117:108941.
5. Liu B, Xu T, Meng Y. lncRNA NEAT1 aggravates cerebral ischemia/reperfusion injury by sponging miR-874-3p. J Biol Regul Homeost Agents 2020. [Epub ahead of print].
6. Leong KW, Cheng CW, Wong CM, et al. miR-874-3p is down-regulated in hepatocellular carcinoma and negatively regulates PIN1 expression. Oncotarget 2017;8:11343-55.
7. Feng X, Xue H, Guo S, et al. miR-874-3p suppresses cell proliferation and invasion by targeting ADAM19 in nasopharyngeal carcinoma. Panminerva Med 2021;63:238-9.
8. Huang Y, Han Y, Guo R, et al. Long non-coding RNA FER1L4 promotes osteogenic differentiation of human periodontal ligament stromal cells via miR-874-3p and vascular endothelial growth factor A. Stem Cell Res Ther 2020;11:5.
9. Song S, Yan Z, Wu W. miR-874-3p inhibits osteogenic differentiation of human periodontal ligament fibroblasts through regulating Wnt/β-catenin pathway. J Dent Sci 2021;16:1146-53.
10. Dai Y, Gao M, Li L, et al. MicroRNA-874-3p Aggravates Doxorubicin-Induced Renal Podocyte Injury via Targeting Methionine Sulfoxide Reductase B3. Oxid Med Cell Longev 2020;2020:9481841.
11. Suento WJ, Kunisawa K, Wulaer B, et al. Prefrontal cortex miR-874-3p prevents lipopolysaccharide-induced depression-like behavior through inhibition of indoleamine 2,3-dioxygenase 1 expression in mice. J Neurochem 2021;157:1963-78.
12. Que K, Tong Y, Que G, et al. Downregulation of miR-874-3p promotes chemotherapeutic resistance in colorectal cancer via inactivation of the Hippo signaling pathway. Oncol Rep 2017;38:3376-86.
13. Wang Y, Chen H, Wei X. Circ_0007142 downregulates miR-874-3p-mediated GDPD5 on colorectal cancer cells. Eur J Clin Invest 2021;51:e13541.
14. Wu G, Zhou H, Li D, et al. LncRNA DANCR upregulation induced by TUFT1 promotes malignant progression in triple negative breast cancer via miR-874-3p-SOX2 axis. Exp Cell Res 2020;396:112331.
15. Xie K, Cai Y, Yang P, et al. Upregulating microRNA-874-3p inhibits CXCL12 expression to promote angiogenesis and suppress inflammatory response in ischemic stroke. Am J Physiol Cell Physiol 2020;319:C579-88.
16. Zhang Z, Yang W, Li N, et al. LncRNA MCF2L-AS1 aggravates proliferation, invasion and glycolysis of colorectal cancer cells via the crosstalk with miR-874-3p/FOXM1 signaling axis. Carcinogenesis 2021;42:263-71.
17. Yang H, Dong Y, Zhou Y, et al. Overexpression of miR-874-3p alleviates LPS-induced apoptosis and inflammation in alveolar epithelial cell by targeting EGR3/NF-κB. Acta Biochim Pol 2021;68:231-8.
18. Bowles RD, Setton LA. Biomaterials for intervertebral disc regeneration and repair. Biomaterials 2017;129:54-67.
19. Newell N, Little JP, Christou A, et al. Biomechanics of the human intervertebral disc: A review of testing techniques and results. J Mech Behav Biomed Mater 2017;69:420-34.
20. Desmoulin GT, Pradhan V, Milner TE. Mechanical Aspects of Intervertebral Disc Injury and Implications on Biomechanics. Spine (Phila Pa 1976) 2020;45:E457-64.
21. D'Este M, Eglin D, Alini M. Lessons to be learned and future directions for intervertebral disc biomaterials. Acta Biomater 2018;78:13-22.
22. Liu D, Kang H, Gao M, et al. Exosome-transmitted circ_MMP2 promotes hepatocellular carcinoma metastasis by upregulating MMP2. Mol Oncol 2020;14:1365-80.
23. Chen LH, Chiu KL, Hsia TC, et al. Significant Association of MMP2 Promoter Genotypes to Asthma Susceptibility in Taiwan. In Vivo 2020;34:3181-6.
24. Wu DM, Deng SH, Liu T, et al. TGF-β-mediated exosomal lnc-MMP2-2 regulates migration and invasion of

lung cancer cells to the vasculature by promoting MMP2 expression. Cancer Med 2018;7:5118-29.
25. Ren X, Xu H, Barker RG, et al. Elevated MMP2 abundance and activity in mdx mice are alleviated by prenatal taurine supplementation. Am J Physiol Cell Physiol 2020;318:C1083-91.
26. Wang X, Yang B, She Y, et al. The lncRNA TP73-AS1 promotes ovarian cancer cell proliferation and metastasis via modulation of MMP2 and MMP9. J Cell Biochem 2018;119:7790-9.
27. Han L, Sheng B, Zeng Q, et al. Correlation between MMP2 expression in lung cancer tissues and clinical parameters: a retrospective clinical analysis. BMC Pulm Med 2020;20:283.
28. Zhu Y, Yan L, Zhu W, et al. MMP2/3 promote the growth and migration of laryngeal squamous cell carcinoma via PI3K/Akt-NF-κB-mediated epithelial-mesenchymal transformation. J Cell Physiol 2019. [Epub ahead of print].
29. Ma L, Zheng LH, Zhang DG, et al. CHCHD2 decreases docetaxel sensitivity in breast cancer via activating MMP2. Eur Rev Med Pharmacol Sci 2020;24:6426-33.
30. Kim KM, Chung KW, Jeong HO, et al. MMP2-A2M interaction increases ECM accumulation in aged rat kidney and its modulation by calorie restriction. Oncotarget 2018;9:5588-99.
31. Wang HL, Zhou PY, Zhang Y, et al. Relationships between abnormal MMP2 expression and prognosis in gastric cancer: a meta-analysis of cohort studies. Cancer Biother Radiopharm 2014;29:166-72.
32. Ruiz-Gómez G, Vogel S, Möller S, et al. Glycosaminoglycans influence enzyme activity of MMP2 and MMP2/TIMP3 complex formation - Insights at cellular and molecular level. Sci Rep 2019;9:4905.
33. Ren F, Wang D, Wang Y, et al. SPOCK2 Affects the Biological Behavior of Endometrial Cancer Cells by Regulation of MT1-MMP and MMP2. Reprod Sci 2020;27:1391-9.
34. Xie Z, Fang T. The expression and significance of NF-κB, MMP1, and MMP2 in rats with abdominal aortic aneurysm. Cell Mol Biol (Noisy-le-grand) 2020;66:17-23.
35. 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;9:4084-100.
36. Cheng X, Zhang G, Zhang L, et al. Mesenchymal stem cells deliver exogenous miR-21 via exosomes to inhibit nucleus pulposus cell apoptosis and reduce intervertebral disc degeneration. J Cell Mol Med 2018;22:261-76.
37. Xu X, Wang D, Zheng C, et al. Progerin accumulation in nucleus pulposus cells impairs mitochondrial function and induces intervertebral disc degeneration and therapeutic effects of sulforaphane. Theranostics 2019;9:2252-67.
38. 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;8:108.
39. Cazzanelli P, Wuertz-Kozak K. MicroRNAs in Intervertebral Disc Degeneration, Apoptosis, Inflammation, and Mechanobiology. Int J Mol Sci 2020;21:3601.
40. Chen D, Xia D, Pan Z, et al. Metformin protects against apoptosis and senescence in nucleus pulposus cells and ameliorates disc degeneration in vivo. Cell Death Dis 2016;7:e2441.

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